Bacterial β-Ketoacyl-Acyl Carrier Protein Synthase III (FabH): An Attractive Target for the Design of New Broad-Spectrum Antimicrobial Agents

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Abstract: The emergence of drug resistant strains of important human pathogens has made urgent the necessity of finding new targets and novel antimicrobial agents. One of the most promising targets is FabH. In this review we summarize the progress made in the design of FabH inhibitors and the role played by the 3D-structure of the enzyme in the drug design process.

Key Words: FabH, FAS, FabH inhibitors, drug design.

INTRODUCTION

Bacterial diseases have not yet been overcome. More than one-third of the world population is likely infected by bacterial pathogens and two million fatalities occur per year from bacterial infections [1]. Antibiotic resistance has increased over the past two decades, achieving today almost every human pathogen and every class of antimicrobials in clinical use [2]. Contributing to the dilemma, only two new classes of antibiotics have been introduced over the past 30 years: the oxazolidinones, represented by linezolid, and the lipopeptide daptomycin [3]. The relatively small amount of antibiotic classes currently in use is directed toward a small subset of essential bacterial targets. Clearly, new approaches to the discovery of novel antibacterial are required and innovative strategies will be necessary to identify novel and effective candidates.

During the last decade, with the beginning of the genomics era was assumed that the genomics would provide a plethora of novel targets and hence a flood of new therapeutic agents [4]. Nevertheless, not all proteins in the bacteria genome can serve as target for broad spectrum antimicrobial agents because they do not fulfill with the desirable properties for an ideal bacterial target such as: the protein must be essential for cell survival; it should be present in multiple bacterial species and must be selective or not to have close homologue in the human genome.

Historically, different targets in key areas of the bacterial cell cycle have been studied such as those related with the transcription and translation of genetic code; cell wall biosynthesis; metabolic pathways; cell division; virulence factors; resistance mechanism and so on [5]. Among them, one of the most attractive biochemical pathways to be used as the target for new antibacterial agents is the fatty acid biosynthesis (FAS). This pathway have been demonstrated to be essential for the bacteria cell survival [6], and differs considerably from human FAS pathway. While in humans fatty acid synthesis occurs in a homodimeric multifunctional enzyme [7, 8], in bacteria the pathway is composed of various discrete enzymes and each one can be considered a putative molecular target. Those features make the type II FAS pathway a potential target for new antimicrobial agents.

A key enzyme in this pathway is the β-ketoacyl-acyl carrier protein synthase III (FabH), which is the responsible enzyme of the first pathway reaction and play an important regulatory role. FabH has also been demonstrated to be essential for organism survival and it is presented in a wide number of important human pathogens. Furthermore, some chemical compounds has shown to inhibit FabH from diverse microorganisms, including multi-drug resistant strains These facts support the idea that FabH can be used as an effective molecular target for the development of new antimicrobial agents.

Taking into consideration the recent availability of determined three-dimensional (3D) structures of FabH from different microorganisms, in this review we attempt to summarize the recent progress made in the field of FabH inhibitors and the implications of those crystal structures for the structure-based drug design of new broad-spectrum antibacterial agents.

BACTERIAL TYPE II FAS: THE BIOLOGICAL PROCESS

In bacteria, each enzyme catalyzes a particular reaction and some steps in the pathway can be catalyzed by more than one enzyme, while the acyl carrier protein (ACP) is the responsible for the transfer of substrates along the whole pathway. The full pathway have been extensively reviewed elsewhere [9, 10] and widely characterized in *E. coli* [11, 12]. A schematic view of the entire pathway is depicted in Fig. (**1**).

The first step in the fatty acid biosynthetic pathway is the synthesis of malonyl-ACP from ACP and malonyl-CoA. This reaction is catalyzed by malonyl-CoA: ACP transacyclase

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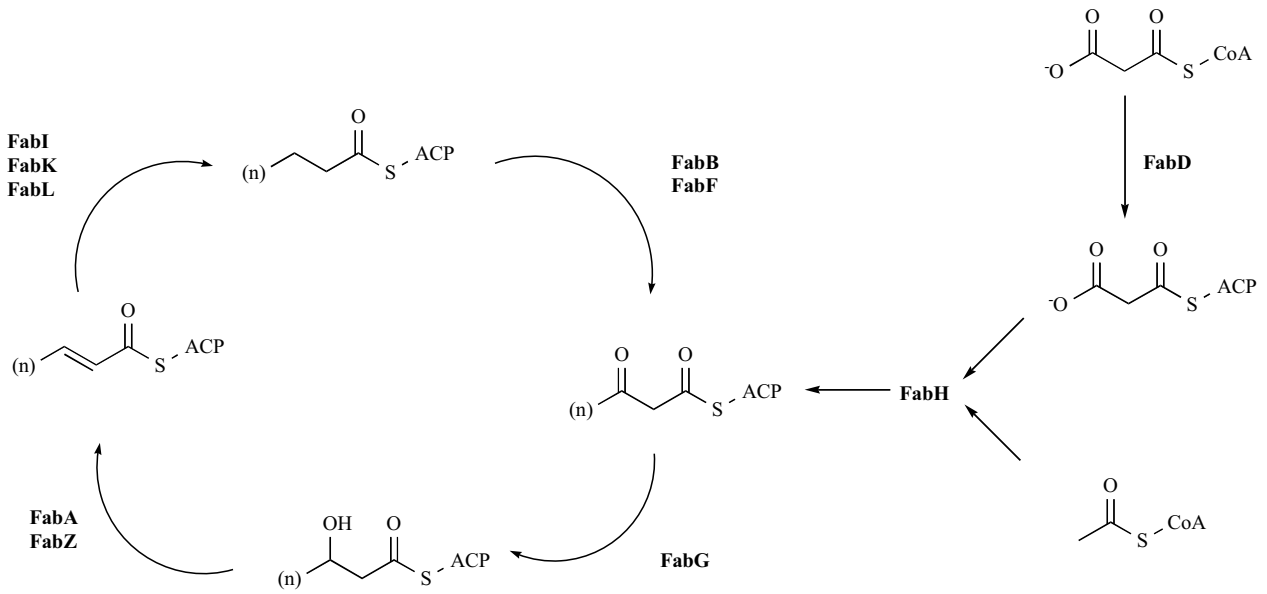

Fig. (1). Type II FAS pathway in bacteria. FabH is the initiating enzyme in the elongation cycle and plays a key regulatory role in the whole pathway. The same reaction step can be catalyzed by more than one enzyme. In some microorganism more than one enzyme catalyzing certain step can be found. On the other hand, FabH is present in a wide range of pathogens and no other enzyme can make its function.

(FabD) [13]. Malonyl-ACP is then condensed with acetyl-CoA by β-ketoacyl-ACP synthase III (FabH) yielding acetoacetyl-ACP [14, 15], which initiates the elongation cycle and is reduced by β-ketoacyl-ACP reductase (FabG) [16]. The β-hydroxybutyryl-ACP is dehydrated later by either FabA or FabZ to form trans-2-enoyl-ACP [17], which is further reduced by enoyl-ACP reductase (FabI) to form acyl-ACP [18]. In subsequent steps malonyl-ACP is condensed with acyl-ACP either by FabB or FabF [14, 19, 20]. Mature acyl-ACPs are obtained through further cycles while the cycle is regulated by the feedback inhibition of FabH and FabI [17, 20].

All the enzymes involved in fatty acid biosynthetic pathway have been successfully used as drug targets for the development of new potent antibacterial agents and have been previously reviewed [10, 21-23]. Inhibitors have been developed targeting FabD [24], the condensing enzymes FabB and FabF [25-31], the reductase FabG [32], dehydratases FabA [33] and FabZ [34], enoyl-ACP reductases I (FabI) [35-40] and II (FabK) [39] and β-ketoacyl-acyl carrier protein synthase III (FabH) [41-45].

FABH AS A DRUG TARGET: DIFFERENCES AND ADVANTAGES

As shown in Fig. (**1**), FabH initiates the elongation cycles catalyzing the condensation of acyl-CoA and malonyl-ACP to form acetoacetyl-ACP [14] and regulates the entire pathway *via* a feedback inhibition mechanism by long chain acyl-ACP [46]. FabH is also an essential enzyme [47, 48]. This enzyme differs, in sequence and structure, from the rest of the condensing enzymes (FabB and FabF) and its catalytic triad (Cys, His, Asn) is different compared with the FabB and FabF enzymes (Cys, His, His) [49-52]. FabH has no close homologous in humans and its selectivity for acetyl-CoA over acyl-ACP diverge from the other condensing enzymes [14, 53].

On the other hand, FabH is present in many important human pathogens, most of them having multiresistant strains, such as *Escherichia coli* (*ec*FabH), *Staphylococcus aureus* (*sa*FabH), *Mycobacterium tuberculosis* (*mt*FabH), *Enteroccoccus faecium* (*ef*FabH), *Streptococcus pneumoniae* (*sp*FabH), *Pseudomonas aeruginosa* (*pa*FabH), *Neisseria meningitidis* (*nm*FabH) and *Haemophilus influenzae* (*hi*-FabH) [14, 44, 54-57]. In Table **1** appear the most important residues involved in ligands binding in *E. coli* and the corresponding amino acids in various microorganisms. The numbering of residues corresponds to *E. coli* and is the same numbering scheme used in the rest of the paper.

From Table **1** is clear that most residues involved in ligands binding are conserved across species or are mutated through synonym mutations. The most important difference is the mutation of Phe87 which determines the active site tunnel size and the substrate specificity across species. These differences and their implications for the design of new FabH inhibitors will be widely explained in this review.

All the FabH attributes, previously discussed, strongly suggest that this enzyme is a potential target for the development of new potent antibacterial agents having broad-spectrum activity.

THREE DIMENSIONAL STRUCTURE OF FABH

FabH in *E. Coli* is a 35kDa protein which is active, in solution, as a 70kDa homodimer [58, 59]. Crystal structure of FabH has been solved for various species. Microorganisms for which the three dimensional structures are available at the Protein Data Bank (PDB) are *E. Coli* [41, 49-51], *M. tuberculosis* [52, 60, 61], *S. aureus* [62] and *T. thermophilus*.

Table 1. Conservation of the Residues in the Binding Tunnel of FabH Across Different Species. *Catalytic Residues are Marked as Italic and Mutated Ones are Marked in Bold. Numbering Corresponds to that of E. coli and Amino Acids are Represented Through the Standard one Letter Code. Remarkable is that Most Residues are Strictly Conserved or are Synonyms Mutations. The Major Mutation Influencing Substrate Specificity and Differences in Inhibitors Potency Across Species is which Occur at F87*

Organism	R36	T37	W32	R151	I155	I156	F157	N274	M207	L189	G209	N210	C112
E. Coli	R	T	W	R	I	I	F	*N*	M	L	G	N	*C*
H. influenzae	R	T	W	R	**V**	**L**	F	*N*	M	L	G	N	*C*
M. tuberculosis	R	T	W	R	**F**	I	F	*N*	**L**	**I**	G	**P**	*C*
N. meningitidis	R	T	W	R	**V**	**L**	F	*N*	M	L	G	**P**	*C*
P. aeruginosa	R	T	W	R	**A**	**L**	F	*N*	M	L	G	**R**	*C*
S. aureus	**M**	T	W	R	**V**	**L**	F	*N*	M	L	G	**R**	*C*
S. pneumoniae	R	T	W	R	**V**	**L**	F	*N*	M	L	G	**R**	*C*
E. faecium	R	T	W	R	**V**	**L**	F	*N*	M	L	G	**R**	*C*
Organism	**G305**	**G306**	**F304**	**R249**	**N247**	**A246**	**F213**	**V212**	**A216**	**H244**	**I250**	**F87**	
E. Coli	G	G	F	R	N	A	F	V	A	*H*	I	F	
H. influenzae	G	G	F	R	N	A	F	**T**	A	*H*	I	**Y**	
M. tuberculosis	G	**A**	**Y**	R	N	A	F	V	A	*H*	I	**T**	
N. meningitidis	G	G	F	R	N	A	F	V	A	*H*	I	F	
P. aeruginosa	G	**A**	F	R	N	A	F	V	A	*H*	I	C	
S. aureus	G	G	F	R	N	A	F	V	A	*H*	I	F	
S. pneumoniae	G	G	**I**	R	N	A	F	V	A	*H*	I	**M**	
E. faecium	G	G	**Y**	R	N	A	F	**I**	A	*H*	I	**T**	

The overall structure of the enzyme monomer is the same for these four microorganisms, as shown in Fig. (**2a**).

The FabH monomer structure can be reduced to the duplication of two almost identical parts (N and C terminals), despite the only 11% sequence identity of both. In *E. coli* each part corresponds to residues 1-171 and 172-317, respectively. The overall structure of *E. Coli* FabH is a mixed α/β structure make up of five β-strands and three α-helices, where the five β-strands forming a mixed β-sheet. In the dimer, the third β-strand of the N-terminal part of one monomer interacts with the same strand from the other monomer to form a ten stranded β-sheet (see Fig. (**2b**)). On the other hand, the fold of this enzyme has been determined to be very similar to the thiolases [63, 64].

The crystal structures of *E. coli* FabH is available in the *apo* form [50], as well as forming a complex among the acetylated enzyme and the enzyme with CoA and malonyl-CoA [49, 51]. Also the crystal structure of the *E. coli* FabH in complex with a bound inhibitor has been determined [41]. These crystal structures have revealed that the active site of the enzyme is a deep and hydrophobic tunnel where the catalytic residues (Cys112, His244 and Asn274) are positioned at the end of such tunnel.

In the dimer form, two active sites are located in opposite sides of the molecule and each one is mainly formed by residues of its own subunit. In the case of *E. coli* FabH the size of the hydrophobic tunnel is limited by the presence of a phenylalanine residue in the 87 position of the other chain and consequently, the size of the acyl chain that can be bind is limited to four carbons. This is different for the *M. tuberculosis* FabH where acyl chains of 16 carbons can bind due to the mutation of Phe87 to threonine (see Table **1**) [52]. This mutation opens a new channel for this microorganism that in *E. coli* remains blocked by the presence of the phenylalanine which can bind long chain acyl substrates. In Fig. (**3**) are represented the binding channels of the primer in *E. coli* and *M. tuberculosis*. As will be discussed in the next section, the presence of Phe87 is one of the main factors influencing the differences in substrate specificity among different species.

SUBSTRATE BINDING AND SPECIFICITY

As discussed before, the length of acyl-CoA primers that can be bound to FabH range from two to eighteen carbons across species [44, 58, 62, 65, 66]. In the same way, in some microorganism FabH only bind straight chain acyl-CoA sub-

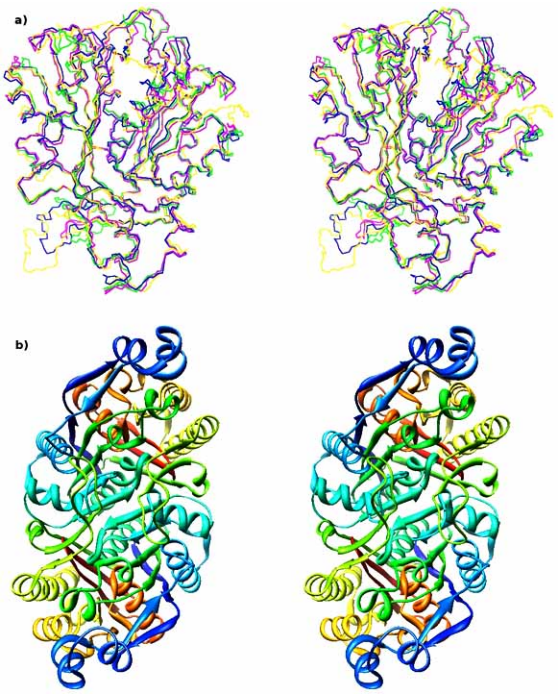

Fig. (2). FabH structure. a) Stereo view of the superposition of FabH monomer backbone from *E. coli* (PDB code 1EBL, blue), *S. aureus* (1ZOW, purple), *T. termophylus* (1UB7, green) and *M. tuberculosis* (1U6E, yellow) showing the identical overall fold for the four species for which crystal structures are available.

b) Ribbon diagram of *E. coli* FabH dimmer, the central β sheet corresponds to the continuos ten stranded beta sheet formed upon dimmer formation. Both images were generated using the USCF CHIMERA package [67]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper).

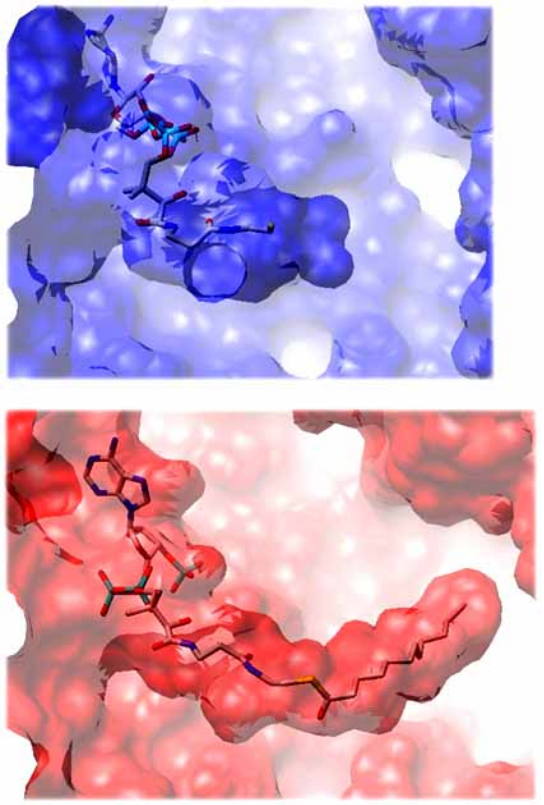

Fig. (3). Comparison of the size of the FabH primer binding pocket in *E. coli* and *M. tuberculosis*. Cross section of FabH from *E. coli* (upper image, PDB code 1EBL) and *M. tuberculosis* (lower image, PDB code 1U6S) showing the acyl primer binding pocket in both microorganisms. Before surface generation the two enzymes were superposed, so the proteins are represented at the same scale. Stick lines inside the binding tunnels represent acetyl-CoA in ecFabH and lauroyl-CoA in mtFabH. As shown in the picture the primer binding pocket in mtFabH is much larger than in ecFabH. The picture was produced using the UCSF CHIMERA package [67].

strates and in others, such as *S. aureus*, where branched chain fatty acids are needed for survival, FabH is capable of using branched chain acyl-CoA primers as substrate; although the amino acids in direct contact with the acyl primer are the same as those for the *ec*FabH [65].

The determination of the three dimensional structure of the *S. aureus* FabH was crucial for the explanation of this fact. As discussed by Qiu *et al*. [62], the acyl primer binding pocket in *S. aureus* FabH is larger than for the *E. coli* enzyme, as a consequence of a shift in residues in contact with the substrate with respect to their position in the *E. coli* enzyme. As a consequence of this shift, the side chain of Phe87 moves 3.0 Å away from the position of this residue in the *E. coli* structure. This shift can be explained on the basis of the differences in sequences between the two species at the dimer interface residues that allows an increased volume of the primer binding pocket.

Additional information provided by the crystal structures is related to the binding mode of the acyl-CoA primer. In all cases the pantethiene moiety of CoA fits in the active site tunnel and the phosphate groups of CoA form salt bridges with the conserved Arg36 and possibly with Arg151. Furthermore the adenine group of CoA stacks between Trp32 and Arg151, while the acyl group side chain is accommodate in the bottom of the tunnel.

CATHALYTIC MECHANISM AND SUBSTRATE BINDING

In all organisms the FabH enzyme catalyzes the condensation of malonyl-ACP with acyl-CoA by a unique mechanism that consists of a two steps (ping-pong) Claisen condensation reaction [14, 15]. The first step is the transfer of the acyl group to the cysteine active site and the release of CoA, while the second one is the condensation of the cysteine bound acyl group with malonyl-ACP yielding acetoacyl-ACP.

The general mechanism of the reaction have been extensively studied and is the same for all bacteria, despite of the differences in substrate specificity that exist among species [49, 51, 61, 62]. The first half of the whole reaction includes the binding of the acyl-CoA primer to the enzyme, the deprotonation of the active site cysteine where the essential thiol group for all condensing enzymes is provided [68], and the nucleophilic attack to the acyl-CoA by the thiolate anion of

the deprotonated cysteine, the subsequent acylation of that cysteine and the release of CoA-SH. This first half reaction is represented in Fig (**4**).

The mechanism responsible for the deprotonation of the active site cysteine differs considerably from one to other enzymes that form acetylated intermediates, where a hydrogen bond between such cysteine and a hydrogen bond acceptor residue promotes the deprotonation of the active cysteine [69, 70]. In the case of FabH, both the active site histidine (His244) and asparagine (Asn274) are to far from Cys112 to promote it deprotonation. Furthermore, site directed mutagenesis studies of the *E. coli* enzyme have confirmed the non essential role of His244 and Asn274 for the transacylation halve reaction [49]. In this study, H244A and N274A mutants showed increased transacylation activities when compared with the wild type enzyme. On the basis of those results and the crystallographic structure of the enzyme that shows that Cys112 is located at the N-terminus of a long α helix, Davies *et al*. [38] proposed a mechanism by which the nucleophilicity of the cysteine active site is due to the effect of the α helix dipole moment [71] and in this position Cys112 can receive the benefit of a half unit of positive charge generated by the helix dipole.

More recently, site directed mutagenesis experiments conducted against *M. tuberculosis* FabH has shown certain influence of the active site histidine (His258) on the transacylation reaction [61]. Unlike the above mentioned results in *E. coli*, the *M. tuberculosis* H258A FabH mutant only retains a 22.6% of the activity of the wild type enzyme. Using this result and considering the presence of a water molecule in the crystallographic structure located between His258 and the active site cysteine (Cys122), a new role for His258 was proposed in which the epsilon nitrogen (Nε) of His258 is capable of extracting a proton from the water molecule and the formed hydroxyl anion can deprotonate Cys122. The fact that the H258A mutant still retains some transacylation activity shows that this residue helps in the deprotonation of Cys122, but transacylation appear to be partially promoted *via* the α helix dipole moment as occur in *E. coli*.

Other important feature of FabH is the so-called oxyanion hole, a region of the active site formed by the backbone amide groups of Cys112 and Gly306. The function of this region is to stabilize the developing negative charge of the tetrahedral transition state during the formation of the acetyl enzyme intermediate (Fig. (**4b**)). In various FabH crystal structures an electronic density is observed in the oxyanion hole and in one of them this density is identified as a water molecule or a hydroxyl anion that makes hydrogen bond interactions with the nitrogen backbone atoms of Cys112 and Gly306 and that can share a proton whit the thiol group of Cys112 [51]. Thus, this water molecule has also been proposed to be relevant for the deprotonation of Cys112.

Once the acyl enzyme intermediate is formed, the carbonyl oxygen of the acetyl-thioester intermediate displaces the water molecule of the oxyanion hole and forms strong hydrogen bonds with the amides of Cys112 and Gly306. Then CoA is released and malony-ACP, that serve as the other substrate for the condensation reaction and have been shown to bind preferentially to the acetylated form of FabH [53], binds to FabH. This last step of the acetylation half reaction is shown in Fig. (**4c**).

In both *E. coli* and *M. tuberculosis* His244 and Ans274 have been demonstrated to be essential for the second half of the reaction, specifically for the decarboxylation reaction [49, 62]. The crystal structures of FabH shows how, as a

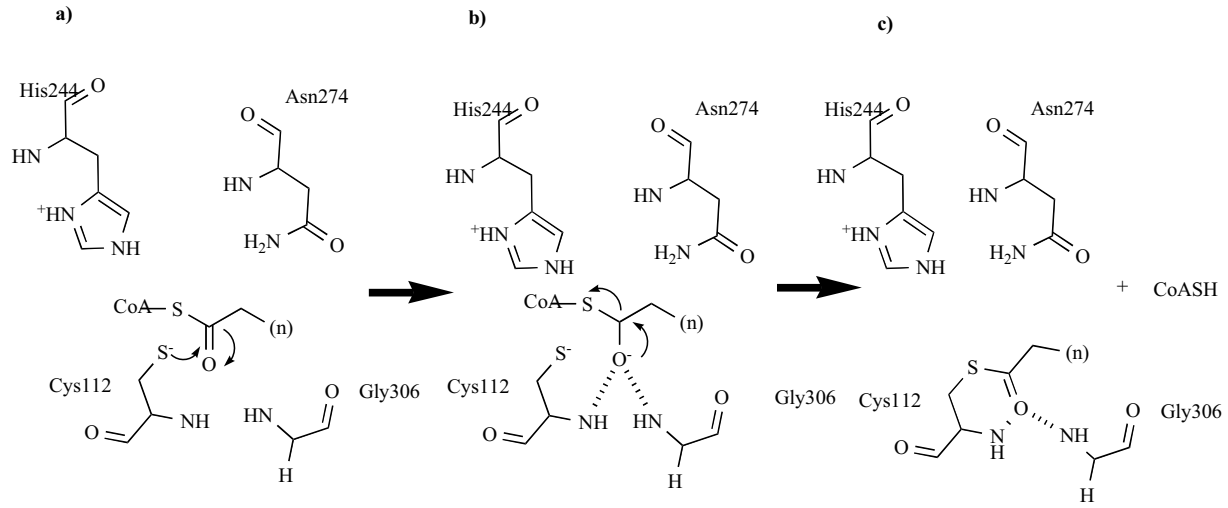

Fig. (4). Acetylation half reaction mechanism.

a) The first step is the binding of the acyl-CoA primer to the enzyme, the deprotonation of the cysteine active site and the nucleophilic attack to the acyl-CoA by the thiolate anion of the cysteine active site.

b) The developing negative charge of the transition state becomes stabilized by the oxyanion hole, formed by the backbone amides of Cys112 and Gly306.

c) Finally, the enzyme becomes acetylated and CoA-SH is released from the enzyme that is then ready to enter the second half of the condensation reaction. The oxyanion hole serves to stabilize the carbonyl group of the acetylated cysteine.

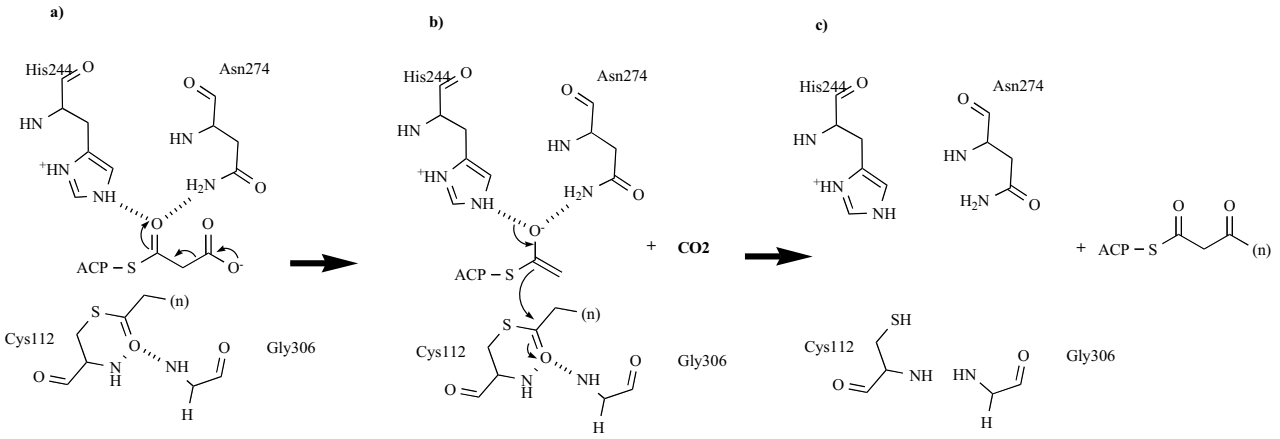

Fig. (5). Second half of the FabH condensation reaction.

a) A network of hydrogen bonds locates Asn274 and His244 pointing to the same position in the active site.

b) The configuration of His244 and Ans274 "mimics" the oxyanion hole and help to stabilize the developing negative charge on the malonyl-ACP thioester carbonyl to promote the formation of the enol intermediate and the formation of a carbanion on the C2 of malonate that attacks the acyl group bound to the active site cysteine and the resulting tetrahedral transition state is again stabilized by the oxyanion hole and a CO_2 molecule is released.

c) Finally, acetoacyl-ACP is released.

consequence of a network of hydrogen bonds, the Nε atom of His244 and the Nδ Ans274 points towards the same location in the active site. This configuration of the catalytic residues Asn274 and His244 "mimics" the oxyanion hole and help to stabilize the developing negative charge on the malonyl-ACP thioester carbonyl to promote the formation of the enol intermediate and the formation of a carbanion on the C2 of malonate. This carbanion then attacks the acyl group bound to the active site cysteine and the resulting tetrahedral transition state is again stabilized by the oxyanion hole. As a final result of the whole reaction acetoacyl-ACP and CO_2 are produced. This second half of the FabH whole reaction is shown in Fig. (**5**).

FABH INHIBITORS

Many research groups have focused on the search of new chemical compounds that can selectivity inhibit FabH from various microorganisms. Some inhibitors have been found that inhibits the enzyme of various species, including important human pathogens such as *E. coli, H. influenzae, S. pneumoniae, M. tuberculosis, E. faecalis* and *S. aureus*. On the other hand, the determination of the crystal structure of this enzyme from various species have aided in the structure-based design of new potent inhibitors. Four basic types of chemical compounds have been demonstrated to inhibit FabH (Fig. (**6**)).

One of the compounds that weakly inhibit FabH is thiolactomycin (TLM, compound (**1**), Fig. (**6**)), a natural product produced by actinomycetes [72]. TLM inhibits the three condensing enzymes, although FabH have been shown to be less sensitive to this compound. The IC_{50} of TLM against *E. coli* FabH have been determined to be of 110 μM [73] and recent works have evidenced that potency of TLM differs from one specie to other [44, 66]. In that sense *M. tuberculosis* FabH have been shown to be 3 times more sen-

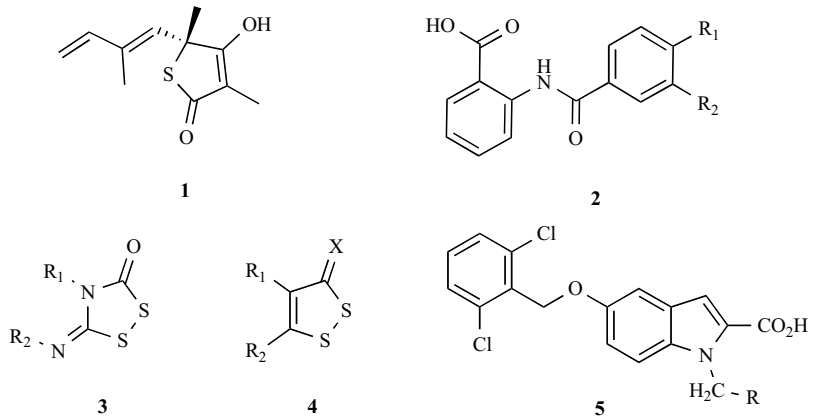

Fig. (6). Four known types of FabH inhibitors. Thiolactomycin (1), benzoylaminobenzoic acids (2), 1,2-dithiole-3-ones (3 and 4) and indole inhibitors (5).

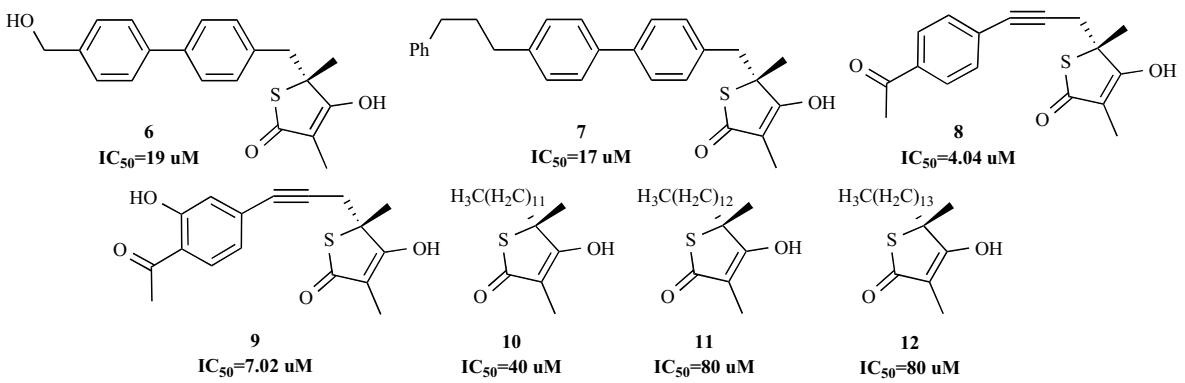

Fig. (7). More potent TLM analogous FabH inhibitors. IC_{50} values are reported against mtFabH [74-76].

sitive to TLM than the *E. coli* enzyme [66], while the enzymes from *S. pneumoniae* and *H. influenzae* are found to be 4-5 fold more susceptible to TLM [44], compared with *E. coli* FabH.

The results achieved with TLM were used as starting point for the design of TLM analogous with improved activity against *M. tuberculosis* FabH [74, 75]. Biphenyl and acetylene based analogous of TLM have been synthesized and assayed against *mt*FabH. The inhibition values achieved for these analogous were improved compared with those obtained with TLM for both acetylene and biphenyl based analogous. In Fig. (**7**) are depicted the TLM analogous more actives against *mt*FabH described by Senior *et al.* (compounds **6-9**) [74, 75].

Kim *et al.* have also investigated the influence of different substitutions at the position 5 of TLM [76]. Only three out of 31 substitutions tested showed modest improved activity against *M. tuberculosis* FabH, while none of the TLM variants showed activity against *E. coli* FabH. Only the variants able to increase the IC_{50} value of TLM were those that included long aliphatic chains (12-14 carbon atoms) at the 5 position (Fig. (**7**), compounds **10-12**). In this work was also demonstrated the essentiality of each of the two conjugated double bonds adjacent to the thiolactone ring.

Despite no experimental or theoretical evidence support this fact, a bulky substituent as the biphenyl group at the 5 position of TLM will only fit the active site of an enzyme with a large volume in the cavity, which is the case of *M. tuberculosis* FabH. In the same way, a long aliphatic chain at the position 5 of TLM could perfectly fit the channel that extends from the active site cysteine in *M. tuberculosis* and accommodates the long chain acyl primers. This is consistent with the fact that no inhibition is reported for the *E. coli* FabH when these variants of TLM are assayed.

Nie *et al.* found a novel series of benzoylaminobenzoic acids as potent inhibitors of *E. faecalis* FabH (Fig. (**6**), compound (**2**)) [45]. The authors proposed an approach that started filtering three million chemicals from various commercial sources using computational filters, including substructure, 2D similarity, phamacophore with size and shape constraints, and docking with FlexX. After applying all filters about 2500 compounds were selected for the primary FabH inhibition screening and 27 exhibited *in vitro* activities below 10 μM against *E. faecalis* and *H. influenzae* FabH. These 27 compounds were grouped in four structurally diverse series and benzoylaminobenzoic acids were selected for further optimization.

Co-crystallization studies were carried out with the most potent compound (Fig. (**8**), compound **13**), and the *E. faecalis* FabH (structure not deposited in the PDB). After the crystallographic analysis two fundamental interactions were found to guide the binding process: an ionic interaction with the protonated nitrogen of the active site histidine and a hydrogen bond with amine group of the side chain of the catalytic asparagine. On the bases of these results, a new campaign of structure based drug design was carried out and as a final result a set of potent *in vitro* inhibitors of FabH were proposed, many of them having good antibacterial activity when were evaluated against various Gram-positive and Gram-negative pathogens. The most potent compound (**14**) *in vitro* after all optimization steps is shown in Fig. (**8**).

More interestingly, the inhibitors proposed by Nie *et al.* showed good inhibitory potency when tested *in vivo* against *E. coli, S. pneumoniae, S. pyogenes, E. faecalis, N. meningitidis* and a Methicillin resistant *S. aureus* strain (MRSA) (see Fig. (**8**), compound (**14a**)).

This experimental data from Nie *et al.* have been used by Ashek and Cho for docking and 3D QSAR studies [77]. These authors found a correlation between the calculated docking scores and the inhibitory activities reported by Nie *et al*. They were also able to describe in details and quantify the influence of each substitution in the activity of benzoylaminobenzoic acids and to find a good correspondence between the CoMSIA field and the receptor three dimensional structures when they were overlapped.

A virtual screening effort for the identification of FabH inhibitors has recently been reported [42]. This work led to the identification of nine compounds, four of them were 1,2-dithiole-3-ones (Fig. (**6**), compounds (**3,4**)). In a second screening effort 13 more analogous of 1,2-dithiole-3-ones were identified and assayed for FabH inhibition. In Fig. (**9**) are represented the more potent 1,2-dithiole-3-ones "*in vitro"* assayed against *E. coli* and *S. aureus* FabH.

The reactivity of 1,2-dithiole-3-ones can make them to target multiple cellular processes, limiting the specificity of

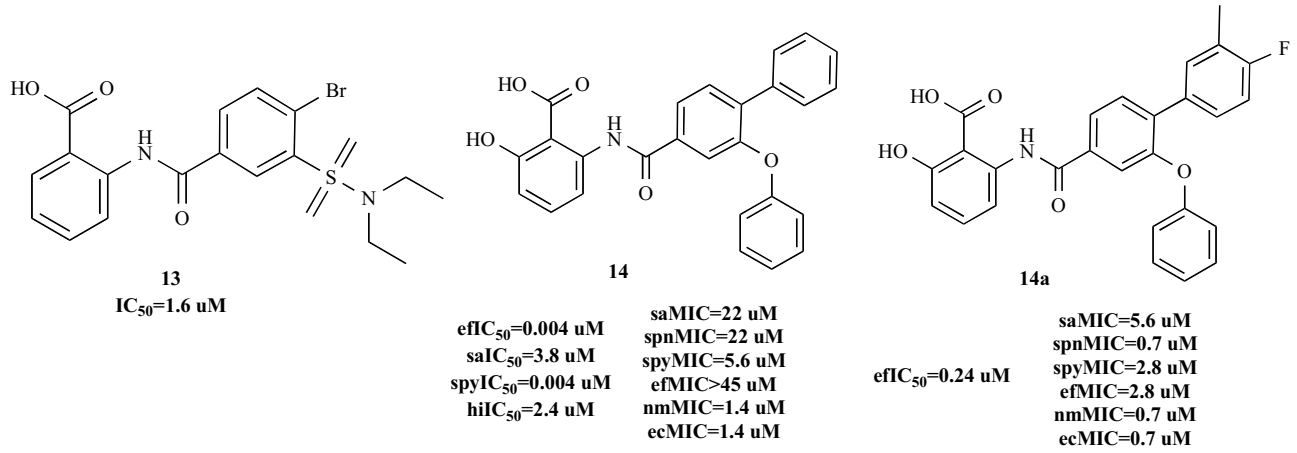

Fig. (8). Benzoylaminobenzoic acids FabH inhibitors [45], compound 13 was co-crystallized with efFabH as starting point for the further structure based design of inhibitors. The most potent "*in vitro*" inhibitor was 14 with a IC_{50} value of 4 nM against efFabH, nevertheless one of the most potent "*in vivo*" inhibitors is (14a). Reported values are IC_{50} for efFabH ($efIC_{50}$), saFabH ($saIC_{50}$), *S. pyogenes* FabH (spyFabH, $spyIC_{50}$) and hiFabH ($hiIC_{50}$). Also are reported the Minimum Inhibitory Concentration for the "*in vivo*" assays against *S. aureus* (saMIC), *S. pneumoniae* (spnMIC), *S. pyogenes* (spyMIC), *E. faecalis* (efMIC), *N. meningitides* (nmMIC) and *E. coli* (ecMIC).

this kind of inhibitors. Furthermore, the mechanism by which 1,2-dithiole-3-ones inhibit FabH is not clear and it can involves the covalent linkage of the compound to the enzyme through the opening of the ring at the S-2 position because of a nucleophilic attack by Cys112.

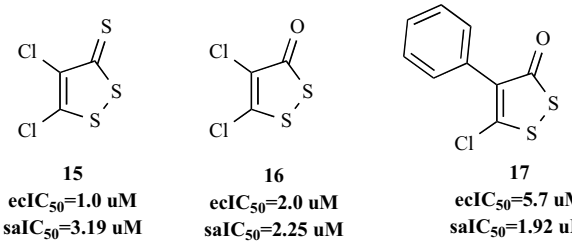

Fig. (9). Potent 1,2-dithiole-3-ones FabH inhibitors proposed by He et al. [42], $ecIC_{50}$ and $ecIC_{50}$ represents the minimum inhibitory concentrations when assayed against ecFabH and saFabH, respectively.

The only crystallographic structure publicly available of FabH in complex with an inhibitor molecule was solved by Daines *et al.* [41]. Indole inhibitors (Fig. (**6**), compound (**5**)) of FabH were first discovered in a high throughput screening effort against *S. pneumoniae* FabH. Attempts were made for the co-crystallization of compound (**18**), shown in Fig. (**10**), and either *E. coli* or *S. pneumoniae* FabH, but no crystal could be obtained, mainly due to the poor solubility of (**18**). Then a structure-based drug design study was carried out and eight compounds with improved solubility and unmodified key substituents were assayed against *E. coli* and *S. pneumoniae* FabH. Finally, a co-crystal structure of FabH and an inhibitor (compound (**19**), Fig. (**10**)) was solved and the predicted binding mode of indole inhibitors was experimentally corroborated. Interestingly, this type of inhibitors does not interact directly with the catalytic triad but with the rest of the active site tunnel.

The results achieved in the development of FabH inhibitors discussed above, shows that this protein remains as a valid target for the development of new broad spectrum antibacterial agents. A key result that support this fact is that obtained by Nie *et al.* [45] (see compound (14a) in Fig. (**10**)). In this study many microorganisms were used in the investigation of FabH inhibitors. Although differences in potency of FabH inhibitors due to different active site architecture, compound (14a) remains active against the six important human pathogens used in this study.

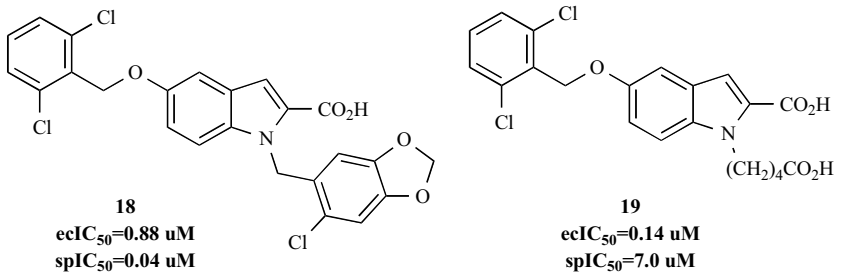

Fig. (10). Potent FabH indole inhibitors proposed by Daines et al. [41] Compound (18) serve as starting point for structure based design of a series of indole analogs with improved solubility. Compound (19) was finally co-crystallized with ecFabH. Inhibition values refer to ecFabH ($ecIC_{50}$) and spFabH ($spIC_{50}$).

PRELIMINARY RESULTS IN THE DEVELOPMENT OF A FABH MODEL SUITABLE FOR VIRTUAL SCREENING

Our research group is currently working in the structure-based drug design of FabH inhibitors. The aim of our work is to develop a suitable docking model for the virtual screening of databases of chemical compounds. As it is the best structurally characterized enzyme, we have chosen the *E. coli* enzyme as the starting point. Among all the available crystallographic structures of *E. coli* FabH we decided to use that where the enzyme forms a complex with a dichlorobenzyloxy-indole-carboxylic acid inhibitor (PDB code 1MZS), since it is the only public structure of a complex FabH-inhibitor. For all the docking calculations we have used DOCK 6.0 [78, 79].

In the PDB only is deposited one of the two subunits of the dimer. As the dichlorobenzyloxy-indole-carboxylic acid inhibitor does not interact with Phe87, no influence of one subunit of the homodimer is observed in the ligand binding to the other subunit. For our virtual screening purposes the influence of Phe87 in ligand binding must be considered and for this reason before any docking calculation the dimer is generated and used as the target structure in the rest of the modeling. The other important thing to be considered is that the active site His244 is protonated in the delta nitrogen.

The preliminary results achieved show that with the set of parameters selected we were able to reproduce the crystallographic pose of the inhibitor with a random middle square deviation (RMSD) of 0.96 Å. When the eight compounds reported as *E. coli* and *S. pneuminiae* FabH inhibitors by Daines *et al.* [41] were mixed with the random set of ~1000 drug-like compounds used in the validation of molecular modeling procedures from Accelrys Corp., the proposed docking scheme was able to rank this eight compounds in the top 40 best scored compounds.

These 40 compounds were rescored, considering the binding pocket and the ligand flexible, using the Amber scoring function implemented in DOCK 6.0. Seven out of eight known inhibitors were positioned in the 20 top scoring compounds, decreasing notably the false positive rate. These preliminary results will be considerably improved in further researches, which will be related with the inclusion of others compounds that have been demonstrated to inhibit *E. Coli* FabH, as those assayed by Nie *et al.* [45], and the inclusion of other target crystallographic structures in the docking in order to, in some way, take into account the receptor flexibility at the first stages of the design.

CONCLUDING REMARKS

As discussed in this review, many advances have been reported in the development of new FabH inhibitors in the last few years and various research groups are working in the search of novel inhibitors for such enzyme. The results achieved in the design of new FabH inhibitors shows that, despite of differences in the three dimensional structure and substrate specificity of the enzyme among different species, the same compounds can inhibit FabH for more than one microorganism.

The availability of crystal structures of FabH of various species in complex with natural substrates and, more important, with inhibitors, will undoubtedly be the major factor which in the next years will influence the process of development of new FabH inhibitors that can serve as new broad spectrum antibacterial agents.

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