Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 10, 2014

Review Article

# DRUG DISCOVERY OF NEWER ANALOGS OF ANTI-MICROBIALS THROUGH ENZYME-INHIBITION: A REVIEW

**MAYURA KALE[1*], MOHAMMAD SAYEED SHAIKH[1]**

[1]Department of Pharmaceutical Chemistry, Government College of Pharmacy, Aurangabad, Maharashtra, India.
Email: kale_mayura@yahoo.com

*Received: 13 Aug 2014 Revised and Accepted: 15 Sep 2014*

**ABSTRACT**

There is a growing interest towards the development of new antibiotics from last decades due to emergence of newer pathogenic bacterial strains with high resistance to powerful antibiotics of last resort. This has caused decline in research for developing newer antibacterial agents. Hence, there is continuous need to develop newer antibiotics that interact with essential mechanisms in bacteria. Recently, enzymes responsible for bio synthesis of the essential amino acid lysine in bacteria have been targeted and it has augmented interest to develop novel antibiotics and to enhance lysine yields in over-producing organisms. Peptidoglycan layer consists of a beta-1,4-linked polysaccharide of alternating N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) sugar units, cross linked by short pentapeptide (muramyl residues) side chain of general structure L-Ala-g-D-Glu-X- D-Ala-D-Ala, where X is either L–Lysine or meso-DAP. Formation of the cross-links makes bacterial cell wall resistant to lysis by intracellular osmotic pressure. Compounds which inhibit lysine or DAP biosynthesis could therefore be very effective antibiotics and novel targets. Lysine is a constituent in gram-positive bacteria while meso-*DAP* occurs in gram negative ones. In this review, substrate-based inhibitors of enzymes in the *DAP* pathway and inhibitors that allow better understanding of enzymology of the targets and provide insight for design of new inhibitors have been discussed. Resistant bacterial strains can be inhibited by using synthetic enzyme inhibitors of *DAP* pathway that are less toxic to mammals. Newer antimicrobial drugs can be thus developed by targeting the enzymes involved in this pathway.

**Keywords:** L-lysine, Antimicrobial resistance, Diaminopimelic acid, Enzyme inhibitors, Diaminopimelate epimerase.

## INTRODUCTION

Literally, the word 'antibiotic' refers to the compound that opposes life and typically antibacterial drug is the one which interferes with the structures or processes essential for the growth or survival of microorganisms. Today, antibiotic resistance is widespread and ever increasing. Hence, there is a need to continuously develop newer antibacterial agents which shall combat with the bacterial survival strategies. However, clinically significant antibacterial resistance develops in periods of few months to years. For penicillin resistance, the phenomenon was noted within two years after introduction of penicillin in the mid 1940s [1-3].

A serious attention in the search for novel antibacterial compounds has developed due to the recent emergence of mutated bacterial strains resistant to currently available antibiotics. These compounds should be targeted toward biomolecules that are essential for bacterial viability but are absent in mammals [4, 5]. Both of these criteria are met by the diaminopimelic acid (DAP) and lysine biosynthesis pathway, thereby presenting multiple targets for novel antimicrobial agents [6, 7]. Lysine is an essential amino acid required in protein synthesis and is also a constituent of the peptidoglycan layer of cell walls in gram positive bacteria. The lysine biosynthesis also produces D,L-DAP, (*meso*-DAP), which is a component of the peptidoglycan layer of gram negative bacteria and mycobacterial cell walls (Figure 1a). This review focuses on current research which is being carried out on structure-based drug discovery of antimicrobial drugs [8, 9]. DAP is a symmetrical α,α-diaminodicarboxylic acid and exists in three stereoisomeric forms as shown in (Figure 1b).

Among these, *meso*-DAP and (S,S)-DAP and serve as the precursors in the biosynthesis of L-lysine [10]. Peptidoglycan layer consists of a beta-1,4-linked polysaccharide of alternating N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) sugar units as building blocks of cell wall. Attached to the lactyl side chain of NAM unit is a pentapeptide (muramyl residues) side chain of general structure L-Ala-g-D-Glu-X- D-Ala-D-Ala, where X is either L–Lysine or meso-DAP [11-13]. Formation of the cross links makes the bacterial cell wall resistant to lysis. Compounds which inhibit lysine or DAP biosynthesis could therefore be very effective antibiotics, if targeted towards cell wall biosynthesis. The enzymes which catalyze the synthesis of L-lysine in plants and bacteria have attracted interest from two directions; firstly from those interested in inhibiting lysine biosynthesis as a strategy for the development of novel antibiotic or herbicidal compounds and secondly to enhance lysine yields in over-producing organisms. Certain advances in genetic studies have also permitted the researchers to compare protein sequences from many dissimilar organisms and has ultimately resulted in cloning of the biosynthetic genes and their over expression [14]. More than hundred tons of L-lysine are produced annually with *C. glutamicum* mutant strains. Genes directly involved in the synthesis of L-lysine are primary targets to improve the overall fermentation process. *C. glutamicum* genome sequencing has been identified and characterized, *dap*F and *dap*C genes have showed significant effects on L–lysine production when over expressed in *C. glutamicum* strain [15]. Many biological effects such as cytotoxicity, antitumor activities, angiotensin converting enzyme (ACE) inhibitory, immunostimulant and sleep-inducing effects are shown by a range of peptidoglycan monomers such as the potent toxin from *B. pertussis* and *N. gonorrhoeae* and similar DAP containing peptides [16-18].

### Lysine biosynthetic pathway in bacteria

The synthesis of lysine begins with the phosphorylation of L-aspartate to form L-aspartylphosphate catalyzed by *aspartate kinase*. Both *B. subtilis* and *E. coli* genomes encoding show three aspartokinase isozymes, required for different biosynthetic pathways starting from aspartate. *E. coli* has two bifunctional aspartokinase/homo-serine dehydrogenases, *Thr*A and *Met*L, and a monofunctional aspartokinase *Lys*C, which are involved in the threonine, methionine and lysine synthesis; respectively. Transcription of the aspartokinase genes in *E. coli* is regulated by appropriate concentrations of the corresponding amino acids. In addition, *Thr*A and *Lys*C are feedback inhibited by threonine and lysine; respectively [19,20]. Aspartate semialdehyde dehydrogenase converts the L-aspartylphosphate to aspartate semialdehyde. The first two steps of the DAP pathway are catalyzed by *aspartokinase* and aspartate semialdehyde dehydrogenase are common for the biosynthesis of amino acids of the aspartate family, like lysine, threonine and methionine [21]. Dihydrodipicolinate synthase

catalyses the condensation of pyruvate (PYR) and aspartate semialdehyde (ASA) to form 4-hydroxy-2,3,4,5-tetrahydro-L,L-dipicolinic acid (HTPA). This enzyme belongs to the family of lyases, specifically the hydrolases, which cleaves carbon-oxygen bonds [22].

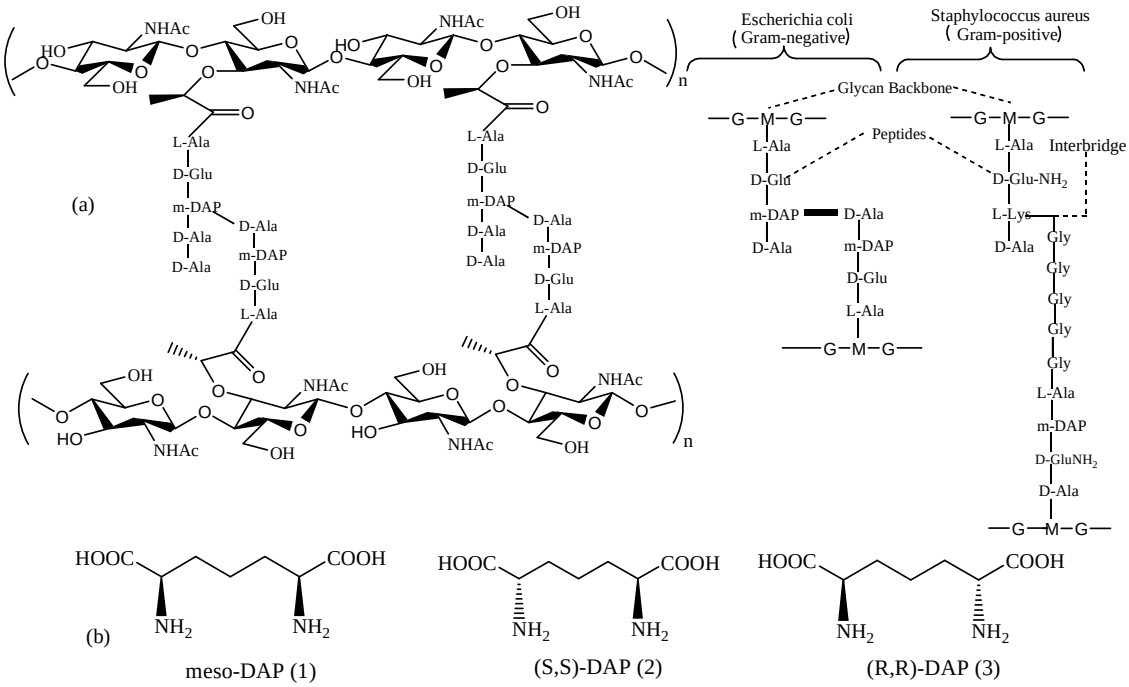


**Fig. 1: (a) Peptidoglycan structure with peptide cross-linking in gram positive and gram negative bacteria. (b) Stereoisomers of diaminopimelic acid (DAP).**

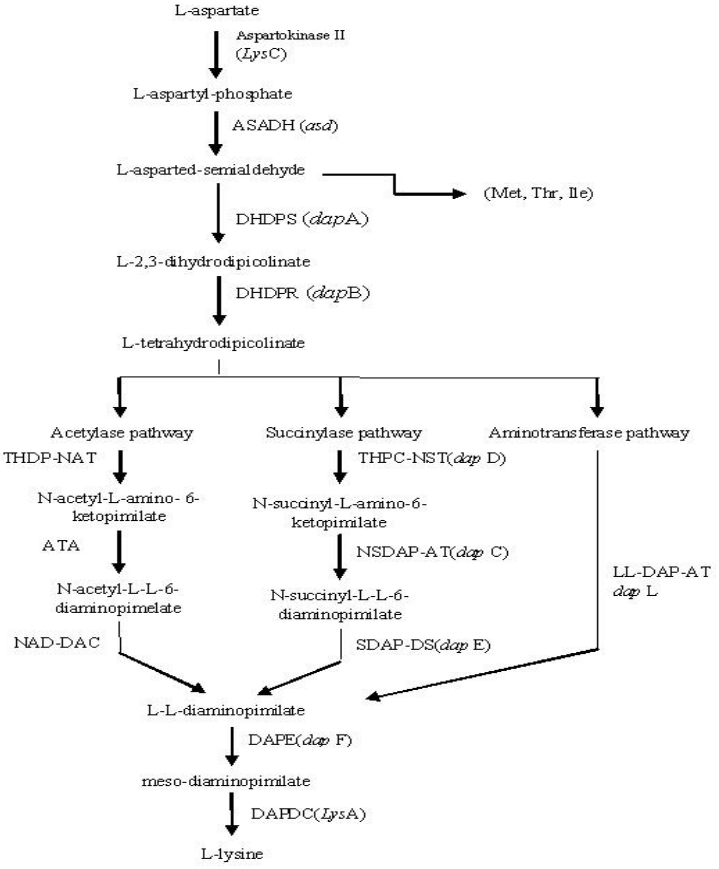


**Fig. 2: Enzymes of the lysine biosynthesis in bacteria operating through acetylase, succinylase and aminotransferase pathways**

The studies using [13]C-labelled pyruvate demonstrate that the product is the unstable heterocycle HTPA. Rapid decomposition of the [13]C-NMR signals of HTPA following its production indicates that formation of L-dihydrodipicolinate (DHDP) occurs via a nonenzymatic step. Dihydrodipicolinate reductase (DHDPR) catalyses the pyridine nucleotide-dependent reduction of DHDP to form L-2,3,4,5,-tetrahydrodipicolinate (THDP). THDP and DHDP are synthesized from aspartate semialdehyde by the products of the *dap*A and *dap*B genes (Figure 2). However, the metabolic pathway then diverges into three sub-pathways depending on the species, namely the succinylase, acetylase and aminotransferase pathways from tetrahydrodipicolinate in bacteria. The presence of multiple biosynthetic pathways is probably a result of the importance of DAP and lysine to bacterial survival [23]. The most common of the alternative metabolic routes is the succinylase pathway, which is inherent to many bacterial species including *E. coli* This sub-pathway begins with the conversion of tetrahydrodipicolinate to *N*-succinyl-L-2-amino-6-ketopimelate (NSAKP) and is catalyzed by 2,3,4,5-tetrahydropyridine-2-carboxylate *N*-succinyltransferase (*dap*D, THPC-NST). NSAKP is then converted to *N*-succinyl-L,L-2,6,-diaminopimelate (NSDAP) by *N*-succinyldiaminopimelate aminotransferase (*Dap*C, NSDAP-AT). Subsequently, it is desuccinylated by succinyldiaminopimelate desuccinylase (*dap*E, SDAP-DS) to form L,L-2,6-diaminopimelate (LL-DAP). The acetylase pathway also involves four enzymatic steps, but incorporates *N*-acetyl groups rather than *N*-succinyl moieties. This pathway is common to several bacillus species, including *B. subtilis* and *B. anthracis* [24]. The succinylase pathway is utilized by all gram negative and many gram positive bacteria, while the acetylase pathway appears to be limited to certain bacillus species. There is one additional sub-pathway which is less common to bacteria which is called as the aminotransferase pathway This is catalyzed by the enzyme diaminopimelate aminotransferase (LL-DAP-AT) which transforms L-tetrahydrodipicolinic acid (L-THDP) directly to LL-DAP. Accordingly, after the formation of L,L-DAP, which is common in all three pathways, the enzyme L,L-diaminopimelate epimerase (DAPE, *dap*F) catalyzes the epimerization of L,L-DAP to form *meso*-DAP. All alternative pathways then converge to utilize the same enzyme for the final step of lysine biosynthesis, namely diaminopimelate decarboxylase (DAPDC, *Lys*A) which catalyses the decarboxylation of *meso*-DAP to yield lysine and carbon dioxide. This step is important for the overall regulation of the lysine biosynthesis. Comparative analysis of genes and regulatory elements identify the lysine-specific RNA element, named as the LYS element, in the regulatory regions of bacterial genes which are involved in biosynthesis and transport of lysine. The LYS element includes regions of lysine-constitutive mutations previously identified in *E. coli and B. subtilis*.The lysine biosynthetic pathway has a special interest for pharmacology, since the absence of DAP in mammalian cells allows for the use of the lysine biosynthetic genes as a bacteria-specific drug target.

This review describes the essential details of the key enzymes functioning in the lysine biosynthetic pathway. Enzymes of this pathway are the products of essential bacterial genes that are not expressed in humans. The pathway is of interest to antibiotic discovery research. Accordingly, it also gives the current status of rational drug design initiatives targeting essential enzymes of the lysine biosynthesis pathway in resistant strains of pathogenic bacteria. More recently, cloning and expression of the DAP pathway components has facilitated detailed investigations and structures of the enzymes have been determined by X-ray crystallography [25].

**Succinylase pathway**

**Tetrahydrodipicolinate N-succinyltransferase (THPC-NST, EC 2.3.1.117, *dap*D)**

The crystal structure of THPC-NST from *M. bovisat* 2.2$^0$A resolution revealed a trimeric structure with each protomer consisting of three domains: α-helical N-terminal domain, α,β-sheet C-terminal domain and a distinctive β-helix middle domain. THPC-NST is the the essential gene product of *dapD* and this enzyme is a member of the hexapeptide acyltransferase superfamily, the enzymes containing repeated copies of an imperfect hexapeptide sequence, (LIV)-(GAED)-X2-(STAV)-X (in single letter code for amino acids, denote alternative amino acids at the position, X indicates any amino acid). Crystal structures with bound substrates and CoA have identified the active site of an enzyme located at the interface between two left-handed parallel β-helices (LβH) of the trimeric enzyme. Comparison with the structure of the apoenzyme from *M. bovis* and *E. coli* reveals the C-terminus of the enzyme forms an α-helix, which blocks the CoA binding site. Upon binding to CoA, this helix moves 2$^0$ A to participate in substrate binding. Structure of the *E. coli*, *M. bovis* and Mtb-DapD reveal the different orientation and conformational changes responsible for the cooperative binding of CoA and substrate by the enzyme [26]. Electron density maps around the active site glutamate Glu199 might be interpreted as evidence for a covalent reaction intermediate between the succinyl group of the cofactor and the carboxylate side chain of the glutamate. Finally, Mtb-DapD shows water-filled channel systems, which allow access to the metal ions bound between the three molecules of the trimer [27].

***N*-Succinyl- L,L-diaminopimelate aminotransferase (NSDAP-AT, EC 2.6.1.17, *dap*C)**

NSDAP-AT catalyzes the transfer of an amino group from L-glutamate to NSAKP to generate NSDAP. NSDAP-AT is a family of class I PLP-dependent aminotransferase, which exists as a homodimer (in M. tuberculosis NSDAP-AT). The reaction begins by the formation of a Schiff base linkage between an active site lysine and the cofactor pyridoxal-5'-phosphate (PLP). The gene encoding NSDAP-AT (*dap*C), is found in a large number of bacterial species including *Bordetella pertussis, C. glutamicum, E. coli,* and *M. tuberculosis.* In *E. coli*, the gene encoding NSDAP-AT is annotated arg D. The *dap*C gene in *B. pertussis, C. glutamicum,* and *E. coli* has been found to map in close proximity to the *dap*D gene on the chromosome. Sequence analyses have shown that NSDAP-AT consists of approximately 400 residues and shares greater than 26% identity across species. The monomer subunit is comprised of an α-helical N-terminal extension and central domain comprising an 8 stranded β-sheet surrounded by 8 α-helices, and a C-terminal domain consisting of a four stranded β-sheet flanked by 4 α-helices. The active site of each subunit is located at the dimer interface with residues from both subunits contributing to the architecture of the active sites. PLP is bound to the active site Lys232, presumably via a Schiff base, and makes a number of noncovalent contacts with other residues within the active site via a hydrogen bond network. The predicted *Dap*C protein of *C. glutamicum* shared 29% identical amino acids with *Dap*C from *B. pertussis*. However, overexpression of the *dap*C gene in *C. glutamicum* resulted in a 9-fold increase of the specific aminotransferase activity [38]. Many substrate analogues (Figure 4) of NSDAP-AT have synthesized with different *N*-acyl substituents. DAP-AT has quite strict requirements for substrate recognition, But it will accept compounds with an aromatic ring in place of the terminal succinyl carboxyl group in 4a. the compound 5a and 5b hydrazine analogues are the most potent slow-binding inhibitors NSDAP-AT enzyme reported so far [29]. Dipeptide substrates of DAP-AT have prepared as hydrazines analogues 6 and 7. The hydrazino-dipeptides showed potent slow binding inhibition of DAP-AT from *E. coli*. Biological activity of these synthetic peptides is a longer term strategy to develop peptide libraries and peptide-mimetics. DAP-AT has been shown to be a suitable target for the development of novel antimicrobial compounds [30, 31].

***N*-Succinyl-L,L-diaminopimelic acid desuccinylase (E. C. 3.5.1.18, SDAP-DS, *dap*E)**

SDAP-DS catalyzes the hydrolysis of NSDAP to form L,L-DAP. The enzyme requires a divalent metal ion for activity, preferably zinc or cobalt and has sequence similarity to a number of other metal-dependent enzymes, A molecular size of 43 kDa [32]. The gene encoding SDAP-DS, *dap*E is present in a large number of bacterial species including, *M. tuberculosis, E. coli, Y. pestis, V. cholerae, H. pylori, H. influenzae, S. aureus* and many more. The fact that the *Dap*E gene has been discovered in several multi-drug-resistant bacteria suggests that inhibitors of *Dap*E enzymes may provide a new class of broad-spectrum antibiotics. Alignment of the *Dap*E proteins listed above show a minimum of 49% sequence Identity across bacterial species. The SDAP-DS amino acid sequences show conservation of

histidine and glutamate metal binding residues that are characteristic of metal-dependent amidases. It is hypothesised that NSDAP adopts an extended conformation when bound to the active site of the enzyme. The NSDAP amide carbonyl coordinates to an active site $Zn^{2+}$ ion and becomes avaliable for nucleophilic attack. This binding event displaces a bridging water molecule, resulting in its hydrolysis by Glu134 and the generation of a zinc bound nucleophilic hydroxide. The hydroxide then attacks the target carbonyl carbon to form a η-1-μ-transition-state complex, which then resolves to release DAP and succinate. The crystal structure of zinc bound SDAP-DS shows that the enzyme forms a homodimer, with each monomer subunit containing a catalytic domain and a dimerisation domain. The core of the catalytic domain is composed of an eight-stranded twisted β-sheet that is sandwiched between seven α-helices. The dimerisation domain adopts a two layer α+β sandwich fold and is comprised of a four stranded antiparallel β-sheet and two α-helices [33]. A screen biased toward compounds containing zinc-binding groups (ZBG's) including thiols, carboxylic acids, boronic acids, phosphonates and hydroxamates has delivered a number of micromolar inhibitors of *Dap*E from *H. influenzae*, including the low micromolar inhibitor L-captopril (IC 50 = 3.3l M, IC50 = 1.8l M). In vitro antimicrobial activity was demonstrated for L-captopril against *E. coli*. Boronic acid tested as inhibitors of *Dap*E [34, 35].

**Aminotransferase pathway**

Function of diaminopimelate aminotransferase (LL-DAP-AT, EC 2.6.1.83, *Dap*L): LL-DAP-AT is a PLP-dependant enzyme that catalyses the conversion of L-2,3,4,5,-tetrahydrodipicolinate (THDP) to LL-DAP (Figure 3). This transamination reaction utilises glutamate as an amino donor to yield A-ketoglutarate. Studies of crude cell extracts have shown that plants do not catalyse reactions specific to the succinylase, acetylase or dehydrogenase pathway [36]. This confirmed that annotated plant genomes, including *A. thaliana*, lack some or all genes associated with the three classical pathway [37]. Bacteroides fragilis and Clostridium thermocellum were recently found to synthesize diaminopimelate (DAP) by way of LL-DAP aminotransferase. Both species also contain an ortholog of meso-diaminopimelate dehydrogenase (Ddh), suggesting that they may have redundant pathways for DAP biosynthesis. Analysis of the microbial genome database revealed that 77% of species with a Ddh ortholog also contain a second pathway for DAP biosynthesis suggesting that Ddh evolved as anancillary mechanism for DAP biosynthesis [38]. The phylogenenetic study of LL-DAP-AT from defferent species shows the existence of two classes of LL-DAP-AT orthologues, namely, DapL1 and DapL2, which differ significantly in primary amino acid sequence. DapL1 and DapL2 are found predominantly in eubacteria and archaea, respectively. LL-DAP-AT has 20% sequence identity when compared to bacterial NSDAP-AT (Dap C)[40] LL-DAP-AT enzymes from plants and Chlamydia belong to the Dap L1 variant of LL-DAP-AT and share approximately 50% amino acid sequence identity. The DapL 2 variant is primarily found in *Archaea* and shares approximately 30% amino acid sequence identity with the DapL 1 variant.[41] LL-DAP-AT enzymes are classified as members of the PLP-dependant protein superfamily of class I/II aminotransferases It is reported that *C. trachomatisand* and *P. amoebophila* also use the LL-DAP-AT pathway, there by supporting an evolutionary relationship between *cyanobacteria* and *Chlamydiales*. *Chlamydia* Species that can cause pneumonia [39,40] urinary tract infections, sexually transmitted infections and may be involved in coronary heart disease [41-43].

**Structure of LL-DAP-AT**

The crystal structure of LL-DAP-AT from different species like *A. thaliana, C. trachomatis* and *M. tuberculosis* (Mtb) has been identified. LL-DAP-AT-Mtb is active as a homodimer with each subunit having PLP in the active site [44]. The crystal structure of LL-DAP-AT consists of two domains, the large domain (LD) and the small domain (SD) (Figure 3). The LD (Asn48 to Gln294) consists of 246 amino acid residues and folds into an α–β–α sandwich. This domain acts as the PLP binding scaffold and is involved in the majority of the dimer-forming inter actions. The SD (Met1 to Leu4 7 plus Leu29 5 to Ser394) consists of the N-terminal arm region and the last 100 residues of the C-terminus and this domain forms an α–β complex. The active site is situated in a groove between the two domains of the monomer (Figure 3). Structural Study of the apo and ligand-bound forms of *C. trachomatis* LL-DAP-AT have revealed that the enzyme adopts an open and closed conformation [45]. In the absence of ligand, the enzyme assumes an open state, whereby the active site is exposed to solvent. Upon PLP binding, the enzyme adopts a closed conformation. Within the active site, PLP is covalently linked to Lys236 via a Schiff base and is stabilized through an aromatic stacking interaction with Tyr128. PLP also forms a network of hydrogen bonding interactions with residues within the enzyme active site.

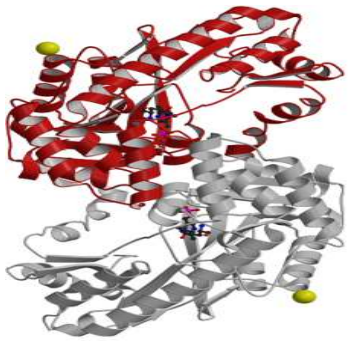

**Fig. 3: The overall structure of the Mtb-DAP-AT homodimer. One monomer is represented in red, the other monomer in grey. The PLP moieties are represented as ball-and-stick models and the bound chloride ions as yellow spheres**

**Inhibition of LL-DAP-AT**

Recently 29,201 inhibitors were screened against LL-DAP-AT. An aryl hydrazide and rhodanine derivatives were further modified to generate analogues that were also tested against LL-DAP-AT [46] the screening of a compound library against LL-DAP-AT identified an o-sulfonamido-arylhydrazide (12) as a reversible inhibitor with IC50 = 5μM, which is derived from the compound (10) (Figure 4). The best inhibitor 34, interact directly with PLP through its free amino group. Compound (12) is selected as lead compound for further development. Structure–activity relationships (SAR) studies based on this lead compound identified key structural features essential for enzyme inhibition and to slightly improved inhibitors. By using SAR study, 16 hydrazide analogues were synthesized based on a lead compound (12). The analogues were tested as inhibitors against LL-DAP-AT from A. thaliana as shown in (Figure 4, compound 10-24) only a few hydrazide analogues were showing promising result. The best inhibitor was found to be an o-sulfonamido-p-fluorophenyl hydrazide (16), with an IC50 value of 2.5 μM [47]. The rhodanine-based inhibitor (9) contains rhodanine ring with furan ring derivative attached, which was chosen as lead compound for SAR studies. It shows that the substituent on the nitrogen has little effect on the activity of these inhibitors, whereas the presence of an electron-donating group on the aromatic ring enhances activity. Both types of inhibitors are still far from becoming effective antimicrobial agents at this point. Future investigations of inhibitors together with structural studies of LL-DAP-AT will be important in developing novel antibiotics targeting LL-DAP-AT.

**Diaminopimelate epimerase (DAPE, EC 5.1.1.7, *dap*F gene) Function of DAPE**

DAPE catalyses the conversion of L,L-2,6-diaminopimelate (LL-DAP) to *meso*-DAP. *meso*-DAP immediate precursor of lysine in the lysine biosynthesis. DAPE is encoded by the *dap*F gene [48]. DAPE was first characterized in 1957 using enzyme derived from crude extracts of *E. coli*. The enzyme specifically recognizes the LL-DAP isomer, whereas the DD-DAP isomer is not a substrate or inhibitor of the enzyme. Early studies noted that DAPE was inhibited by low concentrations of thiol-binding reagents and could be reactivated by reducing agents, suggesting the presence of an essential sulfhydryl group. This finding was subsequently confirmed upon purification of DAPE to homogeneity.

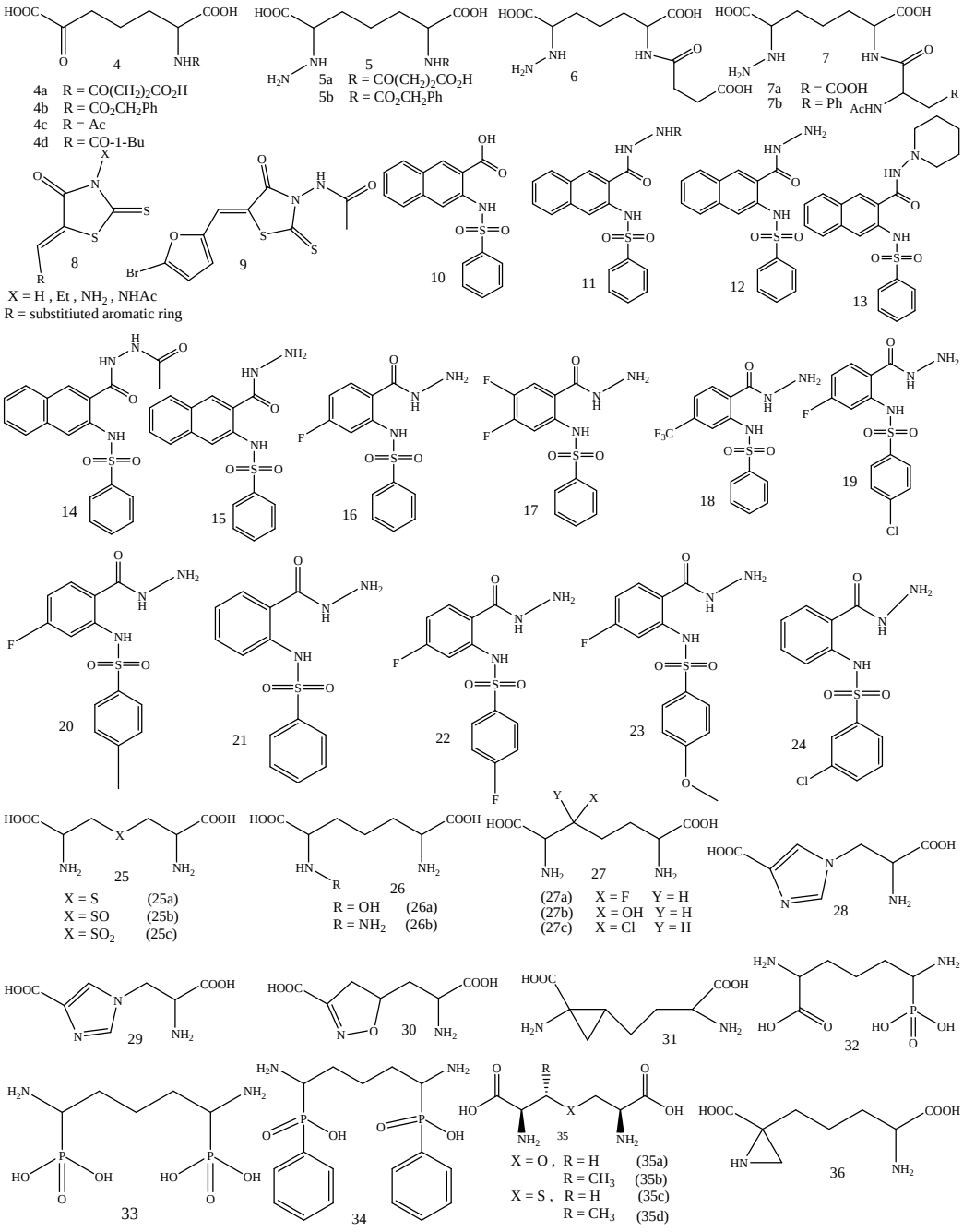


**Fig. 4: Substrate-based analogs of enzymes involve in lysine biosynthesis**

**Mechanism of conversion LL-DAP to *meso*-DAP**

DAPE catalyses the conversion of LL-DAP (37) to meso-DAP (38) by employing a "two-base" mechanism.[49, 50] The reaction involves two active site cysteine residues Cys73 and Cys217, whereas first Cys73 thiolate (73 in *H. influenzae*) acts as base abstracting proton from LL-DAP, while the second Cys217 thiol (217 in *H. influenzae*) reprotonates the molecule the resulting planar carbanionic intermediate (39) from the opposite side to generate *meso*-DAP (Figure 5). Epimerization reaction of L,L-DAP at neutral pH causes these cysteines to exist as a rapidly equilibrating thiolate–thiol pair in the presence of the substrate.

The stereoinversion involves two active-site cysteine residues acting in concert as a base (thiolate) and an acid (thiol). Formation of a rigidly held thiolate–thiol pair from the two cysteines in the active site with exclusion of solvent enhances basicity for removal of the α-hydrogen [51]. The two catalytic residues Cys73 and Cys217 have been confirmed by DFT quantum mechanical computation of the Michaelis complex. All this mechanistic information could be useful for the rational design of new potential antibiotic drugs effective as inhibitors of peptidoglycan biosynthesis.

**Structure of DAPE**

The structures of DAPE from four species have been described these include DAPE from *B. anthracis, H. influenza, M. tuberculosis* and also from the plant species *A. thaliana*. The crystal structures of the apo-forms of DAP epimerase mutants (C73S and C217S) from *H. influenzae* at 2.3 A° and 2.2 A° resolution, respectively. These structures provide a snapshot of the enzyme in the first step of the catalytic cycle. DAPE adopts two distinct conformational states. In the absence of substrate, the enzyme exists in an open conformation, and upon binding substrate adopts a closed conformation. The 'open conformation' of the enzyme prior to substrate binding provide a significant new tools for the rational design and discovery of novel

inhibitors of this important protein drug target.DAP epimerase has symmetrical monomer comprised of two domains with residues 1–117 and 263–274 forming the N-terminal domain and residues 118–262 forming the C-terminal domain; each containing eight β-strands and two-α helices and each domain contributing one active-site cysteine (Figure 6). The DAP epimerase from plants like *A. thaliana* has approximately same structure to the enzyme from bacteria (*H. influenzae*) and shares a similar mechanism [52].

The residues lining the active-site cavity are largely conserved across all of the DAP epimerase sequences isolated from different bacterial species and include: Asn-11, Phe-13, Gln-44, Ty r-60, Asn-64, Val-70, Glu-72, Cys-73, Gly-74, Asn-75, Asn-157, Asn-190, Glu-208, Arg-209, Cys-217, Gly-218, and Ser-219. Some of these residues in the inhibitor bound enzyme form an extensive hydrogen-bonded network surrounding the inhibitor. Electron density maps and crystal structure study revealed the location of the two diastereomeric inhibitors LL-aziDAP (40) and DL-aziDAP (41) in the active site of DAP epimerase (Figure 5), the carboxyl group at the distal site of both inhibitors (40 & 41) forms a salt bridge with the positively charged side chain of Arg-209 along with three hydrogen bonds from side chain $N^{\delta 2}$ atoms of residues Asn-64, Asn-157, and Asn-190. The distal positively charged amino group of the inhibitors forms three hydrogen bonds with side-chain oxygen atoms of Asn-64 and Glu-208 and the carbonyl oxygen of Arg-209. The asymmetric disposition of side chains of Asn-64 and Asn-157 situated on opposite sides of the C-6 carbon of the inhibitor, coupled with tight packing of other active-site residues surrounding the distal site, prevents the binding of a D-isomer at that position.

The binding interactions of the distal L-stereocenter of LL- and DL-aziDAP help in positioning the substrate carbon skeleton correctly for forming the covalent bond from the cysteine sulfur and the methylene carbon of aziDAP. LL-Azi-DAP (40) selectively binds to Cys-73 of the enzyme active site whereas DL-azi-DAP (41) binds to Cys-217 via attack of sulfhydryl on the methylene of the inhibitor aziridine ring. The binding of the aziDAP inhibitors to DAP epimerase induces a large conformational change in the enzyme.

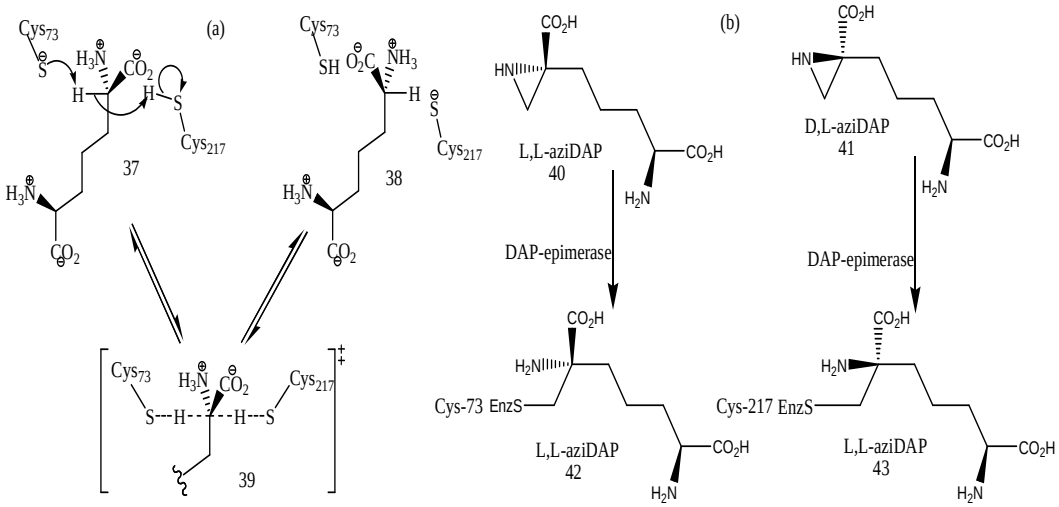


**Fig. 5: (A) Epimerization reaction of L,L-diaminopimelic acid (DAP).(B) Inhibition of dap-epimerase by L,L-azi DAP and D,L-aziDAP.[51,52]**

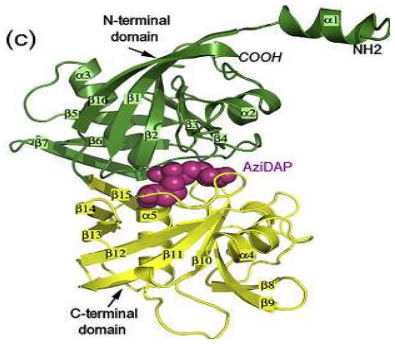


**Fig. 6: Ribbon diagram of DAP epimerase from *A. thaliana*. The two domains (shown in green and yellow), and the substrate-mimic inhibitor AziDAP (spheres; magenta) is bound at the active site located in a cleft between the two domains [54].**

**Substrate requirement for DAPE**

Enzyme inverts the configuration at the α-carbon of an amino acid under physiological conditions without the assistance of any metal ions or cofactors. Recently crystal structures of some of the bacterial PLP-independent racemases including glutamate racemase, aspartate racemase, prolineracemase, and DAPE resolved but DAPE differs from these latter racemases, because it can discriminate between two stereocentres. The docking of the ligand–enzyme complex was studied by means of MD simulations and DFT computations in order to ascertain the optimal structural requirements for the epimerization reaction. MD simulations study shows that the configuration of the distal carbon C–6 of L,L-DAP is critical for complex formation since both amino and carboxylate groups are involved in H–bonding interactions with the active site residues. The interactions occurring between the functional groups bonded to the C–2 and some residues of the binding cavity immobilize the ligand in a position appropriate for the epimerization reaction, i. e. exactly in the middle of the two catalytic residues Cys73 and Cys217 as confirmed by DFT quantum mechanical computation of the Michaelis complex. Specific interactions at the distal site requires only L-configuration. The enzyme discriminates between these two stereocenters and interconverts LL-DAP and DL (*meso*)-DAP, but it cannot further epimerize DL-DAP to DD-DAP.

This shows D,D-DAP is neither a substrate nor inhibitor for this enzyme, Supporting the hypothesis and experimental evidences that the stereochemistry of the non-reacting (distal) C–6 carbon is critical for ligand recognition or activity. DAP analogs lacking an amino or carboxyl group are neither substrates nor effective inhibitors [53].

**Substrate-based inhibitors of DAPE**

These have been developed by using the computational and rational drug design (Figure 4). These analogs show time dependent competitive and non-competitive inactivation of the enzyme as antibacterial activities. Hydroxylated and halogenated analogues of LL-DAP have tested against DAPE. The hydroxylated Compounds

(27b) proved to be very weak inhibitors of DAPE with 50% inhibition. The fluoro analogues (27a) of L,L-DAP, were potent competitive inhibitors of DAPE (IC50 = 4-25 pM). the carbon bearing fluorine, undergoes rapid epimerase-catalyzed elimination of hydrogen fluoride without detectable epimerization [54-56]. Heterocyclic analogues of DAP like oxazole, imidazole isoxazole, aziridine has DAPE inhibitory activity azi-DAP (36) have been shown to be a irreversible inhibitor of DAPE with an IC50 value of 2.88 mM [57-59].

Recently the oxa analogues of azi-DAP was found to irreversibly inhibit DAP epimerase, presumably due to thiol opening of the epoxide moiety. The series of phosphonic acid analogues of this diamino-dicarboxylic acid is synthesized, mono-(32) and diphosphonic acid derivatives (33) as well as their homologues, *N*-alkyl analogues, A phenylphosphinic derivative (34) and some peptidyl derivatives. All the prepared compounds were tested for their antibacterial activity [60, 61]. 4-Oxa-2,6-diaminopimelic acid 35a (oxa-DAP) and its methyl-substituted derivatives 35b and orthogonally protected lanthionine analogues of DAP (lanthionine-DAP) and β-methyllanthionine wherein central oxygen replaces sulfur were prepared. Oxa-DAP and lanthionine-DAP is a substrate of DAPE [62, 63]. A series of di- and tripeptides of individual P-DAP isomers with L-alanine were synthesized to enhance transport into bacterial cells for antimicrobial tests [64, 65]. Possible mechanisms of epimerase inhibition and significance of the DAP pathway as a target for antibiotics are discussed.

## CONCLUSION

The review explores the existing chemical analogs possessing antibacterial activity through enzyme inhibition and draws the need to further develop newer chemical analogs especially by exploiting lysine biosynthetic pathway. It reflects the necessity to develop such analogs to combat with the mutated and resistant bacteria.

These analogs will be target oriented and in a small concentration, will restrain the proteins and enzymes that are mandatory for the survival of bacteria. Such newer antibacterial analogs which selectively inhibit DAP pathway shall be more effective and less toxic to mammals. This type of specific drug discovery approach will open several opportunities and methodologies to treat diseases using antimicrobial therapy. These drugs shall be able to sustain for a longer time in the market.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## ACKNOWLEDGEMENTS

The authors are thankful to the Principal, Government College of Pharmacy, Aurangabad, Maharashtra, India for providing literature survey facility to carry out this review work.

## ABBREVIATIONS

ASADH - Aspartate semialdehyde dehydrogenase

DHDPS-Dihydrodipicolinate synthase

DHDPR - Dihydrodipicolinate reductase

THPC-NST - Tetrahydrodipicolinate *N*-succinyltransferase

NSDAP-AT - *N*-succinyldiaminopimelate aminotransferase

SDAP-DS - Succinyldiaminopimelate desuccinylase

THDP-NAT - Tetrahydrodipicolinate *N*-acetyltransferase

ATA - Aminotransferase A

NAD-DAC - *N*-acetyldiaminopimelate deacetylase

LL-DAP-AT - Diaminopimelate aminotransferase

DAPE - Diaminopimelate epimerase

DAPDC - Diaminopimelate decarboxylase

## REFERENCES

1. Barker J. Antibacterial drug discovery and structure based design. Drug Discovery Today 2006;11:9-10.
2. Christopher Walsh. Cloning of the dapB gene, encoding dihydrodipicolinate reductase, from Mycobacterium tuberculosis. Nature 2000;406:17-20.
3. Simmons KJ, Chopra I, Fishwick WG. Structure-based discovery of antibacterial drugs. Nature Rev Micro 2010;8:501-4.
4. Born TL, Blanchard JS. Structure/function studies on enzymes in the diaminopimelate pathway of bacterial cell wall biosynthesis. Current Opinion Chem Biol 1999;3:607–13.
5. Saito Y, Shinkai T, Yoshimura Y, Takahata H. A straightforward stereoselective synthesis of meso-(S,S)-and (R,R)-2,6-diaminopimelic acids from cis-1,4-diacetoxycyclohept-2-ene. Bioorg Med Chem Letters 2007;17:5894–6.
6. Jurgens AR. Asymmetric synthesis Of differentially protected meso-2,6-diaminopimelic acid. Tetrahedron Letters 1992;33:4727-30.
7. Mayura K, Mohammad SS. Exploration of lysine pathway for developing newer anti-microbial analogs through enzyme inhibition approach. Int J Pharm Sci Rev Res 2014;25 suppl 2:221-30.
8. Kimura K, Bugg TD. Recent advances in antimicrobial nucleoside antibiotics targeting cell wall biosynthesis. Natural Product Report 2003;20:252–73.
9. Girodeau JM, Agouridas C, Masson M, Pineau R, Goffic FL. The Lysine pathway as a target for a new genera of synthetic antibacterial antibiotics. J Med Chem 1986;29:1023-30.
10. Collier PN, Patel I, Taylor RJK. A concise, stereoselective synthesis of meso-2,6-diaminopimelic acid (DAP). Tetrahedron Letters 2001;42:5953–4.
11. Paradisi F, Porzi G, Rinaldi S, Sandri S. A simple asymmetric synthesis of (+)-and (-)-2,6-diaminopimelic acids. Tetrahedron Asymmetry 2000;11:1259-62.
12. Winn M, Goss RJM, Kimura K, Bugg TD. Antimicrobial nucleoside antibiotics targeting cell wall assembly: Recent advances in structure–function studies and nucleoside biosynthesis. Natural Product Reports 2010;27:279–304.
13. Zoeiby A, Sanschagrin F, Levesque RC. Structure and function of the Mur enzymes: development of novel inhibitors. Mol Microbiol 2003;47 suppl 1:1–12.
14. Cox RJ. The DAP pathway to lysine as a target for antimicrobial agents. Natural Product Reports 1996;21:29-43.
15. Hartmann M, Tauch A, Eggeling L, Bathe B. Identification and characterization of the last two unknown genes, dap C and dap F, in the succinylase branch of the L-lysine biosynthesis of Corynebacterium glutamicum. J Biotech 2003;104:199-211.
16. Galeazzi R, Garavelli M, Grandi A, Monari M, Porzi G, Sandri S. Unusual peptides containing the 2,6-diaminopimelic acid framework: Stereocontrolled synthesis, X-ray analysis, and computational modelling. Tetrahedron Asymmetry 2003;14:2639–49.
17. Holcomb RC, Schow S, Kaloustian SA, Powell D. An Asymmetric synthesis of differentially protected meso-2,4-diaminopimelic acid. Tetrahedron Lett 1994;35:7005-8.
18. Chowdhury AR, Boons GJ. The synthesis of diaminopimelic acid containing peptidoglycan fragments using metathesis cross coupling. Tetrahedron Lett 2005;46:1675–8.
19. Grundy FJ, Lehman SC, Henkin TM. The L box regulon: Lysine sensing by leader RNAs of bacterial lysine biosynthesis genes. National Acad Sci 2003;100 suppl 21:12057–62.
20. Cahyanto MN, Kawasaki H, Nagashio M, Fujiyama K, Seki T. Regulation of aspartokinase, aspartate semialdehyde dehydrogenase, dihydrodipicolinate synthase and dihydrodipicolinate reductase in Lactobacillus plantarum. Microbiol 2006;152:105–12.
21. Tsujimoto N, Gunji Y, Miyata Y, Shimaoka M, Yasueda H. l-Lysine biosynthetic pathway of methylophilus methylotrophus and construction of an l-lysine producer. J Biotech 2006;124:327–37.
22. Dobson RCJ, Valegard K, Gerrard JA. The crystal structure of three site-directed mutants of Escherichia coli

dihydrodipicolinate synthase: further evidence for a catalytic triad. J Mol Biol 2004;338:329–39.
23. Rodionov DA, Vitreschak AG, Mironov AA, Gelfand MS. Regulation of lysine biosynthesis and transport genes in bacteria: yet another RNA riboswitch. Nucleic Acids Res 2003;31 suppl 23:6748-57.
24. Dogovski C. Enzymology of bacterial lysine biosynthesis. Biochem 2012;9:225-63.
25. Cox RJ, Sutherland A, Vederas JC. Bacterial diaminopimelate metabolism as a target for antibiotic design. Bioorg Med Chem 2000;8:843-71.
26. Nguyen L, Kozlov G, Gehring K. Structure of *E. coli* tetrahydrodipicolinate N-succinyl transferase reveals the role of a conserved C-terminal helix in cooperative substrate binding. Eur Biochem Soc Lett 2008;582:623–6.
27. Schuldt L, Weyand S, Kefala G, Weiss MS. The three-dimensional structure of a Mycobacterial dapD provides insights into dapD diversity and reveals unexpected particulars about the enzymatic mechanism. J Mol Biol 2009;389:863–79.
28. Hartmann M, Tauch A, Eggeling L, Bathe B, Bettina M. Alfred P, *et al*. Identification and characterization of the last two unknown genes, dap C and dap F, in the succinylase branch of the L-lysine biosynthesis of C. glutamicum. J Biotech 2003;104:199-211.
29. Cox RJ, Sherwin WA, Lam LKP, Vederas JC. Synthesis and evaluation of novel substrates and inhibitors of N-succinyl-LL-diaminopimelate aminotransferase (DAP-AT) from Escherichia coli. J Am Chem Soc 1996;18:7449-60.
30. Cox RJ, Schouten JA, Stentiford RA, Wareing KJ. Peptide inhibitors of N-succinyl diaminopimelic acid aminotransferase (Dap-At): a novel class of antimicrobial compounds. Bioorg Med Chem Lett 1998;8:945-50.
31. Nishida H, Nishiyama M. Evolution of lysine biosynthesis in the phylum deinococcus-thermus. Int J Evol Biol 2012;2012:945-50.
32. Boyena A, Charlier D, Charlier J, Sakanyand V, Mettd I, Glansdorff N. Acetylornithine deacetylase, succinyl diaminopimelate carboxypeptidase G2 are evolutionarily related. Gene 1992;116:1-6.
33. Nocek BP, Gillner DM, Fan Y, Holz RC, Joachimiak A. Structural basis for catalysis by the mono and dimetalated forms of the dap E encoded N-succinyl-L,L diaminopimelic acid desuccinylase. J Mol Biol 2010;397:617–26.
34. Gillner D, Armoush N, Holz RC, Becker DP. Inhibitors of bacterial N-succinyl-L,L-diaminopimelic acid desuccinylase (DapE) and demonstration of *in vitro* antimicrobial activity. Bioorg Med Chem Lett 2009;19:6350–52.
35. Uda NR, Creus M. Selectivity of inhibit ion of N-succiny l-l,l–diaminopimelic acid desuccinylase in bacteria: the product of dapE gene is not the target of l-captopril antimicrobial activity. Bioinorg Chem Appl 2011;11:1-6.
36. Watanabe N, James MNG. Structural insights for the substrate recognition mechanism of LL-diaminopimelate aminotransferase. Biochimica Biophysica Acta 2011;1814:1528–33.
37. Watanabe N, Cherney MM, Belkum MJ, Marcus SL, Flegel MD, Clay MD, *et al*. Structure of LL-siaminopimelate aminotransferase from arabidopsis thaliana: a recently discovered enzyme in the biosynthesis of L-lysine by plants and chlamydia. J Mol Biol 2007;371:685–702.
38. Watanabe N, Clay MD, Belkum MJ, Cherney MM, Vederas JC, James MNG. Mechanism of substrate recognition and PLP-induced conformational changes in LL-diaminopimelate aminotransferase from Arabidopsis thaliana. J Mol Biol 2008;384:1314–29.
39. Kuo CC, Campbell LA. Is infection with *C.* pneumoniae a causative agent in atherosclerosis. Molecular Medicine Today 1998;1357:426-30.
40. He X, Mekasha S, Mavrogiorgos N, Fitzgerald KA, Lien E, *et al*. Inflammation and fibrosis during Chlamydia pneumoniae infection is regulated by IL-1 and the NLRP3/ASC inflammasome. J Immunol 2010;184:5743–54.
41. Lane DR, Takhar SS. Diagnosis and management of urinary tract infection and pyelonephritis. Emergency Medicine Clinics North Am 2011;29 suppl 3:539-52.
42. Zhao X, Bu D, Hayfron K, Pinkerton KE, Bevins CL, Lichtman A, *et al*. A combination of secondhand cigarette smoke and Chlamydia pneumonia accelerates atherosclerosis. Atherosclerosis 2012;222:59–66.
43. Gupta S. Chronic infection in the etiology of atherosclerosis focus on Chlamydia pneumonia. Atherosclerosis 1999;143:1–6.
44. Weyand S, Kefala G, Weiss MS. The three-dimensional structure of N-succinyldiaminopimelate aminotransferase from Mycobacterium tuberculosis. J Mol Biol 2007;367:825–38.
45. Watanabe N, Clay MD, Belkum MJ, Fan C, Vederas JC, James MN. The structure of LL-diaminopimelate aminotransferase from Chlamydia trachomatis: implications for its broad substrate specificity. J Mol Biol 2011;411:649– 60.
46. Fan C, Clay MD, Deyholos MK, Vederas JC. Exploration of inhibitors for diaminopimelate aminotransferase. Bioorg Med Chem 2010;18:2141–51.
47. Fan C, Vederas JC. Synthesis and structure–activity relationships of o-sulfonamid o-arylhydrazides as inhibitors of LL-diaminopimelate aminotransferase (LL-DAP-AT). Org Biomol Chem 2012;10:5815-9.
48. Park JS, Lee WC, Song JH, Kim S, Lee JC, Cheong C, *et al*. Purification, crystallization and preliminary X-ray crystallographic analysis of diaminopimelate epimerase from acinetobacter baumannii. Acta Crystallographic 2013;69:42–4.
49. Pillai B, Cherney M, Diaper CM, Sutherland A, Blanchard JS, Vederas JC, *et al*. Dynamics of catalysis revealed from the crystal structures of mutants of diaminopimelate epimerase. Biochem Biophys Res Comm 2007;363:547–53.
50. Pillai B, Cherney MM, Diaper CM, Sutherland A, Blanchard JS, Vederas JC, *et al*. Structural insights into stereochemical inversion by diaminopimelate epimerase: an antibacterial drug target. Proc Natl Acad Sci U S A 2006;103:8668–73.
51. Pillai B, Moorthie VA, Belkum M, Marcus SL, Cherney MM, Diaper CM, *et al*. Crystal structure of diaminopimelate epimerase from arabidopsis thaliana, an amino acid racemase critical for L-lysine biosynthesis. J Mol Biol 2009;385:580–94.
52. Brunetti L, Galeazzi R, Orena M, Bottoni A. Catalytic mechanism of L,L-diaminopimelic acid with diaminopimelate epimerase by molecular docking simulations. J Mol Graphics Modelling 2008;26:1082–90.
53. Diaper CM, Sutherland A, Pillai B, James M, Semchuk P, Blanchard JS, *et al*. The stereoselective synthesis of aziridine analogues of diaminopimelic acid (DAP) and their interaction with dap epimerase. Org Biomol Chem 2005;3:4402–11.
54. Williams RM, Fegley GM, Gallegos R, Schaefert F, Pruess DL. Asymmetric syntheses of (2S,3S,6S)-, (2S,3S,6R)-and (2R,3R,6-2,3-methano-2,6-diaminopimelic acids: Studies directed to the design of novel substrate-based inhibitors of L,L-diaminopimelate epimerase. Tetrahedron 1996;52 suppl 4:1149-64.
55. Baumann RJ, Bohme EH, Wiseman JS, Vaal M, Nichols JS. Inhibition of Escherichia coli growth and diaminopimelic acid epimerase by 3-chlorodiaminopimelic acid. Antimicrobial Agents Chemotherapy 1988;32 suppl 8:1119.
56. Celb MH, Lin Y, Pickard MA, Song Y, Vederas JC. Synthesis of 3-fluorodiaminopimelic acid isomers as inhibitors of diaminopimelate epimerase: stereocontrolled enzymatic elimination of hydrogen fluoride. J Am Chem Soc 1990;112:4932-42.
57. Steger M, Young DW. Versatile synthesis of inhibitors of late enzymes in the bacterial pathway to lysine. Tetrahedron 1999;55:7935-56.
58. Gerhart F, Higgins W, Tardif C, Ducep J. 2-(4-Amino-4-carboxybutyl)aziridine-2-carboxylic acid, a potent irreversible inhibitor of diaminopimelic acid epimerase. J Med Chem 1990;33:8-12.
59. Song Y, Niederer D, Bell P, Lam L, Crawley S, Palcic M, *et al*. Stereospecific synthesis of phosphonate analogues of diaminopimelic Acid, their interaction with DAP enzymes and antibacterial activity of peptide derivatives. J Org Chem 1994;59:5784-93.
60. Asschel I, Soroka M, Haemersl A, Hooper M, Blanot D, Heijenoort J. Synthesis and antibacterial evaluation of phosphonic acid analogues of diaminopimelic acid. Eur J Med Chem 1991;26:505-15.

61. Lam LK, Arnold LD, Kalantar TH, Kelland SJ, Bell SP, Palcicg MM, *et al*. Analogs of diaminopimelic acid as inhibitors of meso-diaminopimelate dehydrogenase and LL-diaminopimelate epimerase. J Biol Chem 1988;263 suppl 25:11814-9.
62. Liu H, Pattabiraman V, Vederas J. Stereoselective syntheses of 4-oxa diaminopimelic acid and its protected derivatives via aziridine ring opening. Organic Lett 2007;9 suppl 21:4211-4.
63. Balducci D, Porzi G, Sandri S. Enantioselective synthesis of pseudotripeptides incorporating a γ-methylene derivative of 2,6-diaminopimelic acid. Tetrahedron Asymmetry 2004;15:1085–93.
64. Paradisi F, Piccinelli F, Porzi G, Sandri S. Enantioselective synthesis of 2,6-diaminopimelic acid derivatives. Tetrahedron Asymmetry 2002;13:497–502.
65. Balducci D, Crupi S, Galeazzi R, Piccinelli F, Porzi G, Sandri S. Stereoselective approach to uncommon tripeptides incorporating a 2,6-diaminopimelic acid framework: X-ray analysis and conformational studies. Tetrahedron Asymmetry 2005;16:1103–12.