Chapter 15

Vitamin K_2 Biosynthesis: Drug Targets for New Antibacterials

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Additional information is available at the end of the chapter

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Abstract

In prokaryotes, vitamin K_2 (menaquinone) transfers two electrons in a process of aerobic or anaerobic respiration. Respiration occurs in the cell membrane of prokaryotic cells. Electron donors transfer two electrons to menaquinone (MK). Menaquinone in turn transfers these electrons to an electron acceptor. Menaquinones are vital for the electron transport chain. In the spectrum of Gram-positive bacteria and *Mycobacterium* spp., vitamin K_2 serves as the only quinone molecule in their electron shuffling systems. Hence, the bacterial enzymes associated with biosynthesis of the menaquinone(s) serve as potential target molecules for the development of new antibacterial drugs. This chapter summarizes the effects of vitamin K_2 in bacteria and describes in more detail the aspects of menaquinone in bacterial electron transport in general, while also featuring the discoveries of menaquinone biosynthesis inhibitors.

Keywords: vitamin K_1, vitamin K_2, menaquinone, menaquinone biosynthesis, electron transport systems, ATP biosynthesis, Gram-positive bacteria, *Mycobacterium tuberculosis*, antibacterial agents

1. Introduction

Vitamin K is a lipid-soluble vitamin that facilitates the process of blood clot formation. Henrick Dam and Edward Doisy were co-awarded the Nobel Prize in 1943 for the discovery of vitamin Ks and the discovery of their structure. Naturally existing forms of vitamin K, vitamin K_1 (i.e., phylloquinone), and vitamin K_2 (i.e., menaquinone) can be found in the human liver (some 10% of phylloquinone of the total vitamin K contents), as well as in other tissues in rather low amounts; vitamin K_1 accumulates in the liver, while vitamin K_2 is effectively distributed to other body organs [1]. Phylloquinone stems from the dietary intake, while menaquinones are synthesized

by bacteria of the intestine. However, no direct evidence can be established for the biological effects of menaquinones in humans; however, it is surmised that menaquinones mainly are utilized in the synthesis of blood-clotting factors upon the depletion of phylloquinone [2]. Additionally, menaquinones have proven to be more efficient than phylloquinone as a bioactive molecule in processes such as osteroclast differentiation, lowering of blood cholesterol levels, as well as the slowing down of atherosclerotic progression.

The menaquinones have proven to be important in several biological reactions, for example electron transport, active transport, oxidative phosphorylation, as well as endospore formation in bacteria. Furthermore, variations in the inherent molecular structures of the menaquinones and their skewed distributions among bacterial strains are considered as an important marker in bacterial taxonomy [3]. The biosynthesis of menaquinone has been subject to considerable attention in the quest for drug targets displaying multidrug resistance toward Gram-positive pathogenic microorganisms, including *Mycobacterium tuberculosis*.

2. Vitamin K: general molecular structures

There are two naturally occurring forms of vitamin K. Plants and some cyanobacteria synthesize phylloquinone, which is also known as vitamin K_1. Bacteria synthesize a range of vitamin K, but not vitamin K_1, using the different lipophilic side chains derived from isoprene (5-carbon) units. Bacterial vitamin K(s) are designated menaquinone-n (MK-n), where "n" stands for the number of isoprenoid (5-carbon) units. Vitamin K_1 and K_2 share the common molecular structure, belonging to the 2-methyl-1,4-naphthoquinone system, and they appear to be differ structurally in their number of isoprene units within their side chain, as well as their degree of unsaturation [4] (**Figure 1**). Menaquinones, displaying side chains containing up to 15 isoprene units have been identified. For instance, MK-8 is predominantly seen in *E. coli*, while *M. tuberculosis* mainly utilizes MK-9 as the preferred electron carrier. The menaquinones, which possess from 2 to 13 isoprene units, have been detected in both human and animal tissues. A plethora of synthetic vitamin K(s) are now commercially available, and biochemically fabricated vitamin K(s), in the form of vitamin K_3, K_4, and K_5, are applied in several areas, which include pet food industry (e.g., VK_3), as well as human supplements (e.g., VK_5).

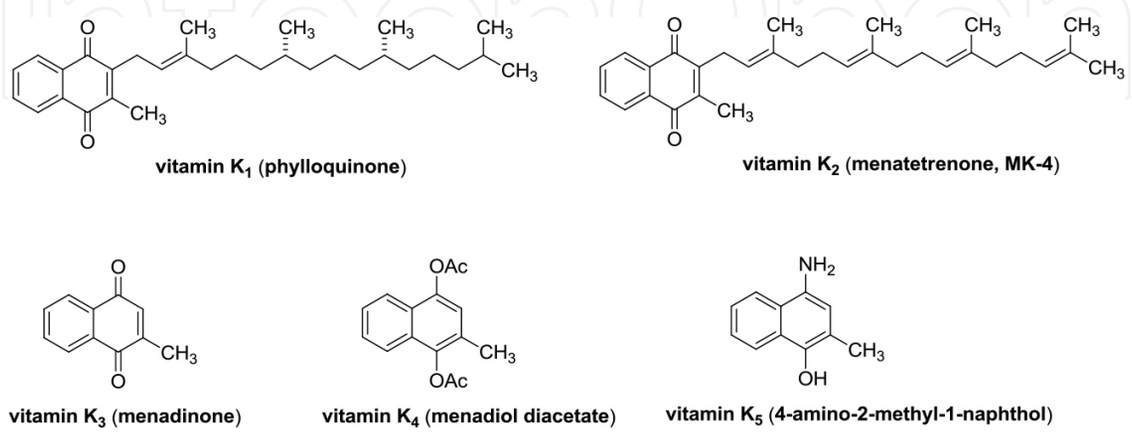

Figure 1. Structures of vitamin K_1 and K_2 and synthetic vitamin Ks (vitamin K_3, K_4, and K_5).

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3. Vitamin K in humans

The role of vitamin K as a cofactor in blood coagulation stems from the post-translational modification of a number of plasma proteins such as factors II, VII, IX, X, proteins C, S, Gla (γ-carboxyglutamic acid) proteins has been well-documented. In addition to the essential role of vitamin K in the blood-clotting cascade, the potential role in the increase of bone mass [5, 6], antioxidant mechanisms [7], the biosynthesis of cholesterol and steroid hormones [8], and anticancer effects have been reported [9, 10].

Vitamin K is taken up by organs such as the liver and bones, but abundantly distributed in other organs such as the brain, the kidneys, and gonadal tissues [11]. However, the exact role of vitamin K in the present tissues is not well described. The distribution of vitamin K moieties is varying, which depends on the molecular structure of the side chain. As for humans, vitamin K_1 is distributed to all tissues and organs, with relatively large amounts to the liver, the heart, and the pancreas (some 10.6, 9.3, 28.4 pmol/g wet tissue weight, respectively). However, low levels (<2 pmol/g) were measured in the brain, kidney, and lung tissue specimens. Menaquinone-4 (MK-4) also seems to be distributed to a plethora of other tissues, that is, its levels exceed the levels of vitamin K_1 detected in the brain and kidneys (2.8 ng/g), which equals to that found in the pancreas. However, some organs, such as the liver, heart, and lung remain low in terms of MK-4 contents. The less known MK-6 ~ 11 menaquinone species are also found in the liver, while only small amounts of MK-6 ~ 9 are detected in organs such as the heart and pancreas. Finally, the total amount of vitamin K reported in human plasma was in the range of 0.47 ~ 1.19 nmol/L [1, 12].

Vitamin K can be absorbed well from diet; however, total vitamin K levels are depend greatly on the gut or digestive health. The intestinal bacteria (i.e., microbiota) influence human nutrition and metabolism in diverse ways. The gut microbiota produces menaquinone; thus, it is considered that vitamin K deficiency is quite rare for healthy humans. In addition, the vitamin K_1 is absolutely abundant in leafy and salad vegetables, and herbs. It is easy to understand that newborn babies are born with a vitamin K deficiency, giving newborns a vitamin K injection upon birth or oral vitamin K drops is established to prevent bleeding or a hemorrhagic disease development. In general, vitamin K deficiency results from extremely inadequate intake of fat (malabsorption) or use of coumarin anticoagulants.

4. The role of vitamin K_2 in electron transport system

Function of ubiquinone (Q) (coenzyme Q_{10}) as a component of the mitochondrial respiratory chain in human is well established ("the chemiosmotic theory," Mitchell, 1978). In prokaryotes, especially in Gram-positive bacteria, vitamin K_2 (menaquinone) will transfer two electrons in a process of aerobic or anaerobic respiration. Respiration occurs in the cell membrane of prokaryotic cells. Electron donors transfer two electrons to menaquinone. Menaquinone in turn transfer these electrons to an electron acceptor. Schematic electron flow mediated by menaquinone in *M. tuberculosis* is illustrated in **Figure 2A**. The exact organization of enzymes

in respiratory chains will vary among different bacteria. Nicotinamide adenine dinucleotide phosphate (NADH) is the most important electron donor in eukaryotes (**Figure 2B**); however, bacteria can use a number of different electron donors, dehydrogenases, oxidases and reductases, and electron acceptors. Electrons are transported along the membrane through menaquinone and a series of protein carriers.

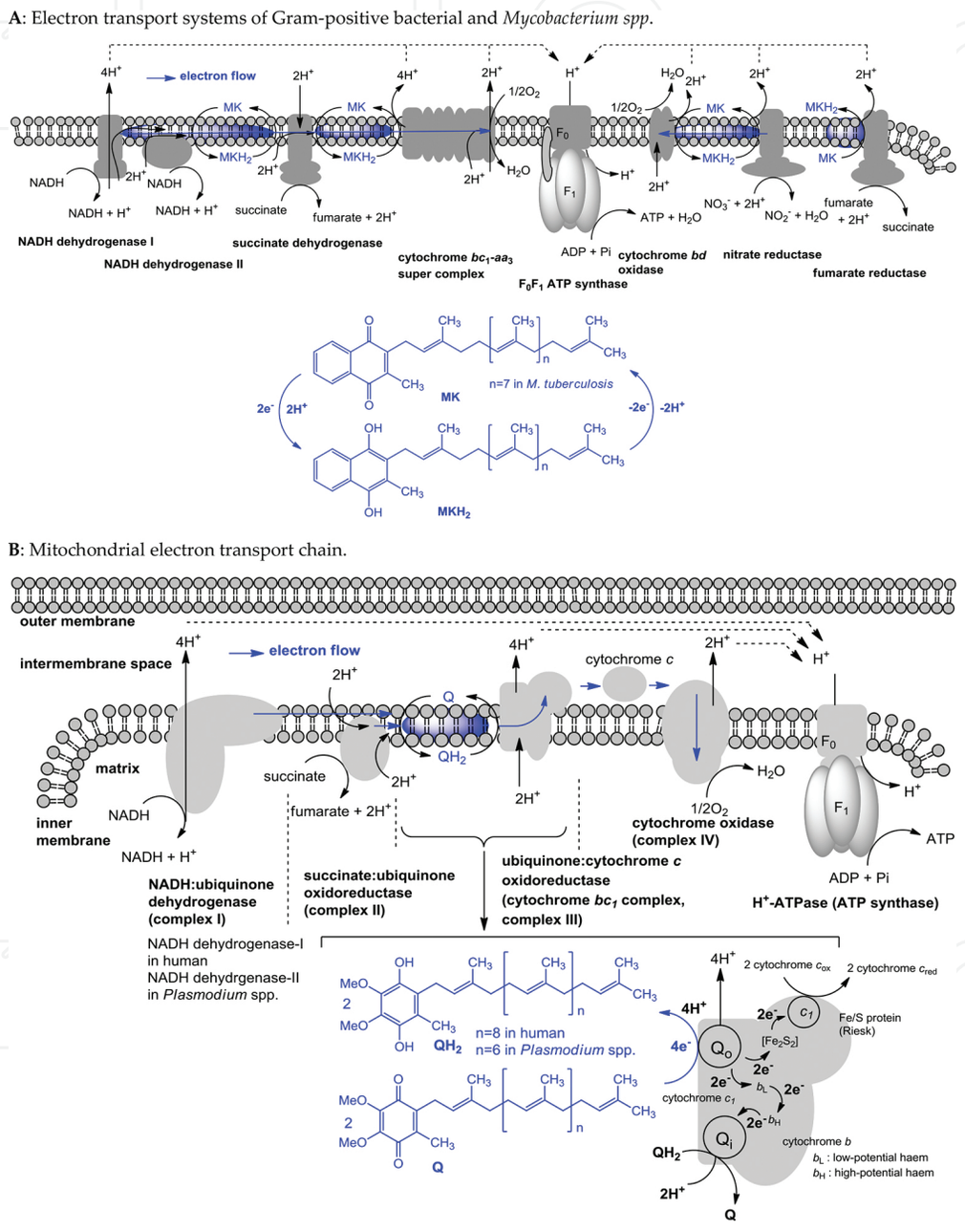

Figure 2. Schematic electron flow systems. (A) Electron transport systems of Gram-positive bacterial and *Mycobacterium* spp. (B) Mitochondrial electron transport chain.

Protons are translocated across the cell membrane (from the cytoplasm to the periplasmic space) concomitantly. Synthesis of Adenosine triphosphate (ATP) from ADP and phosphate

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is coupled with protons move through these complexes (**Figure 2**). Therefore, CoQ_{10} and menaquinone occupy a central and essential role in Adenosine triphosphate (ATP) synthesis [13, 14]. From the taxonomic studies, it is evident that the majority of Gram-positive bacteria including *Mycobacterium* spp. utilize only menaquinone in their electron transport systems [15], and menaquinone biosynthesis is essential for survival of Gram-positive bacteria [16, 17]. Several *in vitro* studies indicated that exogenous menaquinone did not rescue the bacteria treated with selective menaquinone biosynthesis inhibitors [18]. Menaquinone biosynthesis has been extensively studied in *E. coli*. A plethora of Gram-negative organisms use CoQ in their electron transport systems when aerobic conditions prevail, but menaquinone under anaerobic conditions. However, the reaction chain facilitating electron transport humans does not use menaquinone. Unquestionably, the chain of reactions funneling the transport of electrons serves as a central component in the synthesis of Adenosine triphosphate (ATP) and the subsequent multiplication of bacteria (**Figure 2**). Hence, inhibitors of the biosynthesis of menaquinone or inhibitors specifically targeting the enzymes linked to electron transport systems display the potential for the development of novel and selective drugs against multidrug-resistant (MDR) Gram-positive bacteria. Although the functions of vitamin K_1 in humans and vitamin K_2 in bacteria are entirely different, drug discovery targeting vitamin K_2 or its biosynthesis requires careful consideration of vitamin K distribution in tissue and selectivity against the target protein because essential vitamin K-dependent protein(s) may be interfered by vitamin K biosynthesis inhibitors. Nonetheless, many evidences support that menaquinone biosynthesis inhibitors can be developed into selective antibacterial agents for infections caused by Gram-positive bacteria and *Mycobacterium* spp.

5. Biosynthesis of menaquinone

Menaquinones play an important role in electron transport, and oxidative phosphorylation. In addition, they are responsible for active transport, and endospore formation in some *Bacillus* species [19]. The biosynthetic steps leading to menaquinone have been studied in *E. coli* (*vide supra*) [19, 20]. The synthesis of menaquinone is accomplished by MenA-MenG as illustrated in **Figure 3**. These enzymes are encoded by two clusters of genes. The men gene cluster consists of the *MenB, C, D, E, and F* and a separate cluster containing *MenA* and *MenG* [21, 22]. The biosynthesis of menaquinone is initiated from chorismate and proceeds through a series of menaquinone-specific reactions. MenF is isomerizing to chorismate in order to form isochorismate. MenD (a thiamine diphosphate-dependent enzyme) catalyzes a Stetter-like conjugate addition (a 1,4-addition of an carbonyl molecule to α β-unsaturated compound) of α-ketoglutarate with isochorismate, forming 2-succinyl-5-enolpyruvyl-6-hydroxy-3-cyclohexadiene-1-carboxylate. Its pyruvate moiety is eliminated by MenH to yield 2-succinyl-6-hydroxy-2,4-cyclohexadiene-1-carboxylate. MenC catalyzes aromatization of 2-succinyl-6-hydroxy-2,4-cyclohexadiene-1-carboxylate, forming *o*-succinylbenzoate. MenE is an *o*-succinylbenzoate-CoA ligase, which converts *o*-succinylbenzoate to *o*-succinylbenzoate-CoA. Thereafter, MenB catalyzes a formal Dieckmann type of condensation of *o*-succinylbenzoate-CoA to yield 1,4-dihydroxy-2-naphthoyl-CoA, which, in turn, is being hydrolyzed to 1,4-

dihydroxy-2-naphthoate (DHNA) thioeter-splitting enzyme encoded by *yfbB*. In contrast, the prenyl diphosphate with appropriate size (i.e., n = 7 in *E. coli*) is being biosynthesized by the iterative reaction of allyl diphosphate in the presence of isopentenyl diphosphate. Then, DHNA is prenylated and methylated by MenA and MenG, respectively, yielding menaquinones as the end product. The side chains of menaquinones vary in different species and even within the same organisms. The more common of menaquinones display 7, 8, and 9 isoprene (C-5) units; MK-7 serves as the major menaquinone entity in several Gram-positive spore-forming bacteria. MK-8 can be found in *E. coli*, while MK-9 in common in *M. tuberculosis*. However, menaquinones containing 4, 5, 6, 10, 11, 12, and 13 isoprene units have been reported in bacteria.

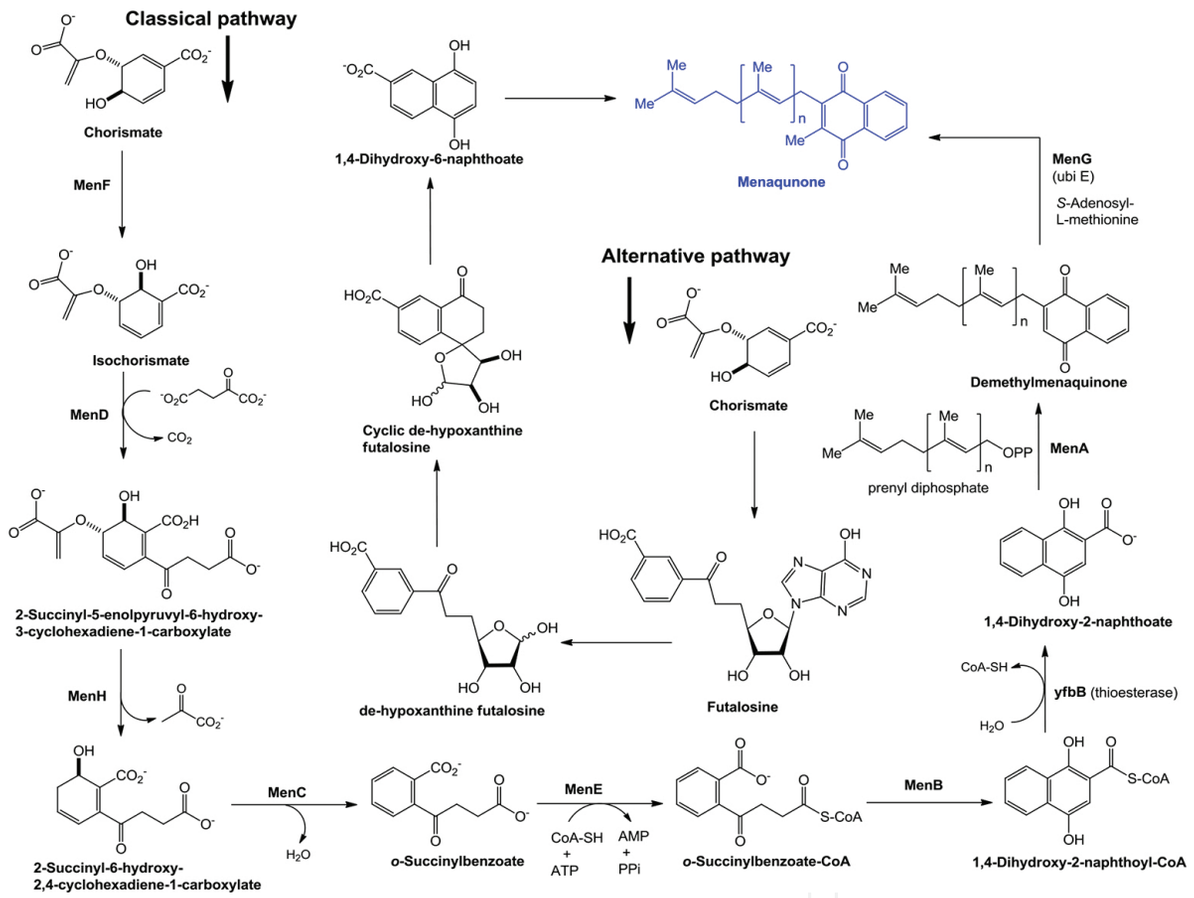

Figure 3. Biosynthesis of menaquinone.

Menaquinones are the predominant isoprenoid lipoquinones of Gram-positive bacteria, whereas Gram-negative bacteria and enterobacteria use menaquinone (MK), demethylmenaquinone (DMK), and ubiquinone (Q) in their electron transport chains (**Figure 2**). Recent studies have shown that several γ-proteobacteria appear to share the similar electron transport system to that observed in *E. coli* [23–26]. Several studies indicated that the regulation of menaquinone biosynthesis of aerobically growing bacteria is different from those of bacteria under anaerobic respiratory conditions being controlled by FNR as the general regulator.

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Additionally, the MK/DMK ratio is not dependent on the fur locus, and substantial amounts of naphthoquinones (MK and DMK) are retrieved only during anaerobic conditions in *E. coli*. The menaquinones were detected almost no exception within the bacterial membrane. The total amount of naphthoquinones was found to be some 0.60 ~ 1.09 μmol/g cell. It has become evident that several bacterial species do not have methylase (or MenG) and thus produce DMK as their sole quinone [19]. Conversion from MK to DMK is the last step in the biosynthesis. The activity of the DMK methylase is likely to be regulated by the presence or absence of the electron carriers or by the supply of *S*-adenosylmethionine [20]. A bioinformatic analysis of whole-genome sequences suggested that some microorganisms, including *Helicobacter pylori* and *Campylobacter jejuni*, and lactobacilli do not have orthologs of the men genes, although they synthesize menaquinone. These bacteria synthesize menaquinones in an alternative pathway via futalosine via alternative pathway (**Figure 3**) [23].

6. Antibacterial drug discovery by targeting menaquinone biosynthesis

Menaquinone is the sole quinone in the electron transport chain in the majority of Gram-positive bacteria including *Mycobacterium* spp. The biosynthetic pathway leading to menaquinone is absent in humans; therefore, the bacterial enzymes responsible for menaquinone biosynthesis are potential drug targets for development of novel antibacterial agents. It is speculated that dormant (non-replicating) *M. tuberculosis* displays a less active metabolism and also diminished energy reserves; however, Adenosine triphosphate (ATP) synthesis during oxidative phosphorylation is active during the dormant state. Therefore, inhibition of the menaquinone biosynthesis might exert serious effects on the maintenance of dormancy in *M. tuberculosis*. This concept emphasized the reports that phenothiazines block the type II Nicotinamide adenine dinucleotide phosphate (NADH): menaquinone oxidoreductase (**Figure 4**) in the bacterial respiratory chain and also were effective in killing non-replicating *M. tuberculosis* [27]. Interestingly, it was demonstrated that inhibition of MenA (1,4-dihydroxy-2-naphthoate prenyltransferase) (**Figure 3**) showed significant growth inhibitory activities against drug-resistant *Mycobacterium* spp. and Gram-positive bacteria [28]. MenA inhibitors

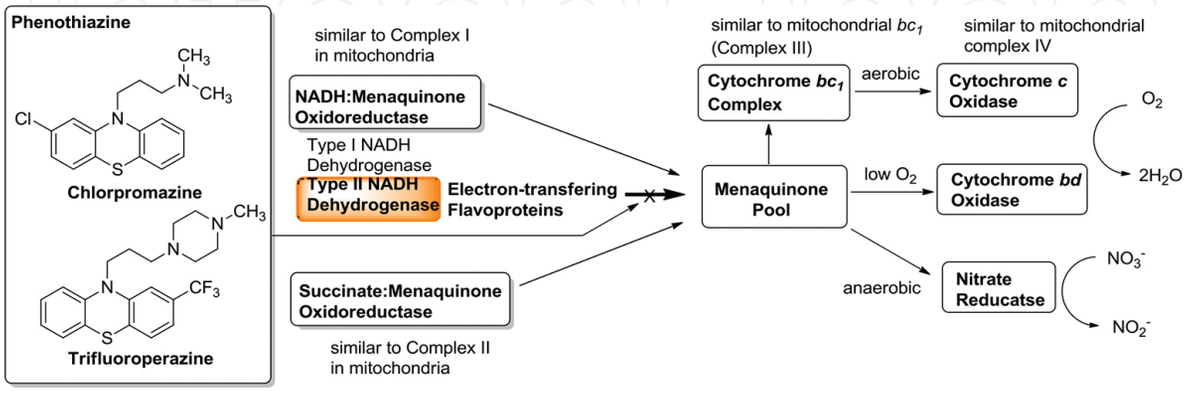

Figure 4. Electron flow in *M. tuberculosis* and type II Nicotinamide adenine dinucleotide phosphate (NADH) dehydrogenase inhibitors.

effectively killed non-replicating *M. tuberculosis in vitro*. Several other promising biological date were generated on MenA as a new antibacterial drug target; (1) *M. tuberculosis* growth *in vitro* could not be rescued by exogenous vitamin K_2 supplementation, (2) all Gram-positive bacteria tested (e.g., *Staphylococcus aureus*, *Enterococcus faecalis,* and *Clostridium difficile*) were susceptible to MenA inhibitors, whereas Gram-negative bacteria (*E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*) were not susceptible under aerobic conditions, and (3) MenA inhibitors are effective in killing *E. coli* under anaerobic conditions [18]. To date, several menaquinone biosynthesis enzymes have been studies for the development of novel antibacterial agents.

7. Menaquinone biosynthesis inhibitors

7.1. MenA inhibitors

Among the menaquinone biosynthesis enzymes, MenA is a membrane-associated protein that catalyzes prenylation of demethylmenaquinone (DMK), forming 1,4-dihydroxy-2-naphtoate (DHNA). Analyses of the amino acid sequence of MenA were revealed that MenA displays five transmembrane segments, and that there exists highly conserved aspartate (D), which would be localized to the inner-plasma membrane, which was being predicted by the aid of a prediction program (Sosui) [14]. The activity is totally dependent on the presence of divalent cations, such as Mg^{2+}. It is therefore likely that these divalent cations produce ion pairs with Asp residues contained within the catalytic site within MenA. A library of DMMK mimics possessing the amino group(s) were generated and evaluated in an enzymatic assay *in vitro* (IC_{50}) against *Mtb* MenA. Identified MenA inhibitors were evaluated in bacterial growth inhibitory assays (MIC). The *tertiary* or *secondary* amine-containing benzophenone derivatives **1** and **2** are the first-generation MenA inhibitors that exhibited bactericidal activities against *M. tuberculosis* (MIC 1–1.5 μg/mL for **1**) and Methicillin-resistant Staphylococcus aureus (MRSA) (MIC 4.0 μg/mL for **2**), respectively. These molecules are inhibited growth of drug-resistant *Mycobacterium* spp. and drug-resistant Gram-positive bacteria (vancomycin-resistant *S. aureus*, vancomycin-resistant *E. faecalis*, and linezolid-resistant Methicillin-resistant Staphylococcus aureus (MRSA)) at low concentrations [29, 30]. Significantly, the MenA

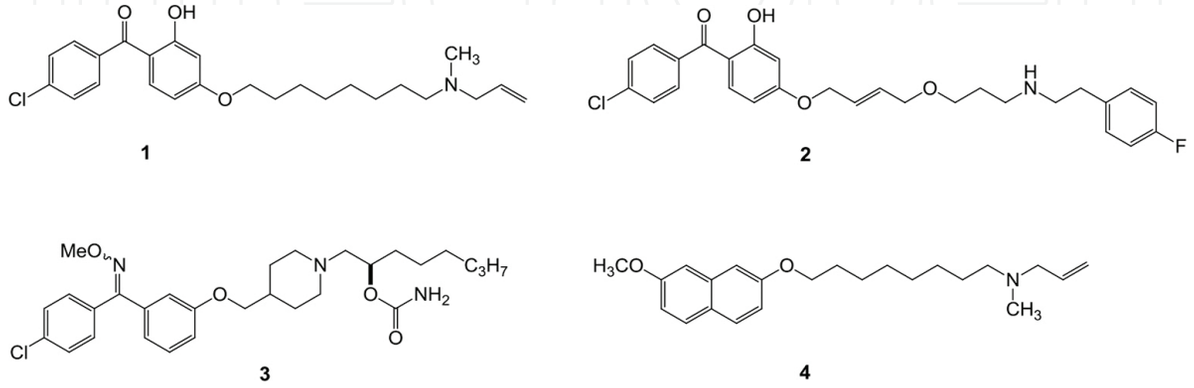

Figure 5. MenA inhibitors.

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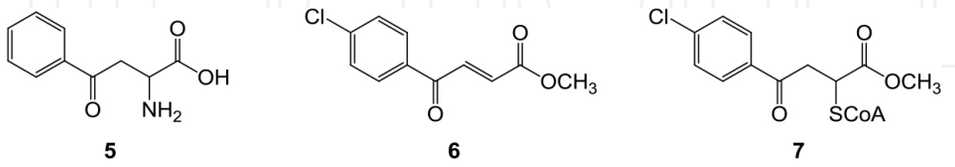
inhibitor **1** killed non-replicating *M. tuberculosis* much faster than first-line Tuberculosis (TB) drug, rifampicin at lower concentrations. Later, the same group developed selective antimycobacterial MenA inhibitor **3**. The optically pure **3** is the most active molecule in killing non-replicating Mtb. *In vitro* data with selective MenA inhibitors suggested that menaquinone biosynthesis is important in maintaining mycobacterial viability under conditions of restricted oxygen [18]. MenA inhibitors are likely to block the electron flow, consequently inhibiting the bacterial growth. The other group identified 7-methoxy-2-naphthol-based MenA inhibitors (e.g., **4**) that killed *M. tuberculosis* and Gram-positive bacteria with the MIC level of 3–25 μg/mL (**Figure 5**) [31].

7.2. MenB inhibitors

One of at least seven enzymes in menaquinone biosynthesis, MenB (1,4-dihydroxy-2-naphthoyl-CoA synthase) forms the bicyclic ring system by catalyzing the Dieckmann type reaction of *o*-succinylbenzoyl-coenzyme A to 1,4-dihydroxy-2-naphthoic acid. A high-resolution co-crystal structure of *E. coli* MenB with a stabilized analog of *o*-succinylbenzoylCoA was successfully diffracted. The MenB X-ray structure provides important insight into the catalytic mechanism. Similarly, the X-ray structure of MenB from *M. tuberculosis* was characterized. A high-throughput screen (HTS) against *M. tuberculosis* MenB led to the discovery of 2-amino-4-oxo-4-phenylbutanoic acid (**5**) that inhibits MenB at low nanomolar concentrations [32]. Later, methyl 4-(4-chlorophenyl)-4-oxobut-2-enoate (**6**) was reported to inhibit MenB by forming the CoA adduct, **7**. The adduct **7** binds to the *S. aureus* MenB with a K_d value of 2 μM, and also killed drug-sensitive and drug-resistant *S. aureus* strains at 0.35–0.75 μg/mL concentrations [33]. Bacterial growth inhibitory assays of **6** against a battery of bacteria concluded that **6** is effective only against bacteria that utilize menaquinone for respiration. *In vivo* efficacy of **1** using the mouse models of Methicillin-resistant Staphylococcus aureus (MRSA) infection revealed that **6** increased survival in a systemic infection model and resulted in a dose-dependent decrease in bacterial load [34]. These *in vitro* and *in vivo* studies came to the conclusion that MenB is a valid target for the development of new anti-Methicillin-resistant Staphylococcus aureus drug and infections caused by Gram-positive bacterial infections (**Figure 6**).



5 **6** **7**

Figure 6. MenB inhibitors.

7.3. MenD inhibitors

MenD (2-succinyl-5-enolpyruvyl-6-hydroxy-3-cyclohexadiene-1-carboxylate synthase) catalyzes a thiamin diphosphate-dependent decarboxylative carboligation of α-ketoglutarate and isochorismate via a Stetter-like conjugate addition. MenD is also essential for menaquinone

biosynthesis in some bacteria and has been recognized as an antibacterial drug target. A succinylphosphonate ester, 4-(methoxyoxidophosphoryl)-4-oxobutanoate (**8**) was reported to be a competitive inhibitor of MenD (Ki values ~700 nM) [35]. An analog of the cofactor, thiamine diphosphate, oxythiamine **9** was reported to exhibit MenD enzyme and *S. aureus* growth inhibitory activities (**Figure 7**) [36].

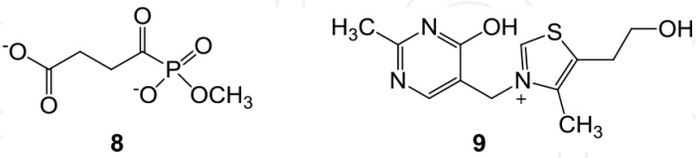


Figure 7. MenD inhibitors.

7.4. MenE inhibitors

MenE (*o*-succinylbenzoate-CoA synthetase) is an essential adenylate-forming enzyme that is also a promising target for development of novel antibiotics in the menaquinone biosynthesis. Adenosylsulfonamide, adenosylsulfamate, and adenosylsulfamide analogs **10**, **11**, and **12** were developed as inhibitors of MenE enzymes based on the structure of *o*-succinylbenzoate-CoA. The vinyl sulfonamide **10** was found to be the most potent MenE inhibitor (IC_{50} ~ 5.7 μM against *M. tuberculosis* MenE) [37]. The vinyl sulfonamide **10** is a competitive inhibitor of *M. tuberculosis* MenE with respect to Adenosine triphosphate (ATP) (Ki = 5.4 ± 0.1 nM) (**Figure 8**).

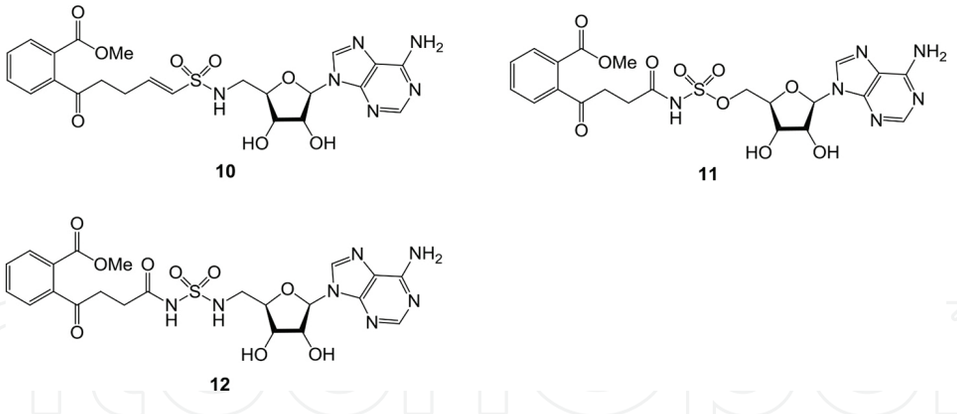


Figure 8. MenE inhibitors.

8. Conclusion

Bacterial Adenosine triphosphate (ATP) synthase, F_1F_0-ATPase, is a viable target for treatment of *M. tuberculosis* infections. Diarylquinolone, an inhibitor of *M. tuberculosis* Adenosine triphosphate (ATP) synthase exhibited a remarkable activity against *Mycobacterium* spp. Electron transport systems associated with Adenosine triphosphate (ATP) synthases are

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established drug targets for antibacterial and antiprotozoal infections. For example, Nicotinamide adenine dinucleotide phosphate (NADH) hydrogenase is a sustainable drug target for the malaria parasite and is also a promising target for *M. tuberculosis* infections. On the other hand, menaquinone biosynthesis has recently been received attention for the development of novel antibacterial agents. Menaquinone is a key component of the electron transport systems in the majority of Gram-positive bacteria including *M. tuberculosis*. As summarized in Chapter 6, inhibitors of menaquinone biosynthesis have been identified and several compounds are also effective inhibitors of bacterial growth. In development of new drugs for *M. tuberculosis* infections, it is the ultimate goal to discover a Tuberculosis (TB) drug that is effective against human latent tuberculosis infection. Among the menaquinone biosynthesis enzymes, MenA has been extensively studies as drug target for *M. tuberculosis* and Gram-positive bacteria. Selective MenA inhibitors inhibit the growth of non-replicating *M. tuberculosis*, suggesting that menaquinone is essential in maintaining the bacterial viability during conditions of restricted levels of oxygen. A large set of data suggest that the DosR/DosS/DosT signaling pathway is mandatory for *M. tuberculosis'* genetic response to hypoxic conditions and nitric oxide, in the adaptation of *M. tuberculosis* to conditions triggering a reversible bacteriostasis. In this way, the DosR/DosS/DosT signaling pathway may contribute to the latency seen *in vivo* [38]. MenA inhibitors display the ability to block or hamper the flow of electrons without inducing a dormancy response in *M. tuberculosis*. Consequently, menaquinone biosynthesis inhibitors have the potential to kill *M. tuberculosis* at any states by inhibiting Adenosine triphosphate (ATP) synthesis non-directory.

Recently, a narrow-spectrum antibiotic, siamycin I was reported to kill *Helicobacter* and *Campylobacter* by inhibiting the futalosine (alternative) pathway (**Figure 3**). Branched fatty acids (12- or 13-methyltetradecanoic acids) also inhibit the biosynthesis of menaquinone biosynthesis of *H. pylori*. Because of advances of biological assays of menaquinone biosynthesis, new inhibitor molecules that possess drug-like characteristics will be identified. It is import to prove the efficacy of menaquinone biosynthesis inhibitor using an appropriate infected animal model. To date, *in vivo* efficacy of a MenB inhibitor was demonstrated using the mouse model of Methicillin-resistant Staphylococcus aureus (MRSA) infection. A Tuberculosis (TB) drug, SQ109, a strong inhibitor of trehalose monomycolate (TMM) transporter, was reported to exhibit inhibitory activities of MenA and MenG enzymes from *M. tuberculosis*. Discovery of a pharmacologically acceptable menaquinone biosynthesis inhibitor, which possesses a significant antibacterial activity against replicating and non-replicating *M. tuberculosis*, has been highlighted in Tuberculosis (TB) drug development. It is worthwhile mentioning that menaquinone biosynthesis inhibitors are also promising agents to kill Gram-negative bacteria growing under oxygen-depleted or anaerobic conditions in which menaquinone is utilized for their respiration.

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