Review

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Menaquinone as a potential target of antibacterial agents

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Summary **The current trend of increasing infections by multidrug-resistant pathogens requires the discovery of novel antimicrobial agents with new target and selective toxicity towards pathogens. Menaquinone is a component of electron transport chains in a majority of anaerobic bacteria and Gram-positive bacteria. Due to its exclusivity in bacteria, menaquinone is thought to be a potential target for development of therapeutically effective antibacterial agents without side effects. In this review, we summarize inhibitors of menaquinone biosynthesis and antibiotics directly targeting menaquinone in bacteria.**

Keywords: Menaquinone, lysocin E, antibacterial agent

1. Menaquinone, its role and distribution

Bacteria use isoprenoid quinones such as ubiquinone (UQ) or menaquinone (MK) or demethylmenaquinone (DMK) (Figure 1) for their electron transport systems, which are found exclusively in cytoplasmic membranes (*1,2*). These quinones are important for the respiratory chain and play vital roles in cellular respiration, oxidative phosphorylation and formation of transmembrane potential in bacteria (*3*). Some bacteria have more than one of these quinones which they utilize according to growth conditions (*4*). MK, 2-methyl-3-polyprenyl-1,4-naphthoquinone, is the sole quinone in anaerobically growing bacteria, mycobacteria and most of the Gram-positive bacteria (*2*). MK exists in different forms according to the number of isoprene units which vary from 4 to 13 (*2*). In addition, some microbes require MK for virulence (*5*), regulation of certain gene expression such as nitrogen fixation (*6*), and during endospore and cytochrome formation (*7-9*).

Mammals utilize UQ as a sole quinone for respiration whereas MK is utilized for blood coagulation (*10*), bone metabolism (*11*), cell-cycle regulation (*12*) *etc*. The major source of MK in humans is either the diet or gut flora. Although UBIAD1, an enzyme that can catalyze the conversion of plant phylloquinone to MK-4, has been reported in humans, humans are not capable of *de novo* biosynthesis of MK (*13*). Therefore, it is expected that MK and its biosynthetic pathway serve as a platform for selectively targeting infections caused by pathogens that utilize MK. In this review, we summarize antimicrobial agents that either inhibit MK biosynthesis or directly interact with MK.

2. MK biosynthesis and its inhibition by small molecules

MK biosynthesis has been extensively studied. MK is synthesized from chorismate using either a classical or an alternative pathway. The recent understanding of MK biosynthesis and its critical roles for microbial growth has made it a potential target of antimicrobial agents and inhibitors of biosynthetic enzymes have been identified. Most of the inhibitors are analogues of either the substrate or cofactors of the enzymes.

The classical pathway involves enzymes, namely MenF, MenD, MenH, MenC, MenE, MenB, yfbB (MenI), MenA and MenG (Figure 2) (*3,14-17*). Targeting these enzymes that exist in bacteria and not in humans, can open up an avenue for novel antimicrobial agents with therapeutic potential. A number of inhibitors of these enzymes have already been identified (Figure 3). Some microorganisms such as *Helicobacter pylori*, *Wolinella succinogenes*, *Campylobacter jejuni*, *Geobacter sulfurreducens*, *Streptomyces coelicolor*, *Streptomyces avermitilis*, *Thermus thermophilus, Deinococcus radiodurans* and so on, synthesize MK using an alternative pathway

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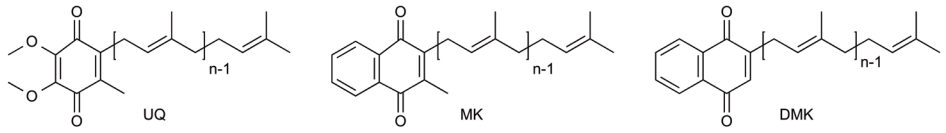


Figure 1. Quinones in bacterial electron transport systems

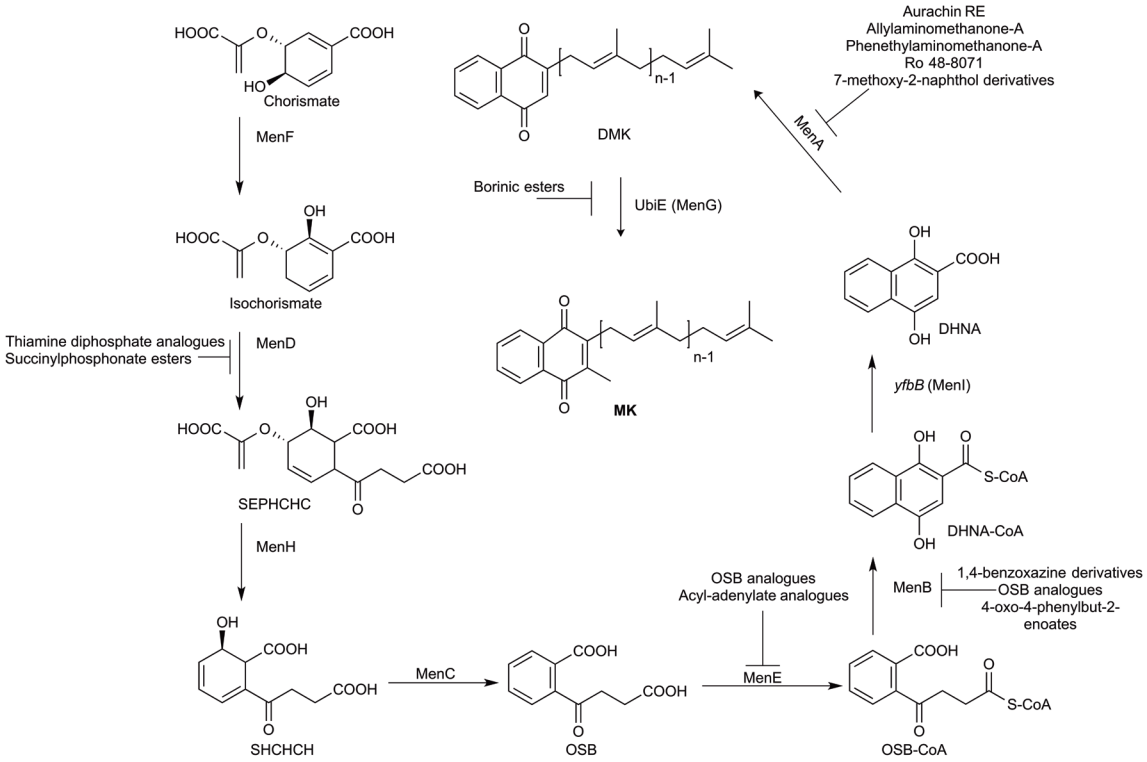


Figure 2. The classical MK biosynthesis pathway and inhibitors (SEPHCHC: 2-succinyl-5-enolpyruvyl-6-hydroxy-3-cyclohexene-1-carboxylate; SHCHC: 2-succinyl-6-hydroxy-2,4-cyclohexadiene-1-carboxylate; OSB: o-succinylbenzoate; DHNA: 1,4-dihydroxy-2-naphthoyl).

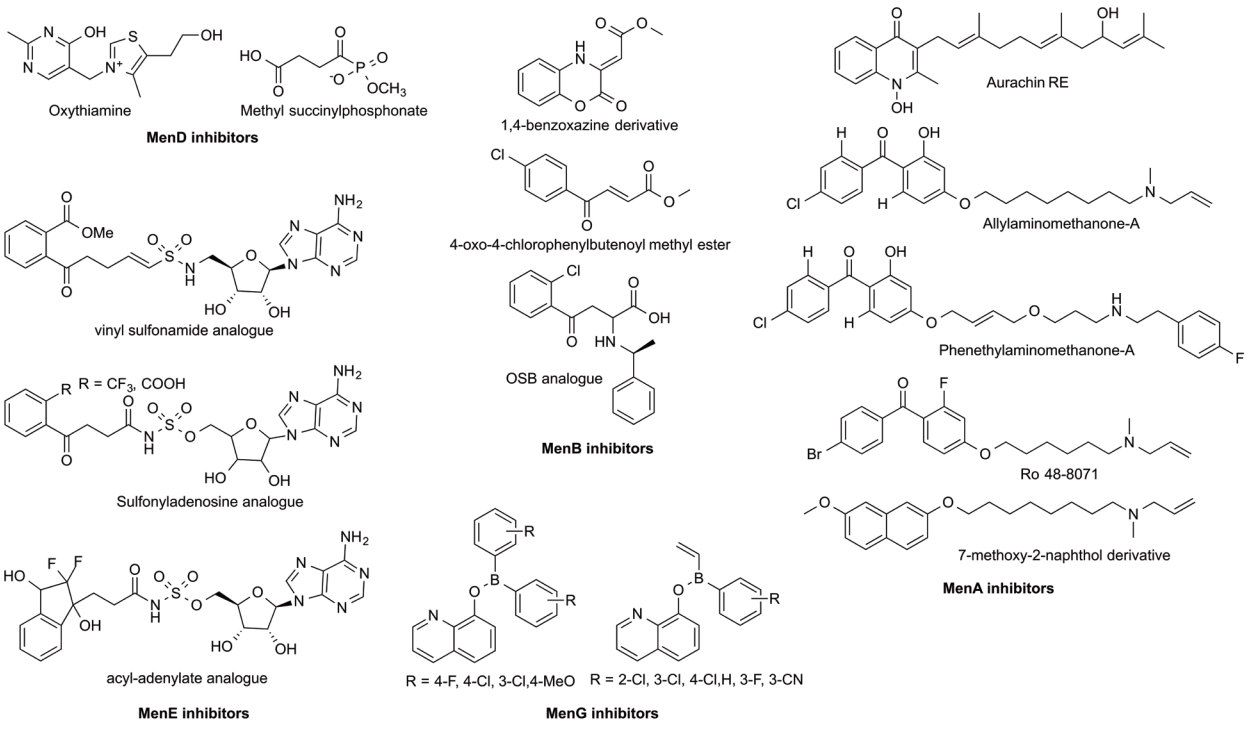


Figure 3. Inhibitors of classical MK biosynthetic pathway

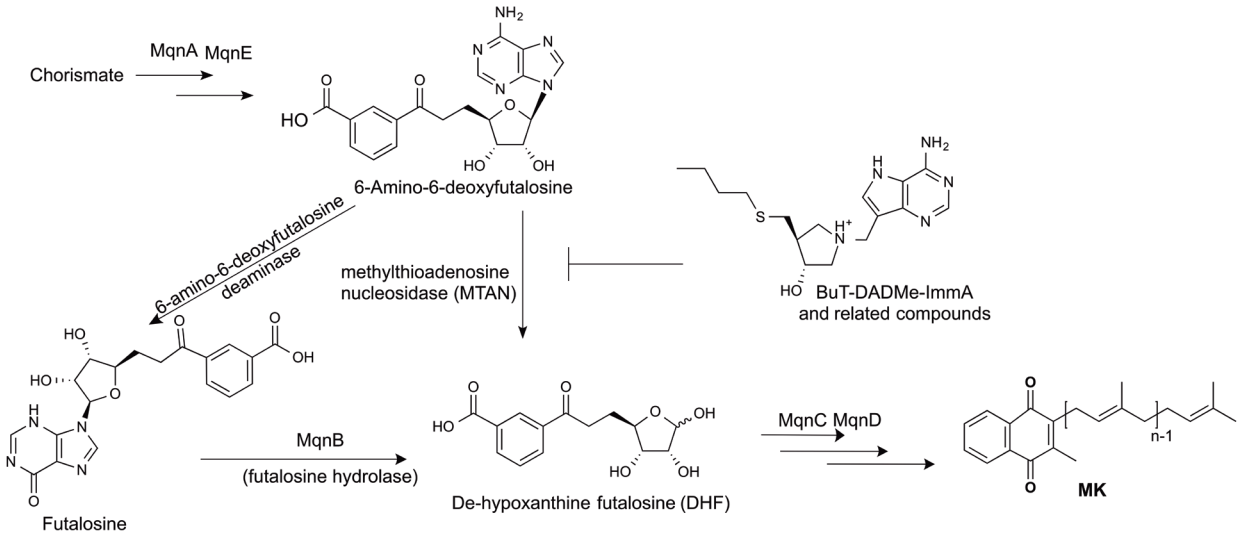


Figure 4. The alternative pathway for MK biosynthesis and inhibitors.

Table 1. MK biosynthesis inhibitors and their action

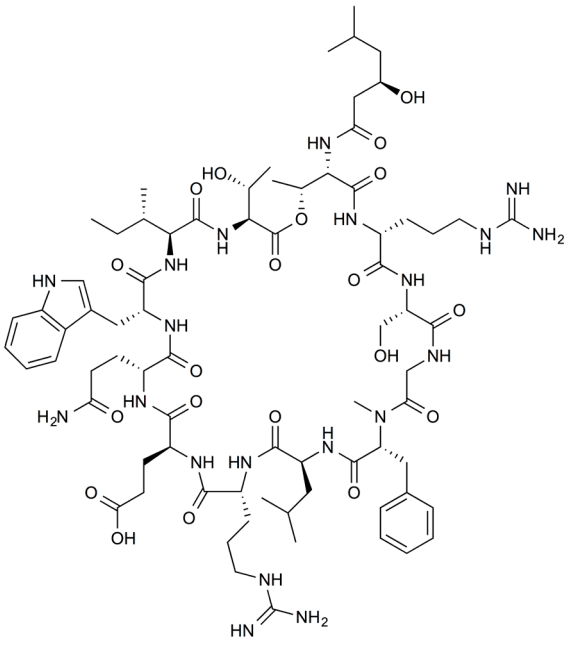
Target enzyme	Inhibitors (*Ref.*)	Growth inhibition
MenD	Thaimine diphosphate analogues (*24*)	+
	Succinyl phosphonate esters (*25*)	-
MenE	OSB analogues	
	Vinyl sulphonamide analogues (*26*)	-
	Sulfonyladenosine analogues (*27,28*)	ND
	Acyl-adenylate analogues (*29*)	+
MenB	OSB analogues (*24*)	+
	1,4-benzoxazine derivatives (*30*)	+
	4-oxo-4-phenylbut-2-enoates (*31*)	+
MenA	Aurachin RE (*17*)	ND
	Allylaminomethanone-A (*32*)	+
	Phenethylaminomethanone-A (*32*)	+
	Selective mycobacterial MenA inhibitor (*17*)	+
	Ro 48-8071 (*33*)	+
	7-methoxy-2-naphthol derivatives (*34*)	+
MenG	Borinic esters (*35*)	+
MTAN	BuT-DADMe-ImmA and analogues (*36,37*)	+

*ND: not determined

(*18,19*) that involves conversion of chorismate into 6-amino-6-deoxyfutalosine by MqnA and MqnE. Another important step of the alternative pathway, conversion of 6-amino-6-deoxyfutalosine to de-hypoxanthine futalosine (DHF), occurs by either a single step reaction catalyzed by methylthioadenosine nucleosidase (MTAN) as in *H. pylori* and *C. jejuni* or a two-step reaction catalyzed by 6-amino-6-deoxyfutalosine deaminase (*20,21*) and MqnB as in *S. coelicolor* and *T. thermophilus* (*18*) (Figure 4). The enzymatic reaction to convert DHF to MK involves MqnC, MqnD and possibly MenA and MenG (*22*). In the alternative pathway, an inhibitor of MTAN has been reported (Figure 4). Among the known inhibitors of MK biosynthesis, a MenB inhibitor 4-oxo-4-chlorophenylbutenoyl methyl ester showed therapeutic effects in a mouse model (*31*). Of note, not all the inhibitors of enzymes showed antimicrobial activity against microorganisms (Table 1).

3. Antibiotics interacting directly with MK

Lysocin E, a cyclic lipopeptide produced by Lysobacter sp. RH2180-5, directly interacts with MK and is the first antibiotic whose target is MK (*23*). It was found to directly bind to MK with a dissociation constant of 4.5 μM. The striking feature that makes lysocin E unique from other known antibiotics is its potent bactericidal



Microorganisms	MIC (μg/ml)	Quinone
Staphylococcus aureus	2-4	MK
Bacillus spp.	2	MK
Listeria monocytogenes	0.5	MK
Mycobacterium spp.	8	MK
Serratia marcescens	>100	UQ
Pseudomonas aeruginosa	>100	UQ
Candida spp.	>100	UQ
Cryptococcus neoformans	>100	UQ
Escherichia coli W3110	>100	UQ, MK, DMK
Streptococcus spp.	>128	None

Figure 5. Lysocin E, its MIC against various microorganisms (*23*), and quinones present in the microorganisms (*2*). MK: menaquinone, UQ: ubiquinone, DMK: demethylmenaquinone.

activity. *Staphylococcus aureus* showed rapid loss in absorbance at 600 nm in the presence of lysocin E indicating the lysis of bacteria. Besides bacteriolysis, potassium ion leakage from membranes and a rapid loss of bacterial membrane potential in *S. aureus* were observed in the presence of lysocin E. Spontaneous mutants resistant to lysocin E showed decreased production of MK and knockout mutants of the genes involved in the MK biosynthetic pathway, Δ*menA* and Δ*menB*, showed resistance to lysocin E. Moreover, antibacterial activity of lysocin E was decreased in the presence of MK, but not UQ, in the culture medium. This is probably due to the binding of lysocin E to the excessive amount of MK in the medium, leaving a small pool of lysocin E for binding with MK present in the bacterial membrane. The disruption of synthetic liposomes by lysocin E was dependent on the presence of MK. Further, Δ*menA* and Δ*menB* mutants of *S. aureus* showed repressed potassium leakage from their membranes. Thus, lysocin E directly targets MK, not the enzymes involved in MK biosynthesis. Lysocin E does not show antibacterial activity against *Escherichia coli* although the bacteria has MK in its cytoplasmic membrane. Membrane permeability might be the limiting factor for this Gram-negative bacteria (Figure 5). Lysocin E targets MK in the bacterial cytoplasmic membrane and causes membrane disruption, ultimately leading to cell death. Moreover, lysocin E was non-toxic to mice (acute toxic dose: > 400 mg/kg) and showed potent therapeutic activity in mice infected with MRSA (ED_{50}: 0.5 mg/kg). Little acute toxicity and potent therapeutic activity of lysocin E in animal infection models suggested that lysocin E has a potential for clinical application.

4. Conclusion

The respiration and electron transport chains are important for organisms. Since, most of the Gram-positive bacteria utilize MK and mammals utilize UQ as the sole cofactor in their electron transport system, inhibitors of MK are expected to show selective toxicity towards these bacteria. Many inhibitors of the enzymes of MK biosynthetic pathway have been developed and recent advances in the understanding of MK biosynthesis have attracted attention for MK as a target of antibacterial agents. Moreover, the discovery of MK targeting antibiotic, lysocin E, is a breakthrough in this field broadening the importance of MK as a potential target of antibacterial agents with therapeutic potential for the treatment of infectious diseases.

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