

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Medicinal Chemistry of Vitamin K Derivatives and Metabolites

Shinya Fujii and Hiroyuki Kagechika

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/63511>

Abstract

Vitamin K acts as a cofactor for γ -glutamyl carboxylase. Recently, various biological activities of vitamin K have been reported. Anti-proliferative activities of vitamin K, especially in vitamin K₃, are well known. In addition, various physiological and pharmacological functions of vitamin K₂, such as transcription modulators as nuclear steroid and xenobiotic receptor (SXR) ligands and anti-inflammatory effects, have been revealed in the past decade. Characterization of vitamin K metabolites is also important for clinical application of vitamin K and its derivatives. In this chapter, recent progress on the medicinal chemistry of vitamin K derivatives and metabolites is discussed.

Keywords: vitamin K derivative, metabolite, antitumor activity, anti-inflammatory activity, steroid and xenobiotic receptor/pregnane X receptor

1. Introduction

Vitamin K is a specific cofactor for γ -glutamyl carboxylase (GGCX), which catalyzes formation of γ -carboxyglutamyl (Gla) residues in vitamin K-dependent proteins (**Figure 1**) [1]. Various other biological activities of vitamin K and its derivatives have also been reported. For example, vitamin K₃ (menadione), a vitamin K homologue that was considered as a synthetic vitamin K, has antitumor activity [2–5], as does vitamin K₂ (menaquinone) [6, 7]. Among the homologues of vitamin K₂, menaquinone-4 (MK-4), which contains four isoprene units, has been intensively investigated. It binds to nuclear receptor human pregnane X receptor (PXR), which is also called steroid and xenobiotic receptor (SXR), and regulates transcription of osteoblastic genes [8, 9]. It also exhibits anti-inflammatory activity by suppressing the NF- κ B pathway [10], and has an inhibitory effect on arteriosclerosis [11]. It binds 17 β -hydroxysteroid dehydrogenase 4 and

modulates estrogen metabolism [12]. Further, it enhances testosterone production [13, 14], and shows growth-inhibitory activity toward hepatocellular carcinoma (HCC) cells [6, 7]. These biological activities of vitamin K and its analogues are attractive targets of drug discovery, and the activities of vitamin K metabolites have also attracted much interest. A great many natural and synthetic biologically active 1,4-naphthoquinone derivatives (i.e., vitamin K derivatives) have been reported. In this chapter, we will focus on three medicinal-chemistry studies of vitamin K activities.

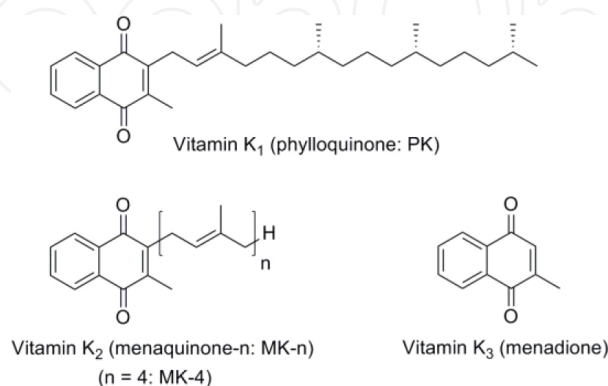


Figure 1. Structures of vitamin K homologues.

2. Menadione derivatives as antitumor agents

The antitumor activity of thioether derivatives is one of the most intensively investigated fields in the medicinal chemistry of menadione derivatives. Several series of naphthoquinone derivatives and benzoquinone derivatives bearing an alkyl, alkoxy, or alkylthio group as a side chain have been synthesized and biologically evaluated by assay of growth-inhibitory activity toward human hepatoma cell line HepB3. Almost all of the tested compounds, as well as the parent menadione, exhibited significant inhibitory activity, and the alkylthio derivatives were more potent than the corresponding alkyl and alkoxy derivatives. Among these compounds, a 2-hydroxyethylthio derivative Cpd 5 (compound 5; NSC 672121) exhibited the most potent activity (**Figure 2**) [15]. Subsequent studies revealed that Cpd 5 irreversibly inhibits growth-regulatory phosphatase Cdc25 by arylating a cysteine residue in the catalytic site, causing cell-cycle arrest [16–19].

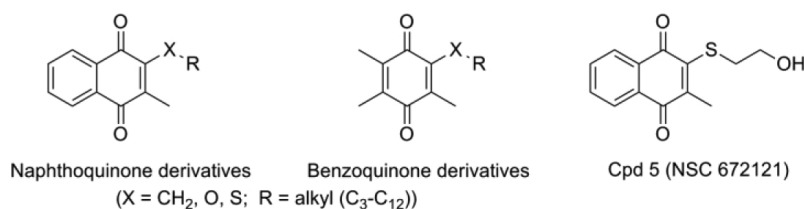


Figure 2. Compounds tested in the initial work on development of Cpd5.

Based on the finding that Cpd5 inhibits Cdc25 and exerts antitumor activities, various menadione derivatives have been developed as candidate antitumor compounds. Bis(2-hydroxyethylthio)naphthoquinone derivative NSC 95397 (**Figure 3**) showed potent Cdc25-inhibitory activity and inhibited proliferation of several cancer cell lines with greater potency than that of Cpd 5 [20]. Hydroxylated NSC 95397 derivatives exhibited enhanced Cdc25-inhibitory activity and inhibited growth of several cancer cell lines [21]. Fluorinated Cpd 5 was three times more potent than Cpd-5 itself in Hep3B growth inhibition and induced phosphorylation of ERK1/2, JNK1/2 and p38 in HepB3 cells [22]. Calculations suggested that fluorinated Cpd 5 cannot generate reactive oxygen species because of its modified redox profile, and therefore, the compound appears to function as a pure arylating agent [23]. Modification of the core structure afforded a maleimide derivative PM-20 with a submicromolar IC₅₀ value for HepB3 growth inhibition. Structure-activity relationship study indicated that the biphenyl structure of PM-20 is essential for activity (**Figure 3**) [24].

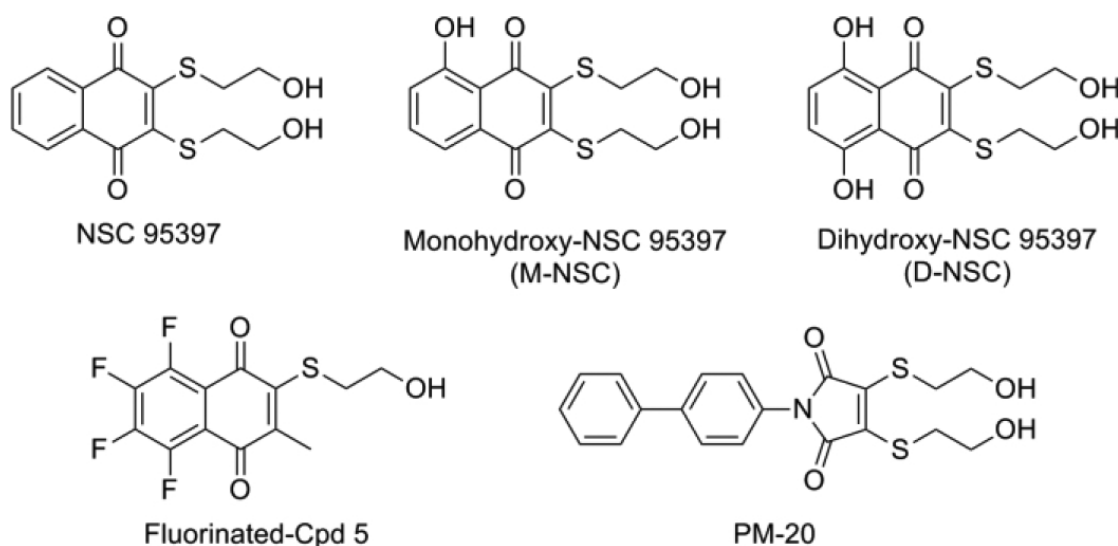


Figure 3. Structures of Cpd 5 derivatives bearing a 2-hydroxyethylthio moiety.

Modification of the hydroxyethyl side chain of Cpd-5 and NSC 95397 was also investigated. Carboxylic acid derivatives such as compounds **1**, **3**, and **4** (**Figure 4**) were designed to interact with arginine residues in the catalytic site of Cdc25B, and indeed, they exhibited potent Cdc25B3-inhibitory activity [25, 26]. Though the cytotoxic activities of these carboxylic acid derivatives, especially dicarboxylic acid **4**, were low, prodrug-type benzyl ester derivatives exhibited enhanced growth-inhibitory activity toward HeLa cells. It was also found that Cpd 5 derivatives bearing a modified terminal, such as **6**, showed selective cytotoxicity toward neuroblastoma cell lines, whereas the parent menadione and Cpd 5 exhibited cytotoxicity toward both neuroblastoma cells and normal cell lines [27]. Aminoalkylmenadione derivatives such as **7** showed angiogenesis-inhibitory activity (**Figure 4**) [28].

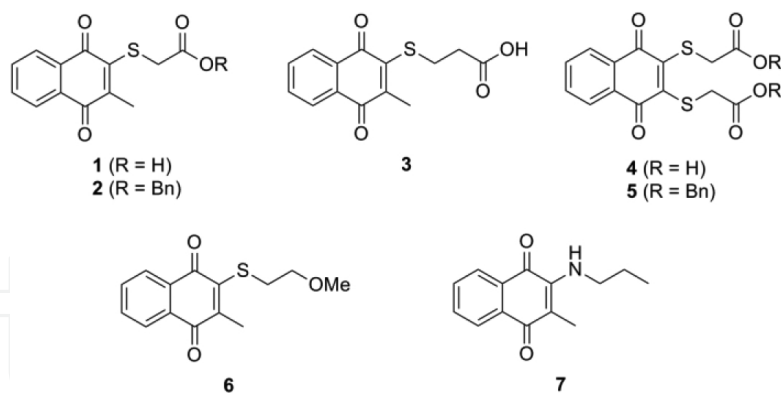


Figure 4. Examples of side chain-modified Cpd 5 derivatives.

A natural product, plumbagin (5-hydroxymenadione, **Figure 5**), shows anticancer and antiproliferative activities [29]. It suppresses the NF- κ B activation pathway by modulating p65 and I κ B α kinase activation to potentiate cytokine- and drug-induced apoptosis [30]. Structurally related naphthoquinone derivatives juglone and 1,4-naphthoquinone exerted similar TNF α -induced NF- κ B inhibitory activities, whereas menadione did not [30]. Another natural product, lapachol, which has a hydroxyl group instead of the methyl group of MK-1, has anticancer activity [31]. A synthetic analogue **8** bearing two isoprene units also exerted antitumor activity (**Figure 5**) [32], and various biologically active lapachol derivatives have been developed [33]. The 2-hydroxy-1,4-naphthoquinone structure has distinct chemistry; for example, it has the characteristics of 1,2-naphthoquinone (e.g., lapachol can cyclize to form α -lapachone or β -lapachone), in contrast to 2-methyl-1,4-naphthoquinone.

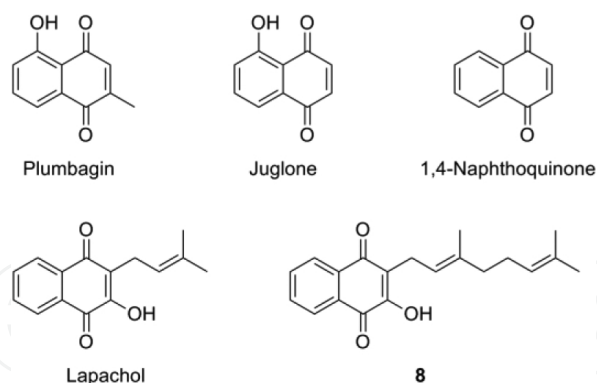


Figure 5. Some vitamin K-related naphthoquinone derivatives with antitumor activity.

3. Structure-activity relationship of MK-4 derivatives as nuclear SXR ligands

In the early twenty-first century, it was found that MK-4 binds a nuclear receptor, steroid, and xenobiotic receptor (SXR), which is a human homologue of pregnane X receptor (PXR), and

regulates transcription of osteoblastic genes [8, 9]. Structure-activity relationships of MK-4 as an SXR ligand were intensively investigated by Suhara et al., using deuterated derivatives (**Figure 6**). Saturation of double bond(s) in the side chain significantly reduced the SXR agonistic activity. Triene derivative **9** bearing a 6,7-saturated side chain exerted only moderate activity, and diene **10**, monoene **11** (phyloquinone-d₇), and alkyl derivative **12** were inactive. Removal of methyl groups also reduced the activity, but demethylated compounds **13–16** still retained significant activity [34].

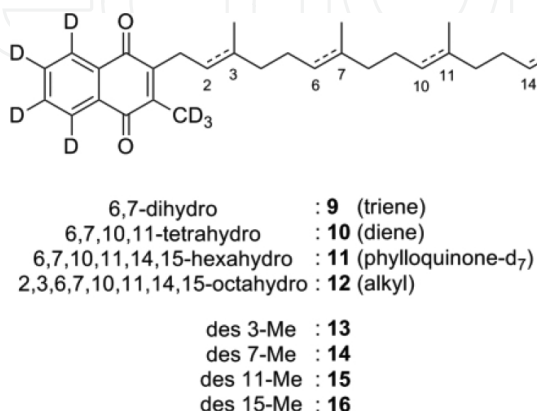


Figure 6. Compounds used in SAR study of SXR.

The length of the side chain is important for the SXR activity of menaquinones. MK-1 bearing one prenyl group showed little ligand potency, while MK-2, MK-3, and MK-4 were more active. In the SXR-GAL4 one hybrid assay system, MK-3 was the most potent compound, and MK-2 and MK-4 showed somewhat lower activity. In the assay system using SXRE, MK-2, and MK-3 were the most potent compounds [35]. “Double side chain” vitamin K analogues bearing the same side chains at the 2-position and 3-position of the naphthoquinone ring were also designed and synthesized. MK-1-W and MK-2-W were as potent as MK-3 and MK-4, whereas MK-3-W, MK-4-W, and PK-W showed little activity (**Figure 7**) [35].

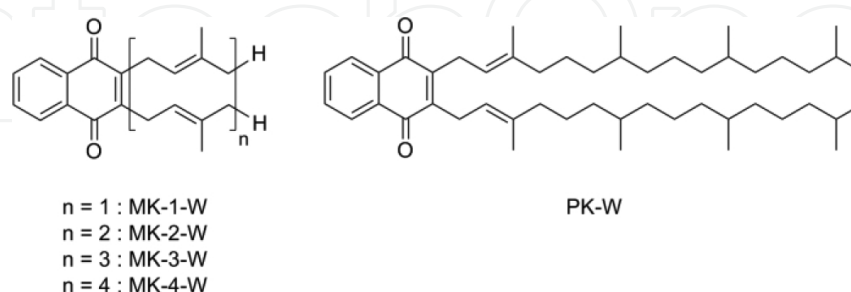


Figure 7. Structures of double side chain vitamin K analogs.

Substitution at the terminal of the side chain of menaquinones significantly affects SXR ligand potency. Hydroxylated derivatives MK-2- ω -OH, MK-3- ω -OH, and MK-4- ω -OH showed little activity in the SXR-GAL4 one hybrid assay system, whereas compounds **17** and **18** bearing a

terminal phenyl group exhibited more potent activity than the parent menaquinones (**Figure 8**). Compounds **17** and **18** also exhibited potent activity in the SXRE assay system [36]. Thus, a suitable hydrophobic side chain is essential for SXR activity of menaquinones.

Interestingly, Suhara et al. also found that menaquinone derivatives bearing a terminal hydrophobic substituent have the ability to induce selective neuronal differentiation of neuronal progenitor cells. The most potent compound **19** was twice as effective as the EtOH control, based on quantitation of Map2 mRNA (**Figure 8**) [37].

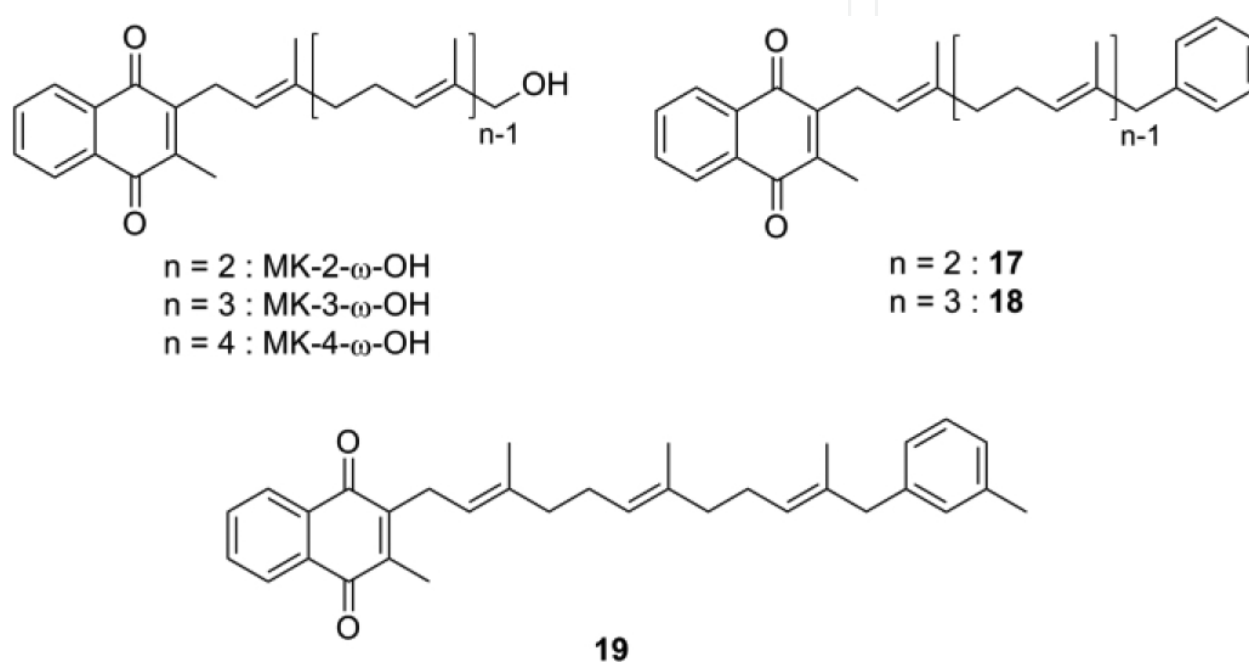


Figure 8. Structures of menaquinone derivatives with modified terminal.

4. Synthesis and biological activity of menaquinone metabolites

The biological activities of metabolites of vitamin K are also important. MK-4 is one of the most interesting vitamin K homologues because of its multifunctional properties, and ω -carboxyl homologues of MK-4 (MK-4- ω -COOH), K acid I, K acid II and their glucuronides have been identified as metabolites [38–42]. It is considered that MK-4 is initially metabolized to MK-4- ω -COOH by ω -oxidation, followed by β -oxidation to afford intermediary carboxylic acids (**Figure 9**) [43]. These carboxylic acids can be categorized into two groups; MK- n - ω -COOH derivatives bearing a α,β -unsaturated carboxy group and MK- n -(ω -2)-COOH derivatives bearing a γ,δ -unsaturated carboxy group. Chemical synthesis of these metabolites is essential for evaluation of their properties, and several synthetic routes have been reported.

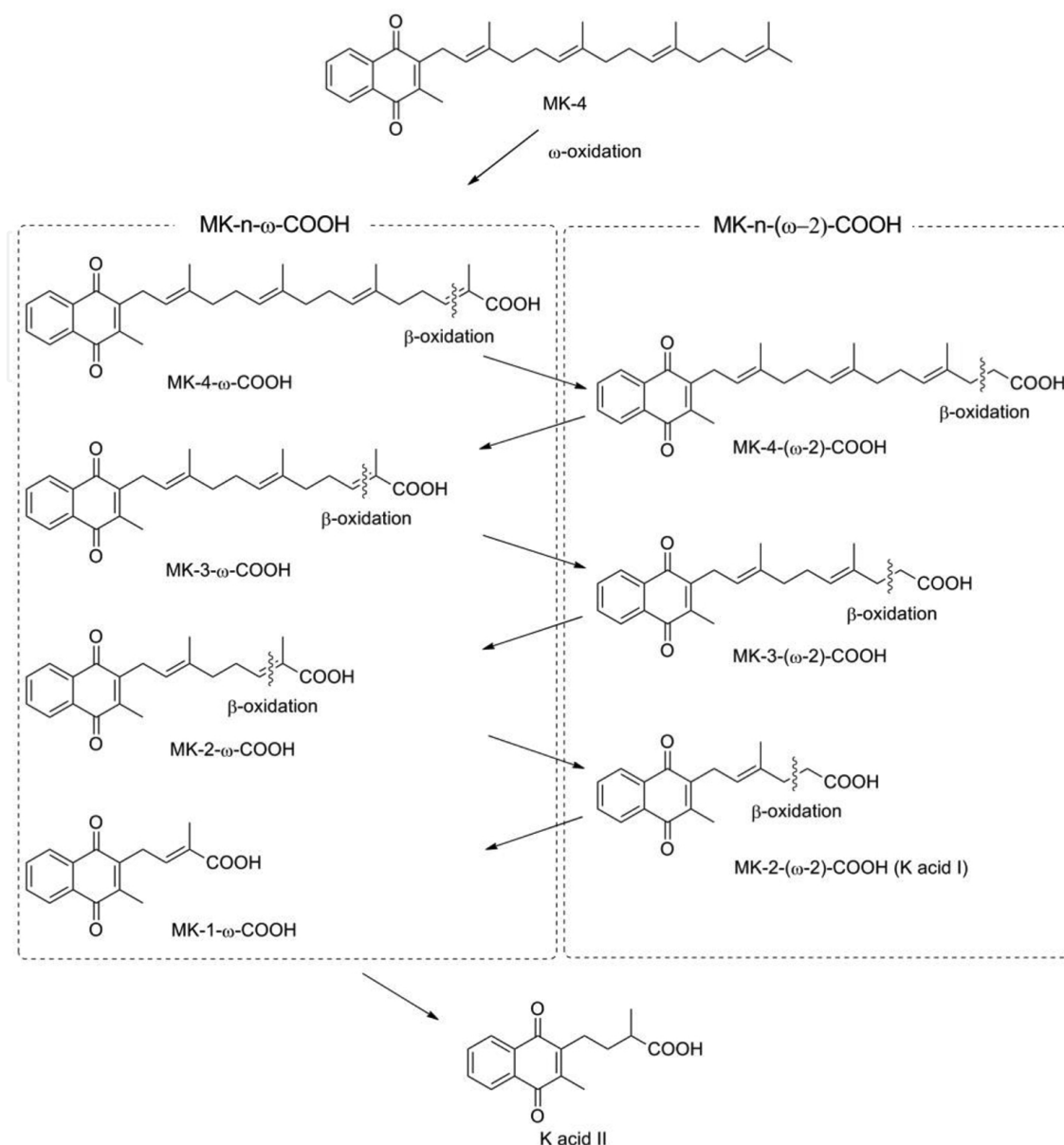


Figure 9. Putative catabolic pathways of MK-4.

4.1. Synthesis of menaquinone metabolites

The MK-4 metabolites K acid I and K acid II are also metabolites of phyloquinone (vitamin K_1). Several chemical syntheses of K acid I and K acid II have been reported. Watanabe et al. synthesized K acid I by direct addition of a carboxy side chain to the naphthoquinone framework using BF_3 etherate [44]. A route involving a malonyl derivative and decarboxylation was also investigated (**Figure 10**) [45]. They also synthesized K acid II. Addition of a side chain moiety by Friedel-Crafts acylation, followed by Clemmensen reduction, afforded naphthylcarboxylic acid, and oxidation of the naphthol moiety using Fremy's salt gave K acid II. Direct alkylation of naphthoquinone using peroxide also afforded K acid II (**Figure 11**) [44].

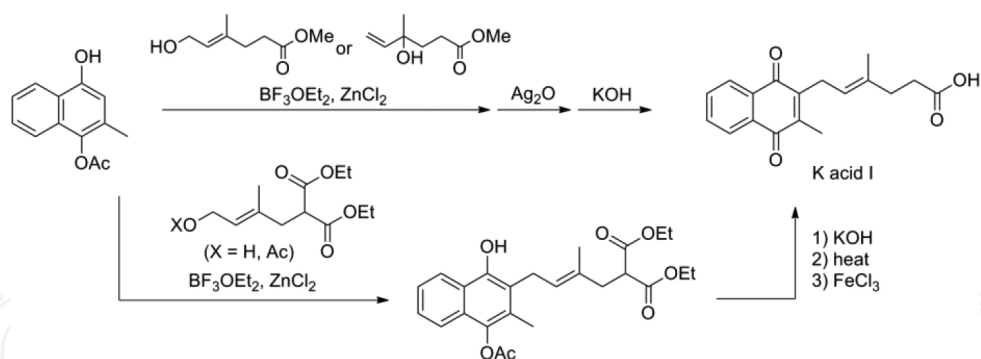


Figure 10. Synthetic route to K acid I (Watanabe et al.).

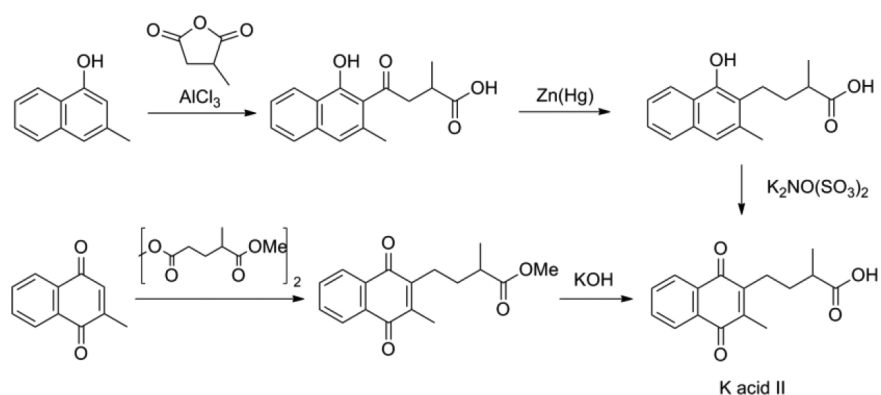


Figure 11. Synthetic route of K acid II (Watanabe et al.).

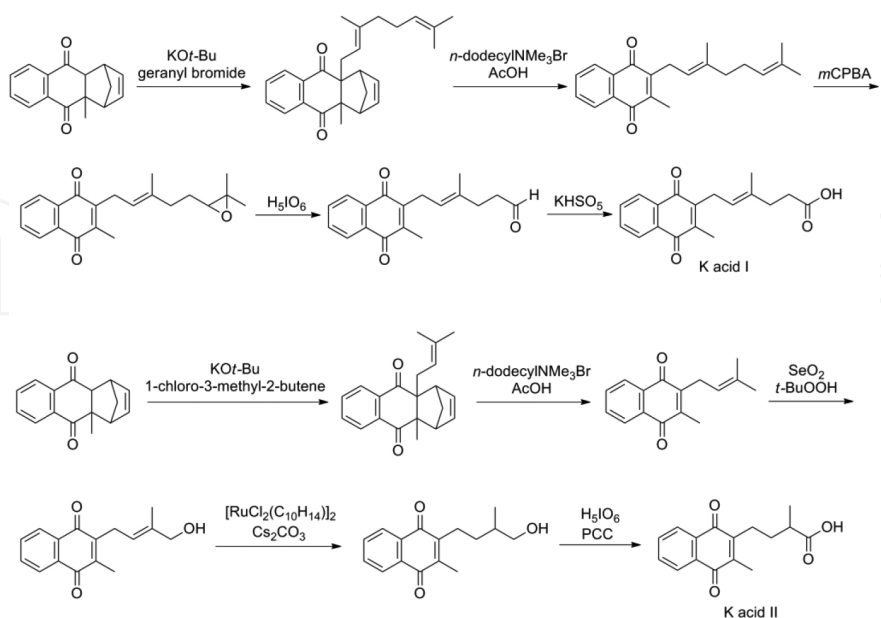


Figure 12. Synthetic routes of K acid I and K acid II (Teitelbaum et al.).

Teitelbaum et al. synthesized K acid I and K acid II by oxidation of MK-2 and MK-1, respectively. They prepared intermediary MK-n using a menadione-cyclopentadiene adduct as the same starting material (**Figure 12**) [46].

Okamoto et al. synthesized MK-1- ω -COOH by using Wittig reaction as a key step. To prepare the intermediary aldehyde, they employed alkylation and oxidative cleavage (**Figure 13**) [47].

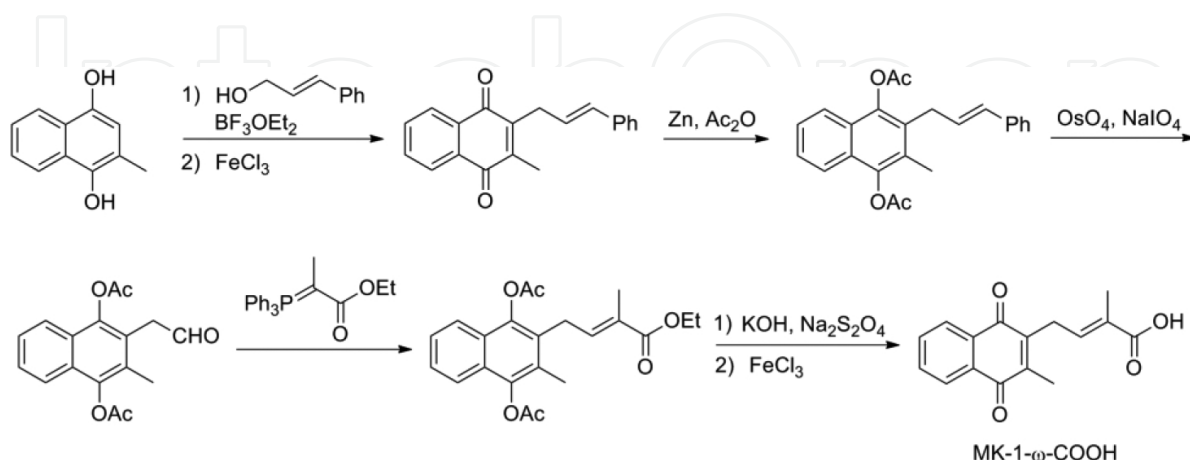


Figure 13. Synthesis of MK-1- ω -COOH (Okamoto et al.).

Terao et al. synthesized MK-3-(ω -2)-COOH and MK-4-(ω -2)-COOH using Claisen rearrangement as a key reaction. Claisen reaction of triethyl orthoacetate and MK-n derivative gave two-carbon-atom-extended carboxylic acid esters, and then hydration afforded MK-n-(ω -2)-COOH derivatives (**Figure 14**) [48].

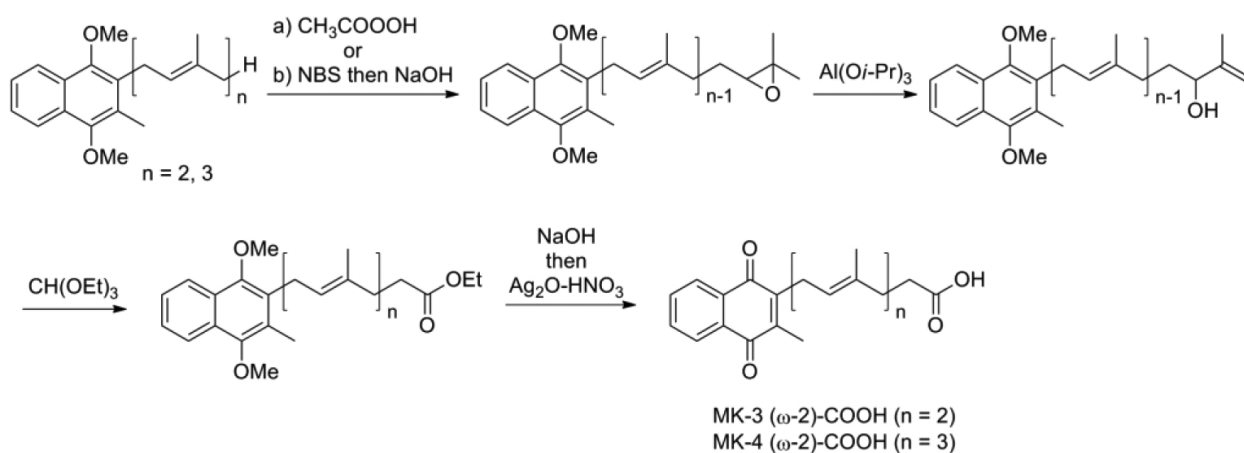


Figure 14. Synthesis of MK-n-(ω -2)-COOH derivatives (Terao et al.).

Masaki et al. employed sulfur-contractive anionic [2,3]-sigmatropic rearrangement for side chain elongation. Treatment of allyl sulfide with base afforded two-carbon-atom-extended carboxylic acid esters in one pot (**Figure 15**). MK-2-(ω -2)-COOH and MK-3-(ω -2)-COOH were obtained in this way [49].

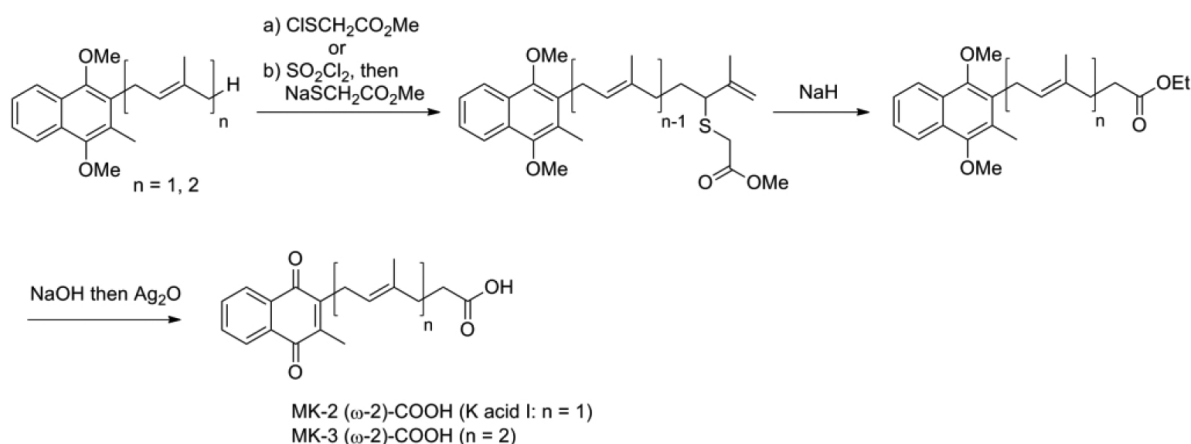


Figure 15. Synthesis of MK- n -(ω -2)-COOH derivatives (Masaki et al.).

Fujii et al. reported systematic synthesis of menaquinone metabolites. MK- n - ω -COOH derivatives were synthesized by oxidation of the terminal carbon of MK- n derivatives. Stereoselective oxidation with selenium oxide, followed by stepwise oxidation, gave MK- n - ω -COOH derivatives. K acid II was synthesized by hydrogenation of MK-1- ω -COOH (**Figure 16**) [50]. MK- n -(ω -2)-COOH derivatives were synthesized by oxidative cleavage of MK- n derivatives. Epoxidation of terminal olefin followed by perchloric acid treatment afforded 1,2-diols. Oxidative cleavage of the diol moiety followed by oxidative reactions gave MK- n -(ω -2)-COOH derivatives (**Figure 17**) [50]. These synthetic schemes correspond to the putative catabolic pathways of menaquinones, that is, ω -oxidation and β -oxidation.

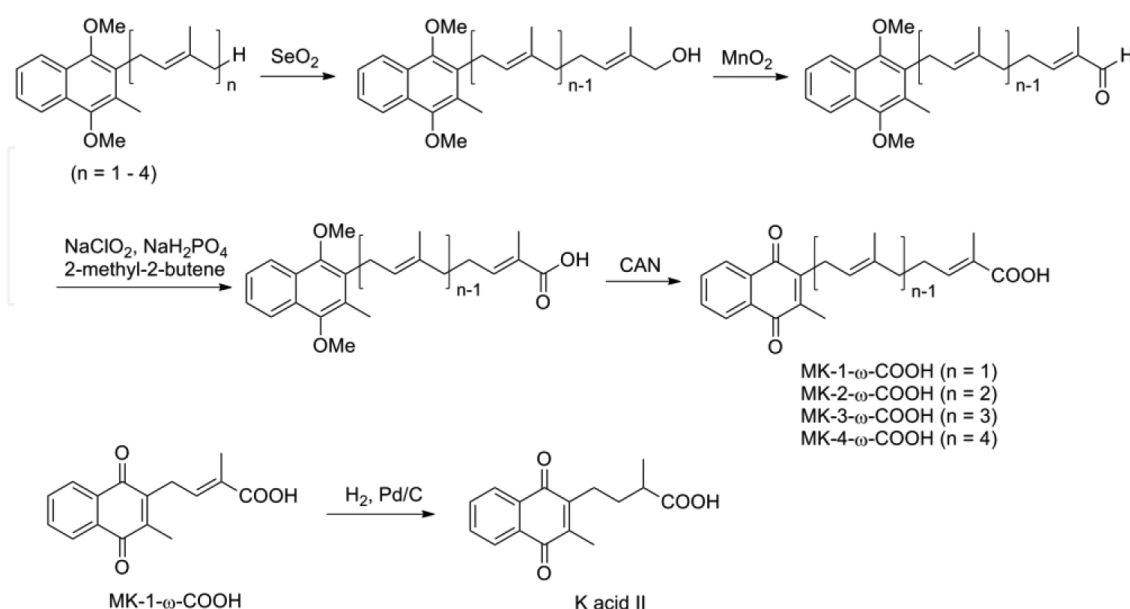


Figure 16. Synthesis of MK- n - ω -COOH derivatives (Fujii et al.).

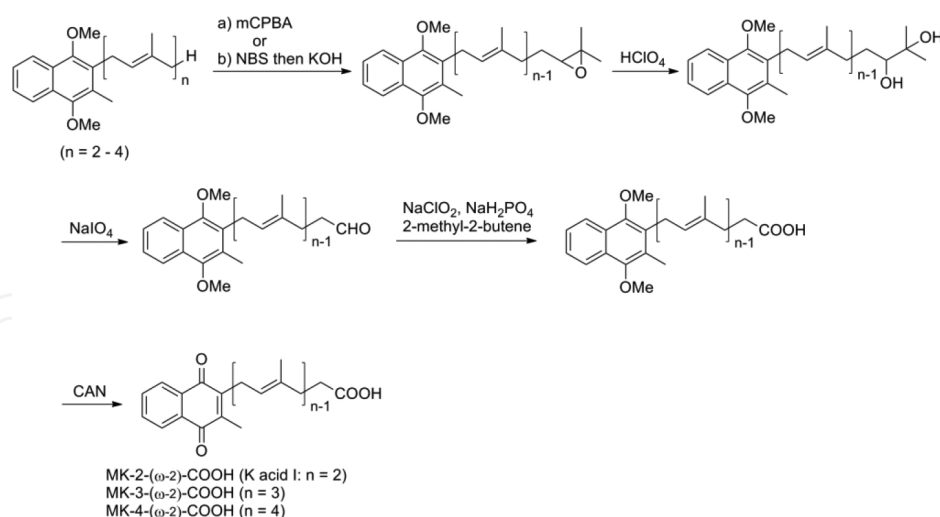


Figure 17. Synthesis of MK- n -(ω -2)-COOH derivatives (Fujii et al.).

Suhara et al. designed and synthesized ω -hydroxy derivatives (ω -alcohols) and ω -formyl derivatives (ω -aldehydes) as menaquinone metabolite analogs. ω -Oxidized side chain moieties were prepared from corresponding isoprene derivatives, and the side chain parts were introduced into the naphthalene core. Oxidation to quinone form afforded ω -alcohols, and then PDC oxidation afforded ω -aldehydes (**Figure 18**) [51, 52].

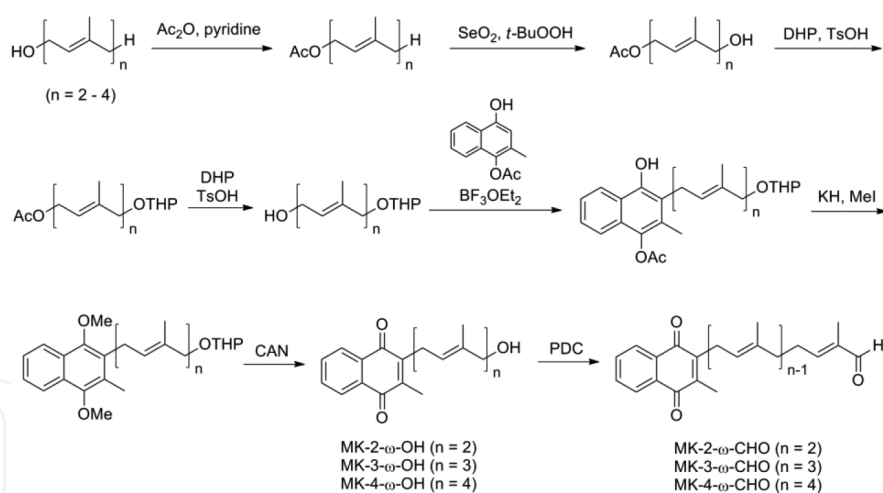


Figure 18. Synthesis of MK- n - ω -alcohols and MK- n - ω -aldehydes (Suhara et al.).

4.2. Biological activities of menaquinone metabolites

These menaquinone carboxylic acid derivatives and related quinone carboxylic acids, including ubiquinone derivatives and tocopheryl derivatives, show lysosomal membrane-stabilizing activity [45, 47]. Appropriate hydrophobicity of the side chain appears to be essential for this activity. Some of these compounds also exert inhibitory effects on the generation of the slow-reacting substance of anaphylaxis [48].

MK-4 has various biological activities, such as anti-inflammatory activity and antitumor activity, and these activities of the menaquinone metabolites were also investigated. All tested menaquinone metabolites inhibited LPS-induced production of proinflammatory cytokines in RAW264.7 cells [50]. It is suggested that naphthoquinone structure is essential for the anti-inflammatory activity of menaquinone derivatives. Regarding antitumor activity, several carboxylic acids, such as MK-2- ω -COOH, significantly inhibited proliferation of JHH7 and HepG2 hepatocellular carcinoma cell lines. On the other hand, MK-2- ω -COOH did not inhibit proliferation of normal hepatic cells. Anti-proliferative activity may be associated with caspase/transglutaminase-related pathways [53].

The ω -alcohols and ω -aldehydes showed apoptosis-inducing activity toward human leukemia cell line HL-60 and human osteosarcoma cell line MG-63. The ω -aldehydes were more potent than the corresponding ω -alcohols [51, 52]. The vitamin K potency of MK-4- ω -OH, that is, its coenzyme activity for GGCX, was also evaluated. MK-4- ω -OH showed a larger V_{\max}/K_m value than that of intact MK-4, indicating that MK-4- ω -OH has greater coenzyme activity than MK-4 [52].

5. Future perspective

Vitamin Ks are attractive lead compounds for drug discovery. One of the most promising applications is as candidate antitumor agents, though the mechanism of action of Cpd 5 could be different from that of intact vitamin Ks. In addition, bone homeostasis and neural effects are also possible targets of vitamin K derivatives. Vitamin K may also be used as a food supplement, and therefore, characterization of its metabolites is important. It is noteworthy that some menaquinone metabolites have characteristic activities distinct from those of intact vitamin K₂. Though a clinical study of MK-4 as an agent to prevent recurrence of hepatocellular carcinoma was terminated [54], the metabolites and their analogs still represent potential drug candidates.

Author details

Shinya Fujii^{1,2} and Hiroyuki Kagechika^{1*}

*Address all correspondence to: kage.chem@tmd.ac.jp

1 Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, Kanda-Surugadai, Chiyoda-ku, Tokyo, Japan

2 Institute of Molecular and Cellular Biosciences, the University of Tokyo, Yayoi, Bunkyo-ku, Tokyo, Japan

References

- [1] Litwack G. ed. Vitamin K, vitamins and hormones, vol. 78, Elsevier Academic Press, New York, 2008.
- [2] Prasad K. N., Edwards-Prasad J., Sakamoto A. Vitamin K₃ (menadione) inhibits the growth of mammalian tumor cells in culture. *Life Sci.*, 29, 1387–1392 (1981).
- [3] Ngo E. O., Sun T.-P., Chang J.-Y., Wang C.-C., Chi K.-H., Cheng A.-L., Nutter L. M. Menadione-induced DNA damage in a human tumor cell line. *Biochem. Pharmacol.*, 42, 1961–1968 (1991).
- [4] Wu F. Y.-H., Chang N.-T., Chen W.-J., Juan C.-C. Vitamin K₃-induced cell cycle arrest and apoptotic cell death are accompanied by altered expression of c-fos and c-myc in nasopharyngeal carcinoma cells. *Oncogene*, 8, 2237–2244 (1993).
- [5] Juan C.-C., Wu F. Y.-H. Vitamin K₃ inhibits growth of human hepatoma HepG2 cells by decreasing activities of both p34-Cdc2 kinase and phosphatase. *Biochem. Biophys. Res. Commun.*, 190, 907–913 (1993).
- [6] Otsuka M., Kato N., Shao R. X., Hoshida Y., Ijichi H., Koike Y., Taniguchi H., Moriyama M., Shiratori Y., Kawabe T., Omata M. Vitamin K₂ inhibits the growth and invasiveness of hepatocellular carcinoma cells via protein kinase A activation. *Hepatology*, 40, 243–251 (2004).
- [7] Habu D., Shiomi S., Tamori A., Takeda T., Tanaka T., Kubo S., Nishiguchi S. Role of vitamin K₂ in the development of hepatocellular carcinoma in women with viral cirrhosis of the liver. *JAMA*, 292, 358–361 (2004).
- [8] Tabb M. M., Sun A., Zhou C., Grün F., Errandi J., Romero K., Pham H., Inoue S., Mallick S., Lin M., Forman B. M., Blumberg B. Vitamin K₂ regulation of bone homeostasis is mediated by the steroid and xenobiotic receptor SXR. *J. Biol. Chem.*, 278, 43919–43927 (2003).
- [9] Ichikawa T., Horie-Inoue K., Ikeda K., Blumberg B., Inoue S. Steroid and Xenobiotic receptor SXR mediates vitamin K₂-activated transcription of extracellular matrix-related genes and collagen accumulation in osteoblastic cells. *J. Biol. Chem.*, 281, 16927–16934 (2006).
- [10] Ohsaki Y., Shirakawa H., Miura A., Giriwono P. E., Sato S., Ohashi A., Iribe M., Goto T., Komai M. Vitamin K suppresses the lipopolysaccharide-induced expression of inflammatory cytokines in cultured macrophage-like cells via the inhibition of the activation of nuclear factor κ B through the repression of IKK α/β phosphorylation. *J. Nutr. Biochem.*, 21, 1120–1126 (2010).
- [11] Spronk H. M. H., Soute B. A. M., Schurgers L. J., Thijssen H. H. W., De Mey J. G. R., Vermeer C. Tissue-specific utilization of menaquinone-4 results in the prevention of arterial calcification in warfarin-treated rats. *J. Vasc. Res.*, 40, 531–537 (2003).

- [12] Otsuka M., Kato N., Ichimura T., Abe S., Tanaka Y., Taniguchi H., Hoshida Y., Moriyama M., Wang Y., Shao R.-X., Narayan D., Muroyama R., Kanai F., Kawabe T., Isobe T., Omata M. Vitamin K₂ binds 17 β -hydroxysteroid dehydrogenase 4 and modulates estrogen metabolism. *Life Sci.* 76, 2473–2482 (2005).
- [13] Shirakawa H, Ohsaki Y, Minegishi Y, Takumi N, Ohinata K, Furukawa Y, Mizutani T., Komai M. Vitamin K deficiency reduces testosterone production in the testis through down-regulation of the Cyp11a a cholesterol side chain cleavage enzyme in rats. *Biochim Biophys Acta*, 1760, 1482–1488 (2006).
- [14] Ito A., Shirakawa H., Takumi N., Minegishi Y., Ohashi A., Howlader Z. H., Ohsaki Y., Sato T., Goto T., Komai M. Menaquinone-4 enhances testosterone production in rats and testis-derived tumor cells. *Lipids Health Dis.*, 10, 158 (2011).
- [15] Nishikawa Y., Carr B. I., Wang M., Kar S., Finn F., Dowd P., Zheng Z. B., Kerns J., Naganathan S. Growth inhibition of hepatoma cells induced by vitamin K and its analogs. *J. Biol. Chem.*, 270, 28304–28310 (1995).
- [16] Ni R., Nishikawa Y., Carr B. I. Cell growth inhibition by a novel vitamin K is associated with induction of protein tyrosine phosphorylation. *J. Biol. Chem.*, 273, 9906–9911 (1998).
- [17] Nishikawa Y., Wang Z., Kerns J., Wilcox C. S., Carr B. I. Inhibition of hepatoma cell growth in vitro by arylating and non-arylated K vitamin analogs. Significance of protein tyrosine phosphatase inhibition. *J. Biol. Chem.*, 274, 34803–34810 (1999).
- [18] Tamura K., Southwick E. C., Kerns J., Rosi K., Carr B. I., Wilcox C., Lazo J. S. Cdc25 inhibition and cell cycle arrest by a synthetic thioalkyl vitamin K analogue. *Cancer Res.*, 60, 1317–1325 (2000).
- [19] Kar S., Lefterov I. M., Wang M., Lazo J. S., Scott C. N., Wilcox C. S., Carr B. I. Binding and inhibition of Cdc25 phosphatase by vitamin K analogues. *Biochemistry*, 42, 10490–10497 (2003).
- [20] Lazo J. S., Nemoto K, Pestell K. E., Cooley K., Southwick E. C., Mitchell D. A., Furey W., Gussio R., Zaharevitz D. W., Joo B., Wipf P. Identification of a potent and selective pharmacophore for Cdc25 dual specificity phosphatase inhibitors. *Mol Pharmacol.*, 61, 720–728 (2002).
- [21] Peyregne V. P., Kar S., Ham S. W., Wang M., Wang Z., Carr B. I. Novel hydroxyl naphthoquinones with potent Cdc25 antagonizing and growth inhibitory properties. *Mol Cancer Ther.*, 4, 595–602 (2005).
- [22] Kar S., Wang M., Ham S. W., Carr B. I. Fluorinated Cpd 5, a pure arylating K-vitamin derivative, inhibits human hepatoma cell growth by inhibiting Cdc25 and activating MAPK. *Biochem. Pharmacol.*, 72, 1217–1227 (2006).
- [23] Ham S. W., Choe J.-I., Wang M.-F., Peyregne V., Carr B. I. Fluorinated quinoid inhibitor: possible “pure” arylator predicted by the simple theoretical calculation. *Bioorg. Med. Chem. Lett.*, 14, 4103–4105 (2004).

- [24] Kar S., Wang M., Yao W., Michejda C. J., Carr B. I. PM-20, a novel inhibitor of Cdc25A, induces extracellular signal-regulated kinase 1/2 phosphorylation and inhibits hepatocellular carcinoma growth in vitro and in vivo. *Mol. Cancer Ther.*, 5, 1511–1519 (2006).
- [25] Brun M.-P., Braud E., Angotti D., Mondésert O., Quaranta M., Montes M., Miteva M., Gresh N., Ducommun B., Garbay C. Design, synthesis, and biological evaluation of novel naphthoquinone derivatives with CDC25 phosphatase inhibitory activity. *Bioorg. Med. Chem.*, 13, 4871–4879 (2005).
- [26] Braud E., Goddard M.-L., Kolb S., Brun M.-P., Mondésert O., Quaranta M., Gresh N., Ducommun B., Garbay C. Novel naphthoquinone and quinolinedione inhibitors of CDC25 phosphatase activity with antiproliferative properties. *Bioorg. Med. Chem.*, 16, 9040–9049 (2008).
- [27] Kitano T., Yoda H., Tabata K., Miura M., Toriyama M., Motohashi S., Suzuki T. Vitamin K₃ analogs induce selective tumor cytotoxicity in neuroblastoma. *Biol. Pharm. Bull.*, 35, 617–623 (2012).
- [28] Kayashima T., Mori M., Mizutani R., Nishio K., Kuramochi K., Tsubaki K., Yoshida H., Mizushima Y., Matsubara K. Synthesis and biological evaluation of vitamin K derivatives as angiogenesis inhibitor. *Bioorg. Med. Chem.*, 18, 6305–6309 (2010).
- [29] Krishnaswamy M., Purushothaman K. K. Plumbagin: a study of its anticancer, antibacterial and antifungal properties. *Indian J. Exp. Biol.*, 18, 876–877 (1980).
- [30] Sandur S. K., Ichikawa H., Sethi G., Ahn K. S., Aggarwal B. B. Plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone) suppresses NF- κ B activation and NF- κ B-regulated gene products through modulation of p65 and I κ B α kinase activation, leading to potentiation of apoptosis induced by cytokine and chemotherapeutic agents. *J. Biol. Chem.*, 281, 17023–17033 (2006).
- [31] Rao K. V., McBride T. J., Oleson J. J. Recognition and evaluation of lapachol as an antitumor agent. *Cancer Res.*, 28, 1952–1954 (1968).
- [32] Hartwell J. L., Abbott B. J. Antineoplastic principles in plants: recent developments in the field. *Adv. Pharmacol. Chemother.*, 7, 117–209 (1969).
- [33] For recent examples: (i) Fiorito S., Epifano F., Bruyere C., Mathieu V., Kiss R. Genovese S. Growth inhibitory activity for cancer cell lines of lapachol and its natural and semi-synthetic derivatives. *Bioorg. Med. Chem. Lett.*, 24, 454–457 (2014). (ii) Wang S.-H., Lo C.-Y., Gwo Z.-H., Lin H.-J., Chen L.-G., Kuo C.-D., Wu J.-Y. Synthesis and biological evaluation of lipophilic 1, 4-naphthoquinone derivatives against human cancer cell lines. *Molecules*, 20, 11994–12015 (2015).
- [34] Suhara Y., Hanada N., Okitsu T., Sakai M., Watanabe M., Nakagawa K., Wada A., Takeda K., Takahashi K., Tokiwa H., Okano T. Structure-activity relationship of novel menaquinone-4 analogues: modification of the side chain affects their biological activities. *J. Med. Chem.* 55, 1553–1558 (2012).

- [35] Suhara Y., Watanabe M., Motoyoshi S., Nakagawa K., Wada A., Takeda K., Takahashi K., Tokiwa H., Okano T. Synthesis of new vitamin K analogues as steroid and xenobiotic receptor (SXR) agonists: insights into the biological role of the side chain part of vitamin K. *J. Med. Chem.* 54, 4918–4922 (2011).
- [36] Suhara Y., Watanabe M., Nakagawa K., Wada A., Ito Y., Takeda K., Takahashi K., Okano T. Synthesis of novel vitamin K₂ analogues with modification at the ω -terminal position and their biological evaluation as potent steroid and xenobiotic receptor (SXR) agonists. *J. Med. Chem.* 54, 4269–4273 (2011).
- [37] Suhara Y., Hirota Y., Hanada N., Nishina S., Eguchi S., Sakane R., Nakagawa K., Wada A., Takahashi K., Tokiwa H., Okano T. Synthetic small molecules derived from natural vitamin K homologues that induce selective neuronal differentiation of neuronal progenitor cells. *J. Med. Chem.* 58, 7088–7092 (2015).
- [38] Wiss O., Gloor U. Absorption, distribution, storage, and metabolites of vitamins K and related quinones. *Vitam. Horm.*, 24, 575–586 (1966).
- [39] Watanabe M., Toyoda M., Imada I., Morimoto H. Ubiquinone and related compounds. XXVI. The urinary metabolites of phylloquinone and α -tocopherol. *Chem. Pharm. Bull.*, 22, 176–182 (1974).
- [40] McBurney A., Shearer M. J., Barkhan P. Preparative isolation and characterization of the urinary aglycones of vitamin K₁ (phylloquinone) in man. *Biochem. Med.*, 24, 250–267 (1980).
- [41] Tadano K., Yuzuriha T., Sato T., Fujita T., Shimada K., Hashimoto K., Satoh T. Identification of menaquinone-4 metabolites in the rat. *J. Pharmacobiodyn.*, 12, 640–645 (1989).
- [42] Harrington D. J., Soper R., Edwards C., Savidge G. F., Hodges S. J., Shearer M. J. Determination of the urinary aglycone metabolites of vitamin K by HPLC with redox-mode electrochemical detection. *J. Lipid. Res.*, 46, 1053–1060 (2005).
- [43] Edson K. Z., Prasad B., Unadkat J. D., Suhara Y., Okano T., Guengerich F. P., Rettie A. E. Cytochrome P450-dependent catabolism of vitamin K: ω -hydroxylation catalyzed by human CYP4F2 and CYP4F11. *Biochemistry*, 52, 8276–8285 (2013).
- [44] Watanabe M., Kawada M., Nishikawa M., Imada I., Morimoto H. Ubiquinone and related compounds. XXVII. Synthesis of urinary metabolites of phylloquinone and α -tocopherol. *Chem. Pharm. Bull.*, 22, 566–575 (1974).
- [45] Watanabe M., Okamoto K., Imada I., Morimoto H. Ubiquinone and related compounds. XXXI. Synthesis of urinary metabolites of ubiquinone, phylloquinone, α -tocopherol and related compounds. *Chem. Pharm. Bull.*, 26, 774–783 (1978).
- [46] Teitelbaum A. M., Scian M., Nelson W. L., Rettie A. E. Efficient syntheses of vitamin K chain-shortened acid metabolites. *Synthesis*, 47, 944–948 (2015).
- [47] Okamoto K., Watanabe M., Kawada M., Goto G., Ashida Y., Oda K., Yajima A., Imada I., Morimoto H. Synthesis of quinones having carboxy- and hydroxy-alkyl side chains,

and their effects on rat-liver lysosomal membrane. *Chem. Pharm. Bull.*, 30, 2797–2819 (1982).

- [48] Terao S., Shiraishi M., Kato K., Ohkawa S., Ashida Y., Maki Y. Quinones. Part 2. General synthetic routes to quinone derivatives with modified polyprenyl side chains and the inhibitory effects of these quinones on the generation of the slow reacting substance of anaphylaxis (SRS-A). *J. Chem. Soc. Perkin Trans 1*, 2909–2920. (1982).
- [49] Masaki Y., Sakuma K., Kaji K. Regio- and stereoselective terminal allylic carboxymethylation of *gem*-dimethyl olefins. Synthesis of biologically important linear degraded terpenoids. *Chem. Pharm. Bull.*, 33, 1930–1940 (1985).
- [50] Fujii S., Shimizu A., Takeda N., Oguchi K., Katsurai T., Shirakawa H., Komai M., Kagechika H. Systematic synthesis and anti-inflammatory activity of ω -carboxylated menaquinone derivatives – Investigation on identified and putative vitamin K metabolites. *Bioorg. Med. Chem.*, 23, 2344–2352 (2015).
- [51] Suhara Y., Murakami A., Kamao M., Mimatsu S., Nakagawa K., Tsugawa N., Okano T. Efficient synthesis and biological evaluation of ω -oxygenated analogues of vitamin K₂: study of modification and structure-activity relationship of vitamin K₂ metabolites. *Bioorg. Med. Chem. Lett.*, 17, 1622–1625 (2007).
- [52] Suhara Y., Hirota Y., Nakagawa K., Kamao M., Tsugawa N., Okano T. Design and synthesis of biologically active analogues of vitamin K₂: evaluation of their biological activities with cultured human cell lines. *Bioorg. Med. Chem.*, 16, 3108–3117 (2008).
- [53] Qin X.-Y., Fujii S., Shimizu A., Kagechika H., Kojima S. Carboxylic derivatives of vitamin K₂ inhibit hepatocellular carcinoma cell growth through caspase/transglutaminase-related signaling pathways. *J. Nutrit. Sci. Vitaminol.*, 61, 285–290 (2015).
- [54] Yoshida H., Shiratori Y., Kudo M., Shiina S., Mizuta T., Kojiro M., Yamamoto K., Koike Y., Saito K., Koyanagi N., Kawabe T., Kawazoe S., Kobashi H., Kasugai H., Osaki Y., Araki Y., Izumi N., Oka H., Tsuji K., Toyota J., Seki T., Osawa T., Masaki N., Ichinose M., Seike M., Ishikawa A., Ueno Y., Tagawa K., Kuromatsu R., Sakisaka S., Ikeda H., Kuroda H., Kokuryu H., Yamashita T., Sakaida I., Katamoto T., Kikuchi K., Nomoto M., Omata M. Effect of vitamin K₂ on the recurrence of hepatocellular carcinoma. *Hepatology*, 54, 532–540 (2011).

