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BRIEF ARTICLE

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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

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Supporting Information

ABSTRACT: Vitamin K_2 has been demonstrated to induce gene expression related to bone formation through a nuclear steroid and xenobiotic receptor (SXR). We synthesized new vitamin K analogues with the same isoprene side chains symmetrically introduced at the 2 and 3 positions of 1,4-naphthoquinone and evaluated the transcriptional activity of the target gene. The transcriptional activity was related to the length of the side chain which allowed optimal interaction with ligand-binding domain of SXR.

INTRODUCTION

Vitamin K serves as an essential cofactor of the γ-carboxylase, an enzyme that transforms glutamic acid residues within specific substrate proteins into γ-carboxyglutamic acid (Gla) residues, and is therefore involved in the activation of the cascade of proteins for blood coagulation.[1] Recent investigations indicate that vitamin K is also required for the synthesis of other calcium-binding proteins such as bone Gla protein (osteocalcin), matrix Gla protein, protein S, and gas6.[2−4] The two molecular forms of "natural" vitamin K can be distinguished by the differences of the alkyl side chains at the 3-position of the common 2-methyl-1,4-naphthoquinone group (Figure 1). Plant-derived vitamin K_1 (**1**) (phylloquinone, PK) contains the phytyl group as the side chain, while the bacterial-derived vitamin K_2 (menaquinone-*n*, MK-*n*) (**2** (MK-1), **3** (MK-2), **4** (MK-3), and **5** (MK-4)) carries a polyisoprenyl side chain. Vitamin K_3 (**6**) (menadione, 2-methyl-1,4-naphthoquinone) has been defined as a "synthetic" vitamin K analogue that lacks the side chain at the C-3 position.

Menaquinone-4 (MK-4), one of the menaquinone homologues, showed additional biological activities related to gene transcription through the steroid and xenobiotic receptor (SXR).[5−10] MK-4 also protected oligodendrocyte precursors and immature fetal cortical neurons from oxidative injury, independent of the vitamin K-dependent γ-carboxylative reaction.[11] There is consistent evidence that the MK-4 is produced by conversion from other dietary vitamin K homologues.[12] We recently confirmed that dietary PK was converted into MK-4 and then was accumulated in various tissues in a high concentration.[13]

This background information prompted us to focus on the SXR-mediated transcriptional activity and the biological roles of the side chains of vitamin K analogues. No studies have examined the biological activities of structure-modified vitamin K analogues even though many reports have investigated natural vitamin K homologues. We predicted that the structure of the side chain would play an important role in biological activities, since the structural differences of the homologues are confined to only the isoprene units. To evaluate the effects of the side chains of vitamin K, we investigated SXR-mediated transcriptional activity of new vitamin K analogues in comparison with vitamin K homologues. We considered that the introduction of dual side chains at the C-2 and C-3 positions of vitamin K homologues (Figure 2) would significantly increased activity. Indeed, some of the synthetic analogues may have biological activity that exceeds that of the natural homologues. In addition, if the new compounds prove to be useful substrates, they may contribute to future investigations of the mechanism that converts vitamin K analogues into MK-4 and the compounds may clarify other biological actions of vitamin K. In this study, we report the synthesis of new vitamin K analogues and the results of SXR-mediated gene transcription.

CHEMISTRY: SYNTHESIS OF VITAMIN K ANALOGUES

Vitamin K homologues **1**−**5** were a generous gift of Eisai Co., Ltd. We first planned to synthesize the requisite vitamin K analogues through coupling of 1,4-naphthoquinone (**12**) and the desired side chain moiety as outlined in Scheme 1. For the synthesis of the analogues, we chose phyltol (**14**), prenol (**15**), geraniol (**16**), farnesol (**17**), geranylgeraniol (**18**), and **12** as starting materials, and the coupling reactions with the naphthoquinone part were carried using our reported method.[14] **12** was vigorously stirred with a 10% sodium hydrosulfite solution (aq) and Et_2O to form hydroquinone **13**, to which 2 equiv of isoprene units **14**−**18** were successively coupled in the presence of a catalytic amount of $BF_3 \cdot Et_2O$. The resulting hydroquinone derivatives were oxidized

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to the respective quinones under atmospheric conditions. Thus, the corresponding "double side chain" vitamin K analogues (PK-W and MK-*n*-W) (**7** (PK-W), **8** (MK-1-W), **9** (MK-2-W), **10** (MK-3-W), and **11** (MK-4-W)) were obtained in 37−57% yields. When the side chain was longer than geranylfarnesol, the chemical yield was not satisfactory. Presumably, the bulkiness of the side chain moiety affected the reactivity with **12**.

RESULTS

Evaluation of SXR-Mediated Transcriptional Activity. All the synthetic ligands and the vitamin K homologues were tested in assays that measured the SXR-mediated transcriptional activity. Luciferase-based assays of SXR-GAL4 and CYP3A4 promoter were carried out with HepG2 human hepatocellular carcinoma cells. SXR is well-known to control the gene expression of CYP3A4. The cells were transfected with pM-SXR and pGVP2-GAL2 luciferase reporter vector and with pRL-CMV using Lipofectamine[15] in the case of the SXR-GAL4 assay. The cells were also treated with pcDNA3.1-FLAG-SXR and pGL4.10-CYP3A4-pro luciferase reporter vector and with pRL-CMV using Lipofectamine in the case of the CYP3A4 promoter assay. The SXR-GAL4 assay measured SXR ligand-binding domain (LBD) driven transcription, while the CYP3A4 promoter assay evaluated SXR/RXR heterodimer-driven transcription.

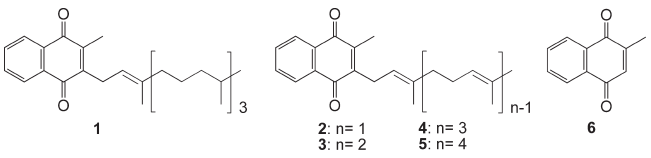


Figure 1. Structures of vitamin K homologues phylloquinone (**1**), menaquinones **2**−**5**, and menadione (**6**).

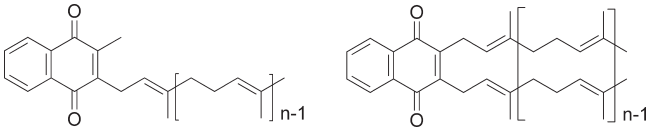
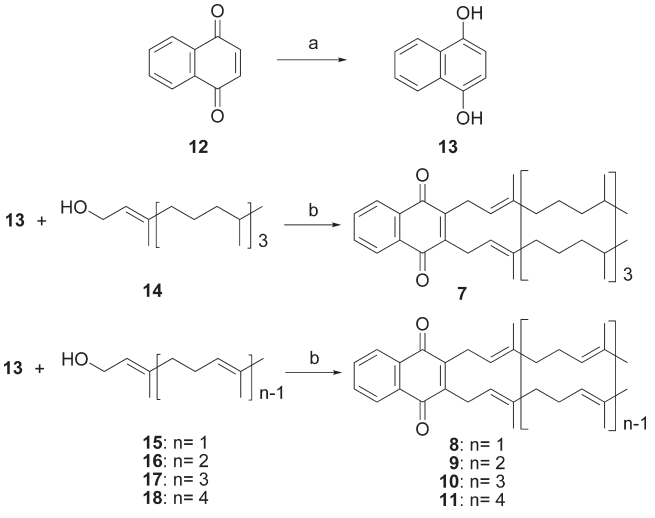


Figure 2. Structures of vitamin K homologues and our synthesized "double side chain" analogues.

Scheme 1. Synthesis of Vitamin K Analogues That Have Double Side Chain Part[a]



[a] Reagents and conditions: (a) 10% $Na_2S_2O_4$ aq, ether, quantitative; (b) $BF_3 \cdot OEt_2$, dioxane/ethyl acetate, 37−57%.

All compounds were tested in the SXR-GAL4 assay at a fixed concentration of 5×10^{-6} M. Among natural vitamin K homologues, the transcriptional activity as an indicator of binding to SXR increased in the order of **2** < **3** < **4** and then slightly decreased in the case of **5**. Among the double side chain analogues, **8** and **9** exhibited the highest activity. **10**, **11**, **1**, and **7** had almost no effect.

The analogues had similar effects on the transcriptional activity of CYP3A4 promoter as shown in Figure 4. **3** and **4** followed by **5** exhibited the most potent activity among the vitamin K homologues. **8** and **9** were the most effective vitamin K analogues.

These findings suggest that the menaquinones **3**−**5** might be favorable ligands for SXR while **8** and **9** may be the most favorable of the double side chain analogues. The data also indicate that the side chain length at the C-2 and C-3 positions in vitamin K analogues affected the binding affinity to SXR, with side chains of geranyl units as optimum size.

Docking Studies. We performed in silico binding analysis of MK-*n* and MK-*n*-W to the SXR-LBD[17] based on the ASEDock method using the MOE (Molecular Operating Environment) program package (see Computational Details). The docking results (Figure 5A) clearly show that **9** has the highest docking score. In the stable docking pose of **9** (Figure 5B), the side chain part and the naphthalene moiety of **9** stably interact with Ser247 and His407. On the other hand, **10** has a low docking score, since the strain of the molecule was large enough. The low docking score for **11** suggests that the formation of a stable complex with the ligand-binding pocket (LBP) of SXR would be difficult.

DISCUSSION

We describe the synthesis and the pharmacological profile related to SXR-mediated transcriptional activities of new vitamin K analogues in which the same side chain moiety was symmetrically introduced at both the 2 and 3 positions of 1,4-naphthoquinone.

SXR is a master gene orchestrating the expression of a large family of genes involved in uptake, metabolism, and disposal of a number of endo- and xenobiotics, including drugs, bile acids, steroid hormones, and metabolic intermediates in mammalian cells. Following ligand binding, SXR forms a heterodimer with the retinoid X receptor (RXR) that binds to SXR response elements (SXREs) located in the 5′-flanking regions of SXR target genes, resulting in their transcriptional activation.

To determine if vitamin K analogues have a role in regulating SXR, we evaluated the transcriptional activity of the new analogues and vitamin K homologues by two luciferase-based assay methods. The one-hybrid luciferase assay with SXR-GAL4 permitted us to examine only the general effects of ligand-binding affinity to SXR in the transcription and excluded any influence of heterodimerization with RXR and binding to SXREs. The second luciferase-based assay including SXRE with CYP3A4 promoter allowed us to examine the influences of heterodimerization with RXR and binding to SXREs. With respect to ligand binding (Figure 3), the activity of natural vitamin K_2 homologues **3**−**5** increased in a manner dependent on the length of the side chain moiety, being greatest with **4**, bearing a farnesyl unit, having the greatest effect. Among all the double side chain analogues, **8** and **9** showed the most potent activity.

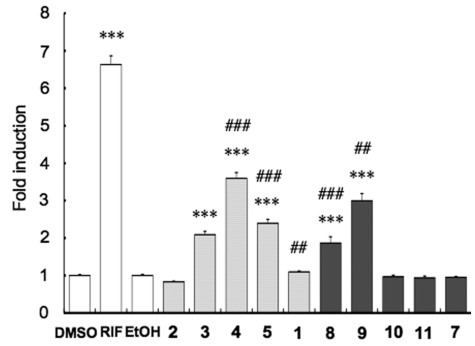


Figure 3. Transcriptional activity with a one-hybrid luciferase assay with SXR-GAL4. HepG2 cells were treated with each compound at 5.0×10^{-6} M. DMSO or EtOH indicates control cells that were treated with the solvent, without ligands. RIF indicates cells treated with rifampicin, a known ligand for SXR.[16] The histogram data are expressed as the mean obtained from three independent experiments; the error bars indicate the SD. Significant difference between control group and each compounds group is as follows: ∗∗∗, $p < 0.001$ (by Dunnett's test). Significant differences between the MK-*n* group and the MK-*n*-W group are as follows: ###, $p < 0.001$; ##, $p < 0.01$ (by Student's *t* test).

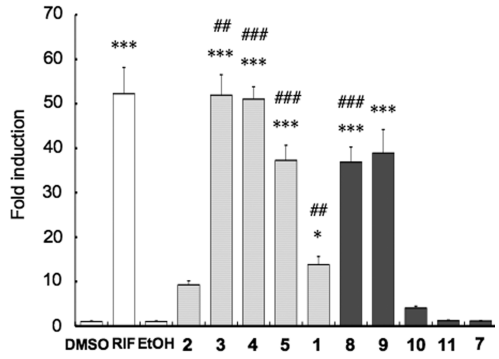


Figure 4. Transcriptional activity with luciferase assay including SXRE with CYP3A4 promoter. HepG2 cells were treated with each compound at 5.0×10^{-6} M. DMSO or EtOH indicates control cells that were treated with the solvent, without ligands. RIF indicates cells treated with rifampicin, a known ligand for SXR.[16] The histogram data indicate the mean obtained from three independent experiments; the error bars indicate the SD. Significant differences between control group and each compounds group are as follows: ∗∗∗, $p < 0.001$; ∗, $p < 0.05$ (by Dunnett's test). Significant differences between the MK-*n* group and the MK-*n*-W group are as follows: ###, $p < 0.001$; ##, $p < 0.01$ (by Student's *t* test).

Similar tendencies were noted in the assay that evaluated heterodimerization and binding to SXREs (Figure 4). Compounds **8** and **9** effectively increased the expression of a well-characterized SXR target gene, CYP3A4, in a human hepatocyte cell line, compared with other analogues. **3** and **4** showed strong activity comparable to that of the established SXR ligand rifampicin.

To explore the docking state of **8** and **9** exhibited potent transcriptional activities, we examined molecular modeling of **9** with the LBD of SXR. The docking study showed hydrophilic interactions between the oxygen atom of **9** and His407 and Ser247, a part of the constituent amino acids of SXR (Figure 5).

Of the menaquinones, **3**−**5** may be the most favorable ligands for SXR, and in the same way, **8** and **9** may be the most favorable of the double side chain analogues. Since the transcriptional

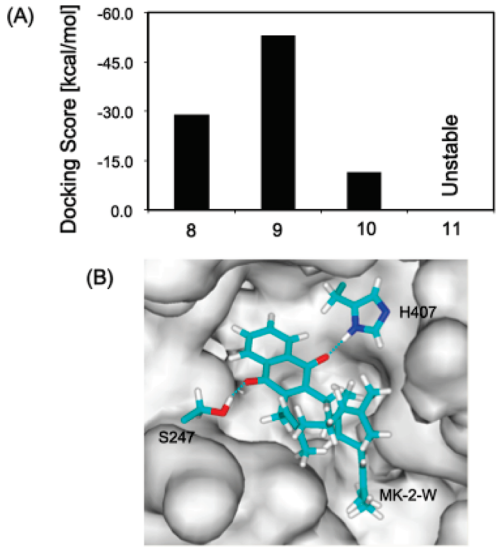


Figure 5. SXR-LBD docking analysis based on ASEDock of MOE: (A) the docking score (U-dock) of the MK-*n*-W/SXR complex; (B) the docking pose of the **9**/SXR-LBD complex. The LBP of the SXR-LBD is shown in white. **9** and key residues (Ser247 and His407) are shown in stick model.

activity of **3** and **4** corresponds to that of **8** and **9**, respectively, the side chain length affected the transcriptional activity. The size of the cavity of the LBP might be in the range of the size of **9** because the activity of **10** was remarkably decreased. On the other hand, in a comparison of **5** with **1**, their side chain lengths were almost the same; however, the transcriptional activities were quite different. From these findings, we considered that the spatial configuration of the side chain part was important. Consequently, the binding affinity to SXR depended on the length of the side chain part and the fitting of the quinone part of vitamin K analogues to the LBP.

Currently, as an effective treatment for osteoporosis and prevention of fractures, vitamin K including MK-4 is frequently prescribed for osteoporosis in Japan.[18,19] The daily dosage of MK-4 for osteoporotic patients is 45 mg, affording a body concentration that corresponds to 10^{-6} M, and the tissue levels are much higher ($>10^{-5}$ M).[20,21] These concentrations are well correlated with those used in our in vitro transcriptional assay. Recent studies have demonstrated that SXR also up-regulated the expression of osteoblastic markers and the factors related to bone formation such as a small leucine-rich proteoglycan named tsukushi, an extracellular matrix protein matrilin-2, CD14 antigen,[9] and Msx2 gene.[22] Therefore, strong SXR agonists based on vitamin K analogues may be a source for entirely new therapeutic drugs for osteoporosis in both aspects of transcriptional activity and coenzyme activity of γ-carboxylative action.

■ CONCLUSION

We report for the first time the synthesis of new vitamin K_2 analogues that have additional side chains introduced at the 1,4-naphthoquinone, and we demonstrate their effects on the induction of transcription. Compound **9** exhibited the most potent activity among the double side chain analogues in separate assays that evaluated ligand binding and the role of heterodimerization in the induction of transcription, and this

compound was as effective as the known substrate rifampicin. These compounds would be useful not only in the development of new biologically active agents but also in the evaluation of still unknown biological roles of vitamin K.

EXPERIMENTAL SECTION

High-resolution ESI-MS spectra were obtained with a Micromass Q-TOF mass spectrometer. ^{1}H NMR spectra were recorded at 500 MHz, and ^{13}C NMR spectra were recorded at 125 MHz using $CDCl_3$ as a solvent unless otherwise specified. Chemical shifts are given in parts per million (δ) using tetramethylsilane (TMS) as the internal standard. Column chromatography was carried out on silica gel 60 (70−230 mesh), and preparative thin layer chromatography (TLC) was run on silica gel 60F_{254}. Unless otherwise noted, all reagents were purchased from commercial suppliers. Purity of compounds was confirmed by a HPLC system.

Synthesis of 7. To a solution of 1,4-naphthoquinone (**12**) (150 mg, 948 μmol) in ether (20 mL) was added a 10% $Na_2S_2O_4$ aqueous solution (20 mL). The mixture was stirred vigorously at 30 °C for 1 h under argon. After the yellow ether layer turned colorless, the mixture was extracted with AcOEt (50 mL × 3). The combined organic layer was washed with brine (50 mL × 3), dried over $MgSO_4$, and concentrated to afford crude hydroquinone **13**. The residue was immediately dissolved in AcOEt (2 mL) and dioxane (2 mL). Then phytol (**14**) (845 mg, 2.85 mmol) and boron trifluoride ether complex (200 μL) were added. The mixture was stirred at 70 °C for 3 h under argon and cooled to room temperature. The mixture was poured into ice−water and extracted with AcOEt (50 mL × 3). The combined organic layer was washed with water (50 mL) and brine (50 mL), dried over $MgSO_4$, and concentrated. The residue was purified by preparative TLC on silica gel (*n*-hexane/AcOEt = 20:1) to afford **7** (373 mg, 55%) as a yellow oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.09−8.06 (2H, m), 7.69−7.67 (2H, m), 5.01 (2H, t, J = 7.0 Hz), 3.37 (4H, d, J = 7.0 Hz), 1.95−1.92 (4H, m), 1.77 (6H, s), 1.55−1.49 (2H, m), 1.36−0.99 (36H, m), 0.88−0.81 (total 24H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 185.1, 146.1, 137.8, 133.3, 132.2, 126.2, 119.5, 40.1, 39.4, 37.5, 37.4, 37.3, 32.8, 32.7, 29.7, 28.0, 26.0, 25.3, 24.8, 24.5, 23.5, 22.7, 22.6, 19.7, 16.4; ESI-HRMS (M + H$^+$) m/z calcd for $C_{50}H_{83}O_2$ 715.6388. Found 715.6391.

Synthesis of 8. Similar to the synthesis of **7** from **12**, the crude product **8**, which was obtained from **12** (50 mg, 316 μmol), prenol (82 mg, 949 μmol), and boron trifluoride ether complex (70 μL) in AcOEt (1 mL) and dioxane (1 mL), was purified by preparative TLC on silica gel (*n*-hexane/AcOEt = 20:1), giving **8** (34 mg, 37%) as a yellow oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.09−8.05 (2H, m), 7.70−7.67 (2H, m), 5.03−5.00 (2H, m), 3.36 (4H, d, J = 7.0 Hz), 1.79 (6H, s), 1.69 (6H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 185.2, 150.0, 133.8, 133.3, 132.2, 126.2, 119.9, 26.1, 25.8, 18.1; ESI-HRMS (M + H$^+$) m/z calcd for $C_{20}H_{23}O_2$ 295.1693. Found 295.1693.

Synthesis of 9. Similar to the synthesis of **7** from **12**, the crude product **9**, which was obtained from **12** (50 mg, 316 μmol), geraniol (146 mg, 949 μmol), and boron trifluoride ether complex (70 μL) in AcOEt (1 mL) and dioxane (1 mL), was purified by preparative TLC on silica gel (*n*-hexane/AcOEt = 20:1), giving **9** (78 mg, 57%) as a yellow oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.10−8.06 (2H, m), 7.70−7.67 (2H, m), 5.05−5.00 (4H, m), 3.37 (4H, d, J = 7.0 Hz), 2.08−1.96 (8H, m), 1.78 (6H, s), 1.63 (6H, s), 1.56 (6H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 185.1, 146.1, 137.4, 133.3, 132.2, 131.5, 126.2, 124.1, 119.8, 39.7, 26.6, 25.9, 25.7, 17.7, 16.4; ESI-HRMS (M + H$^+$) m/z calcd for $C_{30}H_{39}O_2$ 431.2945. Found 431.2945.

Synthesis of 10. Similar to the synthesis of **7** from **12**, the crude product **10**, which was obtained from **12** (50 mg, 316 μmol), farnesol (211 mg, 949 μmol), and boron trifluoride ether complex (70 μL) in AcOEt (1 mL) and dioxane (1 mL), was purified by preparative TLC on silica gel (*n*-hexane/AcOEt = 20:1), giving **10** (86 mg, 48%) as a yellow oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.10−8.08 (2H, m), 7.70−7.69 (2H, m), 5.12−5.03 (6H, m), 3.39 (4H, d, J = 7.0 Hz), 2.09−1.91 (16H, m), 1.80 (6H, s), 1.67 (6H, s), 1.57 (12H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 185.1, 146.1, 137.4, 135.2, 133.3, 132.2, 131.2, 126.2, 124.3, 123.9, 119.8, 39.8, 39.7, 26.7, 26.5, 25.9, 25.7, 17.6, 16.5, 16.0; ESI-HRMS (M + H$^+$) m/z calcd for $C_{40}H_{55}O_2$ 567.4196. Found 567.4197.

Synthesis of 11. Similar to the synthesis of **7** from **12**, the crude product **11**, which was obtained from **12** (50 mg, 316 μmol), geranylgeraniol (276 mg, 949 μmol), and boron trifluoride ether complex (70 μL) in AcOEt (1 mL) and dioxane (1 mL), was purified by preparative TLC on silica gel (*n*-hexane/AcOEt = 20:1), giving **11** (89 mg, 40%) as a yellow oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.08−8.06 (2H, m), 7.70−7.67 (2H, m), 5.09−5.01 (8H, m), 3.37 (4H, d, J = 7.0 Hz), 2.09−1.91 (24H, m), 1.79 (6H, s), 1.67 (6H, s), 1.59 (6H, s), 1.56 (12H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 185.1, 146.1, 137.5, 135.2, 134.9, 133.3, 132.2, 131.2, 126.2, 124.4, 124.2, 123.9, 119.7, 39.8, 39.7, 26.8, 26.7, 26.5, 25.9, 25.7, 17.7, 16.5, 16.0; ESI-HRMS (M + H$^+$) m/z calcd for $C_{50}H_{71}O_2$ 703.5449. Found 703.5445.

Analytical Method Used To Determine Purity of the Compounds. HPLC was conducted with a Shimadzu HPLC system (Shimadzu, Kyoto, Japan) consisting of a binary pump (LC-20AD liquid chromatography), an automatic solvent degasser (DGU-20A3 degasser), and a manual injector. Separations were carried out using a reversed-phase C_{18} analytical column (COSMOSIL 5C_{18}-AR-II; 4.6 mm i.d. × 250 mm) (Nakalai Tesque, Kyoto, Japan) with a solvent system consisting of an isocratic solvent. The solvent contained either methanol, ethanol, or water was delivered at 1.0 mL/min. This mobile phase was passed through the column at 1.0 mL/min. The column was maintained at 40 °C with a CTO-20A column oven. Vitamin K analogues were detected at 258 nm with an SPD-M20A diode array detector. The HPLC system was controlled by a CBM-20A system controller (Shimadzu). The purity of the each compound was calculated from a surface integral of detected peaks. Those data satisfied more than 95% purity.[23]

Cloning and Construction of cDNAs. Human steroid and xenobiotic receptor (SXR) expression vector (pcDNA3.1-FLAG-SXR) were generated by PCR using human genomic DNA as templates and inserted in-frame into pcDNA3.1(+) vector (Invitrogen) at EcoRI and XhoI sites. The CYP3A4 luciferase reporter plasmid pGL3-CYP3A4pro was constructed in our laboratory. The CYP3A4 promoter, from base pairs −362 to +53, and a distal enhancer module of CYP3A4 promoter, from base pairs −7876 to −7208, were generated by polymerase chain reaction (PCR) using the DNA template isolated from the human genome DNA. These products were then subcloned into the pGL4.10 vector, a promoter-less luciferase reporter vector at NheI and BamHI sites. Finally, pGL4.10-CYP3A4pro, CYP3A4 luciferase reporter plasmid, containing −7876 to −7208 and −362 to +53 bp of the CYP3A4 proximal promoter was prepared.[15]

Luciferase Assay. Human hepatoma cell line HepG2 cells were maintained in Eagle's modified medium (Nakalai Tesque) supplemented with 1% penicillin, 1% streptomycin, and 10% fetal calf serum (FCS) (Gibco BRL). Luciferase assay was performed using HepG2 cells (2 × 10^5 cells/well in six-well plates) transfected with 0.25 μg of pcDNA3.1-FLAG-SXR, 0.25 μg of pGL4.10-CYP3A4pro luciferase reporter vector (Toyo Ink. Co. Ltd.), and 0.1 μg of pRL-CMV (Promega) using Lipofectamine (Invitrogen). At 24 h after transfection, cells were treated with rifampicin (Nakalai Tesque, Kyoto, Japan), vitamin K compounds, or vehicle (ethanol) for 48 h in fresh medium, and luciferase activities were determined by a Lumat LB9507 luminometer (Berthold Technologies) using the dual-luciferase assay system (Toyo Ink.). Firefly luciferase activity was normalized to Renilla luciferase, which was used as a transfection control. The experiments were repeated three times with similar results.

Computational Details. We built the three-dimensional structure of the SXR-LBD/MK-*n*-W complexes from chain A of the crystal structure of the hSXR-LBD/SR12813 complex (PDB accession code 1NRL[17]). All hydrogen atoms and partially present atoms of Glu270, Arg303, and Gln695 were added on the basis of chain B by using the Sybyl 7.3 molecular modeling system. Our docking analyses of MK-*n*-W to the SXR were carried out using the MOE[24] by the following procedure: (i) 500 trial conformations for each MK-*n*-W were picked up; (ii) the positions of all hydrogen atoms in hSXR-LBD were refined by conjugate gradient method; (iii) each MK-*n*-W was docked to the hSXR-LBD using the ASEDock method. Calculations were performed on a Dell Precision T3500 workstation.

■ ASSOCIATED CONTENT

Supporting Information. ^{1}H and ^{13}C NMR spectra of **7**−**11** and HPLC data. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

Gla, γ-carboxyglutamic acid; gas6, growth arrest specific 6; PK, phylloquinone; MK-*n*, menaquinone-*n*; SXR, steroid and xenobiotic receptor; PXR, pregnane X receptor; PAR, proteinase-activated receptor; CYP3A4, cytochrome P450 3A4; MGP, matrix Gla protein; RXR, retinoid X receptor; SXRE, SXR response element; MOE, Molecular Operating Environment; ESI-HRMS, electrospray ionization high-resolution mass spectrometry; TMS, tetramethylsilane; TLC, thin layer chromatography; PCR, polymerase chain reaction; PDB, Protein Data Bank

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(23) Refer to Supporting Information for details on method and data.

(24) *Molecular Operating Environment (MOE 2010.10)*; Chemical Computing Group, Inc. (1225 Univesity Street, Suite 1600, Montreal, Quebec, Canada, H3B 3X3).