Journal of Medicinal Chemistry

BRIEF ARTICLE

pubs.acs.org/jmc

Synthesis of Novel Vitamin K_2 Analogues with Modification at the ω-Terminal Position and Their Biological Evaluation as Potent Steroid and Xenobiotic Receptor (SXR) Agonists

Yoshitomo Suhara,[†] Masato Watanabe,[§] Kimie Nakagawa,[§] Akimori Wada,[∥] Yoichi Ito,[†] Kazuyoshi Takeda,[‡] Kazuhiko Takahashi,[†] and Toshio Okano*[,§]

[†]Laboratory of Environmental Sciences, and [‡]Department of Medicinal Chemistry, Yokohama College of Pharmacy, 601, Matano-cho, Totsuka-ku, Yokohama 245-0066, Japan

[§]Department of Hygienic Sciences, and [∥]Department of Organic Chemistry for Life Sciences, Kobe Pharmaceutical University, 4-19-1, Motoyamakita-machi, Higashinada-ku, Kobe 658-8558, Japan

Supporting Information

ABSTRACT: Vitamin K_2 is a ligand for a nuclear receptor, steroid and xenobiotic receptor (SXR), that induces the gene expressions of CYP3A4. We synthesized vitamin K_2 analogues with hydroxyl or phenyl groups at the ω-terminal of the side chain. The up-regulation of SXR-mediated transcription of the target gene by the analogues was dependent on the length of the side chain and the hydrophobicity of the ω-terminal residues. Phenyl analogue menaquinone-3 was as active as the known SXR ligand rifampicin.

INTRODUCTION

Vitamin K is a well-known cofactor for the γ-carboxylation reaction that converts specific glutamic acid residues to γ-carboxyglutamic acid residues in proteins related to blood coagulation and bone formation.[1] Vitamin K has two major homologues: the plant-derived vitamin K_1 (**1**) (phylloquinone, PK) and the bacterium-derived vitamin K_2 (**2**) (menaquinone-*n*, MK-*n*) (Figure 1).[2] Recently, additional biological actions of menaquinone-4 (MK-4) that are independent of the vitamin K-dependent γ-carboxylation reaction have been reported.[3−5] One of important actions is the activation of the steroid and xenobiotic receptor (SXR) (also known as PXR,[6] PAR,[7] NR1I2) to regulate transcription of extracellular matrix-related genes that may contribute to collagen assembly.[8] MK-4 and known SXR ligands rifampicin (RIF) and hyperforin up-regulated the target gene, CYP3A4, and other bone markers such as alkaline phosphatase and MGP.[9] Those findings indicated that MK-4 affected bone homeostasis by acting on γ-carboxylation and by modulating MK-4-dependent transcriptional regulation. Furthermore, there is consistent evidence that other dietary vitamin K homologues can be converted into MK-4.[10] We recently confirmed that MK-4 was converted from dietary PK and then was accumulated in various tissues at high concentrations.[11] In certain tissues containing high concentrations of lipids, such as brain tissue, MK-4 would be a preferred form of vitamin K.

This background information prompted us to explore the biological activities of new vitamin K analogues. To develop new analogues, we focused on modification of the side chain moiety. No studies have referred to the biological role of the side chain part of vitamin K even though many reports have described the biological action of PK or MK-4.[3−5,12,13] We predicted that the side chain should play an important role for the various biological activities, since the structural differences among vitamin K homologues are confined to only the isoprene units.[14] To evaluate the role of the side chain of vitamin K, we first investigated SXR-mediated transcriptional activity of new vitamin K_2 analogues in comparison with vitamin K_2 homologues. We anticipated that the introduction of a hydrophilic or hydrophobic group at the ω-terminal position of vitamin K homologues (Figure 2) would result in significant changes in the activity of the analogues. Some of the new analogues might have more potent activity compared with the natural homologues. At the same time, we hoped to gain insights into the mechanism that converts vitamin K analogues to MK-4 if the new compounds were useful as substrates. In this study, we report the synthesis of new vitamin K analogues and we present the results of their effects on SXR-mediated gene transcription.

CHEMISTRY

Synthesis of Vitamin K Analogues. Menaquinones **3**−**6** were generous gifts of Eisai Co., Ltd., and the ω-hydroxylated vitamin K analogues **7**−**9** were synthesized according to our previously reported method.[15,16] The requisite analogues **10** and **11**, with phenyl groups introduced at the ω-terminal position, were obtained by the synthetic method as shown in Scheme 1. Geranyl acetate (**12a**) and farnesyl acetate (**12b**) served as starting materials for **10** and **11** and were modified in a two-step procedure to introduce the tetrahydropyranyl (THP) ether, providing **13a** and **13b**.[15,16] The acetate was hydrolyzed with 1 N NaOH (aq) to give side chain structures **14a** and **14b** in quantative yield. Conversion of the terminal THP group into

Received: January 11, 2011
Published: May 11, 2011

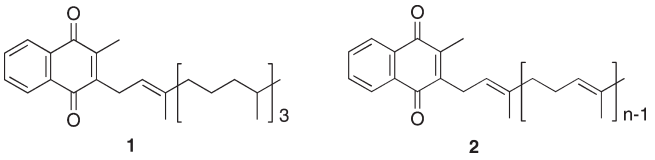


Figure 1. Structures of vitamin K homologues: phylloquinone (**1**) and menaquinones (**2**).

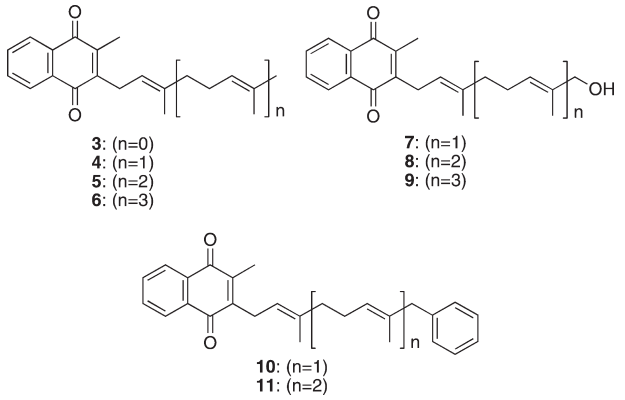


Figure 2. Menaquinones and their analogues.

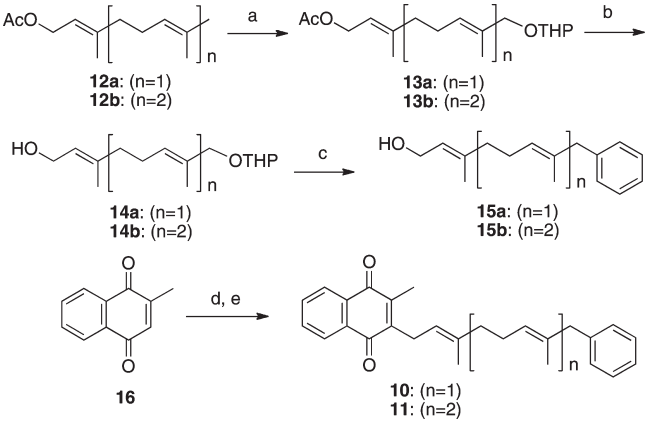
the phenyl group by Grignard reaction by a reported procedure[17] gave **15a** and **15b** in good yields.

We first tried to couple **15a** or **15b** with 1-acetoxy-2-methyl-4-naphthalenol in the presence of $BF_3 \cdot Et_2O$ according to a reported procedure,[16] but the resultant derivatives were quite unstable and easily decomposed. Therefore, we adopted another coupling method.[14] The 1,4-naphthoquinone (**16**) was vigorously stirred with a 10% sodium hydrosulfite (aq) solution and Et_2O to form the hydroquinone derivative, to which an amount of 2 equiv of isoprene unit **15a** or **15b** was successively coupled in the presence of a catalytic amount of $BF_3 \cdot Et_2O$. Finally, the resulting hydroquinone derivatives were immediately oxidized to quinone under atmospheric conditions. Thus, the desired vitamin K analogues **10** and **11** were obtained in 55−65% yield from the oxidation of the quinone, respectively. When an isoprene unit in the side chain part was shorter than geraniol, the chemical yield was not satisfactory. Hence, in this study we were unable to obtain the phenyl analogue menaquinone-1, which had a phenyl group at the ω-terminal position of **3**.

RESULTS

Evaluation of Transcriptional Activities. 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, pGVP2-GAL2 luciferase reporter vector, and pRL-CMV using Lipofectamine[18] in the SXR-GAL4 assay. On the other hand, the cells were treated with pcDNA3.1-FLAG-SXR, pGL4.10-CYP3A4pro luciferase reporter vector, and pRL-CMV using Lipofectamine in the CYP3A4 promoter assay. The SXR-GAL4 assay measured the binding affinity to

Scheme 1. Synthesis of 10 and 11[a]



[a] Reagents and conditions: (a) two steps according to refs 15 and 16; (b) 1 N NaOH (aq), quant; (c) PhMgBr, CuI, 75−85%; (d) 2-methyl-1, 4-naphthoquinone, $Na_2S_2O_4$, Et_2O, quant; (e) **15a** or **15b**, $BF_3 \cdot Et_2O$, 55−65%.

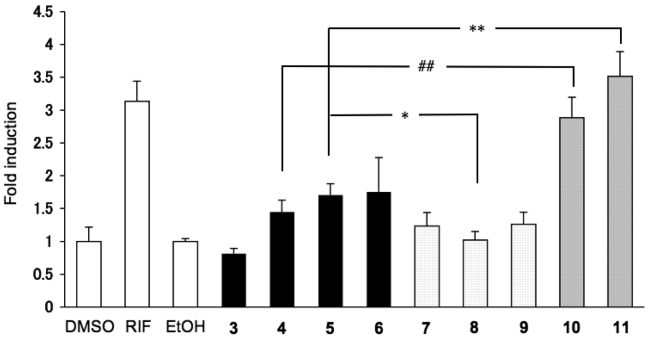


Figure 3. Transcriptional activity with a one-hybrid luciferase assay with SXR-GAL4. HepG2 cells were treated with new vitamin K_2 analogues and natural homologues at 5.0×10^{-6} M. The histogram data are expressed as the mean obtained from three independent experiments. The error bars indicate the SD. Significant difference is as follows: *, $p < 0.05$; **, $p < 0.01$; ##, $p < 0.01$ (by Student's t test).

SXR, while the CYP3A4 promoter assay evaluated SXR-mediated transcription.

To evaluate the effects of ligand binding, we tested all the analogues in the SXR-GAL4 assay at 5.0×10^{-6} M (Figure 3). For purposes of comparison, samples treated with DMSO or EtOH served as baseline, untreated controls, while the sample treated with RIF, a well-known ligand for SXR, served as a positive control.[19] Among the natural vitamin K homologues, the transcriptional activity of **4**, **5**, and **6** exceeded that of **3** in a manner that appeared related to the length of the side chain. For the analogues that contained a hydroxyl group in the side chain, **7**, **8**, and **9**, almost no enhancement in ligand binding activity occurred. On the other hand, the phenyl analogues **10** and **11** exhibited extremely strong activity in comparison with **4** and **5**; **11** had activity comparable to that for RIF. In separate studies, we confirmed that the transcriptional activity as determined by SXR-GAL4 assay showed dose dependency (data not shown).

Similar tendencies were exhibited in the transcriptional activity of the CYP3A4 promoter as shown in Figure 4. Apparent activity of **3**−**6** gradually increased with respect to the length of

dx.doi.org/10.1021/jm200025f | J. Med. Chem. 2011, 54, 4269–4273

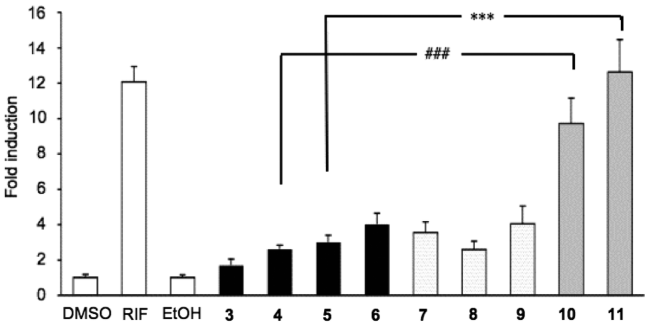


Figure 4. Transcriptional activity with luciferase assay including SXRE with CYP3A4 promoter. HepG2 cells were treated with new vitamin K_2 analogues and natural homologues at 1.0×10^{-6} M. The histogram data indicate the mean obtained from three independent experiments. The error bars indicate the SD. Significant difference is as follows: ***, $p < 0.001$; ###, $p < 0.001$ (by Student's t test).

the side chain, and **7**, **8**, and **9** showed higher induction activity than vehicle controls but slightly lower than **6**. Again, **10** and **11** had the highest activity among the analogues, with **11** showing comparable activity with the known substrate RIF.

Docking Studies. To better understand the binding modes of **10** and **11** to SXR at the atomic level, we performed molecular docking studies on **11** with SXR using the ASEDock docking program, part of the MOE suite (see Computational Details). The docking results positioned **11** within the SXR binding pocket, and the compound with the lowest binding energy displayed an affinity of −29.1 kcal/mol. In this model, the side chain part and the naphthalene moiety of **11** showed hydrophobic interactions with Leu209, Leu240, Met243, Phe281, Phe288, Trp299, Met323, Leu411, and Met246. Especially, a strong interaction between Leu209 and the phenyl group of the side chain moiety was observed (Figure 5). Moreover, the carbonyl groups exerted hydrophilic interaction with Ser247, Gln285, and His407, although no hydrogen bonding was exhibited, providing the complex with an increased predicted stability fully compatible with the experimental biological assays. The binding mode of **10** was similar to that of **11**.

DISCUSSION

In the present report, we describe the synthesis and the pharmacological profile related to SXR-mediated transcriptional activities of new vitamin K analogues modified at the ω-terminal group.

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.[20] 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.[21]

To determine if vitamin K analogues have a role in regulating SXR, we evaluated the transcriptional activity of the new analogues modified at the ω-terminal group by two luciferase-based assay methods. The one-hybrid luciferase assay with SXR-GAL4 permitted us to examine only the 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

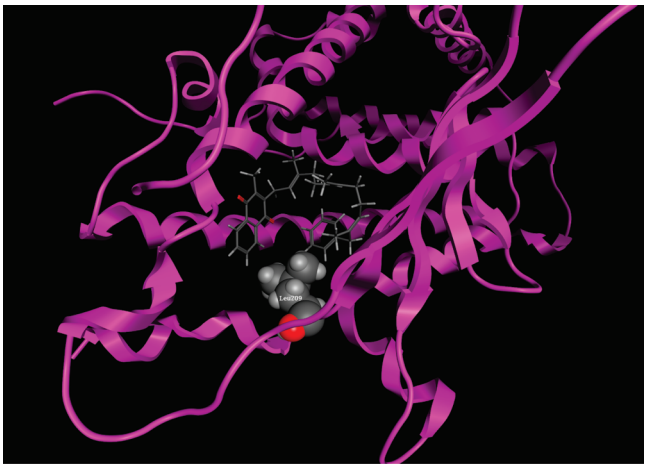


Figure 5. Docked model of **11** bound to SXR (PXR) model (PDB code 1M13, displayed as purple ribbon). **11** is displayed as sticks colored by atom type, while Leu209 is depicted as atom type colored CPK models.

RXR and binding to SXREs. With respect to ligand binding (Figure 3), the activity of natural vitamin K_2 homologues **3**−**6** increased in a manner dependent on the length of side chain moiety, being greatest with **6**. Hydroxylated **7**, **8**, and **9** exhibited only weak activity. Among all the phenyl analogues, **10** and **11** showed the most potent activity, with **11** exhibiting strong activity comparable to that of the established SXR ligand refampicin.

Similar tendencies were noted in the assay that evaluated heterodimerization and binding to SXREs (Figure 4). Compounds **10** and **11** effectively increased the expression of a well-characterized SXR target gene, CYP3A4, in a human hepatocyte cell line, compared with other analogues.

Since the SXR-GAL4 assay measured SXR ligand-binding domain (LBD) driven transcription, we then conducted molecular modeling of **11**, exhibiting potent transcriptional activities, with LBD of SXR. Docking study indicated an additional hydrophobic interaction between the ω-terminal phenyl group of **11** and a part of constituent amino acid (Leu209) of SXR compared with other analogues.

We considered that the ability of the ligands to be absorbed into cells also affects transcriptional activities. Since uptake of the ligands into cells is generally proportional to lipophilicity, introduction of a phenyl group to the molecule would enhance the absorption of **10** and **11**. In contrast, introduction of a hydrophilic group to the molecule, such as for **7**−**9**, would reduce the absorption to cells. These findings suggest that vitamin K analogues with modification at the ω-terminal group can lead to stronger SXR agonists that are more potent than known SXR-ligand such as Taxol, RIF, hyperforin, phenobarbital, etc.

Currently, clinical applications of vitamin K include use of MK-4 as an effective treatment for osteoporosis and preventing fractures, and it is frequently prescribed for osteoporosis in Japan.[22] PK has been used as a therapeutic agent for vitamin K deficiency syndromes such as hypoprothrombinemia in newborn babies and in antibiotic-treated patients.[23,24] The daily dosage of MK-4 for osteoporotic patients is 45 mg, and the body concentration corresponds to 10^{-6} M. Moreover, the tissue levels are much higher ($>10^{-5}$ M).[25,26] 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,[27] and Msx2 gene.[28] Therefore, strong SXR agonists based on vitamin K analogues may be a source for entirely new therapeutic drugs for osteoporosis in 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 with ω-terminal modifications, and we demonstrate their effects on the induction of transcription. The phenyl derivative **11** exhibited the most potent activity 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 RIF. The 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 (ESI-HRMS) was performed 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.

Synthesis of 10 and 11. To a solution of menadion (**16**) (K_3) (150 mg, 871 μmol) in ether (20 mL) was added a 10% $Na_2S_2O_4$ aqueous solution (20 mL), and 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. The residue was immediately dissolved in AcOEt (1 mL) and dioxane (1 mL). Then **15a** (396 mg, 1.72 mmol) or **15b** (519 mg, 1.72 mmol) and boron trifluoride ether complex (50 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 (100 mL) and brine (100 mL), dried over $MgSO_4$, and concentrated. The residue was purified by preparative TLC on silica gel (*n*-hexane/AcOEt = 20:1) to afford **10** or **11** as a yellow oil (**10**, 218 mg, 65%; **11**, 217 mg, 55%). Data for **10**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.10−8.07 (2H, m), 7.70−7.68 (2H, m), 7.28−7.11 (5H, m), 5.17 (1H, t, J = 7.0 Hz), 5.03 (1H, t, J = 7.0 Hz), 3.38 (2H, d, J = 7.0 Hz), 3.21 (2H, s), 2.19 (3H, s), 2.14−2.10 (2H, m), 2.05−2.02 (2H, m), 1.80 (3H, s), 1.50 (3H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 185.5, 184.5, 146.1, 143.4, 140.4, 137.4, 134.6, 133.4, 133.3, 132.19, 132.15, 128.8, 128.1, 126.3, 126.2, 126.0, 125.9, 119.3, 46.2, 39.6, 26.5, 26.0, 16.4, 15.8, 12.7; ESI-HRMS ($M + H^+$) m/z calcd for $C_{27}H_{28}O_2$ 385.2167. Found 385.2165. Data for **11**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.09−8.07 (2H, m), 7.69−7.67 (2H, m), 7.27−7.24 (2H, m), 7.18−7.14 (3H, m), 5.18 (1H, t, J = 7.0 Hz), 5.06 (1H, t, J = 7.0 Hz), 5.02 (1H, t, J = 7.0 Hz), 3.37 (2H, d, J = 7.0 Hz), 3.24 (2H, s), 2.19 (3H, s), 2.09−2.04 (4H, m), 2.01−1.95 (4H, m), 1.80 (3H, s), 1.56 (3H, s), 1.49 (3H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 185.5, 184.5, 146.2, 143.4, 140.5, 137.5, 135.0, 134.2, 133.4, 133.3, 132.19, 132.15, 128.8, 128.1, 126.4, 126.3, 126.2, 125.8, 124.1, 119.1, 46.2, 39.7, 39.6, 26.6, 26.5, 26.0, 16.4, 16.0, 15.7, 12.7; ESI-HRMS ($M + H^+$) m/z calcd for $C_{32}H_{36}O_2$ 453.2793. Found 453.2795.

Cloning and Construction of cDNAs. Human SXR expression vector (pcDNA3.1-FLAG-SXR) was generated by PCR using human genomic DNA as templates, respectively, and then inserted in-frame to pcDNA3.1(+) vector (Invitrogen) at *Eco*RI 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 promoterless luciferase reporter vector, at the NheI and *Bam*HI sites. Finally, pGL4.10-CYP3A4pro, a CYP3A4 luciferase reporter plasmid containing −7876 to −7208 and −362 to +53 bp of the CYP3A4 proximal promoter, was prepared.[18]

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-based assay of SXR-GAL4 was performed using HepG2 cells (2×10^5 cells/well on six-well plates) transfected with 0.1 μg of pM-SXR (constructed in our laboratory), 1.0 μg of pGVP2-GAL2 (extended from Prof. Keiichi Ozono, Osaka University), and 0.05 μg of pRL-CMV (Promega) using Lipofectamine (Invitrogen). In a similar way, luciferase assay of CYP3A4 promoter was conducted using HepG2 cells 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 using Lipofectamine. Twenty-four hours after transfection, cells were treated with RIF (Nakalai Tesque, Kyoto, Japan), vitamin K compounds, or vehicles (EtOH, DMSO) for 48 h in fresh media. The amount of each sample was 2 μL against 2 mL of culture medium. 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 that of Renilla luciferase, which was used as a transfection control. The experiments were repeated three times with similar results.

Computational Details. Calculations were performed on a Dell Precision T3500 workstation. Conformational analyses o ligands, menaquinones, and their analogues were conducted using MOE[29] and MMFF94x molecular mechanics force field. Docking of the minimized energy structure of **11** into the crystal structure of the human pregnane X receptor in complex with hyperforin (PDB code 1M13)[30] was carried out with the ASEDock docking program, part of the MOE suite.

ASSOCIATED CONTENT

Supporting Information. ^{1}H and ^{13}C NMR spectra of **10** and **11**; HPLC data of **3**−**11** used in biological assays; synthesis procedures of **15a** and **15b**. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Phone: +81-78-441-7564. Fax: +81-78-441-7565. E-mail: t-okano@kobepharma-u.ac.jp.

ACKNOWLEDGMENT

We are grateful to Dr. Atsuko Takeuchi and Dr. Chisato Tode for the spectroscopic measurements at Kobe Pharmaceutical University, Japan. We also thank Eisai Co., Ltd. for generously providing the intermediate of the vitamin K analogues. Y.S. acknowledges Takeda Science Foundation. This study was supported in part by a Grant-in-Aid for Scientific Research

(KAKENHI) (C) (Grant No. 20590113 to Y.S.) from the Japan Society for the Promotion of Science.

■ ABBREVIATIONS USED

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-HMRS, electrospray ionization high-resolution mass spectrometry; TMS, tetramethylsilane; TLC, thin layer chromatography; PCR, polymerase chain reaction; PDB, Protein Data Bank

■ REFERENCES

(1) Presnell, R. R.; Stafford, D. W. The vitamin K-dependent carboxylase. *Thromb. Haemostasis* **2002**, *87*, 937–946.

(2) Shearer, M. J. Vitamin K metabolism and nutriture. *Blood Rev.* **1992**, *6*, 92–104.

(3) Li, J.; Lin, J. C.; Wang, H.; Peterson, J. W.; Furie, B. C.; Furie, B.; Booth, S. L.; Volpe, J. J.; Rosenberg, P. A. Novel role of vitamin K in preventing oxidative injury to developing oligodendrocytes and neurons. *J. Neurosci.* **2003**, *23*, 5816–5826.

(4) Otsuka, M.; Kato, N.; Shao, R. X.; Hoshida, Y.; Ijichi, H.; Koike, Y.; Taniguchi, H.; Moriyama, M.; Shiratori, Y.; Kawabe, T.; Omata, M. Vitamin K_2 inhibits the growth and invasiveness of hepatocellular carcinoma cells via protein kinase A. *Hepatology* **2004**, *40*, 243–251.

(5) Lamson, D. W.; Plaza, S. M. The anticancer effects of vitamin K. *Altern. Med. Rev.* **2003**, *8*, 303–318.

(6) Kliewer, S. A.; Moore, J. T.; Wade, L.; Staudinger, J. L.; Jones, M. A.; McKee, D. D.; Oliver, B; M.; Willson, T. M.; Zetterstrom, R. H.; Perlmann, T.; Lehmann, J. An orphan nuclear receptor activated by pregnanes defines a novel steroid signaling pathway. *Cell* **1998**, *92*, 73–82.

(7) Bertilsson, G.; Heidrich, J.; Svensson, K.; Asman, M.; Jendeberg, L.; Sydow Backman, M.; Ohlsson, R.; Postlind, H.; Blomquist, P.; Berkenstam, A. Identification of a human nuclear receptor defines a new signaling pathway for CYP3A induction. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 12208–12213.

(8) Ichikawa, T.; Horie-Inoue, K.; Ikeda, K.; Blumberg, B.; Inoue, S. Steroid and xenobiotic receptor SXR mediates vitamin K_2-activated transcription of extracellular matrix-related genes and collagen accumulation in osteoblastic cells. *J. Biol. Chem.* **2006**, *281*, 16927–16934.

(9) Tabb, M. M.; Sun, A.; Zhou, C.; Grun, F.; Errandi, J.; Romero, K.; Pham, H.; Inoue, S.; Mallick, S.; Lin, M.; Forman, B. M.; Blumberg, B. Vitamin K_2 regulation of bone homeostasis is mediated by the steroid and xenobiotic receptor SXR. *J. Biol. Chem.* **2003**, *278*, 43919–43927.

(10) Yamamoto, R.; Komai, M.; Kojima, K.; Furukawa, Y.; Kimura, S. Menaquinone-4 accumulation in various tissues after an oral administration of phylloquinone in Wistar rats. *J. Nutr. Sci. Vitaminol.* **1997**, *43*, 133–143.

(11) Okano, T.; Shimomura, Y.; Yamane, M.; Suhara, Y.; Kamao, M.; Sugiura, M.; Nakagawa, K. Conversion of phylloquinone (vitamin K_1) into menaquinone-4 (vitamin K_2) in mice: two possible routes for menaquinone-4 accumulation in cerebra of mice. *J. Biol. Chem.* **2008**, *283*, 11270–11279.

(12) Kristensen, M.; Kudsk, J.; Bügel, S. Six weeks phylloquinone supplementation produces undesireble effects on blood lipids with no changes in inflammatory and fibrinolytic markers in postmenopausal women. *Eur. J. Nutr.* **2008**, *47*, 375–379.

(13) Sakamoto, W.; Isomura, H.; Fujie, K.; Iizuka, T.; Nishihara, J.; Tatebe, G.; Takahashi, K.; Osaki, Y.; Komai, M.; Tamai, H. The effect of vitamin K_2 on bone metabolism in aged female rats. *Osteoporosis Int.* **2005**, *16*, 1604–1610.

(14) Suhara, Y.; Wada, A.; Tachibana, Y.; Watanabe, M.; Nakamura, K.; Nakagawa, K.; Okano, T. Structure−activity relationships in the conversion of vitamin K analogues into menaquinone-4. Substrates essential to the synthesis of menaquinone-4 in cultured human cell lines. *Bioorg. Med. Chem.* **2010**, *18*, 3116–3124.

(15) 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 K_2: study of modification and structure−activity relationship of vitamin K_2 metabolites. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1622–1625.

(16) Suhara, Y.; Hirota, Y.; Nakagawa, K.; Kamao, M.; Tsugawa, N.; Okano, T. Design and synthesis of biologically active analogues of vitamin K_2: evaluation of their biological activities with cultured human cell lines. *Bioorg. Med. Chem.* **2008**, *16*, 3108–3117.

(17) Mechelke, M. F.; Wiemer, D. F. Synthesis of farnesol analogues through Cu(I)-mediated displacements of allylic THP ethers by Grignard reagents. *J. Org. Chem.* **1999**, *64*, 4821–4829.

(18) Tirona, R. G.; Lee, W.; Leake, B. F.; Lan, L. B.; Cline, C. B.; Lamba, V.; Parviz, F.; Duncan, S. A.; Inoue, Y.; Gonzalez, F. J.; Schuetz, E. G.; Kim, R. B. The orphan nuclear receptor HNF4alpha determines PXR- and CAR-mediated xenobiotic induction of CYP3A4. *Nat. Med.* **2003**, *9*, 220–224.

(19) Takeshita, A.; Taguchi, M.; Koibuchi, N.; Ozawa, Y. Putative role of the orphan nuclear receptor SXR (steroid and xenobiotic receptor) in the mechanism of CYP3A4 inhibition by xenobiotics. *J. Biol. Chem.* **2002**, *277*, 32453–32458.

(20) Rock, K. L.; Latz, E.; Ontiveros, F.; Kono, H. The sterile inflammatory response. *Annu. Rev. Immunol.* **2010**, *28*, 321–342.

(21) Ma, X.; Shah, Y. M.; Guo, G. L.; Wang, T.; Krausz, K. W.; Idle, J. R.; Gonzalez, F. J. Rifaximin is a gut-specific human pregnane X receptor activator. *J. Pharmacol. Exp. Ther.* **2007**, *322*, 391–398.

(22) Shiraki, M.; Shiraki, Y.; Aoki, C.; Miura, M. Vitamin K_2 (menatetrenone) effectively prevents fractures and sustains lumbar bone mineral density in osteoporosis. *J. Bone Miner. Res.* **2000**, *15*, 515–521.

(23) Booth, S. L.; Tucker, K. L.; Chen, H.; Hannan, M. T.; Gagnon, D. R.; Cupples, L. A.; Wilson, P. W.; Ordovas, J.; Schaefer, E. J.; Dawson-Hughes, B.; Kiel, D. P. Dietary vitamin K intakes are associated with hip fracture but not with bone mineral density in elderly men and women. *Am. J. Clin. Nutr.* **2000**, *71*, 1201–1208.

(24) Van Winckel, M.; De Bruyne, R.; Van De Velde, S.; Van Biervliet, S. Vitamin K, an update for the paediatrician. *Eur. J. Pediatr.* **2009**, *168*, 127–134.

(25) Uematsu, T.; Nagashima, S.; Niwa, M.; Kohno, K.; Sassa, T.; Ishii, M.; Tomono, Y.; Yamato, C; Kanamaru, M. Effect of dietary fat content on oral bioavailability of menatetrenone in humans. *J. Pharm. Sci.* **1996**, *85*, 1012–1016.

(26) Sano, Y.; Tadano, K.; Kaneko, K.; Kikuchi, K.; Yuzuriha, T. Distribution of menaquinone-4, a therapeutic agent for osteoporosis, in bone and other tissues of rats. *J. Nutr. Sci. Vitaminol.* **1995**, *41*, 499–514.

(27) Horie-Inoue, K.; Inoue, S. Steroid and xenobiotic receptor mediates a novel vitamin K_2 signaling pathway in osteoblastic cells. *J. Bone Miner. Metab.* **2008**, *26*, 9–12.

(28) Igarashi, M.; Yogiashi, Y.; Mihara, M.; Takada, I.; Kitagawa, H.; Kato, S. Vitamin K induces osteoblast differentiation through pregnane X receptor-mediated transcriptional control of the Msx2 gene. *Mol. Cell. Biol.* **2007**, *27*, 7947–7954.

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

(30) Watkins, R. E.; Davis-Searles, P. R.; Lambert, M. H.; Redinbo, M. R. Coactivator binding promotes the specific interaction between ligand and the pregnane X receptor. *J. Mol. Biol.* **2003**, *331*, 815–828.