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Brief Article

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Synthesis of Novel Synthetic Vitamin K Analogues Prepared by Introduction of a Heteroatom and a Phenyl Group that Induce Highly Selective Neuronal Differentiation of Neuronal Progenitor Cells

Kimito Kimura,[†] Yoshihisa Hirota,[‡] Shigefumi Kuwahara,[§] Atsuko Takeuchi,[∥] Chisato Tode,[∥] Akimori Wada,[⊥] Naomi Osakabe,[#] and Yoshitomo Suhara[*, †]

[†]*Laboratory of Organic Synthesis and Medicinal Chemistry, Department of Bioscience and Engineering, College of Systems Engineering and Science, Shibaura Institute of Technology, 307 Fukasaku, Minuma-ku, Saitama 337-8570, Japan*

[‡]*Laboratory of Biochemistry, Department of Bioscience and Engineering, College of Systems Engineering and Science, Shibaura Institute of Technology, 307 Fukasaku, Minuma-ku, Saitama 337-8570, Japan*

[§]*Laboratory of Applied Bioorganic Chemistry, Graduate School of Agricultural Science, Tohoku University, 1-1 Tsutsumi-dori-Amamiyamachi, Aoba-ku, Sendai, 981-8555, Japan*

[∥]*Analytical Laboratory, Kobe Pharmaceutical University, 4-19-1 Motoyamakita-machi, Higashinada-ku, Kobe 658-8558, Japan*

[⊥]*Department of Life Science for Organic Chemistry, Kobe Pharmaceutical University, 4-19-1 Motoyamakita-machi, Higashinada-ku, Kobe 658-8558, Japan*

[#]*Food and Nutrition Laboratory, Department of Bioscience and Engineering, College of Systems Engineering and Science, Shibaura Institute of Technology, 307 Fukasaku, Minuma-ku, Saitama 337-8570, Japan*

KEYWORDS. Vitamin K, menaquinone-4, differentiation, neuronal cells, neural progenitor cells

ABSTRACT: We synthesized novel vitamin K_2 analogues that incorporated a heteroatom and an aromatic ring in the side chain and evaluated their effect on the selective differentiation of neuronal progenitor cells into neurons *in vitro*. The result showed that a menaquinone-2 analogue bearing a *p*-fluoroaniline had the most potent activity, which was more than twice as great as the control. In addition, the neuronal selectivity was more than three times greater than the control.

INTRODUCTION

Neural progenitor cells (NPCs) are located in several region of the human adult brain such as the subventricular zone and the dentate gyrus of the hippocampus. NPCs can contribute to neurogenesis in adulthood because they are capable of differentiating into neurons, astrocytes, and oligodendrocytes.[1] Therefore, NPCs would have possible applications for development of stem-cell-based therapies for neurodegenerative diseases.[2] It have been reported that the grafting of NPCs improves neurological deficits in neurodegenerative diseases such as Parkinson's disease, ischemic stroke, and spinal cord injury.[3] However, the induction of new neuronal cells in the damaged brain is relatively low and might be nonfunctional. Otherwise, the transplantation of neural stem cells was expected to repair lesions since they could differentiate into neurons. But transplanted cells also had difficulties of differentiation, maturation, and integration into host neural networks. We have explored a new strategy to repair damaged brain tissues. We focused on "organic compounds" which promote NPCs differentiation and maturation for establishing safe and practical cell therapies. Several natural products have been identified which directly affected differentiation of NPCs into neurons, astrocytes, and oligodendrocytes, however, the compounds demonstrated no selectivity or only weak activity.[4]

We previously reported that menaquinone-4, one of the vitamin K homologues, was biosynthesized and was used in the brain.[5] There are two natural forms of vitamin K homologues, plant-derived vitamin K_1 (phylloquinone, PK) **(1)** and the bacterium-derived vitamin K_2 (**2**) (menaquinone-*n*, MK-*n*) including MK-2 (**3**), MK-3 (**4**), and MK-4 (**5**) (Figure 1). In contrast, vitamin K_3 (menadione) (**6**) is an artificial vitamin K. We have recently revealed that MK-4 had the selective ability to cause differentiation of NPCs, derived from mouse cerebrum, into neuronal cells.[6] Vitamin K homologues also have been reported to play a role in preventing oxidative injury to developing oligodendrocytes and neurons. Based on this findings, we have synthesized new vitamin K analogues modified at the ω-terminal group and several analogues with introduction of

aromatic groups exhibited potent differentiation activity compared to natural vitamin K.[7] The activity of the compound was double that of the control and it was also neuronal selective. This finding showed modification of the side chain of vitamin K could increase the differentiation activity. However, much more potent activity is necessary for application as therapeutic agents. If the analogues exhibited strong differentiation activity in neuronal cells, they may be applicable to elucidate the mechanism of vitamin K in the brain. In this study, we synthesized new analogues by introduction of a heteroatom as well as an aromatic ring in the molecule with the expectation of interaction with target proteins related to differentiation. Here, we report the synthesis and structure-activity relationship of novel vitamin K_2 analogues that selectively induced neuronal differentiation of multipotent neural progenitor cells.

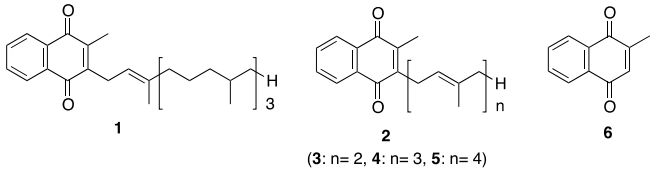
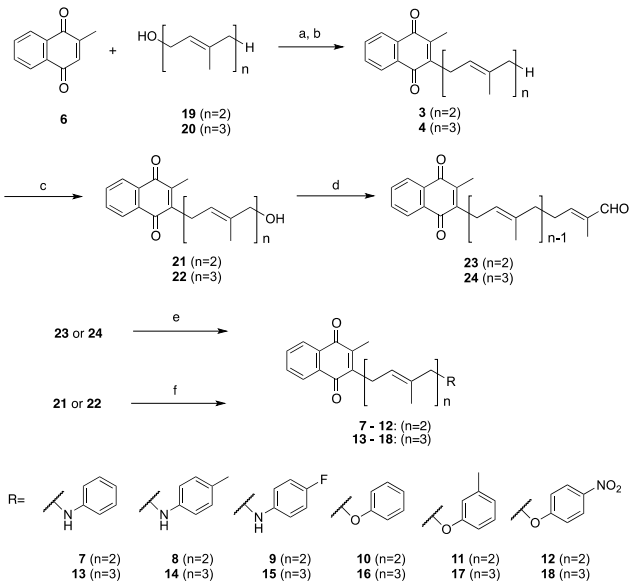


Figure 1. Structure of vitamin K homologues: vitamin K_1 (**1**), vitamin K_2 (**2**) (MK-2 (**3**), MK-3 (**4**), and MK-4 (**5**)), and vitamin K_3 (**6**).

RESULTS AND DISCUSSION

The requisite vitamin K_2 analogues containing aromatic rings and heteroatoms at the ω-terminal position were obtained by the synthetic method as shown in Scheme 1. Compounds were prepared by introducing a nitrogen atom or an oxygen atom as well as phenyl groups. At first, we planned the synthesis of the side-chain by introduction of the nitrogen atom using aniline derivatives, followed by a coupling reaction with vitamin K_3 by Friedel-Crafts alkylation. However, the coupling reaction did not proceed at all. Presumably, the nitrogen atom incorporated into the side chain interrupted the progress of the reaction with vitamin K_3. Therefore, we reconsidered the order of the synthetic coupling reactions between the side chain and vitamin K_3. We first synthesized vitamin K homologues **3** and **4** by a coupling reaction with geraniol (**19**) or farnesol (**20**) and a hydroquinone according to a previously reported method.[8] After **6** was reduced to the hydroquinone derivative with a 10% sodium hydrosulfite (aq) solution in diethyl ether, the isoprene units **19** or **20** were successively coupled with the hydroquinone in the presence of a catalytic amount of BF_3•Et_2O to give **3** and **4** in 34% and 32% yield, respectively. Then, the hydroxyl group was introduced to the carbon atom of ω-terminal side chain in **3** and **4** to give alcohols **21** and **22** in 30% and 5% yield, respectively. Since the side chain length of **4** was longer than that of **3**, the selectivity of introduction of the oxygen at the ω-position of **4** did not proceed very well. The alcohols **21** and **22** were oxidized with MnO_2 to obtain aldehydes **23** and **24** in 78% and 76% yield, respectively.[9] Introduction of the nitrogen atom into the side chain of vitamin K was provided by reductive amination reaction between **23** or **24** and aniline derivatives. Incorporation of an oxygen atom in the side chain was successfully carried out with Mitsunobu reaction between **21** or **22** and phenol derivatives. Thus, the individual desired vitamin K analogues of aniline derivatives **7**-**9** and **13**-**15** were obtained in 52-78% yield. The phenol derivatives **10**-**12** and **16**-**18** were obtained in 43-82 % yield.

Scheme 1. Synthesis of Vitamin K Analogues 7- 18



[a]Reagents and conditions: (a) 10% $Na_2S_2O_4$ aq., Et_2O, quant.; (b) BF_3•Et_2O, 32-34%; (c) SeO_2, salicylic acid, 70% TBHP, 5-30%; (d) MnO_2, 76-78%; (e) aniline derivative, AcOH, $NaBH(OAc)_3$, 52- 78%; (f) phenol derivative, Ph_3P, DIAD, 43-82%.

We investigated the differentiation-inducing activity of the vitamin K analogues using NPCs, which were prepared from embryonic mouse brain. The general method for preparation was as follows as we previously reported[7]: (i) Neural stem cells were dissociated from embryonic day 14 mouse cerebrum and cultured according to the method previously described.[10] (ii) After the cells were seeded and cultured for 24 h, they were treated with 1 μM of the vitamin K analogues every second day for 4 days. We confirmed the differentiation of the NPCs by an immunofluorescence staining method. The specific antigen microtubule-associated protein 2 (Map2) was expressed on the surface of neuronal cells that had successfully differentiated from progenitor cells, and the sample emitted red fluorescence. On the other hand, the expression of glial fibrillary acidic protein (Gfap), indicating differentiation to the astrocyte, was detected as green emission. The differentiation could be evaluated from the resulting fluorescence with fluorescence microscopy (Figure 2). In the ethanol control, we observed the differentiation of NPCs into only neuronal cells. On the other hand, natural menaquinone **5** promoted the differentiation of NPCs into both neuronal cells and astrocytes. The analogues **9** (with a nitrogen substituent in the side chain) and **17** (with a phenol substituent) promoted the selective differentiation of NPCs into neuronal cells since the differentiated cells mostly exhibited red fluorescence, but no green fluorescence.

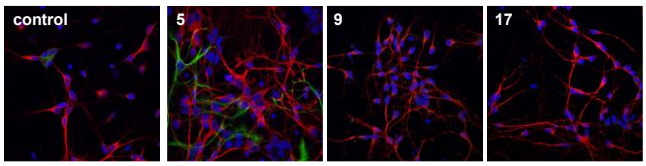
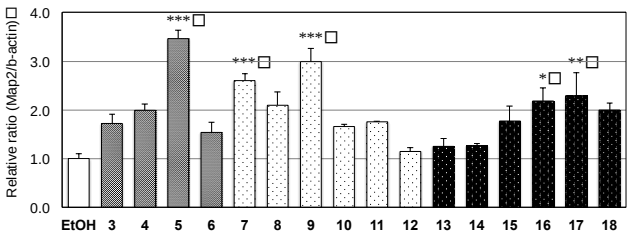


Figure 2. Vitamin K derivatives induce neuronal differentiation of NPCs isolated from an embryonic mouse cerebrum. The cells were treated with the indicated compounds **5**, **9** and **17** at 1 μM. After 48 h, cells were immunostained with markers for neurons (Map2, red) and astrocytes (Gfap, green), and also with DAPI (blue) for nuclei. Mature neurons and a very small amount of astrocytes were respectively observed with Map2 (red) and Gfap (green) after treatment with MK-4 (**5**), a natural menaquinone. On the other hand, the synthesized analogues **9** (bearing a nitrogen) and **17** (bearing a phenol) induced only neuronal differentiation.

Then we quantitated the mRNA of *Map2*, *Gfap* and *β-actin* of the cells using real-time PCR methodology to measure the differentiation activity (Figure 3(A) (B)). The samples treated with EtOH as untreated controls and with menaquinones **3**-**5** as positive controls were used for the purpose of comparison with our compounds. In Figure 3 (A), the data were derived from calculation of the ratio of the mRNA levels of *Map2*/*β-actin*. The ratio obtained for the untreated control (EtOH) was expressed as 1.0 and the values for the analogues were expressed with respect to the control value. Most of the analogues effectively increased the expression of the mRNA level of *Map2*. This result suggests that most compounds enhanced the differentiation-inducing activity toward neuronal cells compared to controls. Among natural menaquinones, **5** showed the highest activity that was more than twice the response of the control in this experiment. Interestingly, the MK-2 analogue **9**, modified with the *p*-fluoroaniline at the ω-terminal position of **3**, showed the most potent activity among the synthetic analogues. It was also more than three times as effective as the EtOH control and displayed almost the same activity as **5**. There were no significant differences between **5** and **9** by Student's *t*-test. While, the activity of the MK-2 analogue **7** was significantly higher than that of the EtOH control. Among the phenol derivatives, compound **16** and compound **17** showed higher activity, but with significantly lower effects than **7** and **9** (Figure 3(A)). On the other hand, Figure 3 (B) was obtained from calculation of the mRNA level of *Gfap*/*β-actin* same as Figure 3 (A). This graph indicated that **5** significantly increased expression of the mRNA level both of *Map2* and *Gfap*, while compounds **7**, **9**, **16**, and **17** promoted only *Map2*.

(A)



(B)

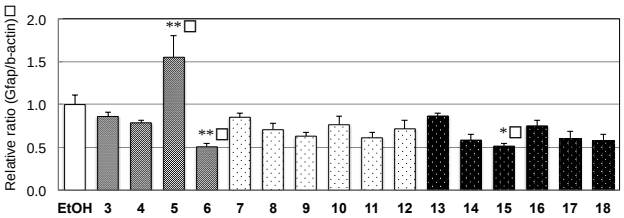


Figure 3. Differentiation-inducing activity of vitamin K_2 analogues from neuronal progenitor cells into neuronal cells as determined by quantitation of mRNA synthesis for *Map2* and *β-actin* (A), *Gfap* and *β-actin* (B) using PCR methodology. The relative ratios were calculated for *Map2*/*β-actin* (A) and *Gfap*/*β-actin* (B), respectively, and then the data were normalized with respect to the EtOH control, which was expressed as 1.0. Cells were treated with the indicated vitamin K_2 analogues as well as natural menaquinones **3**-**5** and menadione **6** at 1.0×10^{-6} M. Compound **7**- **12** were MK-2 analogues and compound **13**- **18** were MK-3 analogues. The histogram data are expressed as the means obtained from three independent experiments; the error bars indicate the SD. Significant difference: ***, $p < 0.001$, **, $p < 0.005$, *, $p < 0.01$, between EtOH and compounds (by Dunnett's *t*-test); There are no significant differences between **5** and **9** (by Student's *t*-test) in (A).

To evaluate selective differentiation activity of vitamin K analogues toward neuronal cells, we also calculated the relative ratio of the mRNA of *Map2*/*Gfap* from the data of Figure 3 (A) and (B) as shown in Figure 4. From this result, **9** and **17** showed significantly higher ratios compared to control (EtOH). Thus, these compounds promoted selective differentiation into neuronal cells rather than into astrocyte cell. Overall, MK-2 analogues bearing an aniline group and MK-3 analogues possessing a phenol tended to exhibit highly selective differentiation activity toward neuronal cells. Specifically, **9** showed the highest effects for both selectivity and activity among the compounds. The selectivity was much higher than that observed with natural vitamin K_2. As compared to the natural menaquinone **5**, compound **9** selectively favored the production of neuronal cells, as confirmed by Student's *t*-test (Figure 4).

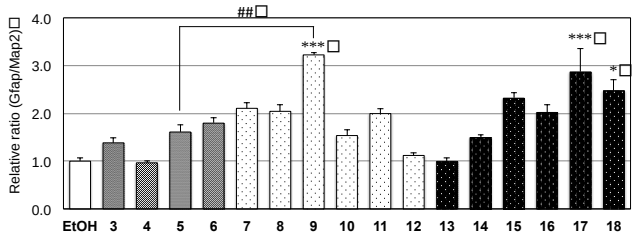


Figure 4. Relative ratios of differentiation-inducing activity of vitamin K_2 analogues to convert neuronal progenitor cells into either neuronal cells or astrocyte cells, as determined by calculation of the relative ratios of *Map2*/*Gfap* from the data of Figure 3 (A) and (B). The data were normalized with respect to that obtained with the EtOH control, which was expressed as 1.0. Cells were treated with the indicated vitamin K_2 analogues **7**- **18** as well

as natural menaquinones **3**-**5** and menadione **6** at 1.0 ×10^{-6} M. Compound **7**- **12** were MK-2 analogues, on the other hand, compound **13**- **18** were MK-3 analogues. The histogram data are expressed as the means obtained from three independent experiments; the error bars indicate the SD. Significant differences: ***, $p < 0.001$, *, $p < 0.01$, between EtOH and the compounds (by Dunnett's *t*-test); #, $p < 0.005$ between **5** and **9** (by Student's *t*-test).

The effects of the vitamin K on NPCs differentiation has not been investigated until now. Recently, it was reported that vitamin E isomer δ-tocopherol enhanced the efficiency of neural stem cell differentiation via the L-type calcium channel.[11] The side chain part of δ-tocopherol and vitamin K resemble each other, suggesting that the mechanism of differentiation might also be mediated through a calcium channel.

CONCLUSION

In conclusion, we successfully introduced nitrogen or oxygen atoms into the side chain of vitamin K derivatives to produce new analogues. We found that some of these compounds had selective and potent activity toward the differential of neuronal progenitor cells into mature neuronal cells. We clarified that significant differences in differentiation activity could be obtained by altering the functional group and the heteroatom of the side chain. A more detailed examination is underway to clarify the mechanism of action of these vitamin K analogues. If the mechanism and the target protein are clarified, it may be possible to design and synthesize much more potent compounds based on our findings.

EXPERIMENTAL SECTION

High-resolution ESI-MS (ESI-HRMS) was performed with a Micro-mass Q-TOF mass spectrometer. ^{1}H NMR spectra were recorded at 400 MHz and ^{13}C NMR spectra were recorded at 100 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). The nuclei were stained with DAPI. Cells were fixed with 10% formaldehyde solution, viewed under a fluorescent microscope and photographed. Unless otherwise noted, all reagents were purchased from commercial suppliers. We confirmed that the purities of the compounds **7**-**18** attained more that 95% as determined by HPLC (high pressure liquid chromatography).

2-((2*E*,6*E*)-3,7-Dimethyl-8-(phenylamino)octa-2,6-dien-1-yl)-3-methylnaphthalene-1,4-dione (7). To a solution of MK-2 ω-aldehyde **23** (51 mg, 158 μmol) in CH_2Cl_2 (2 mL) was added aniline (18 mg, 190 μmol) and acetic acid (11 mg, 190 μmol), and the mixture was stirred in the presence of molecular sieves 4A (MS4A) at room temperature for 30 min under argon. After the mixture was cooled to 0 °C, sodium triacetoxyborohydride (67 mg, 316 μmol) was added to the solution. The mixture was stirred at room temperature for 6 h, then diluted with ethyl acetate (50 mL). The solution was washed with 5% $NaHCO_3$ aq. (50 mL) and brine (50 mL), dried over $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (*n*-hexane/ ethyl acetate= 10: 1) to afford **7** (36 mg, 57%) as a brown oil. ^{1}H NMR (400 MHz, $CDCl_3$); δ 8.09-8.06 (2H, m), 7.69-7.67 (2H, m), 7.17-7.11 (2H, m), 6.69-6.54 (3H, m), 5.36-5.32 (1H, m), 5.03-4.99 (1H, m), 3.74 (1H, br s), 3.57 (2H, s), 3.36 (2H, d, *J* = 6.8 Hz), 2.18 (3H, s), 2.15-2.09 (2H, m), 2.05-1.99 (2H, m), 1.78 (3H, s), 1.63 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$); δ 185.6, 184.6, 148.6, 146.2, 143.5, 137.3, 133.5, 133.4, 132.6, 132.24, 132.22, 129.2, 126.4, 126.3, 125.8, 119.5, 117.2, 112.9, 51.8, 39.4, 26.3, 26.1, 16.5, 14.8, 12.8. HRMS ($[M+H]^+$) m/z calcd for $C_{27}H_{30}NO_2$ 400.2277; found 400.2279.

2-((2E,6E)-3,7-Dimethyl-8-(p-tolylamino)octa-2,6-dien-1-yl)-3-methylnaphthalene-1,4-dione (8). Similar to the synthesis of **7** from **23**, the crude product **8**, which was obtained from reaction of **23** (31 mg, 96 μmol), *p*-toluidine (12 mg, 115 μmol), acetic acid (7 μL, 115 μmol), MS4A, and sodium triacetoxyborohydride (41 mg, 192 μmol) in CH_2Cl_2 (2 mL), was purified by silica gel column chromatography (n-hexane/ ethyl acetate= 10: 1), giving **8** (23 mg, 58%) as a brown oil. ^{1}H NMR (400 MHz, $CDCl_3$); δ 8.09-8.07 (2H, m), 7.69-7.67 (2H, m), 6.97-6.92 (2H, m), 6.55-6.48 (2H, m), 5.35-5.32 (1H, m), 5.02-4.99 (1H, m), 3.55 (2H, s), 3.36 (2H, d, *J* = 7.2 Hz), 2.21 (3H, s), 2.18 (3H, s), 2.14-2.09 (2H, m), 2.04-1.98 (2H, m), 1.78 (3H, s), 1.63 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$); δ 185.5, 184.6, 146.3, 146.2, 143.5, 137.3, 133.5, 133.4, 132.8, 132.2, 129.7, 126.4, 126.3, 125.7, 119.5, 113.1, 52.2, 39.5, 26.3, 26.1, 20.4, 16.5, 14.8, 12.8. HRMS ($[M+H]^+$) m/z calcd for $C_{28}H_{32}NO_2$ 414.2433; found 414.2432.

2-((2E,6E)-8-((4-Fluorophenyl)amino)-3,7-dimethylocta-2,6-dien-1-yl)-3-methylnaphthalene-1,4-dione (9). Similar to the synthesis of **7** from **23**, the crude product **9**, which was obtained from reaction of **23** (92 mg, 285 μmol), *p*-fluoroaniline (33 μL, 342 μmol), acetic acid (20 μL, 342 μmol), MS4A, and sodium triacetoxyborohydride (121 mg, 174 μmol) in CH_2Cl_2 (3 mL), was purified by silica gel column chromatography (*n*-hexane/ ethyl acetate= 10: 1), giving **9** (93 mg, 78%) as a brown oil. ^{1}H NMR (400 MHz, $CDCl_3$); δ 8.07-8.05 (2H, m), 7.68-7.66 (2H, m), 6.88-6.79 (2H, m), 6.55-6.46 (2H, m), 5.34-5.30 (1H, m), 5.02-4.99 (1H, m), 3.66 (1H, br s), 3.52 (2H, s), 3.35 (2H, d, *J* = 6.8 Hz), 2.17 (3H, s), 2.14-2.09 (2H, m), 2.02-1.99 (2H, m), 1.78 (3H, s), 1.62 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$); δ 185.5, 184.6, 156.8, 154.5, 146.1, 144.9, 143.4, 137.2, 133.45, 133.40, 132.5, 132.20, 132.18, 126.4, 126.3, 125.9, 119.5, 115.6, 115.4, 113.7, 113.6, 52.4, 39.4, 26.2, 26.1, 16.5, 14.8, 12.8. HRMS ($[M+H]^+$) m/z calcd for $C_{27}H_{29}FNO_2$ 418.2182; found 418.2182.

2-((2*E*,6*E*)-3,7-Dimethyl-8-phenoxyocta-2,6-dien-1-yl)-3-methylnaphthalene-1,4-dione (10). To a solution of MK-2 ω-alcohol **21** (24 mg, 110 μmol) in THF (2 mL) was added phenol (31 mg, 330 μmol) and triphenylphosphine (87 mg, 330 μmol) at 0ºC under argon. After stirring for 30 min at the same temperature, diisopropyl azodicarboxylate (67 mg, 330 μmol) in THF (1 mL) was added dropwise to the mixture. The reaction mixture was stirred for 1h at room temperature, then diluted with ethyl acetate (50 mL). The mixture was washed with brine (50 mL), dried over $MgSO_4$, and concentrated. The residue was purified with silica gel column chromatography (*n*-hexane/ ethyl acetate= 30: 1) to afford **10** (23 mg, 78%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$); δ 8.09-8.06 (2H, m), 7.69-7.67 (2H, m), 7.26-7.22 (2H, m), 6.93-6.84 (3H, m), 5.49-5.45 (1H, m), 5.05-5.02 (1H, m), 4.31 (2H, s), 3.37 (2H, d, *J* = 6.8 Hz), 2.19 (3H, s), 2.19-2.14 (2H, m), 2.06-2.02 (2H, m), 1.80 (3H, s), 1.70 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$); δ 185.5, 184.6, 159.0, 146.1, 143.5, 137.2, 133.45, 133.40, 132.22, 132.19, 131.3, 129.4, 128.4, 126.4, 126.3, 120.7,

119.6, 114.9, 73.9, 39.2, 26.2, 26.1, 16.5, 14.0, 12.8. HRMS ([M+H]$^+$) m/z calcd for $C_{27}H_{29}O_3$ 401.2117; found 401.2114.

2-((2*E*,6*E*)-3,7-Dimethyl-8-(*m*-tolyloxy)octa-2,6-dien-1-yl)-3-methylnaphthalene-1,4-dione (11). Similar to the synthesis of **10** from **21**, the crude product **11**, which was obtained from the reaction of a mixture of **21** (61 mg, 188 μmol), *m*-cresol (61 mg, 564 μmol), and triphenylphosphine (148 mg, 564 μmol) in THF (4 mL), and diisopropyl azodicarboxylate (114 mg, 564 μmol) in THF (2 mL), was purified by silica gel column chromatography (*n*-hexane/ ethyl acetate= 40: 1), giving **11** (60 mg, 77%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$); δ 8.08-8.06 (2H, m), 7.69-7.65 (2H, m), 7.15-7.10 (1H, m), 6.76-6.65 (3H, m), 5.48-5.44 (1H, m), 5.05-5.02 (1H, m), 4.29 (2H, s), 3.37 (2H, d, *J* = 6.8 Hz), 2.30 (3H, s), 2.19 (3H, s), 2.19-2.14 (2H, m), 2.06-2.02 (2H, m), 1.80 (3H, s), 1.70 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$); δ 185.5, 184.6, 159.0, 146.1, 143.5, 139.4, 137.2, 133.45, 133.40, 132.23, 132.21, 131.4, 129.1, 128.3, 126.4, 126.3, 121.5, 119.6, 115.8, 111.7, 73.9, 39.2, 26.3, 26.1, 21.6, 16.5, 14.0, 12.8. HRMS ([M+Na]$^+$) m/z calcd for $C_{28}H_{30}NaO_3$ 437.2093; found 437.2092.

2-((2*E*,6*E*)-3,7-Dimethyl-8-(4-nitrophenoxy)octa-2,6-dien-1-yl)-3-methylnaphthalene-1,4-dione (12). Similar to the synthesis of **10** from **21**, the crude product **12**, which was obtained from the mixture of **21** (39 mg, 120 μmol), *p*-nitrophenol (50 mg, 360 μmol), and triphenylphosphine (94 mg, 360 μmol) in THF (4 mL), and diisopropyl azodicarboxylate (73 mg, 360 μmol) in THF (1.5 mL), was purified by silica gel column chromatography (*n*-hexane/ ethyl acetate= 10: 1), giving **12** (44 mg, 82%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$); δ 8.20-8.14 (2H, m), 8.08-8.05 (2H, m), 7.71-7.66 (2H, m), 6.92-6.88 (2H, m), 5.51-5.47 (1H, m), 5.05-5.02 (1H, m), 4.40 (2H, s), 3.37 (2H, d, *J* = 6.8 Hz), 2.21-2.16 (2H, m), 2.19 (3H, s), 2.07-2.04 (2H, m), 1.81 (3H, s), 1.70 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$); δ 185.5, 184.6, 164.0, 146.1, 143.5, 141.4, 136.8, 133.5, 133.4, 132.2, 130.0, 129.6, 126.34, 126.31, 125.9, 119.9, 114.8, 74.6, 39.1, 26.1, 16.4, 13.9, 12.8. HRMS ([M+Na]$^+$) m/z calcd for $C_{27}H_{27}NNaO_5$ 468.1787; found 468.1787.

2-Methyl-3-((2*E*,6*E*,10*E*)-3,7,11-trimethyl-12-(phenylamino)dodeca-2,6,10-trien-1-yl)naphthalene-1,4-dione (13). Similar to the synthesis of **7** from **23**, the crude product **13**, which was obtained from MK-3 ω-aldehyde **24** (53 mg, 136 μmol), aniline (14 μL, 150 μmol), acetic acid (10 μL, 150 μmol), MS4A, and sodium triacetoxyborohydride (50 mg, 236 μmol) in CH_2Cl_2 (2 mL), was purified by silica gel column chromatography (*n*-hexane/ ethyl acetate= 10: 1), giving **13** (37 mg, 58%) as a brown oil. ^{1}H NMR (400 MHz, $CDCl_3$); δ 8.09-8.06 (2H, m), 7.69-7.67 (2H, m), 7.16-7.12 (2H, m), 6.68-6.58 (3H, m), 5.36-5.33 (1H, m), 5.02 (2H, dd, *J* = 7.2, 14.4 Hz), 3.77 (1H, br s), 3.60 (2H, s), 3.37 (2H, d, *J* = 6.8 Hz), 2.18 (3H, s), 2.09-1.91 (8H, m), 1.79 (3H, s), 1.62 (3H, s), 1.55 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$); δ 185.6, 184.6, 148.6, 146.2, 143.4, 137.5, 134.9, 133.5, 133.4, 132.3, 132.23, 132.20, 129.2, 126.4, 126.3, 126.2, 124.2, 119.2, 117.2, 112.9, 51.9, 39.8, 39.4, 26.51, 26.45, 26.1, 16.5, 16.1, 14.8, 12.8. HRMS ([M+H]$^+$) m/z calcd for $C_{32}H_{38}NO_2$ 468.2903; found 468.2906.

2-Methyl-3-((2*E*,6*E*,10*E*)-3,7,11-trimethyl-12-(*p*-tolylamino)dodeca-2,6,10-trien-1-yl)naphthalene-1,4-dione (14). Similar to the synthesis of **7** from **23**, the crude product **14**, which was obtained from **24** (45 mg, 115 μmol), *p*-toluidine (15 mg, 138 μmol), acetic acid (8 μL, 138 μmol), MS4A, and sodium triacetoxyborohydride (49 mg, 230 μmol) in CH_2Cl_2 (2 mL), was purified by silica gel column chromatography (*n*-hexane/ ethyl acetate= 10: 1), giving **14** (38 mg, 68%) as a brown oil. ^{1}H NMR (400 MHz, $CDCl_3$); δ 8.09-8.06 (2H, m), 7.70-7.67 (2H, m), 6.95 (2H, d, *J* = 7.6 Hz), 6.53-6.50 (2H, m), 5.35-5.31 (1H, m), 5.05-4.99 (2H, m), 3.57 (2H, s), 3.37 (2H, d, *J* = 6.8 Hz), 2.21 (3H, s), 2.18 (3H, s), 2.08-1.91 (8H, m), 1.80 (3H, s), 1.62 (3H, s), 1.55 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$); δ 185.6, 184.6, 146.4, 146.2, 143.4, 137.6, 135.0, 133.5, 133.4, 132.5, 132.24, 132.20, 129.7, 126.4, 126.3, 126.1, 124.2, 119.2, 113.0, 52.3, 39.8, 39.4, 26.52, 26.45, 26.1, 20.5, 16.5, 16.1, 14.8, 12.8. HRMS ([M+H]$^+$) m/z calcd for $C_{33}H_{40}NO_2$ 482.3059; found 482.3057.

2-((2*E*,6*E*,10*E*)-12-((4-Fluorophenyl)amino)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl)-3-methylnaphthalene-1,4-dione (15). Similar to the synthesis of **7** from **23**, the crude product **15**, which was obtained from **24** (54 mg, 138 μmol), *p*-fluoroaniline (16 μL, 166 μmol), acetic acid (10 μL, 166 μmol), MS4A, and sodium triacetoxyborohydride (59 mg, 276 μmol) in CH_2Cl_2 (2 mL), was purified by silica gel column chromatography (*n*-hexane/ ethyl acetate= 10: 1), giving **15** (45 mg, 67%) as a brown oil. ^{1}H NMR (400 MHz, $CDCl_3$); δ 8.09-8.06 (2H, m), 7.69-7.66 (2H, m), 6.85 (2H, t, *J* = 8.4 Hz), 6.54-6.49 (2H, m), 5.33 (1H, t, *J* = 6.9 Hz), 5.04-4.99 (2H, m), 3.66 (1H, br s), 3.56 (2H, s), 3.37 (2H, d, *J* = 6.9 Hz), 2.18 (3H, s), 2.08-1.91 (8H, m), 1.79 (3H, s), 1.61 (3H, s), 1.55 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$); δ 185.6, 184.6, 156.8, 154.5, 146.2, 144.9, 143.4, 137.5, 134.9, 133.5, 133.4, 132.22, 132.19, 132.15, 126.4, 126.3, 124.2, 119.2, 115.7, 115.4, 113.7, 113.6, 52.5, 39.8, 39.4, 26.5, 26.4, 26.1, 16.5, 16.1, 14.7, 12.8. HRMS ([M+H]$^+$) m/z calcd for $C_{32}H_{37}FNO_2$ 486.2756; found 486.2808.

2-Methyl-3-((2*E*,6*E*,10*E*)-3,7,11-trimethyl-12-phenoxydodeca-2,6,10-trien-1-yl)naphthalene-1,4-dione (16). Similar to the synthesis of **10** from **21**, the crude product **16**, which was obtained from the mixture of MK-3 ω-alcohol **22** (41 mg, 104 μmol), phenol (29 mg, 312 μmol), and triphenylphosphine (82 mg, 312 μmol) in THF (3 mL), and diisopropyl azodicarboxylate (63 mg, 312 μmol) in THF (1.5 mL), was purified by silica gel column chromatography (*n*-hexane/ ethyl acetate= 40: 1), giving **16** (26 mg, 53%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$); δ 8.09-8.06 (2H, m), 7.69-7.67 (2H, m), 7.27-7.23 (2H, m), 6.93-6.89 (3H, m), 5.47 (1H, dd, *J* = 6.0, 6.8 Hz), 5.07-5.00 (2H, m), 4.35 (2H, s), 3.37 (2H, d, *J* = 6.8 Hz), 2.18 (3H, s), 2.14-1.95 (8H, m), 1.79 (3H, s), 1.69 (3H, s), 1.57 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$); δ 185.5, 184.6, 159.1, 146.2, 143.4, 137.5, 134.8, 133.44, 133.38, 132.25, 132.22, 131.0, 129.4, 128.8, 126.4, 126.3, 124.3, 120.7, 119.2, 114.9, 74.0, 39.7, 39.2, 26.5, 26.4, 26.1, 16.5, 16.1, 14.0, 12.8. HRMS ([M+Na]$^+$) m/z calcd for $C_{32}H_{36}NaO_3$ 491.2562; found 491.2562.

2-Methyl-3-((2*E*,6*E*,10*E*)-3,7,11-trimethyl-12-(*m*-tolyloxy)dodeca-2,6,10-trien-1-yl)naphthalene-1,4-dione (17). Similar to the synthesis of **10** from **21**, the crude product **17**, which was obtained from the mixture of **22** (59 mg, 150 μmol), *m*-cresol (49 mg, 450 μmol), and triphenylphosphine (118 mg, 450 μmol) in THF (5 mL), and diisopropyl azodicarboxylate (91 mg, 450 μmol) in THF (1 mL), was purified by silica gel column chromatography (*n*-hexane/ ethyl acetate= 40: 1), giving **17** (31 mg, 43%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$); δ 8.09-8.06 (2H, m), 7.69-7.67 (2H, m), 7.15-

7.11 (1H, m), 6.75-6.69 (3H, m), 5.46 (1H, t, *J* = 6.8 Hz), 5.07-5.00 (2H, m), 4.33 (2H, s), 3.37 (2H, d, *J* = 6.8 Hz), 2.31 (3H, s), 2.19 (3H, s), 2.14-1.95 (8H, m), 1.79 (3H, s), 1.69 (3H, s), 1.57 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$); δ 185.6, 184.6, 159.1, 146.2, 143.4, 139.4, 137.6, 134.8, 133.44, 133.38, 132.3, 132.2, 131.1, 129.1, 128.7, 126.4, 126.3, 124.3, 121.5, 119.2, 115.8, 111.7, 74.0, 39.7, 39.2, 26.5, 26.4, 26.1, 21.6, 16.5, 16.1, 14.0, 12.8. HRMS ($[M+Na]^+$) m/z calcd for $C_{33}H_{38}NaO_3$ 505.2719; found 505.2722.

2-Methyl-3-((2*E*,6*E*,10*E*)-3,7,11-trimethyl-12-(4-nitrophenoxy)dodeca-2,6,10-trien-1-yl)naphthalene-1,4-dione (18). Similar to the synthesis of **10** from **21**, the crude product **18**, which was obtained from the mixture of **22** (46 mg, 117 μmol), *p*-nitrophenol (49 mg, 351 μmol), and triphenylphosphine (92 mg, 351 μmol) in THF (4 mL), and diisopropyl azodicarboxylate (71 mg, 351 μmol) in THF (1.5 mL), was purified by silica gel column chromatography (*n*-hexane/ ethyl acetate= 10: 1), giving **18** (38 mg, 63%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$); δ 8.18-8.15 (2H, m), 8.09-8.06 (2H, m), 7.70-7.68 (2H, m), 6.96-6.93 (2H, m), 5.51-5.48 (1H, m), 5.07-5.00 (2H, m), 4.45 (2H, s), 3.37 (2H, d, *J* = 6.8 Hz), 2.18 (3H, s), 2.16-1.96 (8H, m), 1.79 (3H, s), 1.69 (3H, s), 1.57 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$); δ 185.5, 184.6, 164.1, 146.2, 143.4, 141.4, 137.4, 134.5, 133.44, 133.39, 132.24, 132.21, 130.0, 129.8, 126.4, 126.3, 125.9, 124.5, 119.3, 114.8, 74.8, 39.7, 39.0, 26.5, 26.3, 26.1, 16.5, 16.0, 13.8, 12.8. HRMS ($[M+H]^+$) m/z calcd for $C_{32}H_{36}NO_5$ 514.2593; found 514.2591.

Ethics statement. All animal experimental protocols were performed in accordance with the Guidelines for Animal Experiments at Shibaura Institute of Technology and were approved by the Animal Research and Ethics Committee of Shibaura Institute of Technology, Saitama, Japan. All surgical procedures were performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering.

Isolation of mouse embryonic cerebral-derived neural stem cells. C57BL/6J female mice at 14 days of pregnancy were sacrificed by cervical dislocation, the uterus was taken out, and fetuses were removed from the uterus. The fetuses were decapitated, the scalp and parietal bone were stripped in L-15 medium, and the cerebrum was removed. Under stereoscopic microscope observation, the meninges and blood were completely removed and the cerebrum was washed 5 times with L-15 medium. To 9 mL of L-15 medium was added 1 mL of 2.5 g / L trypsin solution and 100 μL of DNase I solution, and the cerebrum was added and gently stirred at 37 °C for 20 to 25 minutes. DMEM (low glucose) medium containing 10% FCS was added to the trypsin and DNase I-treated cerebrum, the cerebrum was loosened by pipetting, and the cells were dispersed and centrifuged. The supernatant was discarded, DMEM (low glucose) containing 10% FCS medium was added to loosen the cells, and then centrifuged. After this operation was again performed, the cells were suspended in L-DMEM medium containing 10% FCS. A plate-coated glass bottom culture dish was seeded at 3×10^6 cells / dish and cultured at 37 °C for 1 day in the presence of 5% CO_2.

Evaluation of cerebral nerve cell differentiation by immunostaining. The medium was removed from the isolated neural stem cells and replaced with 10% FCS containing DMEM (low glucose) medium supplemented with vitamin K derivative to 10^{-6} M. For control samples, ethanol was used so that the ethanol content in the medium was 0.1%. The cells were cultured for 4 days while changing the medium every 2 days at 37 °C in the presence of 5% CO_2. The medium was removed, the cells were washed with PBS(-), PBS(-) containing 4% paraformaldehyde was added, and the cells were allowed to stand for 20 minutes at room temperature to fix the cells. The cells were then washed 3 times with PBS(-), and then the cells were covered with PBS(-) containing 0.2% Triton X-100 and left at room temperature for 5 minutes. Then, 10% goat serum - containing PBS anti-Map 2 antibody was added dropwise as a primary antibody and allowed to react overnight at 4 °C. After washing three times with PBS(-), CF594 dye was added dropwise as a secondary antibody, the reaction was allowed to proceed for 1 hour at room temperature in a container, and washing was carried out three times with PBS(-) and then the same procedure was carried out using anti Gfap antibody as the primary antibody and CF488A dye as the secondary antibody. The cells were washed three times with PBS(-) and then sealed with an encapsulant containing DAPI. The spectra for DAPI (excitation wavelength 360 nm, fluorescence wavelength 460 nm), CF488A (excitation wavelength 490 nm, fluorescence wavelength 515 nm) and CF594 (excitation wavelength 593 nm, fluorescence wavelength 614 nm) were observed.

Evaluation of differentiation-inducing activity of vitamin K analogues. After the cells were seeded and cultured for 24 h, they were treated with 1 μM of vitamin K analogues as well as control (ethanol) for 4 days. Then the cells were collected and their mRNA were extracted. The mRNA level of *Map2*, *Gfap*, and *β-actin* were quantitated with real-time PCR method. The differentiation activity of vitamin K analogues toward neuronal cells or astrocyte cells was calculated from the ratios of mRNA levels of *Map2*/*β-actin* or *Gfap*/*β-actin*. Furthermore, selective differentiation activity of vitamin K analogues toward neuronal cells was calculated from the relative ratio of the mRNA of *Map2*/*Gfap*. The data were normalized with respect to the EtOH control which was reported as 1.0. The*β-actin* was used for a house-keeping gene.

ASSOCIATED CONTENT

Supporting Information

^{1}H NMR and ^{13}C NMR spectra of **7** - **18**; HPLC data of **7** - **18** used in biological assays. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*For Y.S.: phone, +81-48-720-6043; fax, +81-48-720-6011; E-mail, suhara@shibaura-it.ac.jp

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

NPCs, Neural progenitor cells; Map2, microtubule-associated protein 2; Gfap, glial fibrillary acidic protein

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SYNOPSIS TOC.