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REGULAR ARTICLE

New vitamin K3 (menadione) analogues: synthesis, characterization, antioxidant and catalase inhibition activities

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Abstract. In this study, derivatives of new vitamin K3 were synthesized by the reactions of 2-methyl-1,4-naphthoquinone **1** with some heterocyclic ring substituted nucleophiles: 1-piperonylpiperazine **2**, 1-(2-furoyl)piperazine **5**, 1-(2-aminoethyl)piperidine **8**, 1-(2-aminoethyl)pyrrolidine **10** and 2,6-dimethyl morpholine **12** in chloroform/triethylamine (TEA) or ethanol at room temperature. Their structures were characterized by Fourier transform infrared spectroscopy (FT-IR), ^{1}H nuclear magnetic resonance (^{1}H NMR), attached proton test nuclear magnetic resonance (APT-NMR) and mass spectrometry (MS). Newly synthesized vitamin K3 derivatives (**3**, **4**, **6**, **7**, **9**, **11**, **13**, **14**) have shown catalase inhibition activity and compound **13** has displayed remarkable potency against catalase enzyme. These compounds were also tested for their antioxidant capacity *in vitro* by CUPRAC method.

Keywords. Vitamin K3 (Menadione); heterocyclic ring; CUPRAC method; catalase inhibition activity.

1. Introduction

The quinone compounds are colored compounds due to their conjugation system and are used as natural pigments. The quinone compounds have a wide range of biological activities, and their reactivity and chemical profile is an interesting field of research worldwide.[1] A considerable number of natural and synthetic of quinones especially, 1,4-naphthoquinone derivatives have shown an interesting variety of biological properties, such as antimalarial,[2–4] antifungal,[5–8] antibacterial,[9–12] antitumor,[13,14] and antiallergic activities[15,16] due to their redox potentials.[17]

There are many known natural organic substances contained in the naphthoquinone in their main structure, especially vitamin K groups. Vitamins K, have a basic structure of 2-methyl-1,4-naphthoquinone and different side chain in (C-3) position. Generally, vitamin K regulates blood clotting properties.[18] Vitamin K is naturally found in two forms: Vitamin K1, K2. Vitamin K1 is called phylloquinone, its chemical is "2-methyl-3-suppository-1,4-naphthoquinone, green plants are rich in this vitamin, and vitamin K2 is a group of menaquinone compounds that can be made by bacteria inside the intestine. The chemical name is "2-methyl-3-difarnesyl-1,4-naphthoquinone." There are also other naphthoquinone compounds having a different number of carbon atoms in the side chain and showing vitamin K activity. Vitamin K3 (menadione) or 2-methyl-1,4-naphthoquinone is the synthetic form and same effect of vitamin K group (Figure 1).

In our study, Vitamin K3 (menadione) or 2-methyl-1,4-naphthoquinone was used as a starting material because of their importance of biological activities. We know that piperazine, piperidine, pyrrolidine, morpholine- analogues have drawn great interest in their biological activities in several different

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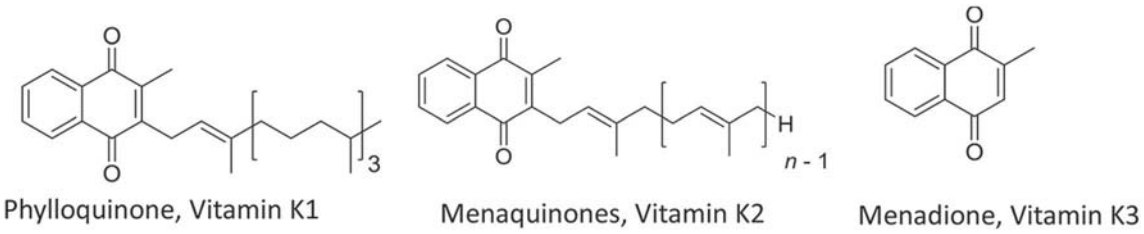


Figure 1. The chemical structures of vitamins K1, K2 and K3.

therapeutic areas. These include anticancer, antifungal, antibacterial, antimalarial and antipsychotic agents,[19] as well as HIV protease inhibitors and antidepressants. Also, the N-carbonyl piperazine moieties exhibit cardio-vascular properties. We aimed that novel K3 derivatives were synthesized from the reactions of menadione with some N-substituted nucleophiles because of that have possessing strong biological properties. Their structures were characterized by FT-IR, ^{1}H NMR, APT-NMR), MS and Elemental analyses.

It is important to measure catalase inhibition activity because it is an abundant enzymatic antioxidant which attenuates the levels of reactive oxygen species (ROS) that cause oxidative stress-related pathological disorders such as cancer, atherosclerosis, diabetes, aging, nutritional deficiency, Parkinson's and Alzheimer' diseases.[20]

2. Experimental

2.1 *Materials and methods*

Melting points were measured on a Büchi B-540 melting point apparatus. FTIR spectra (cm^{-1}) were recorded as KBr pellets in nujol mulls on a Shimadzu IR Prestige 21 model Diamond spectrometer (ATR method). ^{1}H NMR and APT NMR spectrums were obtained using a Varian Unity Inova (500 MHz) spectrometer by using TMS as the internal standard and deuterated chloroform as solvent. Mass spectra were obtained on a hybrid triple quadrupole linear ion trap mass spectrometer (4000 QTRAP, ABSciex). The 4000 QTRAP was operated in the triple quadruple mass spectrometer mode by use of electrospray ionization (ESI) source. Elemental analyses were performed with a Thermo Finnigan Flash EA 1112 elemental analyzer. Products were isolated by column chromatography on silica gel (Fluka Silica gel 60, particle size 63-200 μm). Kieselgel 60 F-254 plates (Merck) were used for thin layer chromatography (TLC). All chemicals were of reagent grade and were used without further purification. Moisture was excluded from the glass apparatus with $CaCl_2$ drying tubes.

2.2 *General procedures*

2.2a *General procedure **1** for the synthesis of compounds (**3**, **4**, **6**, **7**)*: 2-Methyl-1,4-naphthoquinone (**1**) (Vitamin K3) and N-substituted nucleophiles (**2**, **5**) were stirred in chloroform (25 mL) at room temperature for approximately 8 h with the presence of triethylamine (TEA). The reaction mixture was monitoring with (TLC) until the disappearance of starting naphthoquinone. The mixture was extracted using 30 mL of chloroform, washed three times with water, then dried using sodium sulphate (Na_2SO_4). Evaporator system was used to remove the extra amount of solvent. The crude dark residue was purified by column chromatography and dried using a vacuum oven at the end.

2.2b *General procedure **2** of for the synthesis compounds (**9**, **11**, **13**, **14**)*: 2-Methyl-1,4-naphthoquinone (**1**) (Vitamin K3) and N-substituted nucleophiles (**8**, **10**, **12**) were stirred in (25 mL) of absolute ethanol for 4-8 h at room temperature. The reaction mixture was monitoring by (TLC) until the disappearance of starting material. The mixture was extracted using 30 mL of chloroform, washed three times with water, then dried using sodium sulphate (Na_2SO_4). Evaporator system was used to remove the extra amount of solvent. The crude dark residue was purified by column chromatography and dried using a vacuum oven at the end.

*2-(4-((Benzo[d][1,3] dioxol-6-yl)methyl)piperazin-1-yl)-3methylnaph-thalene-1,4-dione (**3**) and 2-(4-((benzo[d][1,3]dioxol-6-yl)methyl)piperazin-1-yl)-3-((4-((benzo[d][1,3] dioxol-6-yl)methyl)piperazin-1-yl)methyl)naphthalene-1,4-dione (**4**):*

According to procedure 1, 1.0 g (5.8 mmol) of 2-methyl-1,4-naphthoquinone **1** was reacted with 1-piperonylpiperazine **2** (1.27 g, 5.7 mmol) in

chloroform with the presence of triethylamine (TEA) (3 mL) at room temperature. The compounds **3** and **4** were synthesized and purified as new compounds.

(**3**): Red oil, yield: 0.351 g, (18%), R_f: 0.54 (EtAc/$CHCl_3$) (1:2 v/v); FT-IR (cm^{-1}): ν = 3093 (C-H_{arom}), 2979, 2902, (C-H_{aliph}), 1661, 1630 (C=O), 1590, 1563 (C=C). ^{1}H-NMR (*ppm*): δ 2.10 (s, 3H, CH_3), 2.55 (br, s, 4H, H_{piper}), 3.55 (t, *J*= 7.3, 4H, H_{piper}), 3.51 (s, 2H, N–CH_2–C_{arom}), 5.96 (s, 2H, O–CH_2–O), 6.59-6.63 (m, 3H, CH_{arom}), 7.56-7.62 (m, 2H, CH_{naphth}), 7.98 (d, *J* = 5.85 Hz, 2H, CH_{naphth}). APT-NMR (*ppm*): δ 11.08 (CH_3), 51.15, 53.31 (CH_2-N-CH_2)$_{piper}$, 62.43 (CH_2), 102.12 (O-CH_2-O), 107.96, 108.01, 122.61, 125.16, 126.16, 128.55, 131.99, 132.62, 133.43 (CH_{arom}, C_{arom}), 146.92, 147.73 (=C-N), 151.52 (=C-O), 180.04, 185.40 (C=O). $C_{23}H_{22}N_2O_4$ (M_w = 390.45 g/mol). Calcd., %: C 70.75; H 5.68; N 7.18. Found, %: C 70.42; H 5.61; N 7.59. MS (+ESI): *m/z* = 391.1 $[M+H]^+$.

(**4**): Red oil, yield: 0.400 g (21%), R_f: 0.72 (EtAc/$CHCl_3$) (1:2 v/v); FT-IR (cm^{-1}): ν = 3069 (C-H_{arom}), 2978, 2900, 2815, 2774 (C-H_{aliph}), 1661, 1630 (C=O), 1587, 1565 (C=C), 1293 (C-N). ^{1}H-NMR (*ppm*): δ 2.61, 3.40 (br, s, 8H, H_{piper}), 3.54, 3.86 (br, s, 8H, H_{piper}), 3.45 (s, 2H, N–CH_2–C_{naphth}), 3.48, 3.50 (s, 4H, N-CH_2-C_{arom}), 5.95 (s, 4H, O–CH_2–O), 6.65-6.70 (m, 6H, CH_{arom}), 7.75 (m, 2H, H_{naphth}), 7.96-8.08 (dd, *J* = 7.32, 0.89 Hz, 2H, CH_{naphth}). APT-NMR (*ppm*): δ 48.03 (N-CH_2-C_{naphth}), 50.98, 51,14, 51,85 (CH_2-N-CH_2)$_{piper}$, 62.89 (N–CH_2-C_{arom}), 101.09 (O-CH_2-O), 109.34, 110.46, 122.44, 125.86, 128.24, 129.68, 130.09, 132.66, 136.17 (CH_{arom}, C_{arom}), 146.93, (=C-N), 150.89, 152.23 (=C-O), 180.31, 183.02 (C=O). $C_{35}H_{36}N_4O_6$ (M_w = 608.70 g/mol). Calcd., %: C 69.09; H 5.96; N 9.20. Found, % : C 69.02; H 5.61; N 8.96. MS (+ESI): *m/z* = 609.0 $[M]^+$.

2-(4-(Furan-2-carbonyl)piperazin-1-yl)-3-methyl-naphthalene-1,4-dione (**6**) and *2-(4-(furan-2-carbonyl)piperazin-1-yl)-3-((4-(furan-2-carbonyl)piperazin-1-yl)methyl) naphthalene-1,4-dione* (**7**):

According to general procedure 1; 1.0 g (5.8 mmol) of 2-methyl-1,4-naphthoquinone (**1**) was reacted with 1-(2-furoyl)piperazine **5** (1.04 g, 5.7 mmol) in chloroform at room temperature with the presence of triethylamine (TEA) (3 mL). The compounds **6** and **7** were obtained and purified as new compounds.

(**6**): Purple oil, yield: 0.563 g (28%), R_f: 0.83 (EtAc/PET) (1:2 v/v); FT-IR (cm^{-1}): ν = 3227 (C-H_{arom}), 2971 (C-H_{aliph}), 1661 (C=O), 1592,1563 (C=C). ^{1}H-NMR (*ppm*): δ 2.41 (s, 3H, CH_3), 2.83 (br, s, 4H, CH_2)$_{piper}$, 3.86 (br, s, 4H, CH_2)$_{piper}$, 6.50 (t, *J* = 7.8 Hz, 1H, CH_{furan}), 7.01 (d, *J* = 9.8 Hz, 1H, CH_{furan}), 7.51 (d, *J* = 9.1 Hz, 1H, CH_{furan}), 7.62-7.68 (m, 2H, CH_{naphth}.), 8.05 (dd, *J* = 8.19, 0.98 Hz, 2H, CH_{naphth}). APT-NMR (*ppm*): δ 14.04 (CH_3), 51.11, 53.76, (N-CH_2) $_{piper}$, 110.54, 112.80, (CH-CH)$_{furan}$, 126.74, 126.75, 128.43, 131.91, 132.69, 133.24, 134.97 (CH_{arom}, C_{arom}), 145.12, 146.66 (C-O-CH)$_{furan}$, 148.42, (=C-N), 160.91, 182.41, 184.03 (C=O). $C_{20}H_{18}N_2O_4$ (M_w = 350.13 g/mol). Calcd., %: C 68.56; H 5.18; N 8.00. Found, % : C 68.32; H 4.88; N 8.09. MS (+ESI): *m/z* = 349.1 $[M-H]^+$.

(7): Red oil, yield: 0.712 g (35%), R_f: 0.33 (EtAc/PET) (1:2 v/v); FT-IR (cm^{-1}): ν = 3116 (C-H_{arom}), 2961, 2912 (C-H_{alipha}), 1667, 1617 (C=O), 1556 (C=C). ^{1}H-NMR (*ppm*): δ 2.68 (t, *J* = 7.8 Hz 2H, N-CH_2)$_{piper}$, 3.12 (s, 2H, CH_2), 3.32 (t, *J* = 7.6 Hz, 2H, N-CH_2)$_{piper}$, 4.05 (t, *J* = 7.8 Hz, 4H, N-CH_2) $_{piper}$, 4.25 (br, s, 4H, N-CH_2)$_{piper}$, 4.51 (t, *J* = 7.3, 2H, N-CH_2)-$_{piper,}$ 6.48 (m, 2H, CH_{furan}), 7.11 (d, *J* = 4.5 Hz, 2H, CH_{furan}), 7.52 (m, 2H, CH_{furan}), 7.68-7.70 (m, 4H, CH_{naphth}.), 7.94 (d, *J* = 7.3 Hz, 1H, CH_{naphth}), 8.00 (d, *J* = 6.4 Hz, 1H, CH_{naphth}). APT-NMR (*ppm*): 45.76 (CH_2), 50.19, 52.88, 55.13, 55.65 (N-CH_2-)$_{piper}$, 112.82, 117.57 (CH-CH)$_{furan}$, 114.06 (C_{naphth}-CH_2), 124.28, 129.55, 132.16, 133.45, 134.97, 136.84, (CH_{arom}, C_{arom}), 145.47 (O-CH)$_{furan}$, 146.72 (C-O)$_{furan}$, 148.05 (=C-N), 161.22, 179.45, 181.96 (C=O). $C_{29}H_{28}N_4O_6$ (M_w = 528.56 g/mol), Calcd., %: C 65.90; H 5.34; N 10.60. Found, % : C 66.02; H 5.61; N 10.59. MS (+ESI): *m/z* =529.1 $[M]^+$.

Synthesis of 2-methyl-3-((2-(piperidin-1-yl)ethyl)amino)naphthalene-1,4-dione (**9**):

According to procedure 2, 1.0 g (5.8 mmol) of 2-methyl-1,4-naphthoquinone **1** was reacted with 1-(2-aminoethyl)piperidine **8** (0.74 g, 5.7 mmol) in ethanol at room temperature. The compound **9** was obtained and purified as a new compound.

(9): Red solid, yield: 0.901 g (53%) R_f: 0.35, (EtAc/hexane) (1:4 v/v); M.p: 61-62 °C; FT-IR (cm^{-1}): ν = 3276 (N-H), 3070 (C-H_{arom}), 2980, 2923, 2855. 2793, 2760 (C-H_{aliph}), 1668 (C=O), 1597, 1561 (C=C). ^{1}H-NMR (*ppm*): δ 1.39 (br, s, 2H $CH_2C\mathbf{H_2}CH_{2piperi}$), 1.55 (m, 4H, $C\mathbf{H_2}CH_2C\mathbf{H_2}_{piperi}$), 2.13 (s, 3H, CH_3), 2.37 (br, s, 4H, $C\mathbf{H_2}$-N-$C\mathbf{H_2}_{piperi}$), 2.52 (br, s, 2H, $CH_2C\mathbf{H_2}$-N), 3.60 (m, 2H, NH-$C\mathbf{H_2}$), 6.37 (s, 1H, NH), 7.46-7.59 (m, 2H, CH_{naphth}), 7.98 (m, 1H, CH_{naphth}.), 8.00 (dd, *J* =7.8, 0.97 Hz, 1H, CH_{naphth} APT-NMR (*ppm*): δ 10.90 (CH_3), 24.34 (C-CH_2-C)$_{piperi}$, 26.01 (C-CH_2-C)$_{piperi}$, 41.84 (HN-CH_2), 45.13 (N-CH_2)$_{piperi}$, 57.64 (N-CH_2). 125.83 (=C-CH_3), 126.86, 126.04, 130.59, 131.86, 133.51. 134.07 (CH_{arom}, C_{arom}), 146.73 (=C-NH), 182.62, 183.31 (C=O). $C_{18}H_{22}N_2O_2$ (M_w =298.38 g/mol). Calcd., %: C 72.46; H 7.43; N 9.39, Found, % : C 72.22; H 7.61; N 9.59. MS (+ESI): *m/z* = 294.2 $[M-4H]^+$.

Synthesis of 2-methyl-3-[(2-(pyrrolidin-1-yl) ethyl)amino]naphthalene-1,4-dione (**11**):

According to general procedure 2; 1.0 g (5.8 mmol) of 2-methyl-1,4-naphthoquinone **1** was reacted with 1-(2-aminoethyl)pyrrolidine **10** (0.74g, 6.4 mmol) in ethanol at room temperature. The compound **11** was obtained and purified as a new compound.

(**11**): Red solid, yield: 0.712 g (43%), R_f: 0.33, ($CHCl_3$/PET) (1:2 v/v); M.p: 83-84 °C; FT-IR (cm^{-1}): ν = 3332 (N-H), 3067 (C-H_{arom}), 2952, 2876, 2794 (C-H_{aliph}), 1661 (C=O), 1596,1563 (C=C). ^{1}H-NMR (*ppm*): δ 1.73 (m, 4H, CH_2-CH_2)$_{pyrroli}$, 2.13 (s, 3H, CH_3), 2.50 (br, s, 4H, CH_2-N-CH_2)$_{pyrroli}$, 2.67 (t, *J*= 6.34 Hz, 2H, $CH_2C\mathbf{H_2}$-N), 3.60 (dd, *J*= 11.7, 5.8, 2H, HN-C$\mathbf{H_2}$), 6.22 (s, 1H, NH), 7.46-7.60 (m, 2H, $CH_{naphth.}$), 7.85 (m, 1H, CH_{naphth}), 7.99 (dd, *J*= 7.8, 0.97 Hz, 1H, CH_{naphth}). APT-NMR (*ppm*): δ 23.60 (CH_3), 43.90 (C-CH_2-C)$_{pyrroli.}$, 53.78 (N-CH_2-)$_{pyrroli}$, 53.78, 55.29 (HN-CH_2-CH_2-N)$_{aliph}$, 112.24 (=C-CH_3), 125.83 130.48, 131.56, 133.47. 134.10 (CH_{arom}, $C_{arom.}$), 146.61 (=C-NH)$_{naphth.}$, 182.51, 183.35 (C=O). $C_{17}H_{20}N_2O_2$ (M_w=284.35 g/mol). Calcd., %: C 71.30; H 7.74; N 9.78. Found, % : C 71.02; H 7.61; N 9.59. MS (+ESI): m/z = 281.6 $[M-2H]^+$.

2-methyl-3-(2,6-dimethylmorpholino)naphthalene-1,4-dione (**13**) *and 2-(2,6-dimethylmorpholino)-3-((2,6-dimethylmorpholino)methyl)naphthalene-1,4-dione* (**14**):

According to procedure 2, 1.0 g (5.8 mmol) of 2-methyl-1,4-naphthoquinone **1** was reacted with 2,6-dimethyl morpholine **12** (0.668 g, 5.8 mmol) in ethanol at room temperature for 6 h. Compounds **13** and **14** and purified were obtained as new compounds.

(**13**): Red oil, yield: 0.362 g (22%); R_f: 0.33 (1:6 EtAc/PET) (1:6 v/v); FT-IR (cm^{-1}): ν = 2971, 2930 (C-H_{aliph}), 1668 (C=O), 1590, 1546 (C=C). ^{1}H-NMR (*ppm*): δ 1.25 (d, *J* = 10.3 Hz, 6H, CH_3)$_{morph}$, 2.30 (s, 3H, CH_3)$_{naphth,}$ 3.27 (dd, *J* = 6.4, 2.5 Hz, 2H, N-CH_2)$_{morph,}$ 3.62 (dd, *J* = 6.1, 2.5 Hz, 2H, N-CH_2)-$_{morph,}$ 3.82-3.87 (m, 2H, CH-O-CH)$_{morph}$, 7.75-7.78 (m, 2H, CH)$_{naphth}$, 8.02 (dd, *J*= 7.52, 4.1 Hz, 2H, CH_{naphth}). APT-NMR (*ppm*): δ 11.63 (CH_3)$_{naphth}$, 18.78 (CH_3)$_{morph}$ 55.43 (CH_2-N-CH_2)$_{morph}$, 67.08 (CH-O-CH)$_{morph,}$ 126.11, (C_{naphth}-CH_3), 127.49, 129.71, 131.36, 132.53, 133.66, 135.91 (CH_{arom}, C_{arom}), 151.78 (=C-N) 182.08, 184.12 (C=O). $C_{17}H_{19}NO_3$ (M_w =285.34 g/mol), Calcd., %: C 71.56; H 6.71; N 4.91. Found, % : C 71.32; H 6.61; N 4.99. MS (+ESI): *m/z* =284.1 $[M-H]^+$.

(**14**): Red oil, yield: 0.352 g (15%); R_f: 0.26 (EtAc/PET) (1:6 v/v); FT-IR (cm^{-1}): ν = 2972, 2929 (C-H_{aliph}), 1785, 1669 (C=O), 1591, 1538 (C=C). ^{1}H-NMR (*ppm*): δ 1.29 (d, 9.7 Hz, 12H, CH_3)$_{morph}$, 2.48, 2.67 (dd, *J* = 6.2, 2.5 Hz, 4H, (CH_2-N-CH_2)$_{morph}$), 2.82, 4.20 (dd, *J* = 6.1, 2.5 Hz, 4H, (CH_2-N-CH_2)$_{morph}$) 2.96 (s, 2H, CH_2)$_{naphth,}$ 3.51 (m, 4H, CH)$_{morph}$, 7.56-7.68 (m, 2H, CH_{naphth}), 8.08 (dd, *J*= 7.52, 4.1 Hz 2H, CH_{naphth}). APT-NMR (*ppm*): δ 18.94 (CH_3)$_{morph,}$ 46.19 (CH_2), 53.82 (CH_2-N- CH_2) $_{morph}$, 58.04 CH_2-(N-(CH_2)$_2$)$_{morph,}$ 69.81, 72.63 (CH-O-CH)$_{morph,}$ 124.02, 127.33, 129.46, 132.65, 133.12, 134.52, 135,83 (CH_{arom}, C_{arom}), 154.11 (=C-N) 182.70, 184.53 (C=O). $C_{23}H_{30}N_2O_4$ (M_w = 398.5 g/mol). Calcd., %: C 69.32; H 7.59; N 7.03. Found, % : C 69.02; H 7.61; N 7.09. MS (+ESI): *m/z* = 399.1 $[M]^+$.

2.3 *Cuprac antioxidant capacity*

The cupric-reducing antioxidant capacity (CUPRAC) method of antioxidant capacity measurement[21] depends on the reduction of a cupric neocuproine complex (Cu(II)-Nc) to the yellow-orange colored cuprous chelate (Cu(I)-Nc) by an antioxidant compound. The CUPRAC reaction mixture comprised of 1 mL of 10 mM $CuCl_2.2H_2O$, 1 mL of 7.5 mM Nc, 1 mL of 1.0 M NH_4Ac buffer solution (pH 7), x mL newly synthesized compound, and H_2O (1.1 – x mL) (total volume: 4.1 mL) in this order. The final mixture was then incubated at room temperature for 30 min. After the incubation, the absorbance at 450 nm was recorded against a reagent blank using a Perkin Elmer Lambda 35 UV–Vis spectrophotometer using a pair of matched quartz cuvettes of 1 cm thickness. Under the described experimental conditions, the calibration curves (absorbance *versus* molar concentration graphs) of each compound were constructed, and their TEAC coefficients $\left(\frac{\varepsilon_{eachcompound}}{\varepsilon_{trolox}};\ \varepsilon : \text{molar absorption coefficient}\right)$ were calculated. The experimental antioxidant capacity results were performed in triplicate.

2.4 *Catalase activity*

The method described by Bekdeser *et al.,* was employed for the determination of catalase enzyme activity.[22] The reaction mixture contained 0.5 mL of 1.0 mM H_2O_2, 1.8 mL of H_2O, 0.1 mL catalase solution (738 U mL^{-1}), and 0.2 mL of 1.0 mM synthesized compound (total volume 2.6 mL). This final mixture was then incubated at room temperature for 30 min. After the incubation period, the optical Cu(II)-Nc-impregnated nafion membrane (optical CUPRAC sensor) was taken out and immersed in a test tube that contained the incubation reaction mixture (2 mL) + EtOH (6.2 mL). After

30 min agitation period, the yellow-orange colored nafion membrane was taken out and its absorbance was recorded at 450 nm against that of a blank membrane without synthesized compound.

3. Results and Discussion

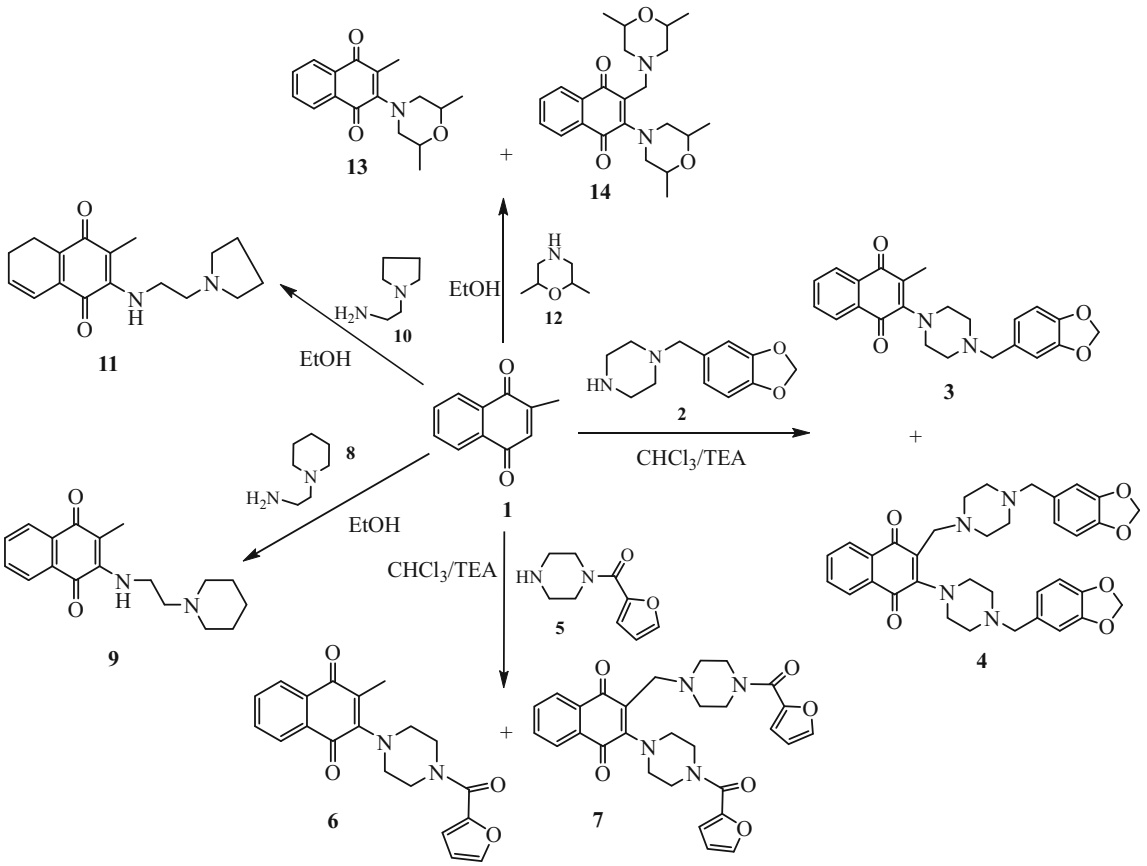
3.1 *Chemistry*

The synthesis of new *N-,* and *N, N*-substituted vitamin K3 derivatives compounds (**3**, **4**, **6**, **7**, **9**, **11**, **13**, **14**) were investigated by the reaction of 2-methyl-1,4-naphthoquinone Vitamin K3 **1** with 1-piperonylpiperazine **2**, 1-(2-furoyl)piperazine **5**, 1-(2-aminoethyl)piperidine **8**, 1-(2-aminoethyl)pyrrolidine **10** and 2,6-dimethyl morpholine **12**, respectively (Scheme 1).

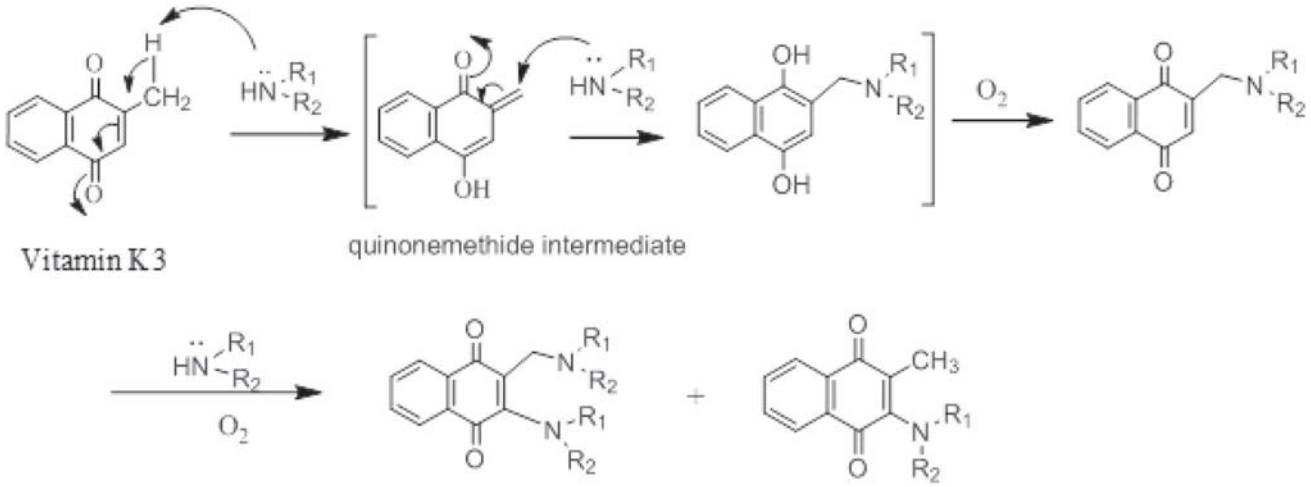
These reactions were carried out at room temperature in chloroform/triethylamine according to procedure 1 or in ethanol according to procedure 2. We obtained expected new compounds (**3**, **6**, **9**, **11**, **13**) by the Michael addition reaction, in other words, the nucleophilic substitution reaction on the naphthoquinone system.[23] Additionally, unexpected new compounds (**4**, **7**, **14**) in these reactions were obtained. It was assumed that the disubstituted products (**4**, **7**, **14**) produced the large excess of the nucleophile. In this case, it is presumed that in the addition-elimination reaction of naphthoquinone system, the methyl group lost a proton to form the quinonemethide intermediate, then an autooxidation steps, followed by nucleophilic attack on naphthoquinone system forming diamino-substituted adducts (**4**,**7**,**14**).[24] A proposed mechanism is shown in Scheme 2.

3.2 *Spectral study*

According to the FT-IR spectrum of compounds **3** and **4**; characteristic stretching bands for (C-H_{arom}) and (C-H_{aliph}) were seen at ν = 3093, 2979, 2902 and 3069, 2978, 2900 cm^{-1}, carbonyl groups bands (C=O) appeared at ν = 1661, respectively. In ^{1}H-NMR spectrum of compound **3**; the chemical shift for methyl protons appeared at δ 2.10 ppm, the singlet at δ 2.55, 3.55 ppm are related to piperazine ring of compound **3**, four proton signals at 2.61, 3.40, 3.54 3.86 ppm are related to piperazine ring of compound **4**. The protons of naphthoquinone around δ 8.00 ppm. The APT-



Scheme 1. The synthesis of new vitamin K3 derivatives (**3**, **4**, **6**, **7**, **9**, **11**, **13**, **14**).



Scheme 2. The proposed general mechanism of compounds **4**, **7**, **14** formation.

NMR showed two signals for carbonyl group at δ 180.04, 185.40 and 180.31, 183.02 ppm for compounds **3** and **4**, respectively.

According to the FT-IR spectrum of compounds **6** and **7**; characteristic stretching bands for carbonyl group were seen at ν = 1661 and 1667, 1617 cm^{-1}, respectively. As we observed in APT-NMR spectrum of compounds **6** and **7**; carbonyl groups gave three small signals at δ 160.91, 182.41, 184.03 and 161.22, 179.45, 181.96 (C=O) ppm, respectively.

In the FT-IR spectra of compounds **9** and **11**, characteristic stretching bands of (NH) were seen at ν = 3276 and 3332 cm^{-1}, respectively. In ^{1}H NMR spectrum of compound **9**; piperidine protons appeared between δ 1.39-2.37 ppm as a broad singlet and (NH) peak was seen at δ 6.37 ppm. The carbons of pyrrolidine in compound **11** gave signals between δ 43.90-55.29 ppm, and carbonyl group gave two signals at δ 182.51, 183.35 ppm as were shown in APT-NMR spectrum.

As was shown in FT-IR spectrum of compound **13**; characteristic stretching bands for (C-H_{aliph}) were observed at ν = 2971, 2930 cm^{-1}, and carbonyl groups at ν = 1668 cm^{-1}. According to the ^{1}H NMR spectrum; methyl groups of morpholine ring and naphthoquinone were seen at δ 1.25, 2.30 ppm as a doublet and singlet, respectively. Morpholine ring protons (CH_2-N-CH_2) and (CH) were found as doublet of doublets and multiplet around δ 3.27, 3.62 and 3.82-3.87 ppm, respectively. Signals at δ 7.75-7.78, and 8.02 ppm are corresponding to naphthoquinone protons. In APT-NMR spectra of compounds **13** and **14**; carbonyl group signals were detected at δ 182.08, 184.12 and 182.70, 184.53 ppm.

In ^{1}H-NMR spectra of compound **14**, protons of $(CH_3)_{morph}$ appeared as doublet at δ 1.29 ppm, whereas, methylene that bonded to quinone ring appeared as a singlet at δ 2.96 ppm. Morpholine ring protons (CH_2-N-CH_2) and $(CH)_{morph}$ were found at δ 2.48, 2.67, 2.82, 4.20 and 3.51 ppm as a doublet of doublets and multiplet, respectively. Signals at δ 7.56-7.68 and 8.08 ppm are corresponding to naphthoquinone protons.

3.3 *CUPRAC antioxidant capacities*

The normal CUPRAC assay (at room temperature) was applied to newly synthesized compounds using trolox (TR) as the reference standard.[21] The slope of the calibration line of the tested compound (CUPRAC molar absorption coefficient: $\varepsilon_{compound}$) divided by that of TR under the same conditions gave the TEAC-CUPRAC coefficients (table 1). RSD and LOD values of CUPRAC method (with respect to synthesized compounds were established between 1.02-3.13 and 1.03-0.22 μM, respectively (n=10). Linear ranges for the tested compound were found as in the range of 4.02-0.018 μM. Amongst the compounds screened for antioxidant capacity, **13** exhibited the highest antioxidant capacity and the TEAC coefficient of this compound was higher than unity ($TEAC_{TR}$ = 1.0) (Table 1).

3.4 *Catalase enzyme inhibition activity of vitamin K3 derivatives*

Catalase (EC 1.11.1.6) is an antioxidant olygomeric enzyme which protects organelles and tissues against

Table 1. Linear equations and correlation coefficients, linear ranges and TEAC coefficients of synthesized compounds with respect to the normal CUPRAC method.

Compounds	Linear range (mol L^{-1})	Calibration equation	r	TEAC[a]
3	1.11×10^{-5}–1.82×10^{-4}	$A = 1.05 \times 10^{4}\ c + 0.07$	0.990	0.63±0.04
4	2.44×10^{-5}–2.05×10^{-4}	$A = 1.07 \times 10^{4}\ c + 0.02$	0.998	0.64±0.01
6	1.05×10^{-5}–2.47×10^{-4}	$A = 9.64 \times 10^{3}\ c + 0.11$	0.991	0.58±0.02
7	1.04×10^{-5}–1.86×10^{-4}	$A = 1.01 \times 10^{4}\ c + 0.02$	0.986	0.60±0.01
9	2.10×10^{-5}–3.36×10^{-4}	$A = 1.09 \times 10^{4}\ c + 0.07$	0.989	0.65±0.03
11	1.90×10^{-5}–2.57×10^{-4}	$A = 1.10 \times 10^{4}\ c + 0.21$	0.980	0.66±0.02
13	4.02×10^{-6}–2.67×10^{-5}	$A = 2.77 \times 10^{4}\ c + 0.12$	0.990	1.66±0.08
14	2.43×10^{-5}–1.83×10^{-4}	$A = 1.17 \times 10^{4}\ c + 0.04$	0.990	0.70±0.03

[a] $TEAC_{compound} = \varepsilon_{compound} / \varepsilon_{TR}$ (TR: trolox); $\varepsilon_{TR} = 1.67 \times 10^{4}$ $Lmol^{-1}cm^{-1}$ (DMSO).

ROS especially hydrogen peroxide (H_2O_2). All compounds were tested *in vitro* for their catalase enzyme inhibition activities[22] and the results are given in -Figure 2. As shown in Figure 2, **13** revealed significant activity. Among eight tested compounds, 7 revealed significant activities (> 0.5 U/mL, Figure 2) and were considered as promising sources of efficient catalase enzyme inhibitor.

In this work, the catalase activity values of newly synthesized compounds were found as U mL^{-1}. Additionally, the IC_{50} (the half-maximal inhibitory concentration) value of compound **13** (having highest UmL^{-1} with respect to the optical sensor based-CUPRAC method) found as (1.31 ± 0.04 μM) with the catalase activity assay[22] was then compared with the well-known catalase inhibitor sodium azide (NaN_3; (1.22 ± 0.01 μM).[25] In this context, a lower concentration of **13** can lead to the catalase enzyme lose its initial activity by 50%.

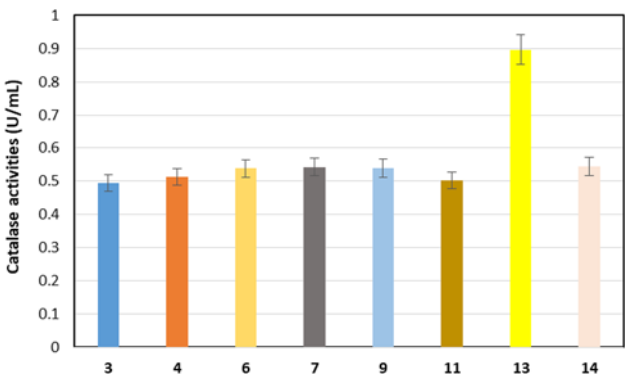


Figure 2. Catalase enzyme activities (U mL^{-1}) of the newly synthesized compounds (**3**, **4**, **6**, **7**, **9**, **11**, **13** and **14**).

Many studies have confirmed that quinone compounds (menadiones *e.g.*,) play a critical role in cellular oxidative damage by generating ROS in the presence of flavoenzymes. Seemingly, menadione -a widely used therapeutic agent for cancer- can also non-enzymatically react with thiol compounds to form ROS resulting in thiol depletion.[26] On the other hand, Wefers and Sies[27] reported that the reaction of menadione and GSH catalyzed by glutathione *S*-transferase (GST: a detoxification enzyme), was not accompanied by the production of ROS. As a result, there may be antioxidant capacity−inhibitive combinations for the newly synthesized MQ compounds.

4. Conclusions

Vitamin K3 (menadione) was chosen as the starting material in this study because of the high biological activity potential of vitamin K3. The new vitamin K3 derivatives that have possessed the potential bioactivity properties were synthesized by the reaction of menadione with heterocyclic ring substituted nucleophiles such as piperazine, piperidine, pyrrolidine and morpholine. Their structures were characterized by Fourier transform infrared spectroscopy (FT-IR), ^1H nuclear magnetic resonance (^1H NMR), attached proton test nuclear magnetic resonance (APT-NMR) and mass spectrometry (MS).

Potential enzyme inhibitors is an active area of research in drugs development. To check their potential, we subjected these compounds to catalase enzyme and these new vitamin K3 derivatives **3**, **4**, **6**, **7**, **9**, **11**, **13** and **14** showed significant activity against catalase enzyme. Especially, the compound **13** has displayed remarkable potency against catalase enzyme.

Supplementary Information (SI)

Supplementary information for this article is available at www.ias.ac.in/chemsci.

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Compliance with ethical standards

Conflict of interest No potential conflict of interest was reported by the authors.

References

1. Kayashima T, Mori M, Yoshida H, Mizushina Y and Matsubara K 2009 1,4-Naphthoquinone is a potent inhibitor of human cancer cell growth and angiogenesis *Cancer Lett*. **278** 34
2. Prescott B 1969 Potential antimalarial agents. Derivatives of 2-chloro-1,4-naphthoquinone *J. Med. Chem*. **12** 181
3. Belorgey D, Lanfranchi D A and Davioud-Charvet E 20131,4-Naphthoquinones and other NADPH-dependent glutathione reductase-catalyzed redox cyclers as antimalarial agents *Curr. Pharm. Design.* **19** 2512
4. Matsumoto K, Choshi T, Hourai M, Zamami Y, Sasaki K, Abe T, Ishikura M, Hatae N, Iwamura T, Tohyama S, Nobuhiro J and Hibino S 2012 Synthesis and antimalarial activity of calothrixins A and B, and their N-alkyl derivatives *Bio. Med. Chem. Lett.* **22** 4762
5. Kurban S, Deniz N G, Sayil C, Ozyurek M, Guçlu K, Stasevych M V, et al. 2019 Synthesis, antimicrobial properties, and inhibition of catalase activity of 1,4-naphtho- and benzoquinone derivatives containing N-, S-, O-substituted *Heteroatom Chem.* **2019** 1
6. Tandon V K, Maurya H K, Mishra N N and Shukla P K 2011 Micelles catalyzed chemoselective synthesis 'in water' and biological evaluation of oxygen containing hetero-1,4-naphthoquinones as potential antifungal agents *Bio. Med. Chem. Lett.* **21** 6398
7. Ryu C K, Lee J Y, Jeong S H and Nho J H 2009 Synthesis and antifungal activity of 1H-pyrrolo[3,2-g]quinoline-4,9-diones and 4,9-dioxo-4,9-dihydro-1H-benzo[f]indoles *Bio. Med. Chem. Lett.* **19** 146
8. Castro M A, Gamito A M and Tangarife-Castano V 2013 Synthesis and antifungal activity of terpenyl-1,4-naphthoquinone and 1,4-anthracenedione derivatives *Eur. J. Med. Chem.* **67** 19
9. Jordao A K, Novais J and Leal B 2013 Synthesis using microwave irradiation and antibacterial evaluation of new N,O-acetals and N,S-acetals derived from 2-amino-1,4-naphthoquinones *Eur. J. Med. Chem.* **63** 196
10. Deniz N G, Ozyurek, M, Tufan A N and Apak M R 2015 One-pot synthesis, characterization, antioxidant capacity of sulfur and oxygen substituted 1,4-naphthoquinones and a structural study *Monatsh. Chem.* **146** 2117
11. Tandon V K, Maurya H K, Verma M K, Kumar R and Shukla P K 2010 On water assisted synthesis and biological evaluation of nitrogen and sulfur containing hetero-1,4-naphthoquinones as potent antifungal and antibacterial agents *Eur. J. Med. Chem.* **45** 2418
12. Ryu C K and Kim D H 1992 The synthesis and antimicrobial activities of some 1,4-naphthoquinones (II) *Arch. Pharm. Res.* **15** 263
13. Kacmaz A, Deniz N G, Aydinli S G, Sayil C, Onay Ucar E, Mertoglu E, et al. 2019 Synthesis and antiproliferative evaluation of some 1,4-naphthoquinone derivatives against human cervical cancer cells *Open Chem.* **17** 337
14. Tip-Pyang S, Limpipatwattana Y, Khumkratok S, Siripong P and Sichaem J 2010 A new cytotoxic 1-azaanthraquinone from the stems of Goniothalamus laoticus *Fitoterapia* **81** 894
15. Huang L J, Chang F C, Lee K H, Wang J P, Teng C M and Kuo S C 1998 Synthesis and antiplatelet, antiinflammatory, and antiallergic activities of substituted 3-chloro-5,8-dimethoxy-1,4-naphthoquinone and related compounds *Bio. Med. Chem.* **6** 2261
16. Huang L J, Lien J C, Lee K H, Wang J P, Teng C M and Kuo S C 1996 Synthesis and antiplatelet, antiinflammatory and antiallergic activities of 2,3-disubstituted-1,4-naphthoquinones *Chem. Pharm. Bull.* **44** 1181
17. Tudor G, Gutierrez P, Aguilera-Gutierrez A and Sausville E A 2003 Cytotoxicity and apoptosis of benzoquinones: redox cycling, cytochrome c release, and BAD protein expression *Biochem. Pharm*. **65** 1061
18. Bingol G 1977 *Vitamins and Enzymes* (Ankara University: Faculty of Pharmacy Publications) p. 46
19. Berkheij M, Sluis L, Sewing C, Boer D J, Terpstra J W, Hiemstra H, Bakker W I I, Hoogenband A and Maarseveena J H 2005 Synthesis of 2-substituted piperazines via direct α-lithiation *Tetrahedron Lett.* **46** 2369
20. Vendemiale G, Grattagliano I and Altomare E 1999 An update on the role of free radicals and antioxidant defense in human disease *Int. J. Clin. Lab. Res*. **29** 49
21. Apak R, Guclu K, Ozyurek M and Karademir S E 2004 Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method *J. Agric. Food. Chem*. **52** 7970
22. Bekdeser B, Ozyurek M, Guclu K, Ustun Alkan F and Apak R 2014 Development of a new catalase activity assay for biological samples using optical CUPRAC sensor *Spectrochim. Acta Part A* **132** 485
23. Cameron D W and Scott P M 1964 Facile loss of C-methyl groups during the amination of quinones *J. Chem. Soc.* **1066** 5569
24. Ohta S, Hinata Y, Yamashita, M and Kawasaki I 1994 One step synthesis of 1,2,3,4-Tetrahydrobenzo[g]quinazoline-5,10-dione derivatives from Vitamin K3 *Chem. Pharm. Bull.* **42** 1730
25. Krych J and Gebicka L 2013 Catalase is inhibited by flavonoids *Int. J. Biol. Macromol.* **58** 148

26. Chung S-H, Chung S-M, Lee J-Y, Kim S-R, Park K-S and Chung J-H 1999 The biological significance of non-enzymatic reaction of menadione with plasma thiols: enhancement of menadione-induced cytotoxicity to platelets by the presence of blood plasma *FEBS Letts.* **449** 235

27. Wefers H and Sies H 1983 Hepatic low-level chemiluminescence during redox cycling of menadione and the menadione-glutathione conjugate: Relation to glutathione and NAD(P)H:quinone reductase (DT-diaphorase) activity *Arch. Biochem. Biophys.* **224** 568