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Evaluation of Cancer Preventive Activity and Structure-Activity Relationships of 3-Demethylubiquinone Q_2, Isolated from the Ascidian *Aplidium glabrum*, and its Synthetic Analogues

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Abstract

Purpose—3-Demethylubiquinone Q_2 (**1**) was isolated from the ascidian *Aplidium glabrum*. The cancer preventive properties and the structure-activity relationship for 3-demethylubiquinone Q2 (**1**) and 12 of its synthetic analogues (**3–14**) are reported.

Methods—Compounds **3**–**14**, having one or several di- or triprenyl substitutions and quinone moieties with methoxyls in different positions, were synthesized. The cancer preventive properties of compounds **1** and **3**–**14** were tested in JB6 Cl41 mouse skin cells, using a variety of assessments, including the MTS assay, flow cytometry, and soft agar assay. Statistical nonparametric methods were used to confirm statistical significance.

Results—All quinones tested were shown to inhibit JB6 Cl41 cell transformation, to induce apoptosis, AP-1 and NF-κB activity, and to inhibit p53 activity. The most promising effects were indicated for compounds containing two isoprene units in a side chain and a methoxyl group at the *para*-position to a polyprenyl substitution.

Conclusions—Quinones **1** and **3**–**14** demonstrated cancer preventive activity in JB6 Cl41 cells, which may be attributed to the induction of p53-independent apoptosis. These activities depended on the length of side chains and on the positions of the methoxyl groups in the quinone part of the molecule.

Keywords

marine prenylated quinones; cancer prevention; apoptosis; nuclear factor; structure-activity relationship

ABBREVIATIONS

3-demethylubiquinone Q2. 2,3-dimethoxy-5-(3′,7′- dimethyl- octa-2′(E),6′- dienyl)-[1,4] benzoquinone (1); EGF, epidermal growth factor; TPA, 12-O-tetradecanoyl-phorbol-13-acetate; FBS, fetal bovine serum; MEM, minimum essential medium

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INTRODUCTION

Interest in developing effective cancer therapies led to the discovery of new important technologies and biomolecules including viral vaccines, microbial-based therapy, interferons, interleucins, and a number of new promising anti-tumor agents (1–4). With respect to anti-tumor agents, some marine secondary metabolites are known to be among the most promising for chemotherapy of cancer (5–7). Polyprenylated 1,4-benzoquinones and hydroquinones are commonly found in a variety of organisms and play an important role in photosynthesis, electron transport, and as antioxidants (8,9). The previously described marine polyprenylbenzoquinones and hydroquinones contain a terpenoid portion ranging from one to nine prenyl units. These quinones have been isolated from brown algae of the order Fucales (10–13), sponges(14–17), alcyonaceans(18), gorgonaceans(19), and ascidians belonging to the genus *Aplidium* (20–25). Brown algae contain diprenyl-, triprenyl-, and tetraprenylquinones and hydroquinones (10–13). Sponges contain prenylated 1,4-benzoquinones and hydroquinones with linear and longer (up to nine prenyl units) terpenoid side chains (14–17). Ascidians of the genus *Aplidium* have previously yielded about a dozen prenylated quinones and related compounds (20–25).

The purpose of the present work was to study the cancer preventive activity and to establish the corresponding structure-activity relationships (SARs) of a group of prenylated quinones resembling ubiquinones in their structures, but differing in that they have shorter polyprenyl side chains and modified quinone moieties. Two of these compounds, 3-demethylubiquinone Q_2 (**1**) and its 2′,3′-*cis*-isomer, 2,3-dimethoxy-5-(3′,7′-dimethyl-octa-2′(Z),6′-dienyl)-[1,4] benzoquinone (**2**) were isolated from the Far-eastern ascidian *Aplidium glabrum* (26), while compounds **3** through **14** are synthetic analogues of **1**. Distinct from previous investigations on related compounds, we studied the pro-apoptotic properties and the cancer preventive activities of these compounds by using mouse cell lines and methods of flow cytometry or DNA laddering for assessing apoptosis and the well-accepted anchorage-independent assay using JB6 P^+ Cl41 cells in soft agar for investigation of the cancer preventive activity.

MATERIALS AND METHODS

General Procedures

^1H and ^{13}C NMR spectra were recorded on a Bruker WM-250 spectrometer at 250 and 62.9 MHz, respectively, Bruker DPX 300 at 300 and 75 MHz, respectively. HREIMS were obtained on an AMD-604S mass spectrometer. HPLC separations were conducted on a DuPont 8800 chromatograph equipped with differential refractometer using an Ultrasphere Si column. The IR spectra were measured on a Bruker FT-IR “Vector 22” spectrophotometer. UV spectra were determined in CCl_4 on a Cecil CE 7200 spectrophotometer. The onset of apoptosis was analyzed by flow cytometry using the Becton Dickinson FACSCalibur (BD Biosciences, San Jose, CA). The MTS reduction assay to determine cell viability was measured using the Multiskan MS microplate reader (Labsystems, Finland). Cell colonies in the anchorage independent transformation assay were scored using the LEICA DM IRB inverted research microscope (Leica Mikroskopie und Systeme GmbH, Germany) and Image-Pro Plus software, version 3.0 for Windows (Media Cybernetics, Silver Spring, MD). The luminescence assay for p53, AP-1 and NF-κB nuclear factor-dependent transcriptional activity was measured using the Luminoscan Ascent Type 392 microplate reader (Labsystems, Finland).

Reagents

Minimum essential medium (MEM) and DMEM were from Gibco Invitrogen Corporation (Carlsbad, CA). Fetal bovine serum (FBS) was from Gemini Bio-Products (Calabasas, CA). Penicillin/streptomycin and gentamycin were from Bio-Whittaker (Walkersville, MD), L-

glutamine was from Mediatech, Inc. (Herndon, Virginia). Epidermal growth factor (EGF) was from Collaborative Research (Bedford, MA). Luciferase assay substrate and Cell Titer 96 Aqueous One Solution Reagent (MTS) for the cell proliferation assay were from Promega (Madison, WI). The Annexin V-FITC Apoptosis Detection Kit was from Medical & Biological Laboratories (Watertown, MA). Silica gel L (40/100 μm) for low-pressure column liquid chromatography was from Chemapol (Praha, Czech Republic). Silica gel plates for thin-layer chromatography (4.5 × 6.0 cm, 5–17 μm) were from Sorbfil (Russia).

Cell Culture

The JB6 P^+ Cl41 mouse epidermal cell line and its stable transfectants Cl41-NF-κB, Cl41-AP-1, Cl41-p53 (PG-13) were cultured in monolayers at 37°C and 5% CO_2 in MEM containing 5% FBS, 2 mM L-glutamine, 100 units/ml penicillin and 100 μg/ml streptomycin.

Syntheses of Quinones 1 – 14

Step *a*—Boric trifluoride etherate (0.5 ml) was added to the stirred mixture containing 1 mmol of the corresponding phenol (**15** – **17**) and 4 mmol of *trans*-geraniol (**18**) or *trans*-farnesol (**19**) in 10 ml of absolute ether. The mixture was kept during 12 h at room temperature and then 30 ml of water were added to the mixture and products were extracted with ether (3×15 ml). The extract was washed with 10% NaCl and dried over Na_2SO_4. The solvent was removed and residue was separated using column chromatography on silica gel. Prenylated phenols were eluted with the solvent system gradient hexane: acetone, 50:1 → 20:1. The yield of mixtures of the purified prenylphenols was about 60%. These mixtures were used for Step 2 of the syntheses. The prenylated phenols **20**, **21**, **23** and **28** were isolated as individual compounds by HPLC on an Ultrasphere Si (Altex), 4.6 mm × 25 cm column in the system hexane: ethyl acetate, 7:1.

Step *b*—A solution of CAN (0.9 mmol) in 3 ml of the mixture CH3CN: H_2O, 1:2 was added to the cooled (0°C) stirred solution of the corresponding prenylated phenol in 7 ml of CH3CN. After being stirred at 0°C for 1–2 h, the mixture was poured into 25 ml of 10% NaCl and extracted with ether (3×15 ml).

The extract was dried over Na_2SO_4 and evaporated. The corresponding prenylated [1,4]-benzoquinones **1** through **10** and **12** through **14** were separated by preparative thin-layer chromatography on silica gel in the system hexane: acetone, 8:1. Yields of target products were about 70% at this stage. Total yield calculated for two stages was about 45%.

Step *c* (synthesis of prenylated hydroquinone 11)—A solution of $Na_2S_2O_4$ (3 mmol) in 3 ml of water was added to 1 mmol of the prenylquinone **7** in 7 ml of acetone. The mixture was stirred for 1 h, diluted with water and extracted with ether (3×15 ml). The extract was dried over Na_2SO_4 and evaporated. As result, hydroquinone **11** was obtained.

3-demethylubiquinone Q2 or 2,3-dimethoxy-5-(3′,7′- dimethyl- octa-2′(E),6′-dienyl)-[1,4]benzoquinone (1): yellow oil, HREIMS *m/z* 304.1655 $[M]^+$, calcd for $C_{18}H_{24}O_4$ 304.1675, IR ($CHCl_3$): 1675, 1657, 1603. 1H NMR ($CDCl_3$, 250MHz) §: 6.34 (t, J=1.7, 1H, H-6); 5.13 (m, 1H, H-2′); 5.08 (m, 1H, H-6′); 4.02 (s, 3H, OMe); 4.00 (s, 3H, OMe); 3.10 (dd, J=7.3, 1.7, 2H, H-1′); 2.09 (m, 2H, H-5′); 2.08 (m, 2H, H-4′); 1.70 (d, J=1.2, 3H, H-8′); 1.62 (d, J=1.2, 3H, H-10′); 1.60 (br.s, 3H, H-9′). ^{13}C NMR ($CDCl_3$, 62.9 MHz) §: 16.20 (q, C-10′), 17.79 (q, C-9′), 25.77 (q, C-8′), 26.52 (t, C-5′), 27.17 (t, C-1′), 39.72 (t, C-4′), 61.20 (q, OMe), 61.30 (q, OMe), 117.78 (d, C-2′), 123.98 (d, C-6′), 130.45 (d, C-6), 131.95 (s, C-7′), 140.17 (s, C-3′), 144.91 (s, C-2 or C-3), 145.16 (s, C-3 or C-2), 146.92 (s, C-5), 184.38 (s, C-4 or C-1), 184.54 (s, C-1 or C-4).

2,3-Dimethoxy-5-(3′,7′-dimethyl-octa-2′(Z),6′-dienyl)-[1,4]benzoquinone (2): yellow oil, HREIMS *m/z* 304.1662 $[M]^+$, calcd for $C_{18}H_{24}O_4$ 304.1675, ^{1}H NMR ($CDCl_3$, 250MHz) §: 6.37 (t, J=1.7, 1H, H-6); 5.13 (m, 1H, H-2′); 5.07 (m, 1H, H-6′); 4.02 (s, 3H, OMe); 4.00 (s, 3H, OMe); 3.11 (br. d, J=7.1, 1.2, 2H, H-1′); 2.04 (m, 2H, H-5′); 2.04 (m, 2H, H-4′); 1.75 (q, J=1.2, 3H, H-10′); 1.66 (br. d, J=1.2, 3H, H-8′); 1.59 (d, J=1.2, 3H, H-9′). ^{13}C NMR ($CDCl_3$, 62.9 MHz) §: 17.79 (q, C-9′), 22.76 (q, C-10′), 25.77 (q, C-8′), 26.52 (t, C-5′), 27.27 (t, C-1′), 32.01 (t, C-4′), 61.20 (q, OMe), 61.30 (q, OMe), 117.78 (d, C-2′), 123.98 (d, C-6′), 130.45 (d, C-6), 131.95 (s, C-7′), 140.17 (s, C-3′), 144.91 (s, C-2 or C-3), 145.16 (s, C-3 or C-2), 146.92 (s, C-5), 184.38 (s, C-4 or C-1), 184.54 (s, C-1 or C-4).

2-Methoxy-3-(3′,7′-dimethyl-octa-2′,6′-dienyl)-[1,4]benzoquinone (3): yellow oil, HREIMS *m/z* 274.1558 $[M]^+$, calcd for $C_{17}H_{22}O_3$ 274.1569, ^{1}H-NMR (250 MHz, $CDCl_3$) § 6.68 (d, J=10.0, 1H, H-5); 6.59 (d, J=10.0, 1H, H-6); 5.05 (m, 2H, H-2′, H-6′); 4.02 (s, 3H, OMe); 3.15 (br. d, J=7.3, 2H, H-1′); 2.01 (m, 4H, H-4′, H-5′); 1.73 (br. s, 3H, Me); 1.65 (br. s, 3H, Me); 1.58 (br. s, 3H, Me).

2-Methoxy-6-(3′,7′-Dimethyl-octa-2′,6′-dienyl)-[1,4]benzoquinone (4): yellow oil, HREIMS *m/z* 274.1576 $[M]^+$, calcd for $C_{17}H_{22}O_3$ 274.1569, ^{1}H-NMR (250 MHz, $CDCl_3$) § 6.45 (q, J=2.1, 1H, H-5); 5.87 (d, J=2.4, 1H, H-6); 5.15 (m, 1H, H-2′); 5.08 (m, 1H, H-6′); 3.82 (s, 3H, OMe); 3.14 (br. d, J=7.3, 2H, H-1′); 2.07 (m, 4H, H-4′, H-5′); 1.70 (br. s, 3H, Me); 1.63 (br. s, 3H, Me); 1.60 (br. s, 3H, Me).

2-Methoxy-5-(3′,7′-dimethyl-octa-2′,6′-dienyl)-[1,4]benzoquinone (5): yellow crystals, HREIMS *m/z* 274.1582 $[M]^+$, calcd for $C_{17}H_{22}O_3$ 274.1569, ^{1}H-NMR (250 MHz, $CDCl_3$) § 6.46 (t, J=1.7, 1H, H-6), 5.92 (s, 1H, H-3), 5.15 (m, 1H, H-2′), 5.08 (m, 1H, H-6′), 3.82 (s, 3H, OMe), 3.14 (br. d, J=7.3, 2H, H-1′), 2.08 (m, 4H, H-4′, H-5′), 1.70 (br. s, 3H, Me), 1.62 (br. s, 3H, Me), 1.60 (br. s, 3H, Me).

2-(3′,7′-Dimethyl-octa-2′,6′-dienyl)-[1,4]benzoquinone (6): yellow oil, HREIMS *m/z* 244.1454 $[M]^+$, calcd for $C_{16}H_{20}O_2$, 244.1463, ^{1}H-NMR (250 MHz, $CDCl_3$) § 6.77 (d, J=10.2, 1H, H-6); 6.69 (dd, J=10.2, 2.3, 1H, H-5); 6.53 (q, J=1.9, 1H, H-3); 5.15 (m, 1H, H-2′); 5.07 (m, 1H, H-6′); 3.13 (br. d, J=7.5, 2H, H-1′); 2.08 (m, 4H, H-4′, H-5′); 1.69 (br. s, 3H, Me); 1.62 (br. s, 3H, Me); 1.60 (br. s, 3H, Me).

2,3-Dimethoxy-5-(3′,7′,11′-trimethyl-dodeca-2′,6,′10′-trienyl)-[1,4]benzoquinone (7): yellow oil, HREIMS *m/z* 372.2316 $[M]^+$, calcd for $C_{23}H_{32}O_4$ 372.2300, ^{1}H-NMR (250 MHz, $CDCl_3$) § 6.34 (t, J=1.8, 1H, H-6); 5.10 (m, 3H, H-2′, H-6′, H-10′); 4.02 (s, 3H, OMe); 4.00 (s, 3H, OMe); 3.11 (br. d, J=7.3, 2H, H-1′); 2.05 (m, 8H, H-4′, H-5′, H-8′, H-9′); 1.68 (br. s, 3H, Me); 1.62 (br. s, 3H, Me); 1.60 (br. s, 6H, 2Me).

2-Methoxy-6-(3′,7′,11′-trimethyl-dodeca-2′,6′,10′-trienyl)-[1,4]benzoquinone (8): yellow oil, HREIMS *m/z* 342.2182 $[M]^+$, calcd for $C_{22}H_{30}O_3$ 342.2195, ^{1}H-NMR (250 MHz, $CDCl_3$) § 6.45 (q, J=2.0, 1H, H-5); 5.87 (d, J=2.4, 1H, H-3); 5.15 (m, 1H, H-2′); 5.10 (m, 2H, H-6′, H-10′); 3.81 (s, 3H, OMe); 3.14(br. d, J=7.3, 2H, H-1′); 2.06 (m, 8H, H-4′, H-5′, H-8′, H-9′); 1.67 (br. s, 3H, Me); 1.63 (br. s, 3H, Me); 1.60 (br. s, 6H, 2Me).

2-Methoxy-5-(3′,7′,11′-trimethyl-dodeca-2′,6′,10′-trienyl)-[1,4]benzoquinone (9): yellow crystals, HREIMS *m/z* 342.2172 $[M]^+$, calcd for $C_{22}H_{30}O_3$ 342.2195, ^{1}H-NMR (250 MHz, $CDCl_3$) § 6.47 (t, J=1.7, 1H, H-6); 5.93 (s, 1H, H-3); 5.16 (m, 1H, H-2′); 5.10 (m, 2H, H-6′, H-10′); 3.82 (s, 3H, OMe); 3.14(br. d, J=7.3, 2H, H-1′); 2.05 (m, 8H, H-4′, H-5′, H-8′, H-9′); 1.68 (br. s, 3H, Me); 1.62 (br. s, 3H, Me); 1.60 (br. s, 6H, 2Me).

2-Methoxy-3-(3′,7′,11′-trimethyl-dodeca-2′,6′,10′-trienyl)-[1,4]benzoquinone (10): yellow oil, HREIMS *m/z* 342.2212 $[M]^+$, calcd for $C_{22}H_{30}O_3$ 342.2195, ^{1}H-NMR (250 MHz, $CDCl_3$) § 6.68 (d, J=10.0, 1H, H-6); 6.57 (d, J=10.0, 1H, H-5); 5.07 (m, 3H, H-2′, H-6′, H-10′); 4.02 (s, 3H, OMe); 3.16(br. d, J=7.3, 2H, H-1′); 2.01 (m, 8H, H-4′, H-5′, H-8′, H-9′); 1.73 (br. s, 3H, Me); 1.67 (br. s, 3H, Me); 1.60 (br. s, 3H, Me); 1.57 (br. s, 3H, Me).

2,3-Dimethoxy-5-(3′,7′,11′-trimethyl-dodeca-2′,6′,10′-trienyl)-benzene-1,4-diol (11): yellow oil, HREIMS *m/z* 374.2472 $[M]^+$, calcd for $C_{23}H_{34}O_4$ 374.2457, ^{1}H-NMR (250 MHz, $CDCl_3$) § 6.49 (s, 1H, H-6); 5.31 (s, 1H, OH); 5.30 (m, 1H, H-2′); 5.17 (s, 1H, OH); 5.12 (m, 2H, H-6′, H-10′); 3.91 (s, 3H, OMe); 3.88 (s, 3H, OMe); 3.28(br. d, J=7.6, 2H, H-1′); 2.05 (m, 8H, H-4′, H-5′, H-8′, H-9′); 1.70 (br. s, 3H, Me); 1.68 (br. s, 3H, Me); 1.60 (br. s, 6H, 2Me).

2,3-Dimethoxy-5,6-bis-(3′,7′-dimethyl-octa-2′,6′-dienyl)-[1,4]benzoquinone (12): yellow oil, HREIMS *m/z* 440.2944 $[M]^+$, calcd for $C_{28}H_{40}O_4$, 440.2927, 1H-NMR (250 MHz, $CDCl_3$) § 5.04 (m, 2H, H-2′, H-2″); 4.94 (m, 2H, H-6′, H-6″); 3.99 (s, 6H, 2OMe); 3.19(br. d, J=6.8, 2H, H-1′, H-1″); 2.00 (m, 8H, H-4′, -5′, H-4″, H-5″); 1.73 (br. s, 6H, 2Me); 1.66 (br. s, 6H, 2Me); 1.58 (br. s, 6H, 2Me).

2-Methoxy-5,6-bis-(3′,7′,11′-trimethyl-dodeca-2′,6′,10′-trienyl)-[1,4]benzoquinone (13): yellow oil, HREIMS *m/z* 546.4048 $[M]^+$, calcd for $C_{37}H_{54}O_3$, 546.4073, 1H-NMR (250 MHz, $CDCl_3$) § 5.87 (s, 1H, H-3); 5.00 (m, 6H, H-2′, H-6′, H-10′, H-2″, H-6″, H-10″); 3.79 (s, 3H, OMe); 3.22(br. d, J=6.8, 4H, H-1′, H-1″); 2.01 (m, 16H, H-4′, H-5′, H-8′, H-9′, H-4″, H-5″, H-8″, H-9″); 1.73 (m, 3H, Me); 1.67 (m, 9H, 3Me); 1.60 (m, 12H, 4Me).

2-Methoxy-3,5-bis-(3′,7′,11′-trimethyl-dodeca-2′,6′,10′-trienyl)-[1,4]benzoquinone (14): yellow oil, HREIMS *m/z* 546.4052 $[M]^+$, calcd for $C_{37}H_{54}O_3$, 546.4073, 1H-NMR (250 MHz, $CDCl_3$) § 6.33 (t, J=1.2, 1H, H-6); 5.09 (m, 6H, H-2′, H-6′, H-10′, H-2″, H-6″, H-10″); 4.00 (s, 3H, OMe); 3.14(m, 4H, H-1′, H-1″); 2.04 (m, 16H, H-4′, H-5′, H-8′, H-9′, H-4″, H-5″, H-8″, H-9″); 1.74 (m, 3H, Me); 1.68 (m, 9H, 3Me); 1.60 (m, 12H, 4Me).

2,3,4-Trimethoxy-6-(3′,7′-dimethyl-octa-2′,6′-dienyl)-phenol (20): pale yellow oil, HREIMS *m/z* 320.1974 $[M]^+$, calcd for $C_{19}H_{28}O_4$ 320.1987, IR (CCl_4): 3541, 2935, 1498, 1464 cm^{-1}. ^{1}H NMR ($CDCl_3$, 250MHz) §: 6.44 (s, 1H, H-5), 5.45 (s, 1H, OH), 5.31 (m, 1H, H-2′), 5.11 (m, 1H, H-6′) 3.95 (s, 3H, OMe), 3.86 (s, 6H, OMe), 3.79 (s, 3H, OMe), 3.31 (br.d, J=7.1, 2H, H-1′), 2.07 (m, 4H, H-4′, H-5′), 1.72 (d, J=1.2, 3H, Me), 1.67 (d, J=1.2, 3H, Me), 1.60 (d, J=0.7, 3H, Me). ^{13}C NMR ($CDCl_3$, 62.9 MHz) §: 16.12 (q, C-10′), 17.66 (q, C-9′), 25.66 (q, C-8′), 26.73 (t, C-5′), 27.90 (t, C-1′), 39.75 (t, C-4′), 56.62 (q, OMe), 60.89 (q, OMe), 61.16 (q, OMe), 108.30 (d, C-5), 121.61 (s, C-6), 121.98 (d, C-2′ or C-6′), 124.20 (d, C-6′ or C-2′), 128.89 (s, C-1), 131.41 (s, C-7′), 136.59 (s, C-4), 140.04 (s, C-3′), 140.81 (s, C-3 or C-2), 146.14 (s, C-2 or C-3).

2,3,4-Trimethoxy-6-(3′,7′,11′-trimethyl-dodeca-2′,6′,10′-trienyl)-phenol (21): pale yellow oil, HREIMS *m/z* 388.2648 $[M]^+$, calcd for $C_{24}H_{36}O_4$ 388.2635, ^{1}H-NMR (250 MHz, $CDCl_3$) § 6.44 (s, 1H, H-5); 5.45 (s, 1H, OH); 5.32 (br. t, J=7.3, 1H, H-2′); 5.12 (m, 2H, H-6′, H-10′); 3.95 (s, 3H, OMe); 3.87 (s, 3H, OMe); 3.79 (s, 3H, OMe); 3.31(br. d, J=7.3, 2H, H-1′); 2.06 (m, 8H, H-4′, H-5′, H-8′, H-9′); 1.72 (br. s, 3H, Me); 1.67 (br. s, 3H, Me); 1.60 (br. s, 6H, 2Me).

3,4-Dimethoxy-6-(3′,7′-dimethyl-octa-2′,6′-dienyl)-phenol (23): pale yellow oil, HREIMS *m/z* 290.1876 $[M]^+$, calcd for $C_{18}H_{26}O_3$ 290.1882, ^{1}H-NMR (250 MHz, $CDCl_3$) § 6.73 (s, 1H, H-2); 6.49 (s, 1H, H-5); 5.27 (br t, J=7.3, 1H, H-2′); 5.15 (s, 1H, OH); 5.10 (m, 1H, H-6′); 3.87 (s, 3H, OMe); 3.79 (s, 3H, OMe); 3.24(br. d, J=7.3, 2H, H-1′); 2.05 (m, 4H, H-4′, H-5′); 1.68 (br. s, 6H, 2Me); 1.60 (br. s, 3H, Me).

2,4-Dimethoxy-6-(3′,7′-dimethyl-octa-2′,6′-dienyl)-phenol (28): pale yellow oil, HREIMS *m/z* 290.1898 $[M]^+$, calcd for $C_{18}H_{26}O_3$ 290.1882, ^{1}H-NMR (250 MHz, $CDCl_3$) § 6.36 (d, J=2.7, 1H, H-3 or H-5); 6.31 (d, J=2.7, 1H, H-3 or H-5); 5.33 (br. t, J=7.1, 1H, H-2′); 5.26 (s, 1H, OH); 5.11 (br. t, J= 6.6, 1H, H-6′); 3.86 (s, 3H, OMe); 3.75 (s, 3H, OMe); 3.35 (br. d, J=7.3, 2H, H-1′); 2.08 (m, 4H, H-4′, H-5′); 1.72 (br. s, 3H, Me); 1.67 (br. s, 3H, Me); 1.60 (br. s, 3H, Me).

Cell Viability Assay

JB6 P^+ Cl41 cells were cultured overnight in 96-well plates (6,000 cells/well) using 5% FBS-MEM. Then the medium was replaced with 0.1% FBS-MEM containing the quinones at different concentrations in a volume of 0.1 ml and the cells were incubated with the quinone solutions for 22 hrs. Then 20 μl of the MTS reagent was added into each well. The MTS reduction was measured 2 hrs later spectrophotometrically at 492 nm and at 690 nm as a background using the Multiskan MS microplate reader (Labsystems, Finland). For each compound, two independent experiments with five samples for each concentration were performed.

Anchorage-Independent Assay

The cancer preventive effects of the quinones were evaluated in 6-well plates using JB6 P^+ Cl41 cells, activated with EGF (10 ng/ml) or TPA (20 ng/ml). JB6 P^+ Cl41 cells (8×10^3/ml) were treated with the indicated concentrations of the quinones in 1 ml of 0.33% BME (basal medium Eagle) agar containing 10% FBS over 3.5 ml of 0.5% BME agar containing 10% FBS and indicated concentrations of the quinones. The cultures were maintained in a 37°C, 5% CO_2 incubator for 1 wk (JB6 P^+ Cl41 cells, activated with EGF) or 2 weeks (JB6 P^+ Cl41 cells, activated with TPA). Cell colonies were then scored using the LEICA DM IRB inverted research microscope (Leica Mikroskopie und Systeme GmbH, Germany) and Image-Pro Plus software, version 3.0 for Windows (Media Cybernetics, Silver Spring, MD). For each compound, two independent experiments in triplicate for each concentration were performed.

Apoptosis Assay Using Flow Cytometry

JB6 P^+ Cl41 cells (3×10^5 cells/dish) were grown in 6-cm dishes for 24 hrs in 5% FBS-MEM. Then cells were treated with different concentrations of the compounds dissolved in 0.1% FBS-medium for 3 hrs. Then the medium was removed and attached cells were harvested with 0.025% trypsin in 0.1% EDTA in PBS. Trypsinization was stopped by adding 2 ml of 5% FBS in PBS. Cells were then washed by centrifugation at 1,000 rpm (170 rcf) for 5 min and processed for detection of apoptosis using Annexin V- FITC and propidium iodide staining according to the manufacturer's protocol. In brief, $1–5\times10^5$ cells were collected after centrifugation, and resuspended in 500 μl of 1x binding buffer (Annexin V-FITC Apoptosis Detection Kit, Medical & Biological Laboratories (Watertown, MA)). Then, 5 μl of Annexin V-FITC and 5 μl of propidium iodide were added and the cells were incubated at room temperature for 5 min in the dark and analyzed by flow cytometry. For each compound, two independent experiments in duplicate for each concentration were performed.

Apoptosis Assay Using DNA Ladder Method

JB6 Cl 41 cells were grown in 10-cm dishes and treated with 3-demethylubiquinone Q2 (**1**) when cells were 80% confluent. The cells were incubated with quinone **1** for 24 h. Then, both detached and attached cells were harvested by scraping followed by centrifugation. The obtained cells were disrupted with lysis buffer (5 mM Tris-HCl, pH 8.0, 20 mM EDTA, and 0.5% Triton X-100) and left on ice for 45 min. After centrifugation at 14,000 rpm (45 min, 4° C), the supernatant fraction containing fragmented DNA was extracted twice with phenol/chloroform/isopropyl alcohol (25:24:1, v/v) and once with chloroform. Then the fragmented

DNA was precipitated overnight at -20°C after addition of two volumes of 100% ethanol and 1/10 volume of 5 M NaCl. The DNA pellet was saved by centrifugation at 14,000 rpm for 45 min, washed once with 70% ethanol, dried, and resuspended in TE buffer (10 mM Tris-HCl, 1mM EDTA, pH 8.0). After addition of 100 μg/ml RNAse A (Sigma), the mixture was incubated at 37°C for 2 h. The DNA fragments were separated by 1.8% agarose gel electrophoresis. DNA laddering in the gel was stained with ethidium bromide and photographed under UV light. Two independent experiments were performed.

The Effect of Quinones 1 and 3 Through 14 on p53-, AP-1- and NF-κB-Dependent Transcriptional Activities

The ability of quinones **1** and **3** through **14** to influence AP-1-, NF-κB- and p53-dependent transcriptional activities in the mouse JB6 Cl41 cell line was evaluated using the luciferase method. Viable JB6-LucPG-13, JB6-LucAP-1, or JB6-LucNF-κB cells (6×10^3) suspended in 100 μl 5% FBS-MEM were added into each well of a 96-well plate. Plates were incubated for 24 h and then treated with various concentrations of quinones in 100 μl of 0.1% FBS-MEM. After incubation with quinones for 24 h, the cells were extracted for 1 h at room temperature with 100 μl/well of lysis buffer (0.1 M potassium phosphate buffer at pH 7.8, 1% Triton X-100, 1 mM DTT, 2 mM EDTA). Then 30 μl of lysate from each well were transferred in a plate used for luminescent analysis and luciferase activity was measured using 100 μl/well of the luciferase assay buffer (1 mM D-luciferase, pH = 6.1 – 6.5; 40 mM Tricin, 2.14 mM magnesium carbonate $(MgCO_3)_4 \times Mg(OH)_2 \times 5H_2O$, 5.34 mM $MgSO_4 \times 7H_2O$, 66.6 mM DTT, 1.06 mM ATP, 0.54 mM COA, 0.2 mM EDTA, pH = 7.8) and the Luminoscan Ascent Type 392 microplate reader (Labsystems, Finland). For each compound, two independent experiments with five samples for each concentration were performed.

Statistics

The statistical computer program, Statistica 6.0 for Windows (StatSoft, Inc., 2001) was used for analysis of the obtained data. Nonparametric Mann-Whitney U Test was used to compare two independent groups of data. Nonparametric Spearman Rank Order Correlations method was used to compute single nonparametric correlations. Method of regressions was used to compute IC_{50} or $INCC_{50}$ in corresponding experiments.

RESULTS

Syntheses of Polyprenylquinones 1 Through 14

Syntheses of polyprenylquinones **1** through **14** were carried out in accordance with Scheme 1. The first stage yielded prenylated phenols through the alkylation of corresponding available methoxyphenols by *trans*-geraniol (**18**) or *trans*-farnesol (**19**) in the presence of boric trifluoride etherate as an acidic catalyst as was described earlier (27). Under these conditions, total yields of the alkylation products were about 60%. The second stage, the oxidative demethylation of obtained fractions with cerium-ammonium nitrate (CAN), yielded mixtures of the corresponding quinones, which were further separated to obtain individual compounds. The third step, reduction of the obtained quinones with $Na_2S_2O_4$, was used only in one case and yielded the corresponding prenylated hydroquinone (**11**).

In particular, prenylquinones **1**, **2**, **7**, **11** and **12** were synthesized from 2,3,4- trimethoxyphenol (**15**, Scheme 2:A), prenylquinones **3**, **5**, **9**, **10** and **14** from 3,4-dimethoxyphenol (**16**, Scheme 2:B), and prenylquinones **4**, **8** and **13** from 2,4-dimethoxyphenol (**17**, Scheme 2:C). The previously known 2-(3′,7′-dimethyl-octa-2′,6′-dienyl)-1,4-benzoquinone (**6) (**not shown on scheme) was synthesized as earlier described (27).

Target 2′-3′-*trans*-prenylquinones **1** and **3**–**14** contained less than 6% of the corresponding 2′-3′-*cis*- isomers as impurities. The formation of these isomers may be explained by the isomerization of geraniol and farnesol or corresponding products of alkylation at the action of boric trifluoride etherate at the stage of prenylation of phenols. As a rule, impurities of *cis*-isomers were not separated from the major products (*trans*-isomers) except for the mixture of **1** and **2**, which was separated by HPLC. To the best of our knowledge, among all the above-mentioned polyprenylquinones, only compounds **1, 6, 7** and **8** were described previously (22,27–29). Intermediate prenylated phenols **20**, **21**, **23** and **28** were obtained as individual substances. The structures of the target prenylquinones **3** – **14** as well as the intermediate prenylphenols **20**, **21**, **23** and **28**, were established using NMR spectroscopy in comparison with spectra of **1** and **2** (26).

3-Demethylubiquinone Q2 and Synthetic Polyprenylquinones 3 Through 14 Inhibit Malignant JB6 P$^+$ Cl41 Cell Transformation

Both natural and synthetic compounds **1** and **3** – **14** were assayed for cancer preventive activity using the anchorage-independent JB6 P+ Cl41 cell transformation assay in a soft agar. Based on our previous experience (30–39), inhibition of cell transformation is a good indication that a compound will have an effective cancer preventive activity. Toxicity of each compound for JB6 Cl41 cells was determined by the MTS cell viability assay. For one of the quinones, natural 3-demethylubiquinone Q2 A (**1**), the corresponding data are shown in Fig. 1 and 2. Using the obtained data and statistical computer program Statistica 6.0, the corresponding regressions were built and the IC_{50} for decreased cell viability and the $INCC_{50}$ (Inhibition of the Number of the Colonies C_{50}) for inhibition of cell transformation were determined for each quinone studied. These data are summarized in Table 1. The obtained results indicated that all quinones studied inhibited cell transformation induced by EGF or TPA in dose-dependent manner in JB6 Cl41 cells. For some of the quinones, the dose that inhibited malignant transformation by 50% was below that which was toxic (Table 1). For example, for 3-demethylubiquinone Q2 the corresponding doses were 7.3 and 11.4 μM. To understand the possible inhibitory signalling pathway activated by the quinones studied, we then investigated whether the JB6 cells were undergoing apoptosis induced by quinones.

The Quinones Induce Apoptosis in JB6 Cl41 Cells

The ability of the quinone compounds to induce apoptosis was determined by flow cytometry. The results indicated that quinones **1** and **3** – **14** induced apoptosis in JB6 Cl41 cells in a dose-dependent manner (Fig. 3). For the natural 3-demethylubiquinone Q2 A (**1**), apoptosis was also demonstrated by DNA laddering in JB6 Cl41 cells (Fig. 4).

Polyprenylquinones 1 and 3 Through 14 Inhibit p53 and Induce AP-1 or NF-κB Transcriptional Activity

Several key transcription factors, including the p53 tumor suppressor protein, AP-1 or NF-κB are often implicated in the induction or inhibition of apoptosis by various stimuli, including chemopreventive compounds or drugs. Therefore, we then studied the effect of quinones **1** and **3** – **14** on these three transcription factors. JB6 Cl41 cell lines stably expressing a luciferase reporter gene controlled by an AP-1, NF-κB, or p53 DNA binding sequence were used. To study the effect of the substances on the nuclear factors-dependent transcriptional activity we used broad range of the concentrations. In the Table 2 we showed only maximal significances of the corresponding induction or inhibition. These significances were achieved at the concentrations of the substances correlated well with their IC_{50} for the corresponding cells. Quinones **1** and **3** – **14** showed a significant (up to an 8-fold) induction of AP-1 or NF-κB-dependent transcriptional activation, and a substantial (up to 4 fold) inhibition of p53-dependent transcriptional activity (see Table 2).

Structure-Activity Relationships for Polyprenylquinones 1 and 3 Through 14

One of the major goals of present investigation was to establish the SARs for the quinones studied. The SARs of quinones **1** and **3** – **14** were studied using statistical analysis (Statistica 6.0). The quinone compounds **1** and **3** – **14** were divided into three groups according to the number of isoprene units included in their terpenoid parts (see Table 1). Group 1 consists of quinones **1** and **3** – **6**, which have two isoprene units (10 carbon atoms) in their side chains. Group 2, quinones **7** – **11** contain three isoprene units (15 carbon atoms) in their terpenoid parts and group 3, quinones **12** – **14,** have four to six isoprene units (20 to 30 carbon atoms) in their side chains. Significant differences and correlations between the data regarding the biological activities obtained for different structural groups of the quinones were determined using the nonparametric Spearman correlation method and the Mann-Whitney U Test and the data in Table 1.

Our results indicated that the biological activity of these quinones depends on the length of the terpenoid side chains in the molecule. Statistical analysis of data from Table 1 indicates that quinones in group 1 averaged an IC_{50} of 20.0 ± 15.2 μM for toxicity in JB6 Cl41 cells. Quinones in group 2 had an average IC_{50} of 9.7 ± 9.0 μM. Finally, the quinones in group 3 showed an average IC_{50} of 84.9 ± 63.8 μM. These results suggest that quinones **7** – **11** (group 2) with three isoprene units in their terpenoid portion are on the whole more toxic against JB6 cells than the quinones in group 1 (quinones **1** and **3**–**6**), which have only two isoprene units in their side chains. The opposite conclusion can be drawn when comparing the IC_{50} of quinones in groups 1 and 3 or for those in groups 2 and 3. The quinones of groups 1 or 2 are significantly more toxic for JB6 Cl41 cells than those of group 3. Therefore, we conclude that when the length of the terpenoid portion increases as from group 1 to group 2 the toxicity of the quinones of this series also increases; but it decreases dramatically by further increasing of the length of the terpenoid portion as from group 2 to group 3.

In the statistical analysis of the effect of these quinones on EGF-induced cell transformation in JB6 Cl41 cells, we found that the quinones in group 1 displayed an average $INCC_{50}$ of 9.4 ± 5.3 μM and the quinones in group 2 averaged an $INCC_{50}$ of 24.0 ± 16.7 μM. Group 3 again possessed minimal activity among all three groups of compounds with an average $INCC_{50}$ of 59.7 ± 33.3 μM (Table 1). Therefore, based on this analysis and the results of the Mann-Whitney U Test, group 1 quinones had the most potent effect on inhibition of cell transformation ($p = 0.0283$ vs. group 2; $p = 0.0253$ vs. group 3; $p = 0.0084$ vs. groups 2 and 3) and group 3 was the least effective. A significant correlation was observed between the length of the terpenoid portion and $INCC_{50}$ ($p = 0.0002$, $R = 0.8556$). These results indicate that when the length of the terpenoid portion increases, the $INCC_{50}$ values for cell transformation also increase.

We then compared toxicity with cell transformation in JB6 P^+ Cl41 cells and found that the quinones in group 1 displayed an average $INCC_{50}$ (inhibition of cell transformation) 1.4 to 4 times less than the IC_{50} (toxicity) for the corresponding cells (Table 1). On the other hand, the majority of quinones in group 2 showed an $INCC_{50}$ (inhibition of cell transformation) at doses 4 to 10 times higher than the IC_{50} values (toxicity) (Table 1). Based on these data, we can conclude that quinones of group 1 are distinctly more toxic to transformed JB6 cells than to normal JB6 cells. In contrast, the quinones in group 2 are more toxic to normal JB6 cells than to those that have been transformed. Therefore, the quinones in group 1 have more potential in respect to cancer preventive activity than the quinones from group 2.

We further showed that the activity of these quinones depends on the position of the methoxy group relative to the terpenoid part. We selected several pairs of structurally similar quinones, which have the methoxy groups in the same position. These pairs are as follows: 1) *orto*-analogues, quinones **3** and **10**; 2) *meta*-analogues, quinones **4** and **8**; and 3) *para*-analogues, quinones **5** and **9**. Based on the data from Tables 1 and 2, we conclude that the cancer preventive

activity and the effect of quinones on AP-1 transcriptional activity, increased in the line of *orto* → *meta* → *para.* The $INCC_{50}$ had following values: for quinones **3**, **10:** 15.1 and 24.6 μM, respectively; quinones **4, 8:** 6.6 and 16.7 μM, respectively; and quinones **5, 9:** 3.1 and 7.4 μM, respectively.

Induction of AP-1 transcriptional activity by the *orto* compounds **3, 10** averaged 133.8% of control and by the *meta* derivatives **4, 8,** the average was 187.7% of control. The *para* derivatives **5** and **9** had the highest induction of AP-1 activation at 486.9% of control. The *orto*-disubstituted quinones **3** and **10** are the least active compounds not only in the induction of AP-1 transcriptional activity, but also in the inhibition of cell transformation compared to the *meta*- and *para*- analogues. Among the *para*–disubstituted derivatives, quinone **5** having two isoprene units in the side chain showed better activities compared with quinone **9** having three isoprene units in the side chain. Indeed, the *para*-disubstituted quinones **5** and **9** showed an $INCC_{50}$ of 3.1 and 7.4 μM, respectively, against EGF-induced JB6 P^+ Cl41 cell transformation. Quinone **5** also demonstrated a higher induction of AP-1 transcriptional activity (721.7%) compared to quinone **9** (252.2%).

DISCUSSION

In spite of the fact that polyprenylated 1,4-benzoquinones and hydroquinones are very common in nature, to the best of our knowledge, only one related marine metabolite, 2-(3-methylbuten-2-yl)-[1,4] hydroquinone (20), was earlier studied for its cancer protective properties. To establish the cancer preventive activity of this metabolite, the modified Ames assay for mutagenicity of benzo(a)-pyrene, aflatoxin B1, or UV against *Salmonella typhimurium* was used (40). However, biological tests examining transformation of animal cells after treatment with tumor promoters were not used in this study. For the first time in the present work, the cancer preventive properties for a large group of the newly synthesized and earlier known polyprenylated benzoquinones were studied using mouse epithelial JB6 P^+ Cl41 cells and MEFs.

In our study the quinones having two isoprene units in the side chain showed specific effects against the malignantly transformed JB6 Cl41 cells compared to normal cells. The active doses differed up to 4-fold.

We established structure-activity relationships for the quinones studied in respect to cytotoxic or cancer preventive properties. Our present study indicated that cytoxicity of quinones increased with the number of carbon atoms from quinones having two prenyl units in their side chain to their analogues having three prenyl units and then decreased for compounds with 4 to 6 isoprene units. The observed SARs do not fully correspond to that earlier established for related non-methoxylated quinones. A series of non-methoxylated prenylated quinones with side chains containing from one to eight prenyl units was synthesized by an Italian research group and studied along with related marine natural compounds (27). Resembling our study, the toxicity of these compounds in brine shrimp and fish lethality assays was shown to first increase and then again to decrease with the length of the terpenoid part. But distinct from our study, quinones containing two isoprene units in the terpenoid portion were reported to be the most toxic among all studied compounds (27). No one has previously established the SARs for any series of prenylquinone compounds in respect to cancer preventive activity. Our study showed that cancer preventive activity decreased when the polyprenyl side chain became longer. The most active cancer preventive polyprenylquinones, among those studied herein, have a side chain containing two isoprene units.

No previous evidence exists in the literature, which shows that any polyprenyl quinone induces apoptosis in any cell line. Using flow cytometry and the DNA laddering method, we showed

that quinones **1** and **3** – **14** induced apoptosis in JB6 Cl41 cells and MEFs. The tumor suppressor protein, p53, which is a part of the cell's emergency team and functions to negatively regulate cell growth following DNA damage, is often involved in apoptosis induced by various stimuli including chemopreventive agents and drugs (41–45). However, in our study quinones **1** and **3** – **14** did not activate p53 but instead, most of the quinones studied demonstrated significant inhibition of p53 dependent transcriptional activity. In addition, these compounds induced a substantial activation of AP-1 or NF-κB-dependent transcriptional activities (Table 2). The AP-1 transcription factor regulates a variety of cellular processes, including proliferation, differentiation, apoptosis and has been considered primarily to be an oncogene.(33,46, 47)[36–38] Recently, some of the AP-1 proteins, such as Jun-B and c-Fos, were shown to have tumor-suppressor activity both *in vitro* and *in vivo* (48,49). Activation of another AP-1 protein, c-Jun, is required for induction of Fas L-mediated apoptosis in PC12 and human leukemia HL-60 cells (50,51). Activation of both AP-1 and NF-κB nuclear factors is necessary for DNA damaging agents- and ceramide-induced apoptosis in T lymphocytes and Jurkat T cells (52, 53). The balance between AP-1 family members, c-Jun and ATF-2 governs the choice between differentiation and apoptosis in PC12 cells (54). Anticancer drugs, such as vinblastine, which inhibit microtubules, activate AP-1 in human KB-3 carcinoma cells. This activation is required for efficient apoptosis induced by these drugs (55,56). NF-κB, a member of a family of highly regulated dimeric transcription factors, is involved in the activation of a large number of genes that respond to infections, inflammation, and other stressful situations. NF-κB is reported to be involved in both induction and inhibition of apoptosis (52,53,57–59). Our study therefore suggests that apoptosis induced by quinones **1** and **3** – **14** occurs independently of p53 activation but instead may be related to the induction of AP-1 and NF-κB transcriptional activity.

CONCLUSION

Our results show that methoxylated polyprenylquinones and their synthetic analogues represent a new prospective group of marine secondary metabolites as cancer preventive compounds. They show also cytotoxic properties and induce apoptosis of JB6 P^+ Cl41 cells and MEFs. The most active of these compounds are potent inducers of AP-1- and NF-κB-activation and, at the same time, inhibitors of p53 transcriptional activities.

In our study, therefore, the cancer preventive effects of quinones **1** and **3** through **14** (Tables 1) may be explained by the induction of p53-independent apoptosis.

We also found that quinones having a side chain of 10-carbon atom length showed specificity in the inhibitory effect for transformed JB6 P+ Cl41 cells in contrast to quinones with 15 or 20 to 30 carbon atoms in the side chain. We conclude therefore that a further search for cancer preventive agents may be promising among diprenylated analogues of the compounds studied. Taking into consideration that these compounds were active against transformation of the epithelial JB6 cells, we suggest that some quinones studied may be used themselves or as models for development of promising skin cancer preventive agents.

Taking into account the data from Table 1, and SAR based on the position of the methoxy-groups, we also conclude that quinone **5,** which has a diprenylated side chain in the *para* position relative to the methoxy-group, is the most potent among all the quinones studied in respect to cancer preventive effect.

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References

1. Herskind C, Fleckenstein K, Lohr J, Li CY, Wenz F, Lohr F. Antitumoral action of interferons and interleukins in combination with radiotherapy. Part I: immunologic basis. Strahlenther Onkol 2004;180:187–93. [PubMed: 15057428]
2. Rogozin EA, Urazova LN, Serebrov VYu. Influence of vaccines of Venezuelan Equine Encephalitis, influenza and mumps viruses on morphological pattern and vitality of human tumor cells in vitro. Journal of BUON 2003;8:39–43. [PubMed: 17415867]
3. Hersey P. Evaluation of vaccinia viral lysates as therapeutic vaccines in the treatment of melanoma. Ann N Y Acad Sci 1993;690:167–77. [PubMed: 8368735]
4. Davis ID, Jefford M, Parente P, Cebon J. Rational approaches to human cancer immunotherapy. J Leukoc Biol 2003;73:3–29. [PubMed: 12525559]
5. Rinehart KL. Antitumor compounds from tunicates. Med Res Rev 2000;20:1–27. [PubMed: 10608919]
6. Haefner B. Drugs from the deep: marine natural products as drug candidates. Drug Discov Today 2003;8:536–44. [PubMed: 12821301]
7. Mayerand AM, Lehmann VK. Marine pharmacology in 1999: antitumor and cytotoxic compounds. Anticancer Res 2001;21:2489–500. [PubMed: 11724312]
8. R. H. Thomson. Naturally Occurring Quinones, Academic Press, London, 1971.
9. J. F. Pennock. The chemistry of isoprenoid quinones. In J. B. Pridham (ed), Terpenoids in plants (J. B. Pridham, ed), Academic Press, London, 1967, pp. 129–146.
10. Faulkner DJ. Marine natural products. Nat Prod Rep 1986;3:1–33. [PubMed: 2872636]
11. Ochi M, Kotsuki H, Inoue S, Taniguchi M, Tokoroyama T. Isolation of 2-(3,7,11-trimethyl-2,6,10-dodecatrienyl) hydroquinone from the brown seaweed *Dictyopteris unulata*. Chem Lett 1979;31–832
12. Capon RJ, Ghisalberti EL, Jefferies PR. Isoprenoid dihydroquinones from a brown alga, *Cystophora sp.* Phytochemistry 1981;20:2598–2600.
13. Gerwick WH, Fenical W. Ichthyotoxic and cytotoxic metabolites of the tropical brown alga *Stypopodium zonale* (Lamouroux) Papenfuss. J Org Chem 1981;46:22–27.
14. W. Fenical. Diterpenoids. In P. J. Scheuer (ed), Marine Natural Products, Chemical and Biological Perspectives, Vol. 2 (P. J. Scheuer, ed), Academic Press, New York, 1978, pp. 238–245.
15. L. Minale. Terpenoids from marine sponges. In P. J. Scheuer (ed), Marine Natural Products, Chemical and Biological Perspectives, Vol. 1 (P. J. Scheuer, ed), Academic Press, New York, 1978, pp. 220–240.
16. De Rosa S, Crispino A, De Giulio A, Iodice C, Milone A. Sulfated polyprenylhydroquinones from the sponge *Ircinia spinulosa*. J Nat Prod 1995;58:1450–1454.
17. Bifulco G, Bruno I, Minale L, Riccio R. Bioactive prenylhydroquinone sulfates and a novel, C_{31} furanoterpene alcohol sulfate from the marine sponge, *Ircinia sp.* J Nat Prod 1995;58:1444–1449.
18. Bowden BF, Coll JC. Studies of Australian soft corals. XXVI tetraprenylbenzoquinone derivatives from a *Nephthea* species of soft coral (Octocorallia, Alcyonacea). Aust J Chem 1981;34:2677–2681.
19. Ravi BN, Wells RJ. Lipid and terpenoid metabolites of the gorgonian *Plexaura flava.* Aust J Chem 1982;35:105–112.
20. Howard BM, Clarkson K, Bernstein RL. Simple prenylated hydroquinone derivatives from the marine urochordate *Aplidium californicum*. Tetrahedron Lett 1979;20:4449–4452.
21. Targettand NM, Keeran WS. A terpenehydroquinone from the marine ascidian Aplidium constellatum. J Nat Prod 1984;47:556–7. [PubMed: 6548254]
22. Guella G, Mancini I, Pietra F. Verapliquinones: novel diprenylquinones from an *Aplidium* sp. (Ascidiacea) of the Ile-Verte waters, Brittany. Helv Chim Acta 1987;70:621–626.
23. Faulkner DJ. Marine natural products. Nat Prod Rep 1993;10:497–539. [PubMed: 8295744]
24. Fu X, Bilayet Hossain M, van der Helm D, Schmitz F. Longithorone A: unprecedented dimeric prenylated quinone from the tunicate *Aplidium longithorax*. J Am Chem Soc 1994;116:12125–12126.
25. Fu X, Bilayet Hossain M, Schmitz F, van der Helm D. Longithorones, unique prenylated para- and metacyclopropane type quinones from the tunicate *Aplidium longithorax*. J Org Chem 1997;62:3810–3819.

26. Shubina LK, Fedorov SN, Radchenko OS, Balaneva NN, Kolexnikova SA, Dmitrenok PS, Bode AM, Dong Z, Stonik VA. Desmethylubiquinone Q_2 from the Far-eastern ascidian *Aplidium glabrum:* structure and synthesis. Tetrahedron Lett 2005;46:559–562.
27. De Rosa S, De Giulio A, Iodice C. Biological effects of prenylated hydroquinones: structure-activity relationship studies in antimicrobial, brine shrimp, and fish lethality assays. J Nat Prod 1994;57:1711–6. [PubMed: 7714539]
28. Misiti D, Moore HW, Folkers K. Identification of plastoquionone-2 from chloroplasts. J Am Chem Soc 1965;87:1402–1403.
29. Sakamoto K, Miyoshi H, Takegami K, Mogi T, Anraku Y, Iwamura H. Probing substrate binding site of the Escherichia coli quinol oxidases using synthetic ubiquinol analogues. J Biol Chem 1996;271:29897–902. [PubMed: 8939932]
30. Colburn NH, Former BF, Nelson KA, Yuspa SH. Tumour promoter induces anchorage independence irreversibly. Nature 1979;281:589–91. [PubMed: 492322]
31. Colburn NH, Wendel EJ, Abruzzo G. Dissociation of mitogenesis and late-stage promotion of tumor cell phenotype by phorbol esters: mitogen-resistant variants are sensitive to promotion. Proc Natl Acad Sci U S A 1981;78:6912–6. [PubMed: 6947266]
32. Dong Z, Cmarik JL, Wendel EJ, Colburn NH. Differential transformation efficiency but not AP-1 induction under anchorage-dependent and -independent conditions. Carcinogenesis 1994;15:1001–4. [PubMed: 8200060]
33. Dong Z, Birrer MJ, Watts RG, Matrisian LM, Colburn NH. Blocking of tumor promoter-induced AP-1 activity inhibits induced transformation in JB6 mouse epidermal cells. Proc Natl Acad Sci U S A 1994;91:609–13. [PubMed: 8290571]
34. Dong Z, Watts SG, Sun Y, Colburn NH. Progressive elevation of AP-1 activity during preneoplastic-to neoplastic progression as modeled in mouse JB6 cell variants. Int J Oncol 1995;7:359–364.
35. Dong Z, Lavrovsky V, Colburn NH. Transformation reversion induced in JB6 RT101 cells by AP-1 inhibitors. Carcinogenesis 1995;16:749–56. [PubMed: 7728951]
36. Dongand Z, Cmarik JL. Harvesting cells under anchorage-independent cell transformation conditions for biochemical analyses. Sci STKE 2002;2002:PL7. [PubMed: 11983939]
37. Lavrovsky V, Dong Z, Ma WY, Colburn N. Drug-induced reversion of progression phenotype is accompanied by reversion of AP-1 phenotype in JB6 cells. In Vitro Cell Dev Biol Anim 1996;32:234–7. [PubMed: 8727048]
38. Strickland J, Sun Y, Dong Z, Colburn NH. Grafting assay distinguishes promotion sensitive from promotion resistant JB6 cells. Carcinogenesis 1997;18:1135–8. [PubMed: 9214594]
39. Sun Y, Nakamura K, Hegamyer G, Dong Z, Colburn N. No point mutation of Ha-ras or p53 genes expressed in preneoplastic-to- neoplastic progression as modeled in mouse JB6 cell variants. Mol Carcinog 1993;8:49–57. [PubMed: 8352891]
40. Ames BN, McCann J, Yamasaki E. Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. Mutat Res 1975;31:347–64. [PubMed: 768755]
41. Fedorov SN, Bode AM, Stonik VA, Gorshkova IA, Schmid PC, Radchenko OS, Berdyshev EV, Dong Z. Marine alkaloid polycarpine and its synthetic derivative dimethylpolycarpine induce apoptosis in JB6 cells through p53- and caspase 3-dependent pathways. Pharm Res 2004;21:2307–19. [PubMed: 15648263]
42. Huang C, Ma WY, Goranson A, Dong Z. Resveratrol suppresses cell transformation and induces apoptosis through a p53-dependent pathway. Carcinogenesis 1999;20:237–42. [PubMed: 10069459]
43. Nomura M, Kaji A, Ma W, Miyamoto K, Dong Z. Suppression of cell transformation and induction of apoptosis by caffeic acid phenethyl ester. Mol Carcinog 2001;31:83–9. [PubMed: 11429785]
44. Huang C, Ma WY, Li J, Hecht SS, Dong Z. Essential role of p53 in phenethyl isothiocyanate-induced apoptosis. Cancer Res 1998;58:4102–6. [PubMed: 9751619]
45. He Z, Ma WY, Hashimoto T, Bode AM, Yang CS, Dong Z. Induction of apoptosis by caffeine is mediated by the p53, Bax, and caspase 3 pathways. Cancer Res 2003;63:4396–401. [PubMed: 12907610]
46. Bernsteinand LR, Colburn NH. AP1/jun function is differentially induced in promotion-sensitive and resistant JB6 cells. Science 1989;244:566–9. [PubMed: 2541502]

47. Young MR, Li JJ, Rincon M, Flavell RA, Sathyanarayana BK, Hunziker R, Colburn N. Transgenic mice demonstrate AP-1 (activator protein-1) transactivation is required for tumor promotion. Proc Natl Acad Sci U S A 1999;96:9827–32. [PubMed: 10449779]

48. Chiu R, Angel P, Karin M. Jun-B differs in its biological properties from, and is a negative regulator of, c-Jun. Cell 1989;59:979–86. [PubMed: 2513128]

49. Passegue E, Jochum W, Schorpp-Kistner M, Mohle-Steinlein U, Wagner EF. Chronic myeloid leukemia with increased granulocyte progenitors in mice lacking junB expression in the myeloid lineage. Cell 2001;104:21–32. [PubMed: 11163237]

50. Le-Niculescu H, Bonfoco E, Kasuya Y, Claret FX, Green DR, Karin M. Withdrawal of survival factors results in activation of the JNK pathway in neuronal cells leading to Fas ligand induction and cell death. Mol Cell Biol 1999;19:751–63. [PubMed: 9858598]

51. Kondo T, Matsuda T, Kitano T, Takahashi A, Tashima M, Ishikura H, Umehara H, Domae N, Uchiyama T, Okazaki T. Role of c-jun expression increased by heat shock- and ceramide-activated caspase-3 in HL-60 cell apoptosis. Possible involvement of ceramide in heat shock-induced apoptosis. J Biol Chem 2000;275:7668–76. [PubMed: 10713077]

52. Kasibhatla S, Brunner T, Genestier L, Echeverri F, Mahboubi A, Green DR. DNA damaging agents induce expression of Fas ligand and subsequent apoptosis in T lymphocytes via the activation of NF-kappa B and AP-1. Mol Cell 1998;1:543–51. [PubMed: 9660938]

53. Manna SK, Sah NK, Aggarwal BB. Protein tyrosine kinase p56lck is required for ceramide-induced but not tumor necrosis factor-induced activation of NF-kappa B, AP-1, JNK, and apoptosis. J Biol Chem 2000;275:13297–306. [PubMed: 10788436]

54. Leppa S, Eriksson M, Saffrich R, Ansorge W, Bohmann D. Complex functions of AP-1 transcription factors in differentiation and survival of PC12 cells. Mol Cell Biol 2001;21:4369–78. [PubMed: 11390664]

55. Berry A, Goodwin M, Moran CL, Chambers TC. AP-1 activation and altered AP-1 composition in association with increased phosphorylation and expression of specific Jun and Fos family proteins induced by vinblastine in KB-3 cells. Biochem Pharmacol 2001;62:581–91. [PubMed: 11585055]

56. Fan M, Goodwin ME, Birrer MJ, Chambers TC. The c-Jun NH(2)-terminal protein kinase/AP-1 pathway is required for efficient apoptosis induced by vinblastine. Cancer Res 2001;61:4450–8. [PubMed: 11389075]

57. Bessho R, Matsubara K, Kubota M, Kuwakado K, Hirota H, Wakazono Y, Lin YW, Okuda A, Kawai M, Nishikomori R, et al. Pyrrolidine dithiocarbamate, a potent inhibitor of nuclear factor kappa B (NF-kappa B) activation, prevents apoptosis in human promyelocytic leukemia HL-60 cells and thymocytes. Biochem Pharmacol 1994;48:1883–9. [PubMed: 7986199]

58. Garcia-Bermejo L, Perez C, Vilaboa NE, de Blas E, Aller P. cAMP increasing agents attenuate the generation of apoptosis by etoposide in promonocytic leukemia cells. J Cell Sci 1998;111(Pt 5):637–44. [PubMed: 9454737]

59. Begand AA, Baltimore D. An essential role for NF-kappaB in preventing TNF-alpha-induced cell death. Science 1996;274:782–4. [PubMed: 8864118]

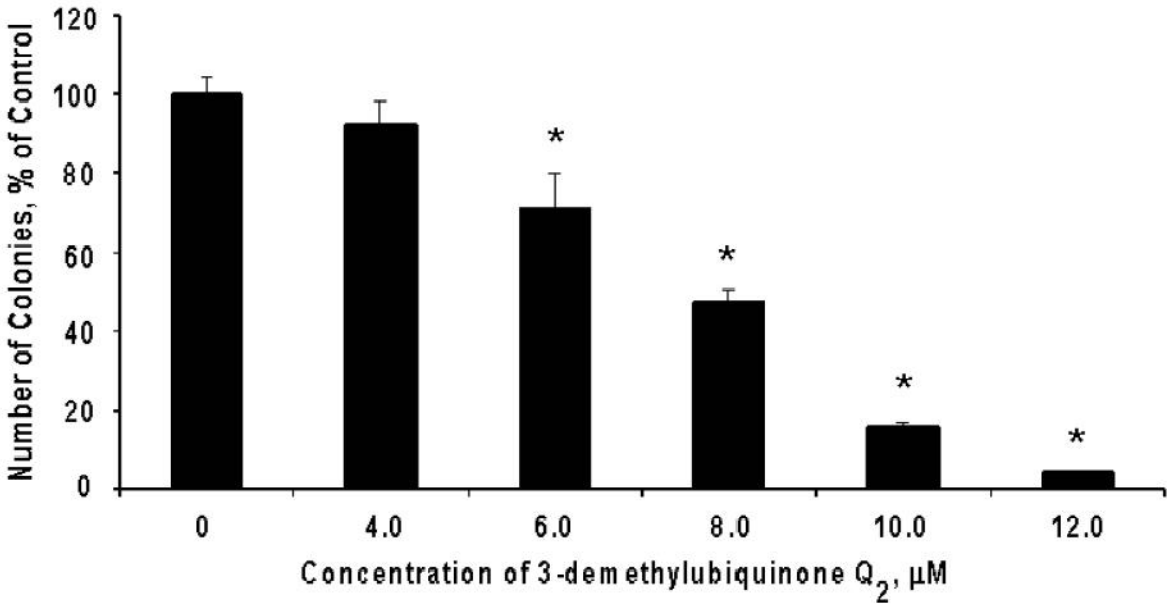


Fig. 1.

The inhibition of EGF-induced JB6 P^+ Cl41 cell transformation by demethylubiquinone Q2 (**1**) in soft agar (anchorage-independent assay). JB6 P+ Cl41 cells (8×10^3/ml in 6-well plates), were activated with EGF (10 ng/ml), treated with the indicated concentrations of quinone (**1**), maintained for 1 week, and cell colonies were then scored as described in "Materials and Methods". Data represent the percentage of EGF-activated, quinone (**1**)-treated cell colonies compared to percentage of EGF-activated, untreated cells. Each *bar* represents the mean ± SD from six samples of two independent experiments. *, indicates a significant inhibition by quinone (**1**) ($p < 0.05$) compared to EGF-activated untreated control.

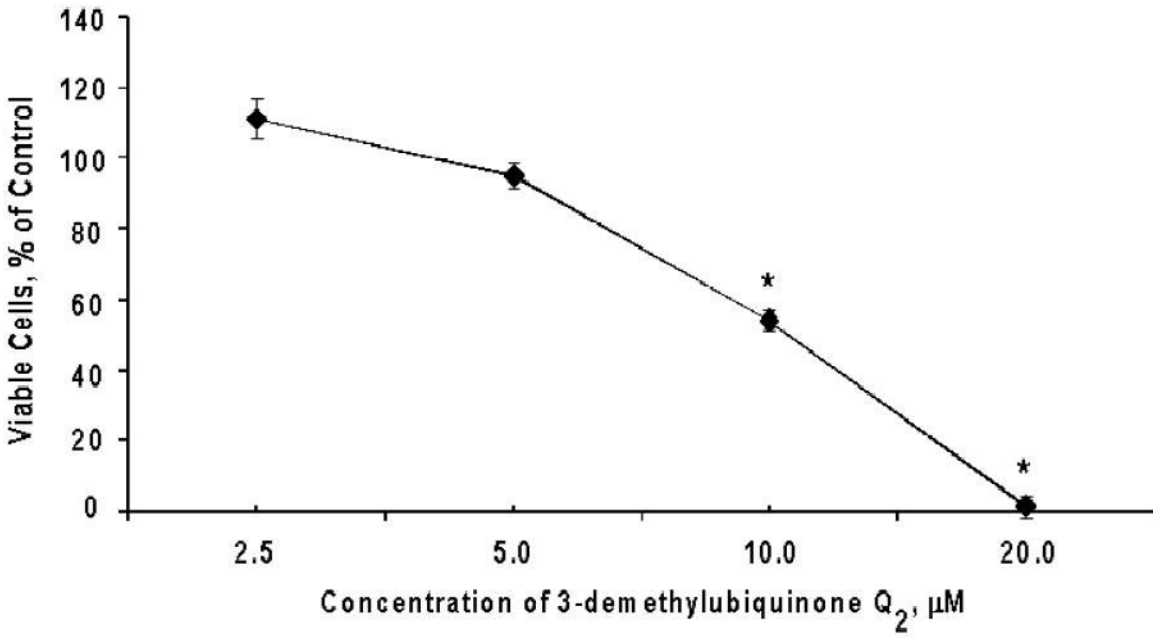


Fig. 2.

The effect of demethylubiquinone Q2 (**1**) on JB6 P^+ Cl41 cell viability. The cells were cultured in 96-well plates, as described in "Materials and Methods". Then, the medium was replaced with 0.1% FBS-MEM containing the indicated concentrations of quinone (**1**). The cells were incubated with the quinone (**1**) for 22 h. The MTS reagent was then added and its reduction was measured spectrophotometrically 2 h later. Data represent the percentage of quinone (**1**)-treated viable cells compared to percentage of untreated control cells. Each data point represents the mean ± SD from ten samples of two independent experiments. *, indicates a significant decrease in viability induced by quinone (**1**) ($p < 0.05$) compared to untreated control cells.

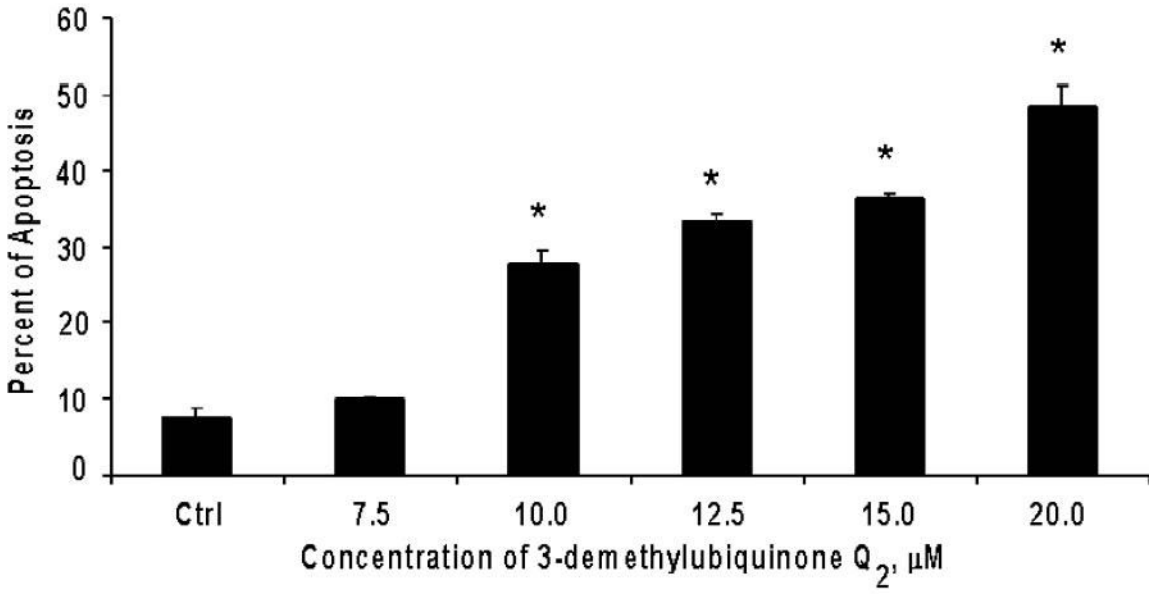


Fig. 3.

The induction of apoptosis by demethylubiquinone Q2 (1) in JB6 P^+ Cl41 cells measured by flow cytometry. The cells (3×10^5/dish) were grown in 6-cm dishes and treated with the indicated concentrations of quinone (1) as described in "Materials and Methods". Cells were harvested and processed for detection of apoptosis using Annexin V- FITC and propidium iodide staining according to the manufacturer's protocol. Each *bar* represents the mean ± SD from four samples of two independent experiments. *, indicates a significant increase in apoptosis by quinone (1) ($p < 0.05$) compared to untreated control cells.

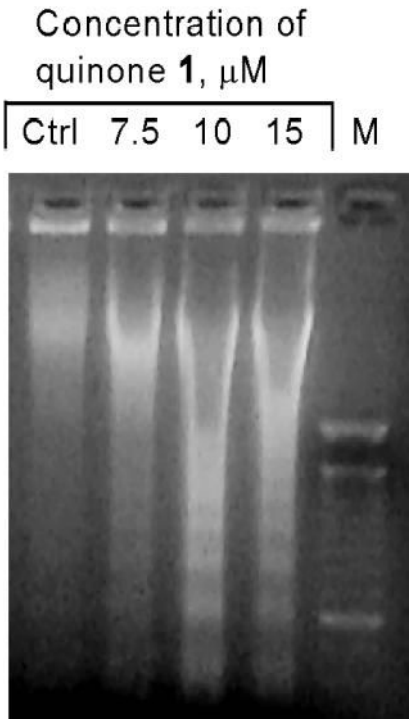
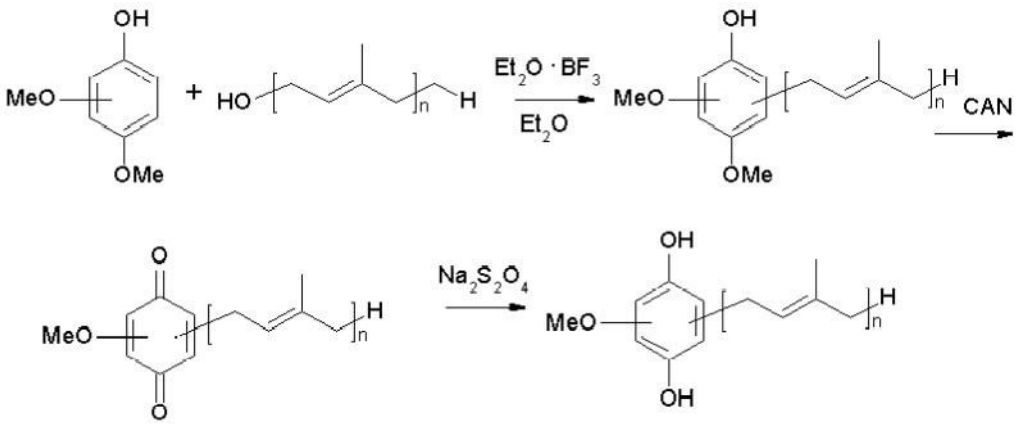


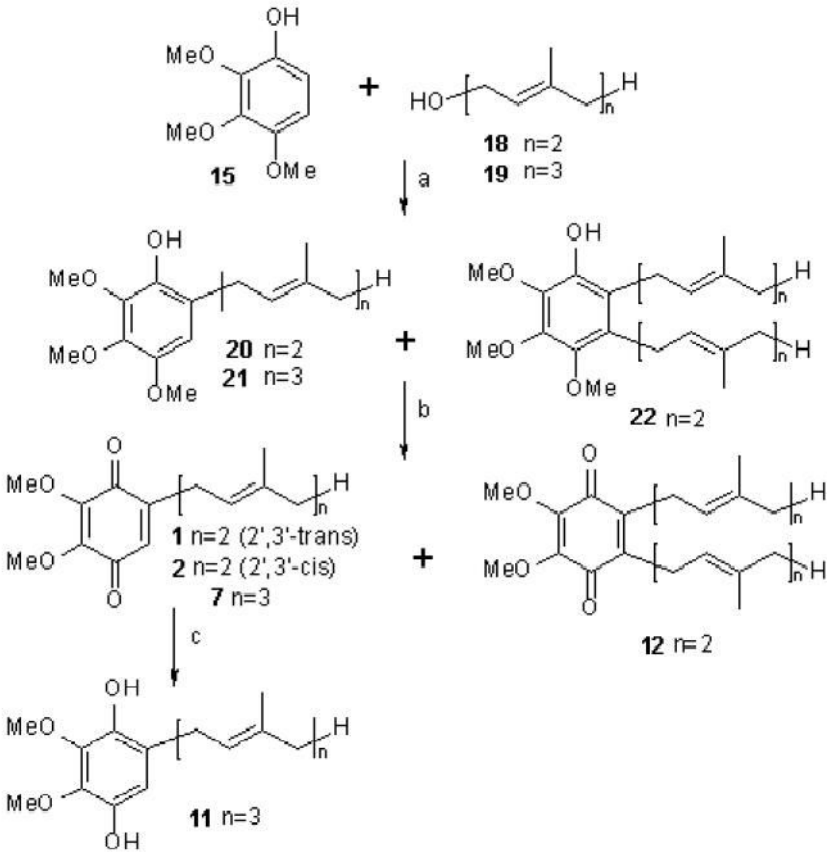
Fig. 4.

The induction of apoptosis by demethylubiquinone Q2 (**1**) in JB6 Cl 41 cells determined by the method of DNA-laddering. JB6 Cl41 cells were grown in 10-cm dishes, treated with the indicated concentrations of quinone (**1**) for 24 h, and harvested as described in “Materials and Methods”. The isolated DNA fragments were separated by 1.8% agarose gel electrophoresis. DNA laddering in the gel was stained with ethidium bromide and photographed under UV light. A representative experiment is shown.

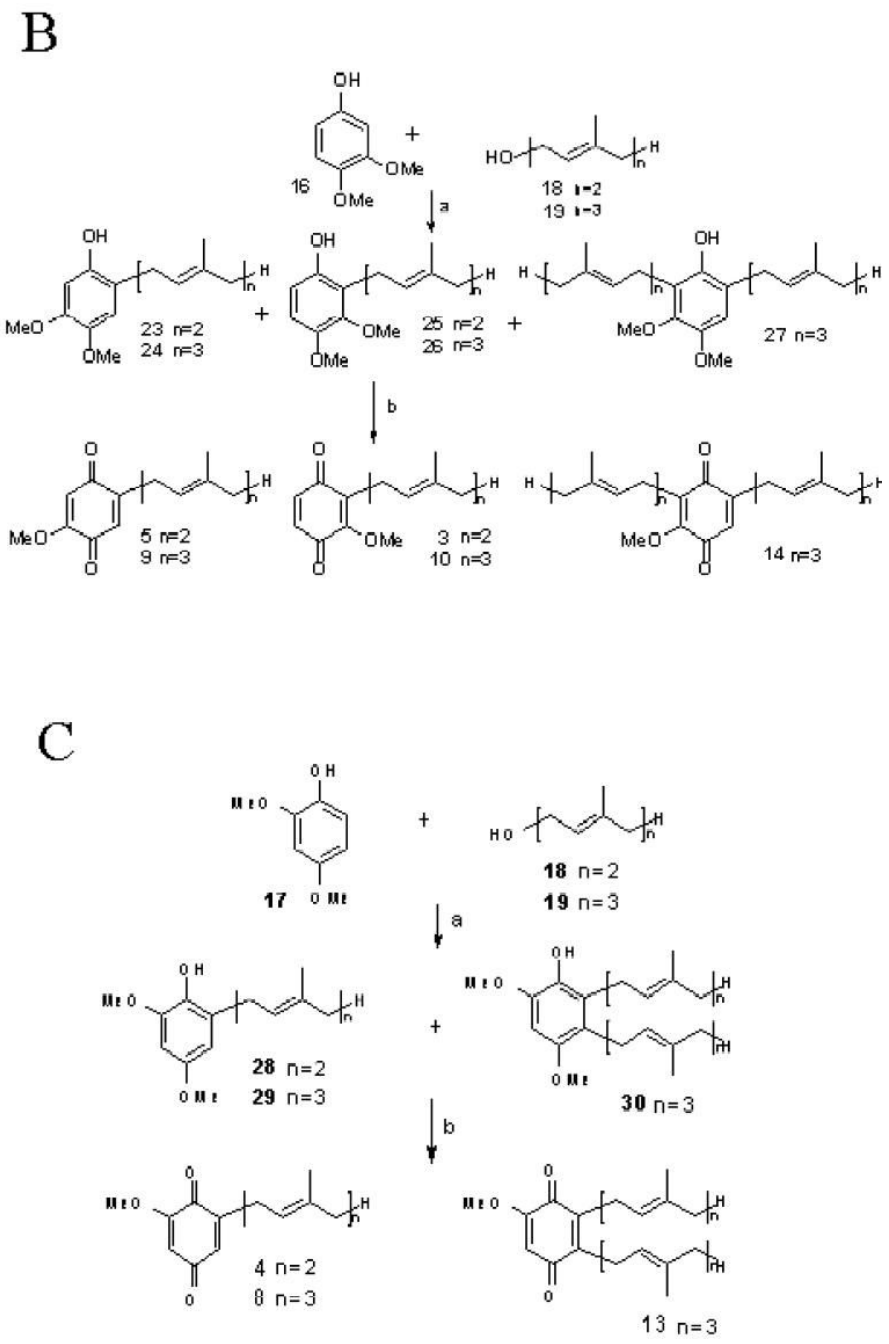


Scheme 1.
The general scheme of synthesis of quinones **1** through **14**.

A



a: $Et_2O\ BF_3$, Et_2O, r.t., 12 h; b: CAN, MeCN-H_2O, 0°C,1.5 h;
c: $Na_2S_2O_4$, acetone-H_2O, r.t., 1h



Scheme 2.
Syntheses of quinones **1**, **2**, **7**, **11**, **12** (A); **3**, **5**, **9**, **10**, **14** (B); **4**, **8**, **13** (C).

Table 1

IC_{50} and $INCC_{50}$ of the quinones **1**, **3 – 14** for JB6 Cl41 P^+ cells.

Compounds	IC_{50}, μM	$INCC_{50}$, μM
Structural Group 1		
1. Quinone **1**	11.4	7.3
2. Quinone **3**	45.1	15.1
3. Quinone **4**	8.3	6.6
4. Quinone **5**	11.8	3.1
5. Quinone **6**	23.4	14.7
Structural Group 2		
6. Quinone **7**	5.1	19.4
7. Quinone **8**	4.7	16.7
8. Quinone **9**	8.6	7.4
9. Quinone **10**	25.5	24.6
10. Quinone **11**	4.6	51.7
Structural Group 3		
11. Quinone **12**	>140*	29.2
12. Quinone **13**	> 15*	54.7
13. Quinone **14**	99.6	95.3

*, in all calculations the designated numbers were used.

Table 2

The effect of the quinones **1**, **3 – 14** on AP-1-, NF-κB-, and p53-dependent transcriptional activity in JB6 Cl41 cells.

Compounds	AP-1-dependent transcriptional activity, % of untreated control	NF-κB-dependent transcriptional activity, % of untreated control	p53-dependent transcriptional activity, % of untreated control
Structural Group 1			
Quinone **1**	190.3	216.9	71.7
Quinone **3**	159.8	194.8	55.8
Quinone **4**	293.3	197.9	36.3
Quinone **5**	721.7	201.2	31.8
Quinone **6**	880.7	421.7	47.0
Structural Group 2			
Quinone **7**	125.0	99.2	24.6
Quinone **8**	97.2	138.8	57.9
Quinone **9**	252.2	223.0	26.9
Quinone **10**	107.7	179.3	31.5
Quinone **11**	84.0	403.5	22.8
Structural Group 3			
Quinone **12**	139.5	225.7	*
Quinone **13**	*	*	*
Quinone **14**	198.5	549.7	36.5

*, significant differences were not determined.