Supplementary Material (1) to "Pharmacological Inhibition of ABCA1 Degradation Increases HDL Biogenesis and Exhibits Antiatherogenesis" by Arakawa et al.

Experimental Procedures for Synthesis and Characterization of Spiroquinone and Diphenoquinone

General Methods

Melting points were determined using a micro hot plate melting point apparatus (YAZAWA Co., Ltd., Tokyo, Japan) without correction. Elemental analysis for C and H was performed using a Vario EL analysis apparatus (Elementar Co., Ltd.). Proton NMR (¹H NMR) spectra were recorded on a JEOL EX-270 spectrometer (JEOL Co., Ltd.) using tetramethylsilane as the internal standard. Mass spectra were obtained in a JEOL JMS-T100GC mass spectrometer system (JEOL Co., Ltd.).

Synthesis of Spiroquinone

A mixture of probucol (250 g), manganese (IV) oxide (125 g) and hexane (400 mL) was vigorously stirred under a light protecting condition at room temperature for 17 hours. The reaction mixture was filtered and the residue was washed with dichloromethane (700 mL). The filtrate was concentrated under reduced pressure. Methanol (400 ml) was added to the residue and concentrated under reduced pressure again. The resulting precipitate was collected, washed with methanol (200 mL X 6) and dried under reduced pressure. Spiroquinone was obtained as yellow crystals, 240.3 g (97 %) of mp 156-158 °C. The results of the mass analysis; for the calculated value $C_{31}H_{46}O_2S_2$: C, 72.32; H, 9.01,the found value was: C, 72.3; H, 9.0.; ¹H NMR (CDCl₃)δ: 1.20 (36H, s), 2.01 (6H, s), 6.88 (4H, s).; MS m/z: 515 (M+1), 473 (M-C₃H₆), 441 (M-C₃H₆S+1), 409 (M-C₃H₆S₂+1), 279 (M-C₁₄H₂₀OS+1), 237 (C₁₄H₂₀OS+1). The purity was verified by HPLC operated in the conditions below. Detection; ultraviolet absorption at 243 nm. Column; Capcell Pak C8 (3.5 X 75 mm). Temperature; 40 °C. Mobile phase; H₂O (0.1% TFA)-CH₃CN (14 : 86). Flow rate; 0.5 mL/min. Retention time of spiroquinone; 12.0 min. (probucol; 6.1 min.). Purity; > 98 % (as peak area %). Stability of spiroquinone was examined as follows being estimated as the HPLC citeria; photostability

testing (2500 Lux X hr) for 480 hours and preservation test in a dark place (40 °C/75 % RH) for 4 weeks, as powdered ingredients above, and stability in a methanolic solution at room temperature (in a light-resistant container and a transparent container) and in a suspension of coconut oil (in a light-resistant container). The results are summarized in the table below. The residual ratio was expressed in percent of the initial value of peak area. Spiroquinone showed a low photo-stability and very stable under light protecting conditions.

State of sample	Powdered ingredients		Methanolic solution		Suspension of coconut oil
Testing condition	2500Lux X hr, RT, 480 hours	40°C /75%RH, 4 weeks, dark place	RT, 24 hours, light-resistant container	RT, 24 hours, transparent container	RT, 24 hours, light-resistant container
Residual ratio of spiroquinone	96.8%	100.2%	100.7%	81.5%	98.8%

Synthesis of diphenoquinone

$$O_2$$
, KOHaq.

 O_3 , KOHaq.

 O_3 , KOHaq.

 O_4 , KOHaq.

 O_5 , KOHaq.

 O_5 , KOHaq.

 O_7 , KOHaq.

 O_8 , KOHaq.

 O_8 , CC(CH₃)₃
 O_8 , CC(CH₃)₃

Diphenoquinone was prepared by the method previously reported (M. S. Kharasch and B. S. Joshi, *J. Org. Chem.*, **22**, 1439, 1957), being describes as follows. To a solution of 2,6-di-*tert*-butylphenol (45.3 g) in *tert*-butyl alcohol (500 mL), potassium hydroxide (44 g, in 3 mL water) was added and the mixture was stirred in an oxygen atmosphere for 4 hours. The reaction mixture was diluted with water and the precipitate was collected, washed with water, and dried under reduced pressure. Diphenoquinone, 40.1 g, was obtained at red-brown plates; ¹H NMR (CDCl₃) δ: 1.37 (36H, s), 7.71 (4H, s). The compound was analyzed by HPLC operated in the conditions as below. Detection; ultraviolet absorption at 420 nm. Column; Capcell Pak C8 (3.5 X 75 mm). Column temperature; 40 °C. Mobile phase; H₂O (0.1% TFA)-CH₃CN (14: 86). Flow rate; 0.5 mL/min. Retention time of diphenoquinone; 9.4 min. Purity of the compound was 89 % (as peak area %). Stability of diphenoquinone was determined in the suspension of coconut oil in a light-resistant container at room temperature for 24 hours, and the residual ratio was 105.1%.