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4,4′-Dihydroxy-*trans*-stilbene, a resveratrol analogue, exhibited enhanced antioxidant activity and cytotoxicity

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ABSTRACT

Resveratrol (3,5,4′-*trans*-trihydroxystibene) is a natural phytoalexin present in grapes and red wine, which possesses a variety of biological activities including antioxidant activity. In order to find more active antioxidant with resveratrol as the lead compound we synthesized 4,4′-dihydroxy-*trans*-stilbene (4,4′-DHS). The antioxidant activities of resveratrol and 4,4′-DHS were evaluated by the reaction kinetics with galvinoxyl radical or Cu(II) ions, and the inhibition effects against free-radical-induced peroxidation of human erythrocyte ghosts. It was found that 4,4′-DHS exhibits remarkably higher antioxidant activity than resveratrol. The oxidative products of resveratrol and 4,4′-DHS in the presence of Cu(II) in acetonitrile were identified as the dihydrofuran dimers by spectroscopic method, and the antioxidant mechanism for 4,4′-DHS was proposed. In addition, 4,4′-DHS exhibits remarkably higher cytotoxicity against human promyelocytic leukemia (HL-60) cells than resveratrol.

1. Introduction

Polyphenols are abundant micronutrients in our diet, and evidence for their role in the prevention of degenerative diseases such as cancer and cardiovascular diseases is emerging. In particular resveratrol (3,5,4′-trihydroxy-*trans*-stilbene), a polyphenol mainly present in grapes and red wine, has been proposed to be the main protagonist for the so-called 'French paradox'.[1] This phenomenon describes the situation that the French were consuming more calories, and more fatty food, than westerners, and experiencing far less heart disease. This natural compound has attracted considerable interest because of its biological activities against a collection of diseases, including heart disease, neurodegenerative disease and cancer.[2,3] One of the most striking biological activities of resveratrol intensely investigated during the last years has been its cancer chemopreventive property.[4] The chemopreventive activity and low-toxicity of resveratrol, associated with its defects such as low bioavailability and rapid clearance from the circulation,[5] offer the promise for designing new and resveratrol-directed chemopreventive agents. The cancer chemoprevention effects elicited by resveratrol can be traced back to its antioxidant activity, because free-radical-mediated peroxidation of membrane lipids and oxidative damage of DNA might play a causative role in cancer.[6] In the antioxidant reaction of resveratrol, the hydrogen abstraction from 4′-OH is more favorable than that from 3-OH or 5-OH as evidenced by its oxidation product.[7] It was also proved by theoretical calculations that the hydroxyl groups at the 4′-position is much easier to subject to oxidation than other hydroxyl groups.[8] The experimental X-ray structure results showed that the 4′-OH is particularly active and the 4′ position relevant to the subsequent biological activity.[9,10] It was reported previously that the 4′-OH in resveratrol is responsible for its biological activities.[11–13] Therefore, we were motivated to synthesize 4,4′-dihydroxy-*trans*-stilbene (4,4′-DHS) by the Wittig–Hornor reaction,[14] which has two hydroxyl group at 4- and 4′-positions, and found the compound is more effective antioxidant[15] and prooxidant[16] than resveratrol.

It is also desirable to see if the same structure-activity relationship (SAR) is also valid in using other radicals and systems, with emphasis placed on the detail mechanism study of SAR. We report herein an in vitro study on the reaction kinetics of 4,4′-DHS and resveratrol (Fig. 1) with galvinoxyl radical (GO·) or Cu(II) ions,

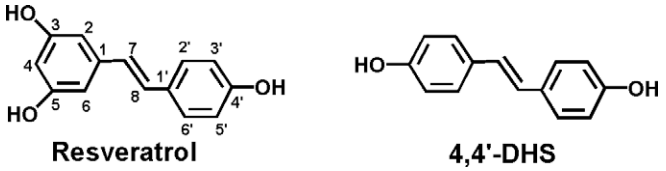

Figure 1. Molecular structures of resveratrol and 4,4′-DHS.

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and the antioxidant effects of them against free-radical-induced peroxidation of human erythrocyte ghosts. The oxidation products of 4,4′-DHS and resveratrol obtained in the presence of Cu(II) ions were also studied to help elucidate the antioxidant mechanism. The cytotoxic effects of 4,4′-DHS and resveratrol for human promyelocytic leukemia (HL-60) cells were also assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method. It was found that 4,4′-DHS exhibited remarkably higher antioxidant activity and cytotoxicity against HL-60 cells than resveratrol.

2. Results and discussion

2.1. Galvinoxyl radical-scavenging reactions of resveratrol and 4,4′-DHS in ethanol

The stable oxygen radical galvinoxyl (GO·) is widely used to evaluate the antioxidant properties of natural and synthetic phenols using alcohols as the most convenient solvents. Upon addition of resveratrol to an ethanol solution of GO·, the absorption band at 428 nm due to GO· disappeared immediately as shown in Figure 2. The decay of GO· was significantly faster in the presence of 4,4′-DHS than resveratrol (the inset of Fig. 2). The decay of GO· obeyed pseudo-first-order kinetics when the concentration of resveratrol was maintained at more than 10-fold excess of the concentration of GO·. Plotting this pseudo-first-order rate constant (k_{obs}) versus the concentration of resveratrol gave a straight line (Fig. 3), from which the second-order rate constant (k) for the GO·-scavenging reaction by resveratrol could be obtained. 4,4′-DHS gave the same second-order kinetics (Fig. 3). The second-order rate constants of resveratrol and 4,4′-DHS were 0.30 and $4.41 \times 10^2\ M^{-1}\ s^{-1}$, suggesting that 4,4′-DHS exhibits remarkably higher activity in the GO·-scavenging reaction than resveratrol.

2.2. Fe^{2+}/ascorbate (VC)-induced lipid peroxidation of human erythrocyte ghosts and its inhibition by resveratrol and 4,4′-DHS

Combination of Fe^{2+} and a reducing reagent is an extensively used system for generating hydroxyl radicals to induce lipid peroxidation.[17] Line a in Figure 4 shows the malondialdehyde (MDA) formation during the Fe^{2+}/VC-induced lipid peroxidation of human erythrocyte ghosts under an aerobic atmosphere. The MDA formation was remarkably inhibited by the addition of 1 μM of resveratrol (line b) during the so-called 'inhibition period'. After the inhibition period, the rate of MDA formation increased to close to the original rate, corresponding to the exhaustion of resveratrol. It is clearly seen from the lines b–e of Figure 4 that resveratrol significantly suppressed the rate of MDA formation and increased the inhibition period in a dose-dependent manner. The MDA formation was completely inhibited by 5 μM of resveratrol (line e). Table 1 shows the inhibition effects of different concentrations of resveratrol and 4,4′-DHS against Fe^{2+}/VC-induced MDA formation. 4,4′-DHS exhibited higher antioxidant activity than resveratrol, similar to that observed in the GO·-scavenging reaction. However, at the higher concentration (3 μM), there is no apparent difference in the inhibition between resveratrol and 4,4′-DHS.

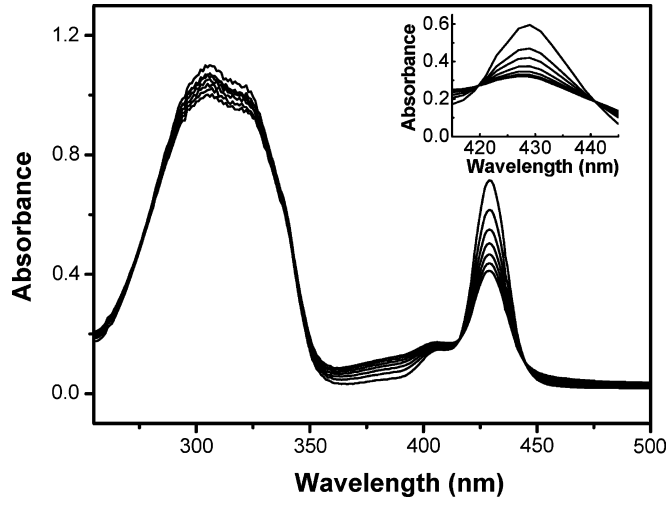


Figure 2. Spectral changes observed upon addition of resveratrol (50 μM) to an ethanol solution of GO· (5 μM) at 298 K (Interval: 3 min.). The inset shows the spectral changes of GO· (5 μM) in the presence of 4,4′-DHS (50 μM) (Interval: 10 s.).

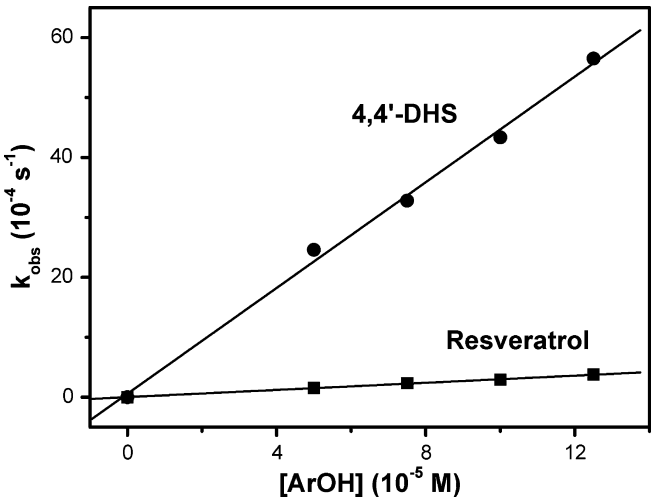


Figure 3. Plot of the pseudo-first-order rate constant (k_{obs}) *vs.* the concentration of resveratrol or 4,4′-DHS.

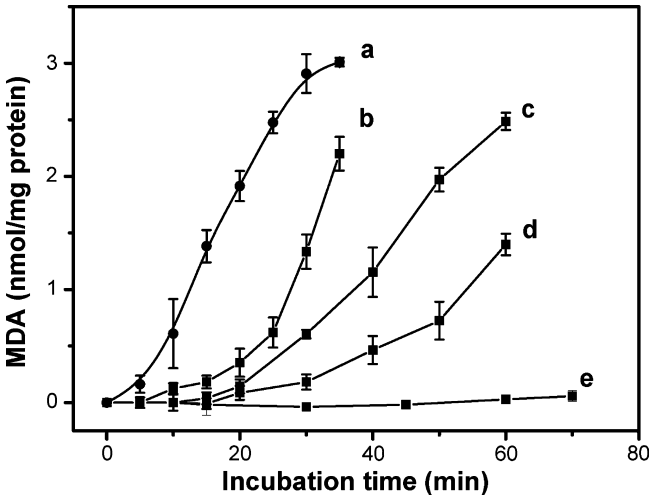


Figure 4. Inhibition of malondialdehyde (MDA) formation during 50 μM Fe^{2+}/200 μM VC-induced peroxidation of human erythrocyte ghosts by resveratrol at 37 °C. The erythrocyte ghosts were incubated at 37 °C in 0.1 M PBS (pH 7.4) with protein concentration of 0.96 mg/mL. Resveratrol was added before the initiation. (a) Native erythrocyte ghosts as blank; and the initial concentrations of resveratrol were (b) 1 μM; (c) 2 μM; (d) 3 μM; (e) 5 μM. Values represent mean ± S.D. of three experiments.

2.3. UV–vis spectral changes and oxidation products of resveratrol and 4,4′-DHS in the presence of Cu(II)

Copper is one of the most redox active metal ions present in cell.[18] Therefore, Cu(II) was used to oxidize resveratrol and 4,4′-

Table 1
Inhibition of MDA formation by resveratrol and 4,4′-DHS during the Fe^{2+}/VC-induced lipid peroxidation of human erythrocyte ghosts[a,b,c]

ArOHs	MDA (nmol/mg protein)
Control	2.91 ± 0.17
Resveratrol (1 μM)	1.33 ± 0.02
Resveratrol (2 μM)	0.61 ± 0.04
Resveratrol (3 μM)	0.18 ± 0.07
4,4′-DHS (1 μM)	0.80 ± 0.02
4,4′-DHS (2 μM)	0.45 ± 0.05
4,4′-DHS (3 μM)	0.17 ± 0.04

[a] The formation of MDA was determined after human erythrocyte ghosts were incubated for 30 min.
[b] The reaction conditions are the same as those described in the captions of Figure 4.
[c] Data are expressed as the mean ± S.D. for three determinations.

DHS. Figure 5 is obtained when Cu(II) was added to resveratrol in acetonitrile under aerobic conditions. It was found that the decrease of the absorbance of resveratrol centered at 305 nm. The addition of Cu(II) to 4,4′-DHS resulted in the decrease of the maximal absorption at 302 nm, accompanied by the appearance of two new peaks at 399 and 425 nm due to the formation of *para*-semiquinone or *para*-quinone (the inset in Fig. 5). The absorption of *para*-semiquinone or *para*-quinone in acetonitrile became clearer than that in phosphate-buffered saline (PBS).[16] It has been reported previously that the semiquinone of 3,5,3′5′-tetra-*tert*-butyl-4,4′-dihydroxystilbene absorbs at 450 nm.[19] The decay of 4,4-DHS in the presence of Cu(II) was significantly faster than that of resveratrol in the presence of Cu(II).

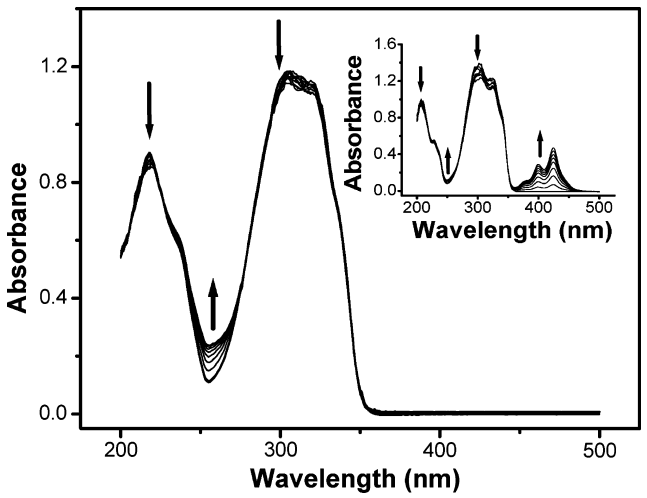
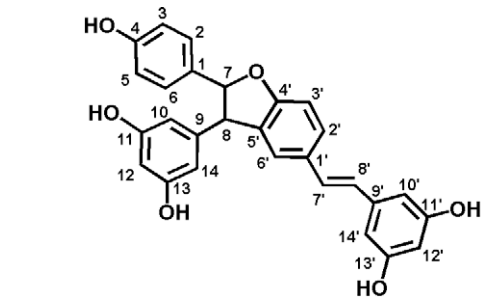


Figure 5. Absorption spectral changes of resveratrol (40 μM) and 4,4′-DHS (40 μM) in the presence of 40 μM of Cu(II) in acetonitrile under air. The spectra were recorded every 3 min for 24 min after the addition of Cu(II).

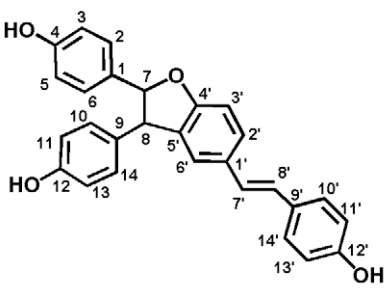
In order to clarify the antioxidant mechanism of resveratrol and 4,4′-DHS, we isolated and identified their oxidative products in the presence of Cu(II) in acetonitrile at room temperature. After the addition of Cu(II) to the acetonitrile-containing resveratrol or 4,4′-DHS, oxidation resulted in the change in the color of the solution from blue to green. The major products were dihydrofuran dimers (Fig. 6) by characterizing with HRMS (ESI) and 1D and 2D NMR (see Supplementary data). As an example, the oxidation of 4,4′-DHS in the presence of Cu(II) and the structural characterization of its dimer will be discussed. The HRMS analysis of the major product showed a $[M+H]^+$ of 423.1595 (calculation: 423.1591), consistent with the structure of a dehydrodimer, and its ^1H NMR spectrum gave some additional information that allowed its identification with the structure. Despite the fact that the product was a dimer, only three groups of aliphatic AA′BB′ protons (two doublets at $\delta = 7.24$ and 6.85, $J = 8.4$ Hz, H-2,6 and H-3,5, two doublets at $\delta = 7.01$ and 6.83, $J = 8.8$ Hz, H-10,14 and H-11,13, two doublets at $\delta = 7.39$ and 6.80, $J = 8.4$ Hz, H-10′,14′ and H-11′,13′) were present, due to a single 4-hydroxybenzoyl moiety. Additional signals were due to two *trans*-olefinic protons (two doublets at $\delta = 6.97$ and 6.96, with a large coupling constant of 18.4 Hz, H-7′ and H-8′) and three aromatic protons. The correct structural assignments could be made on the basis of homonuclear bidimensional correlation, heteronuclear multiple-quantum coherence (^1H, ^{13}C HMQC) and heteronuclear multiple-bond correlation (^1H, ^{13}C HMBC) experiments. The long-range correlation between the doublet at $\delta = 7.24$ (H-2,6) and the aliphatic carbon at $\delta = 94.5$ (related to the doublet at $\delta = 5.43$, C-7) allowed us to establish that hydroxybenzoyl moiety was bonded to C-7. The long-range correlation between the doublet at $\delta = 7.05$ (H-10 and H-14) and the aliphatic carbon at $\delta = 57.5$ (related to the doublet at $\delta = 4.53$, C-8), allowed us to establish that hydroxybenzoyl moiety was bonded to C-8. All these data were consistent with the dihydrofuran dimer formed *via* an oxidative coupling (vide infra). Moreover, more than 8 values for coupling constant (^3J) between H-7 and H-8 in the dimer suggested a predominant pseudo-*trans*-axial arrangement for these two aliphatic protons. Furthermore, enantiomeric composition of the dimer was evaluated by high performance liquid chromatography (HPLC) analysis on a chiral column and it was found that the dimer was a racemic mixture (see Supplementary data).

2.4. Effects of resveratrol and 4,4′-DHS on HL-60 cell proliferation

The cell viability was assessed by the colorimetric MTT assay[20] and results are demonstrated in Figure 7 and summarized in Table 2. It is seen from the figure that resveratrol exhibits dose-depen-



Dimer of resveratrol
(31% conversion and 16% isolated yield)



Dimer of 4,4'-DHS
(39% conversion and 20% isolated yield)

Figure 6. The oxidative products of resveratrol and 4,4′-DHS in the presence of Cu(II) in acetonitrile at room temperature. The molar ratio of ArOHs:Cu(II) was 1:1.1.

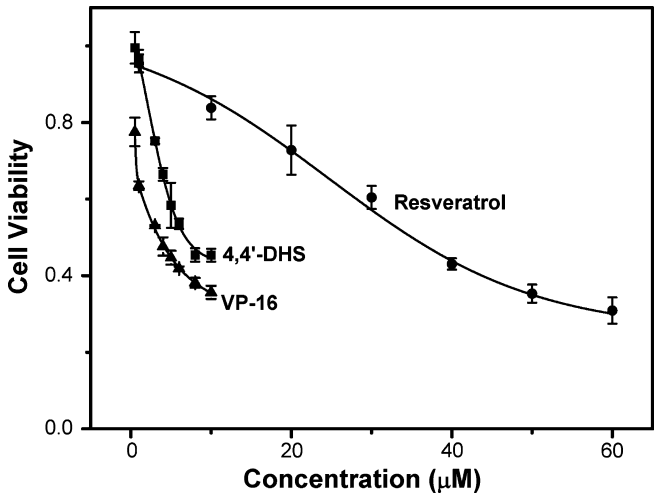


Figure 7. Inhibitory effects of resveratrol and 4,4′-DHS on HL-60 cell viability. HL-60 cells were treated with tested compounds for 48 h and the percent viability was determined using the MTT method. Values are the mean ± S.D. for three independent experiments.

Table 2
In vitro cytotoxicity against HL-60 cells of resveratrol and 4,4′-DHS[a]

ArOHs	IC_{50} (μM)
Resveratrol	36.3 ± 1.8
4,4′-DHS	6.5 ± 0.1
Vp-16	3.3 ± 0.3

[a] Cytotoxicity is expressed as IC_{50}, the concentration for the compound to cause 50% inhibition of the cell viability. Data are expressed as the mean ± S.D. for three determinations.

dent cytotoxic effects on HL-60 cells. The cell viability was reduced to 83%, 72% and 43% after treatment with 10, 20 and 40 μM, respectively, of resveratrol for 48 h. 4,4′-DHS showed more active effect on suppression of the cell viability than resveratrol. It is also noticeable that the activity of 4,4′-DHS is similar to that of etoposide (VP-16, positive control), anticancer drugs. The IC_{50} values for these compounds are shown in Table 2. It indicates that 4,4′-DHS exhibits higher cytotoxicity than resveratrol.

2.5. Mechanism

Resveratrol is a well-known natural antioxidant and cancer chemopreventive agent which has attracted much interest in the past decade. Effective effort has, therefore, been continuously devoted to the synthesis of new resveratrol analogues, aiming to find more effective antioxidant and cancer chemopreventive agent.[21–25]

The oxidative product of resveratrol in the presence of Cu(II) suggests that the hydroxyl group at the 4′-position is much easier to subject to oxidation than other hydroxyl groups. This is consistent with that obtained from both experiments[7] and theoretical calculations.[8,9] Theoretical calculations[9] indicated that the most stable phenolate in resveratrol is obtained after extraction of the proton in the 4′-position, which is ~7 kcal/mol lower than those having extracted the protons in the 3- and 5-position. Therefore, the 4′-OH in resveratrol provides the most acidic hydrogen and its removal from the 4′-OH is highly preferred.

The present study, demonstrates that 4,4′-DHS exhibits remarkably higher antioxidant activity than resveratrol. This can be explained because the *p*-OH phenoxyl radical (*p*-semiquinone), the oxidation intermediate for 4,4′-DHS, can be stabilized by the 4′-OH group by resonance through the *trans* double bond. The theoretical calculation showed that the bond dissociation energy (BDE) of hydroquinone is 5.9 kcal/mol lower than that of phenol.[26] In addition, the *p*-OH phenoxyl radical (*p*-semiquinone) should be more easily further oxidized to form the final product *para*-quinone as depicted in Scheme 1. It is suggested from the oxidative product of 4,4′-DHS that the oxidation reaction results in the formation of phenoxyl radicals or semiquinone ('A', 'B' and 'C'). Successively, the coupling of one radical 'B' and one radical 'C', followed by tautomeric rearrangement and intramolecular nucleophilic attack to the intermediate quinone, gave the dihydrofuran dimer as shown in Scheme 1.

It is also noticeable from Table 2 that 4,4′-DHS exhibits remarkably higher cytotoxicity against HL-60 cells than resveratrol. Of interest, intriguing result was recently reported that 4,4′-DHS, which is nonestrogenic, but significantly antioxidative, could selectively induce the down-regulation of estrogen receptor α (ERα) of MCF-7 breast cancer cells.[27] It was also suggested that binding of 4,4′-DHS to ERα depends on the ability of 4-OH and 4′-OH to from hydrogen bonding with GLu353 and His524, respectively.[28]

3. Conclusions

In summary, the work demonstrates that resveratrol and its analogue, 4,4′-DHS, are effective antioxidants in scavenging GO· and inhibiting Fe^{2+}/VC-induced lipid peroxidation of human erythrocyte ghosts. The oxidative product analysis of resveratrol and 4,4′-DHS in the presence of Cu(II) in acetonitrile suggests free-radical coupling dimerization reaction and the importance of 4′-OH in resveratrol. The observation that 4,4′-DHS possesses remarkably higher antioxidant activity and cytotoxicity against HL-60 cells than resveratrol gives us useful information for antioxidant and chemoprevention drug design.

4. Experimental

4.1. Materials

Resveratrol and 4,4′-DHS were synthesized with reference to the available method.[14] Their structures were fully identified using ^{1}H NMR and EI-MS (see the below). The purity (>98%) of each compound was all checked using a HPLC.

4.1.1. Resveratrol

^{1}H NMR (300 MHz, $(CD_3)_2CO$), δ 6.26 (1H, t, *J* = 1.8 Hz, H-4), 6.54 (2H, d, *J* = 1.8 Hz, H-2 and H-6), 6.83 (2H, d, *J* = 9.0 Hz, H-3′ and H-5′), 6.89, 6.99 (each 1H, d, *J* = 16.5 Hz, H-7 or H-8), 7.40 (2H, d, *J* = 9.0 Hz, H-2′ and H-6′); MS, *m/z* 228 [M^+], 211, 199, 181, 157, 115.

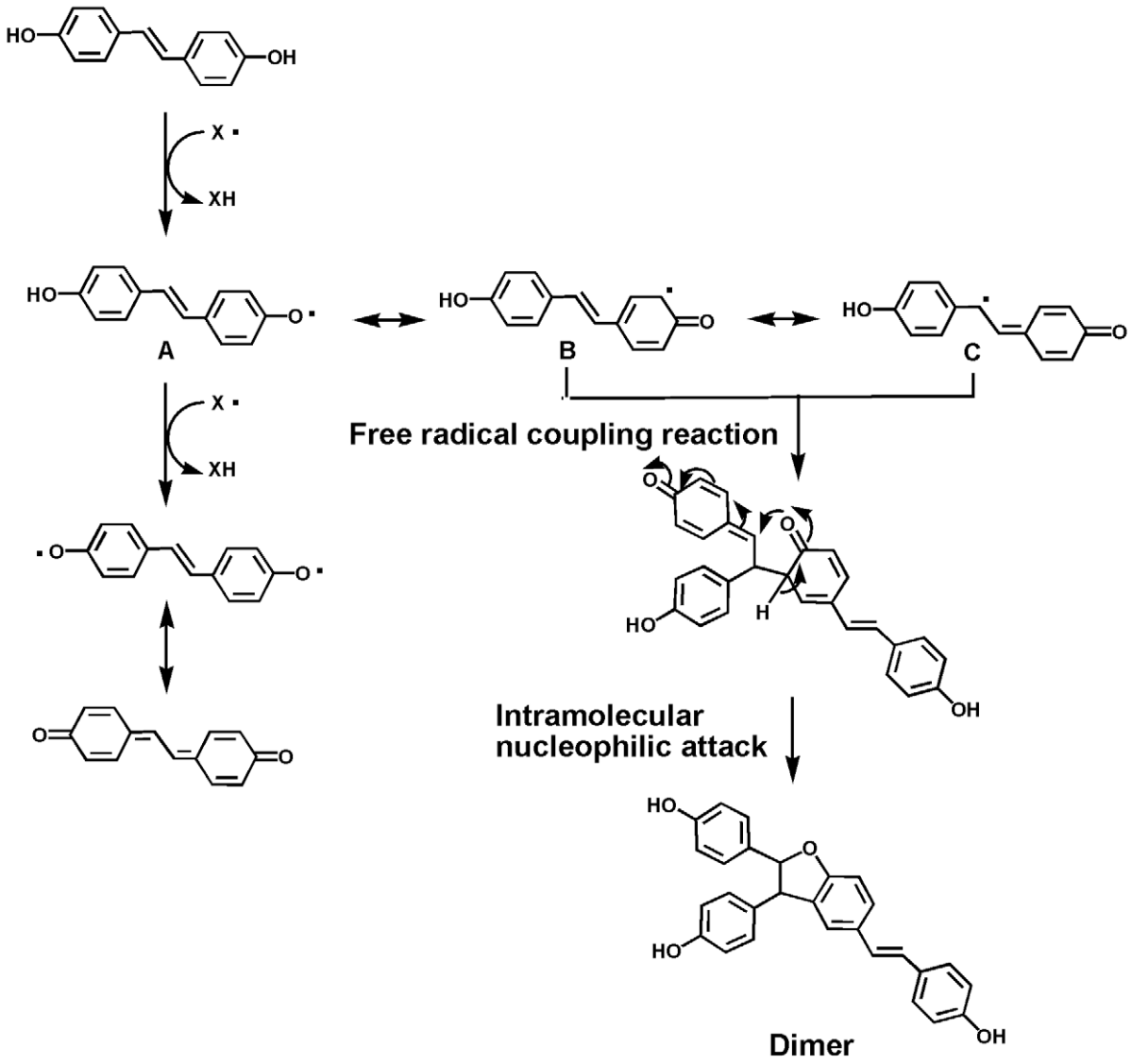
4.1.2. 4,4′-DHS

^{1}H NMR (300 MHz, $(CD_3)_2CO$), δ 6.82 (4H, d, *J* = 8.7 Hz, H-3, H-5, H-3′ and H-5′), 6.96 (2H, s, H-7 and H-8), 7.39 (4H, d, *J* = 8.7 Hz, H-2, H-6, H-2′ and H-6′); MS, *m/z* 212 [M^+], 197, 165, 141, 115, 77.

GO· was purchased from Acros (98%, New Jersey, USA). Thiobarbituric acid (TBA) and butylated hydroxytoluene (BHT) were purchased from Sigma–Aldrich (St. Louis, MO) and used as received.

4.2. Spectral and kinetic measurement

An aliquot of resveratrol or 4,4′-DHS at more than 10-fold excess of the concentration of GO· was added to a quartz (10 mm i.d.) cuvette which contained GO· (5 μM) in ethanol solution. This led to a hydrogen-transfer reaction from ROH to GO·. UV-vis spectra changes associated with this reaction were measured at room temperature with a Hitachi 557 spectrophotometer. The rates of



Scheme 1. Antioxidant mechanism of 4,4′-DHS. X· denotes free radical.

hydrogen transfer were determined by monitoring the absorbance change at 428 nm due to GO·.

4.3. Human erythrocyte ghost preparation

Human erythrocyte ghosts were separated from heparinized blood of a healthy donor from Central Blood Center, Red Cross of Gansu province. The Cells were washed three times in 10 volumes of PBS at pH 7.4, which consisted of 137 mM of NaCl, 2.7 mM of KCl, 8.1 mM of Na_2HPO_4 and 1.5 mM of KH_2PO_4 in distilled water. The supernatant and buffy coat was carefully removed after each wash. The packed erythrocyte ghosts were then suspended in 30 volumes of an ice-cold hypotonic PBS (5 mM, pH 7.6) for lysis.[29] The hemoglobin-free ghosts were pelleted by centrifugation at 20,000g at 4 °C for 20 min and further washed twice with the same hypotonic buffer. The protein concentration in the membranes was determined by the method of Lowry.[30]

4.4. MDA formation measurements

The formation of MDA, due to the formation of thiobarbituric acid reactive substances (TBARS), was used to monitor lipid peroxidation.[31] The erythrocyte ghosts were incubated at 37 °C in 0.1 M PBS (pH 7.4), and made up to a final protein concentration of 0.96 mg/mL. The peroxidation was initiated by Fe^{2+} (50 μM)/VC (200 μM) and inhibited by 4,4′-DHS or resveratrol, and the reaction mixture was gently shaken at 37 °C. Aliquots of the reaction mixture were taken out at specific intervals to a TCA–TBA–HCl stock solution (15% w/v trichloroacetic acid (TCA); 0.375% w/v TBA; 0.25 N HCl), together with 0.02% w/v BHT. This amount of BHT completely prevents the formation of any nonspecific TBARS.[32] The solution was heated in a boiling water bath for 15 min. After cooling, the precipitate was removed by centrifugation. MDA in the supernatant was determined at 532 nm using the extinction coefficient of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$.[31]

4.5. UV–vis spectral measurement

UV-vis spectra were measured at room temperature with a Hitachi 557 spectrophotometer. Acetonitrile containing 40 μM 4,4′-DHS or resveratrol was kept at room temperature, and the spectral tracing was started by addition of 40 μM $CuSO_4$. The spectra were recorded every 3 min after addition of $CuSO_4$.

4.6. Oxidation of resveratrol or 4,4′-DHS in the presence of Cu(II)

A solution of resveratrol or 4,4′-DHS (0.5 mmol) and anhydrous copper sulfate (96 mg, 0.55 mmol) were mixed in anhydrous acetonitrile (50 mL) and stirred for 30–35 h at room temperature, as monitored by silica gel (petroleum ether/acetone, 20/1). The major dimer products were isolated by silica gel chromatography, and were characterized with HRMS (ESI) and 1D and 2D NMR. Enantiomeric composition of the dimer was evaluated by HPLC (Waters 600 instrument with Photodiode Array detector) analysis on a chiral OD column.

4.6.1. The dehydrodimer of resveratrol

^{1}H NMR (400 MHz, $(CD_3)_2CO$), δ 4.62 (d, J = 8.0 Hz, H-8); 5.48 (d, J = 8.0 Hz, H-7); 6.19 (d, J = 2.0 Hz, H-10 and H-14); 6.25 (d, J = 2.0 Hz, H-12 and H-12′); 6.53 (d, J = 2.0 Hz, H-10′ and H-14′); 6.84 (d, J = 8.0 Hz, H-3 and H-5); 6.88 (d, J = 8.0 Hz, H-3′); 6.92 and 7.08 (AB system, d, J = 16.0 Hz, H-7′ and H-8′); 7.24 (d, J = 8.0 Hz, H-2 and H-6); 7.25 (d, J = 8.0 Hz, H-2′); 7.45 (d, J = 2.0 Hz, H-6′). ^{13}C NMR (100 MHz, $(CD_3)_2CO$), δ 57.7 (C-8); 94.2 (C-7); 102.4 (C-12); 102.7 (C-12′); 105.7 (C-10′ and C-14′); 107.4 (C-10 and C-14); 110.1 (C-3′); 116.1 (C-3 and C-5); 123.9 (C-2′); 127.2 (C-7′); 128.5 (C-2, C-6 and C-6′); 129.1 (C-8′); 131.7 (C-1); 132.1 (C-5′); 132.5 (C-1′); 140.7 (C-9′); 145.1 (C-9); 158.4 (C-4); 159.5 (C-11′ and C-13′); 159.7 (C-11 and C-13); 160.5 (C-4′). HRMS (ESI): *m/z calcd* for $[M+H]^+$: 455.1489, found: 455.1481, error = 1.8 ppm.

4.6.2. The dehydrodimer of 4,4′-DHS

^{1}H NMR (400 MHz, $(CD_3)_2CO$), δ 4.53 (d, J = 8.8 Hz, H-8); 5.43 (d, J = 8.8 Hz, H-7); 6.80 (d, J = 8.4 Hz, H-11′ and H-13′); 6.85 (d, J = 8.4 Hz, H-3 and H-5); 6.83 (d, J = 8.8 Hz, H-11 and H-13); 6.87 (d, J = 8.4 Hz, H-3′), 6.96 (d, J = 18.4 Hz, H-8′); 6.97 (d, J = 18.4 Hz, H-7′); 7.05 (d, J = 8.8 Hz, H-10 and H-14); 7.16 (bs, H-6′); 7.24 (d, J = 8.4 Hz, H-2 and H-6); 7.37 (d, J = 8.4 Hz, H-2′); 7.39 (d, J = 8.4 Hz, H-10′ and H-14′). ^{13}C NMR (100 MHz, $(CD_3)_2CO$), δ 57.5 (C-8); 94.5 (C-7); 110.2 (C-3′); 116.2 (C-11′ and C-13′); 116.4 (C-11 and C-13); 116.6 (C-3 and C-5); 123.4 (C-6′); 126.6 (C-7′ and C-8′); 126.9 (C-2′); 128.1 (C-10′ and C-14′); 128.4 (C-2 and C-6); 128.7 (C-9′); 130.3 (C-10 and C-14); 130.5 (C-1); 132.4 (C-1′); 132.9 (C-9); 133.4 (C-5′); 157.6 (C-12); 157.9 (C-4); 158.5 (C-4′); 160.3 (C-12). HRMS (ESI): *m/z calcd* for $[M+H]^+$: 423.1591, found: 423.1595, error = 0.9 ppm.

4.7. Cell culture

HL-60 cell lines were originally obtained from Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, The cells were grown in RPMI-1640 medium supplemented with 10% (v/v) heat inactivated fetal bovine serum, penicillin (100 kU/L), streptomycin (100 mg/L) and 2 mM of glutamine. The cell cultures were maintained at 37 °C in a humidified CO_2 (5%) incubator. Exponentially growing cells were used throughout.

4.8. Assessment of HL-60 cell viability

The HL-60 cells viability was assessed by the MTT colorimetric assay which is based on the reduction of MTT by the mitochondrial succinate dehydrogenase of intact cells to a purple formazan product.[20] Briefly, Aliquots (100 μL) of thymocytes containing 5×10^4 cells/mL were added to each well of a 96-well flatmicrotiter plate and incubated with various amounts of resveratrol or its analogues dissolved in 0.1% dimethylsulfoxide (DMSO). Six replicate wells were used in each point in the experiment. After 48 h incubation at 37 °C, 10 μL MTT solution (5 mg/mL in PBS) was added and incubated for another 4 h at 37 °C in a 5% CO_2 incubator. The resulting MTT-formazan product was dissolved by the same volume of lysis buffer (10% SDS, 5% isopropanol and 0.012 M HCl (w/v/v)), and the incubation was continued overnight at 37 °C. The amount of formazan was determined by measuring the absorbance at 570 nm using a Bio-Rad 550 ELISA microplate reader.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.02.014.

References and notes

1. Renaud, S.; de Lorgeril, M. *Lancet* **1992**, *339*, 1523.
2. Saiko, P.; Szakmary, A.; Jaeger, W.; Szekere, T. *Mutat. Res.* **2008**, *658*, 68.
3. Baur, J. A.; Sinclair, D. A. *Nat. Rev. Drug Discov.* **2006**, *5*, 493.
4. Delmas, D.; Lancon, A.; Colin, D.; Jannin, B.; Latruffe, N. *Curr. Drug Targets* **2006**, *7*, 423.
5. Wenzei, E.; Somoza, V. *Mol. Nutr. Food. Res.* **2005**, *49*, 472.
6. Perwez Hussain, S.; Hofseth, L. J.; Harris, C. C. *Nat. Rev. Cancer* **2003**, *3*, 276.
7. Wang, M.; Jin, Y.; Ho, C.-T. *J. Agric. Food Chem.* **1999**, *47*, 3974.
8. Fukuhara, K.; Nagakawa, M.; Nakanishi, I.; Ohkubo, K.; Imai, K.; Urano, S.; Fukuzumi, S.; Ozawa, T.; Ikota, N.; Mochizuki, M.; Miyata, N.; Okuda, H. *Bioorg. Med. Chem.* **2006**, *14*, 1437.
9. Caruso, F.; Tanski, J.; Villegas-Estrada, A.; Rossi, M. *J. Agric. Food Chem.* **2004**, *52*, 7279.
10. Rossi, M.; Caruso, F.; Opazo, C.; Salciccioli, J. *J. Agric. Food Chem.* **2008**, *56*, 10557.
11. Farines, V.; Monje, M.-C.; Telo, J. P.; Hnawia, E.; Sauvain, M.; Nepveu, F. *Anal. Chim. Acta* **2004**, *513*, 103.
12. Matsuoka, A.; Takeshita, K.; Furuta, A.; Ozaki, M.; Fukuhara, K.; Miyata, N. *Mutat. Res.* **2002**, *521*, 29.
13. Stivala, L.-A.; Savio, M.; Carafoli, F.; Perucca, P.; Bianchi, L.; Maga, G.; Forti, L.; Pagnoni, U. M.; Albini, A.; Prosperi, E.; Vannini, V. *J. Biol. Chem.* **2001**, *276*, 22586.
14. Thakkar, K.; Geahlen, R. L.; Cushman, M. *J. Med Chem.* **1993**, *36*, 2950.
15. Fang, J.-G.; Zhou, B. *J. Agric. Food Chem.* **2008**, *56*, 11458.
16. Zheng, L.-F.; Wei, Q.-Y.; Cai, Y.-J.; Fang, J.-G.; Zhou, B.; Yang, L.; Liu, Z.-L. *Free Radic. Biol. Med.* **2006**, *41*, 1807.
17. Kamat, J. P.; Devasagayam, T. P. A. *Neurosci. Lett.* **1995**, *195*, 179.
18. Yoshida, Y.; Furuta, S.; Niki, E. *Biochim. Biophys. Acta* **1993**, *1210*, 81.
19. Simándi, L.; Simándi, T. L. *J. Mol. Catal. A: Chem.* **1997**, *117*, 299.
20. Hussain, R. F.; Nourin, A. M. E.; Oliver, R. T. D. *J. Immunol. Methods* **1993**, *160*, 89.
21. Cai, Y. J.; Wei, Q. Y.; Fang, J. G.; Yang, L.; Liu, Z.-L.; Wyche, J.-H.; Han, Z. *Anticancer Res.* **2004**, *24*, 999.
22. Murias, M.; Jäger, W.; Handler, N.; Erker, T.; Horvath, Z.; Szekeres, T.; Nohl, H.; Gille, L. *Biochem. Pharmacol.* **2005**, *69*, 903.
23. Wang, Y.; Wang, B.; Cheng, J.; Yang, L.; Liu, Z.-L.; Balan, K.; Pantazis, P.; Wyche, J. H.; Han, Z. *Biochem. Pharmacol.* **2005**, *69*, 249.
24. Gosslau, A.; Chen, M.; Ho, C.-T.; Chen, K.-Y. *Br. J. Cancer* **2005**, *92*, 513.
25. Horvath, Z.; Murias, M.; Saiko, P.; Erker, T.; Handler, N.; Madlener, S.; Jaeger, W.; Grusch, M.; Fritzer-Szekeres, M.; Krupitza, G.; Szekeres, T. *Exp. Henatol.* **2006**, *34*, 1377.
26. Wright, J. S.; Johnson, E. R.; Dilabio, G. A. *J. Am. Chem. Soc.* **2001**, *123*, 1173.
27. Balan, K. V.; Wang, Y.; Chen, S. W.; Chen, J.-C.; Zheng, L.-F.; Yang, L.; Liu, Z.-L.; Pantazis, P.; Wyche, J.-H.; Han, Z. *Biochem. Pharmacol.* **2006**, *72*, 573.
28. Fang, H.; Tong, W.; Shi, L. M.; Blair, R.; Perkins, R.; Branham, W.; Hass, B. S.; Xie, Q.; Dial, S. L.; Moland, C. L.; Sheehan, D. M. *Chem. Res. Toxicol.* **2001**, *14*, 280.
29. Liao, K.-L.; Yin, M.-C. *J. Agric. Food Chem.* **2000**, *48*, 2266.
30. Lowry, O. H.; Rosebrough, N. J.; Farr, A. C.; Randall, R. J. *J. Biol. Chem.* **1951**, *193*, 265.
31. Buege, J. A.; Aust, S. D. *Methods Enzymol.* **1978**, *52*, 302.
32. Palozza, P.; Krinsky, N. I. *Arch. Biochem. Biophys.* **1992**, *297*, 184.