Syntheses of Resveratrol Analogues and Evaluation of Their Antioxidant Activity

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Reactive oxygen species (ROS) referring free radicals with oxygen molecules are produced in the body as by-products during the normal metabolic process which generates energy by the oxidation of carbohydrates, fats, and proteins. Free radicals such as superoxide anion radicals ($O_2^{•-}$), hydroxyl radicals (•OH) and non-free radical species such as hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) are considered as ROS.[1] These ROS not only oxidize membrane lipids but damage nucleic acids, proteins and carbohydrates leading to mutations.[2] If ROS are not scavenged by antioxidants, they could be involved in ageing and various diseases related to oxidative stress.[3]

Resveratrol **1** is a natural phytoalexin found in the skin of grapes, red wines, and peanuts.[4] It has three hydroxyl groups at the *trans*-stilbene structure, in which resorcinol and phenol are bridged by a *trans* double bond (Fig. 1). The recent extensive studies on the resveratrol and its derivatives revealed that they have antioxidant,[5] antimutagenic,[6] anti-

inflammatory,[7] antidiabetic,[8] cardiovascular protective,[9] and anticancer[10] properties. It has been believed that the majority of the biological functions of resveratrol has been attributed to its antioxidant activity.[11,12]

Even though the extensive research to establish the structure-activity relationship of resveratrol has been made, most of them were concentrated on variations in the periphery of the resveratrol. In other words, the effect of the hydroxyl groups on the stilbene ring system has been studied intensively to elucidate the relationship between the antioxidant activity and the structure of resveratrol derivatives.[13-15]

Here we report the design and syntheses of bridging double bond modified resveratrol analogues such as imine type **2**, amide type **3**, (*Z*)-alkene **4**, and alkyne **5** (Fig. 1) and the evaluation of the antioxidant activity of these analogues with three reference compounds, resveratrol **1**, quercetin, and ascorbic acid.

Resveratrol **1** and the amide type compound **3** were synthesized according to the literature by H. S. Lee *et al.*[16] by Rho *et al.*,[17] respectively. The imine compound **2** was synthesized by the similar method published by Aldaba *et al.*[18] as shown in Scheme 1.

Scheme 1. The synthesis of 5-[(4-hydroxyphenylimino) methyl]-benzene-1,3-diol **2**.
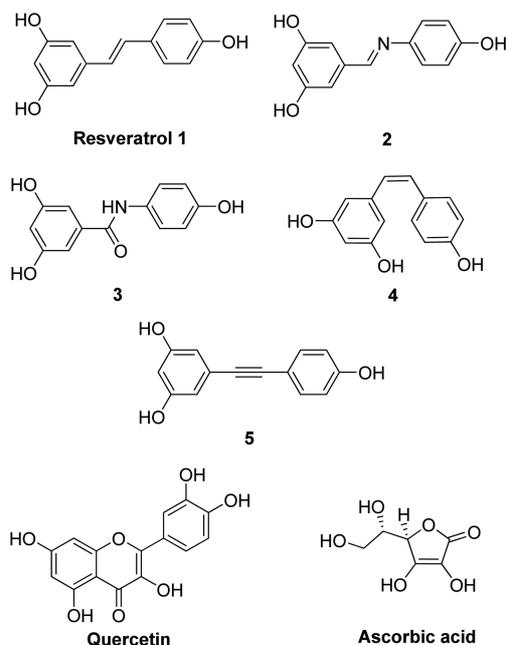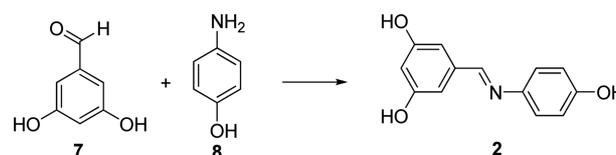

Figure 1. Structures of resveratrol **1**, its bridging double bond modified analogues **2-5** and reference compounds, quercetin and ascorbic acid.

Table 1. The reaction conditions for the synthesis of 5-[(4-hydroxyphenylimino)methyl]benzene-1,3-diol **2**

Entry	Reaction Condition	Solvent	Time	Yield (%)
1	rt	CH_2Cl_2	3 h	60%
2	rt	MeOH	3 h	60%
3	rt	CH_3CN	3 h	60%
4	rt, Na_2SO_4	CH_3CN	3 h	80%
5	rt, Na_2SO_4	CH_2Cl_2	3 h	93%
6	Microwave 700W	MeOH	5 min	–
7	Microwave 700W	MeOH	10 min	–
8	Microwave 700W	MeOH	15 min	–

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Table 2. Optimal isomerization condition from *trans* resveratrol to *cis* resveratrol **4**

| Energy	Temperature	Time	Ratio (*cis*:*trans*)
9 J	room temp.	30 min	78:22
11 J	room temp.	30 min	84:16
13 J	room temp.	30 min	73:27
15 J	room temp.	30 min	57:43
11 J	0 °C	30 min	84:16

Several conditions including microwave reactions were tested to produce the imine analogue **2** from 3,5-dihydroxy-benzaldehyde **7** and 4-hydroxyanline **8** (Table 1). However, due to the low yield and the low stability of the product, it was quite challenging to synthesize the desired imine compound **2**. The compound **2** was unstable on column chromatography and decomposed when heat was applied. The best yield 93% was obtained when the solvent was dichloromethane, the byproduct H_2O was removed by Na_2SO_4, and the product was solidified in acetonitrile.

The compound **4** was synthesized by a similar method published by Montsko *et al.*[19] The optimal irradiation energy for isomerization was 11 J. Temperature and time dependence of isomerization was not observed. Reaction at 0 °C or prolonged irradiation did not make any difference in isomerization ratio (Table 2). Due to the nature of the reversible isomerization between *cis*- and *trans*-configuration, the reaction mixture was used to test ABTS assay without further purifications. However, it is noteworthy that there is little difference in their relative ratio under darkness for several days at room temperature, indicating the relatively long lifetime of the corresponding *cis*-isomer. From the above observations, we were assured that the ratio of the *cis* and *trans* mixture would not change during the ABTS assay which was performed under darkness.

For the compound **9**,[20] the phenolic OH was protected by methoxyethyl (MOE) group at first but the solubility problem renders the MOE protecting group ineffective. Therefore the protecting group was changed to tetrahydropyranyl
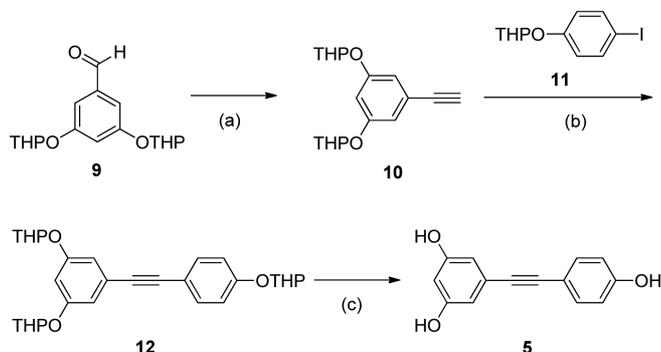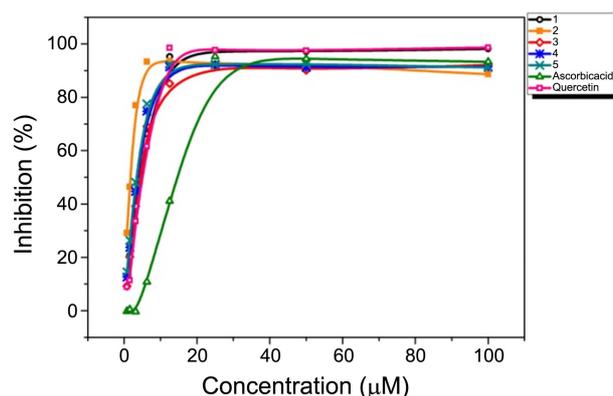
Figure 2. ABTS radical scavenging activity of compounds **1-5**.

(THP) group. For Sonogashira coupling between compound **10** and **11**, the acetylene precursor **10** was synthesized by Colvin rearrangement from the compound **9** using trimethyl-silyldiazomethane and 2 M *n*-butyllithium *via* carbene intermediate by 48% yield. The compound **10** was then coupled with THP protected compound **11** to produce compound **12** by Sonogashira coupling in DMF. The deprotection of the THP groups of compound **12** was done by using Dowex 50X2-100 ion-exchange resin in methanol. The desired product 5-[(4-hydroxyphenyl)ethynyl]-1,3-benzenediol **5** was obtained as a brown solid in 23% yield in 3 steps (Scheme 2).

Antioxidant activity of the synthetic resveratrol **1** and its analogues **2-5** was tested three times for each compound using ABTS. Figure 2 shows the concentration dependent radical scavenging activities of the compound **1-5**. In the Figure 2, it is evident that the antioxidant activity trend of all the synthesized compounds was much better than that of ascorbic acid, one of the reference compounds.

IC_{50} values of the compounds were calculated by using Origin 8 software. Compound **1-5** showed IC_{50} values of 4.5, 1.6, 4.0, 3.4, and 3.1 μM, respectively (Table 3). As reference compounds, ascorbic acid and quercetin, which are known as good antioxidants, were used. Their IC_{50} values were 14.0 μM and 4.7 μM, respectively. All resveratrol analogues **1-5** exhibit much better IC_{50} values than that of ascorbic acid and at least similar IC_{50} values as that of quercetin. Especially, *cis* double bond compound **4** and triple bond compound **5** displayed a little bit better IC_{50} values than those of reference compounds. The IC_{50} value difference between compound **4** and resveratrol **1** could be interpreted in terms of steric strain. The phenoxy radical formation of (*Z*)-resveratrol **4** could release, at least in part,

Scheme 2. The synthesis of 3,4',5-trihydroxydiphenyl-acetylene. Reagents and conditions: (a) TMSiCHN₂ (2.0 eq.), *n*-BuLi (1.8 eq.), THF, −78 °C, 48%; (b) PdCl₂(PPh₃)₂ (0.05 mol %), CuI (0.05 mol %), TEA (2 eq.), DMF, rt, 57%; (c) Dowex 50X2-100 ion-exchange resin (excess), MeOH, 85%.

Table 3. IC_{50} values of compound **1-5** by ABTS assay

Compound	IC_{50} (μM)	Compound	IC_{50} (μM)
1	4.5 ± 0.61	**2**	1.6 ± 0.10
3	4.0 ± 0.10	**4**	3.4 ± 0.19
5	3.1 ± 0.17	**Quercetin**	4.7 ± 0.26
Ascorbic acid	14.0 ± 1.03		

the steric strain resulted from *cis* configuration through resonance. The alkyne compound **5** has a weak second π bond and therefore could be easily broken to accommodate radical. Furthermore, imine type resveratrol analogue **2** showed three times better activity than resveratrol **1** and quercetin. In addition to the stabilization of the radical through conjugation, electron donating effect due to nitrogen atom might enhance the stability of resulting radical furthermore. Amide type compound **3** displayed similar activities compared to resveratrol **1**. The partial double bond character and electron donating inductive effect of the amide group altogether might contribute to the stability of the resulting radical compound.

Therefore, the radical scavenging activity of resveratrol analogues depends on not only conjugation effect but also inductive effect. This argument could be seen clearly in the case of imine type compound **2**.

Experimental

Chemicals and Measurements. All chemicals used were purchased from Aldrich Chemical Co., Junsei or other commercial companies, and used without further purification unless otherwise stated. NMR spectra were recorded at Varian Mercury TM 300 MHz FT-NMR for ^{1}H and 75 MHz for ^{13}C, with the chemical shifts (δ) reported in parts per million (ppm) relative to TMS and the coupling constants (*J*) quoted in Hz. CDCl$_3$, acetone-d_6 and methanol-d_4 were used as solvents with TMS as an internal standard. Bio-Sun UV irradiation system (300W) from Vilber Lourmat was used for the isomerization of *trans* resveratrol to *cis* resveratrol. Flash chromatography was carried out using Silica gel Merck 60 (230-400 mesh). Thin-layer chromatography (TLC) was performed on TLC Silica gel 60 F$_{254}$ (Merck, layer thickness 0.2 mm), glass-backed Silica gel plates, with visualization by UV light (254 nm) or by treatment with *p*-anisaldehyde. Melting points were measured on a MEL-TEMP II apparatus and were uncorrected. UV absorptions were measured on Shimadzu UV-1800 spectrometer and Origin 8 program was used to calculate the IC$_{50}$ values of the compounds.

5-[(4-Hydroxyphenylimino)methyl]benzene-1,3-diol (2). The reaction mixture of 3,5-dihydroxybenzaldehyde **7** (500 mg, 3.62 mmol), 4-hydroxyaniline **8** (395 mg, 3.62 mmol) and Na$_2$SO$_4$ (500 mg) in methylene chloride (20 mL) was stirred for 3 hr at room temperature under argon. After the reaction, the solvent was evaporated. Then the residue was dissolved in acetonitrile and filtered with fritted glass. The filtrate was dried in vacuo. The desired product **2** was obtained as a yellow solid (788 mg, 93%). Physical properties and NMR spectra of the compound **2** were matched to those in the previous report by Albada *et al*.[18]

(Z)-3,4',5-Trihydroxystilbene (4). The solution of (*E*)-3,4',5-trihydroxystilbene (100 mg, 0.438 mmol) in methanol-d_4 (5 mL) in a petri dish was irradiated at 365 nm and 11 J (joule) for 0.5 h at rt under darkness. After the reaction, ^{1}H NMR showed that the reaction mixture was composed of

cis and *trans* resveratrol (*cis*:*trans* = 84:16). Because this mixture was converted to *trans* resveratrol during the further purification such as column chromatography or recrystallization, this mixture was used without further purification. ^{1}H NMR (300 MHz, CD$_3$OD) *cis* δ 7.08 (2H, d, *J* = 8.4 Hz), 6.62 (2H, d, *J* = 8.4 Hz), 6.43 (2H, d, *J* = 12.3 Hz), 6.31 (2H, d, *J* = 12.3 Hz), 6.20 (2H, d, *J* = 2.1 Hz), 6.11 (1H, t, *J* = 2.1 Hz); *trans* δ 7.35 (2H, d, *J* = 8.7 Hz,), 6.97 (1H, d, *J* = 16.2 Hz), 6.80 (1H, d, *J* = 16.2 Hz), 6.76 (2H, d, *J* = 8.7 Hz), 6.45 (2H, d, *J* = 2.1 Hz), 6.15 (1H, t, *J* = 2.1 Hz).

3,5-Bis(tetrahydro-2*H*-pyran-2-yloxy)ethynylbenzene (10). To the solution of (trimethylsilyl)diazomethane (2 M solution in diethyl ether, 6.21 mL, 12.42 mmol) in THF (20 mL) was added *n*-butyl lithium (2 M solution in cyclohexane, 5.59 mL, 11.18 mmol) at −78 °C under nitrogen atmosphere. Then to the above mixture was added 3,5-bis[(tetrahydro-2*H*-pyran-2-yl)oxy]benzaldehyde **9**[20] (1.905 g, 6.218 mmol) in THF (40 mL) dropwise and the resulting mixture was stirred for 1 hour at −78 °C. The reaction was quenched with saturated ammonium chloride solution (25 mL). The organic layer was extracted with diethyl ether, washed with saturated sodium bicarbonate solution and brine, dried over Na$_2$SO$_4$, filtered and dried in vacuo. The resulting residue was applied to silicagel column chromatography (EtOAc:hexanes = 1:1). The desired product **10** was obtained as an orange liquid (839 mg, 48%). R_f 0.62 (EtOAc: hexanes = 1:3); ^{1}H NMR (300 MHz, CDCl$_3$) δ 6.84 (2H, d, *J* = 1.8 Hz), 6.77 (1H, t, *J* = 1.8 Hz), 5.38 (2H, d, *J* = 3.6 Hz), 3.88 (2H, t, *J* = 9.6 Hz), 3.61 (2H, d, *J* = 11.4 Hz), 3.02 (1H, s), 2.00-1.57 (12H, m); ^{13}C NMR (75 MHz, CDCl$_3$) δ 160.19, 125.46, 114.42, 100.13, 96.50, 79.78, 79.07, 61.73, 30.81, 25.70, 19.32.

5-[4-(Tetrahydro-2*H*-pyran-2-yloxy)phenyl]ethynyl-1,3-[bis(tetrahydro-2*H*-pyran-2-yloxy)]benzene (12). To a mixture of 3,5-bis(tetrahydro-2*H*-pyran-2-yloxy)ethynylbenzene **10** (2.569 g, 8.447 mmol), PdCl$_2$(PPh$_3$)$_2$ (0.296 g, 0.422 mmol), 4-[(tetrahydro-2*H*-pyran-2-yl)oxy]iodo-benzene **11**[21] (2.322 g, 9.29 mmol) and CuI (0.064 g, 0.42 mmol) in DMF (13 mL) was added trimethylamine (2.35 mL, 16.89 mmol) under nitrogen atmosphere. The resulting mixture was stirred for 15 h at room temperature under nitrogen atmosphere. The reaction was quenched with water, and the organic layer was extracted with methylene chloride. The organic layer was washed with water, dried over Na$_2$SO$_4$, filtered, and dried in vacuo. The residue was applied to column chromatography (Methylene chloride: hexanes = 1:1). The desired product **12** was obtained as a brown solid (2.304 g, 57%). R_f 0.55 (EtOAc:hexanes = 1:3); mp 204-206 °C; ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.43 (2H, d, *J* = 8.7 Hz), 7.01 (2H, d, *J* = 8.7 Hz), 6.87 (2H, d, *J* = 1.8 Hz), 6.73 (1H, t, *J* = 1.8 Hz), 5.43 (3H, t, *J* = 3.9 Hz), 3.89 (3H, t, *J* = 9.6 Hz), 3.62 (3H, d, *J* = 11.4 Hz), 2.00-1.57 (18H, m); ^{13}C NMR (75 MHz, CDCl$_3$) δ 160.35, 158.06, 134.65, 125.09, 117.83, 114.84, 112.82, 99.86, 98.69, 96.50, 94.88, 61.90, 30.81, 25.70, 19.31.

5-[(4-Hydroxyphenyl)ethynyl]-1,3-benzenediol (5). To a solution of 5-[(4-tetrahydro-2*H*-pyran-2-yloxy)phenyl]

ethynyl-1,3-[bis(tetrahydro-2*H*-pyran-2-yloxy)]benzene **12** (500 mg, 1.04 mmol) in MeOH was added Dowex 50X2-100 ion-exchange resin (excess) and the resulting mixture was stirred for 3 h at room temperature. The reaction mixture was filtered through Celite 545 and the filtrate was evaporated. The residue was applied to silicagel column chromatography (EtOAc:hexanes = 1:1). The desired product **5** was obtained as a brown solid (199 mg, 85%). R_f 0.23 (EtOAc:hexanes = 1:3); mp 235-239 °C; [^1]H NMR (300 MHz, CD$_3$OD) δ 7.30 (2H, d, J = 8.7 Hz), 6.76 (2H, d, J = 8.7 Hz), 6.38 (2H, d, J = 1.8 Hz), 6.32 (1H, t, J = 1.8 Hz); [^13]C NMR (75 MHz, CD$_3$OD) δ 161.62, 158.06, 134.65, 124.51, 117.83, 117.61, 113.01, 104.31, 98.69, 94.88.

Antioxidant Assay using ABTS. The radical cation was prepared by mixing an equal amount of 7 mM ABTS$^{\bullet+}$ (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) stock solution with 2.45 mM potassium persulfate stock solution. This mixture was stored at 0 °C for 12 h under darkness. The above ABTS solution was diluted with methanol appropriately to give UV absorption value of 1.000 at the 734 nm. The compounds **1-5** were dissolved in methanol to prepare 1000 μM stock solutions. Then these solutions were diluted with methanol to 500 μM, 250 μM, 125 μM, 62.5 μM, 31.25 μM, 15.63 μM, 7.81 μM, 3.90 μM, 1.95 μM, and 0.93 μM. In different 3 sets of test tubes, 0.9 mL of the ABTS solution and 0.1 mL of the compound solution were mixed under darkness. After 10 min of incubation, the UV absorption at 734 nm was measured. Control was used the mixture of 0.9 mL ABTS and 0.1 mL methanol. For the reference compound, ascorbic acid (Vitamin C) and quercetin were used. The radical scavenging rates were obtained from these UV absorption data and the resulting IC$_{50}$ values were calculated using Origin 8.

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