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Chemo-enzymatic synthesis of vinyl and L-ascorbyl phenolates and their inhibitory effects on advanced glycation end products

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ABSTRACT

This study successfully established the feasibility of a two-step chemo-enzymatic synthesis of L-ascorbyl phenolates. Intermediate vinyl phenolates were first chemically produced and then underwent *trans*-esterification with L-ascorbic acid in the presence of Novozyme 435® (*Candida Antarctica* lipase B) as a catalyst. Twenty vinyl phenolates and 11 ascorbyl phenolates were subjected to *in vitro* bioassays to investigate their inhibitory activity against advanced glycation end products (AGEs). Among them, vinyl 4-hydroxycinnamate (**17VP**), vinyl 4-hydroxy-3-methoxycinnamate (**18VP**), vinyl 4-hydroxy-3,5-dimethoxycinnamate (**20VP**), ascorbyl 4-hydroxy-3-methoxycinnamate (**18AP**) and ascorbyl 3,4-dimethoxycinnamate (**19AP**) showed 2–10 times stronger inhibitory activities than positive control (aminoguanidine and its precursors). These results indicated that chemo-enzymatically synthesized compounds have AGE inhibitory effect and thus are effective in either preventing or retarding glycation protein formation.

1. Introduction

Glycation is involved in structural and functional alterations of proteins and other cellular components. It is a process that occurs ubiquitously during normal aging, but is accelerated in a diabetic disease state. The final products of the glycation reaction are referred to as advanced glycation end products (AGEs); these fluorescent compounds have been suggested to be a potential cause of diabetes (Abraham, Swamy, & Perry, 1989). The increase in formation of AGEs is a major source of complications related to diabetes (Bonnefont-Rousselot, 2002). Both diabetes and aging are associated with accumulation of AGEs in tissue, increased oxidative stress and decline in antioxidant status. AGE accumulation has been implicated in various conditions such as retinopathy, cataracts, neuropathy, and chronic kidney disease. Intensive treatment to prevent oxidative stress may be a successful strategy for preventing diabetes-related complications.

Polyphenols are secondary plant metabolites and are abundant in fruits, vegetables, and natural beverages; these are thought to reduce the risk of chronic disease. They are an integral part of the human diet and widespread in natural plants and plant products (Pietta, Mauri, Simonetti, & Testolin, 1995). Various derivatives (i.e., phenolic acid, flavonoid, and anthocyanin) have been reported to have beneficial antioxidant effects in diabetes and its complications. The flavonoids isolated from the fruit of *Phellinus linteus* and *Colocasia esculenta* are reported to inhibit AGEs and confer an antioxidant effect (Lee et al., 2008a, 2008b; Li, Hwang, Kang, Hong, & Lim, 2014). Caffeic acid ethylene ester (vinyl caffeate) isolated from *Prunella vulgaris* displayed therapeutic potential in the prevention and treatment of diabetic complications by inhibiting both AGEs and aldose reductase (Li et al., 2012). These results suggest that vinyl phenolates and other phenolate esters could be good candidates for the development of a therapeutic anti-AGE agent.

L-ascorbic acid has long been considered an excellent natural antioxidant in cosmetics as well as a necessary and an important component of diet. It is a water-soluble vitamin and an essential nutrient. L-ascorbic acid is a strong reducing agent and acts as a primary defense against aqueous free radical attack in the blood (Na et al., 2006). Its' function has been widely studied both *in vitro* and *in vivo*, and its role in reducing oxidative stress is of particular interest in various disease states (including diabetes and related complications) (Dracha, Narkiewicz-Michałeka, Sienkiewicza, Szymulaa, & Bravo-Diazb, 2011). Various studies have reported the synthesis of ascorbyl esters catalyzed by lipases in organic solvent systems (Humeau, Girardin, Rovel, & Miclo, 1998) and solid-phase systems (Yan, Bornscheuer, & Schmid,

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1999). For instance, lipase-catalyzed synthesis of L-ascorbyl esters of varied fatty acids is widely reported, although many of the reports have focused specifically on the synthesis of L-ascorbyl-palmitate/linoleate/laurate/oleate. Another area of ongoing research involves L-ascorbyl-palmitate synthesized by *Candida antarctica* and *Bacillus stearothermophilus* SB1 lipase, which may have anticancer effects. This technique has also been successfully used to synthesize derivatives of L-ascorbic acid, with applications in artificial flavoring, cosmetics and as monosaccharides (Claon & Akoh, 1994; Tokiwa, Kitagawa, Raku, Yanagitanib, & Yoshino, 2007; Seino, Seino, Uchibori, Nishitani, & Inamasu, 1984). In this study, we attempted to synthesize phenolate esters, which are the esters of phenolic acids and L-ascorbic acid.

To expand upon previous research, we have attempted chemo-enzymatic *trans*-esterification of phenolic acids with L-ascorbic acid and evaluation using AGEs. To the best of our knowledge, the structure activity relationship (SAR) studies of vinyl and L-ascorbyl phenolates synthesized via chemo-enzymatic transformation of L-ascorbic acid and the AGE-inhibitory activity of phenolic acid have not been reported thus far in the literature. The SAR studies of synthetic compounds were analyzed, in order to assess their potential for further development for use in the food and pharmaceutical industries.

2. Materials and methods

2.1. Apparatus and reagents

Lipase acrylic resin from *Candida antarctica* (Novozyme 435®, 5 U/mg) was purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). One unit represents the microequivalents of fatty acid hydrolyzed from a triglyceride in 1 h at pH 7.2 at 37 °C. Salicylic acid (**1P**), 4-hydroxybenzoic acid (**2P**), 2,5-dihydroxybenzoic acid (**3P**), vanillic acid (**4P**), syringic acid (**5P**), 3,4-dimethoxybenzoic acid (**6P**), 4-chlorophenylacetic acid (**7P**), 4-hydroxyphenylacetic acid (**8P**), 3-phenylpropanoic acid (**9P**), 4-phenylbutyric acid (**10P**), 5-phenylvaleric acid (**11P**), 3-(4-hydroxyphenyl) propionic acid (**12P**), 3-(3,4-dihydroxyphenyl) propanoic acid (**13P**), cinnamic acid (**14P**), 2-hydroxycinnamic acid (**15P**), 3-hydroxycinnamic acid (**16P**), 4-hydroxycinnamic acid (**17P**), 4-hydroxy-3-methoxycinnamic acid (**18P**), 3,4-dimethoxycinnamic acid (**19P**), 4-hydroxy-3,5-dimethoxycinnamic acid (**20P**), vinyl acetate, molecular sieve (MS), *tert*-butanol (*t*-BuOH), L-ascorbic acid (AA), aminoguanidine hydrochloride (AG), quercetin (QC), methylglyoxal (40% aqueous solution), bovine serum albumin (essentially fatty acid free), D-gluconolactone, *N*-acetyl-glycyl-lysine methyl ester acetate salt (G.K. peptide), D-ribose and silica gel 60 F254 glass plates (0.25 mm thick, 20 × 20 cm) were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). Sulfuric acid was purchased from Daejung Chemicals & Metals Co., Ltd. (Siheung, Korea). All solvents and other reagents used in this study, unless otherwise specified, were analytical grade and purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.).

2.2. Preparative synthesis of vinyl and ascorbyl phenolates

Twenty vinyl phenolates (**VP**), including vinyl salicylate (**1VP**), vinyl 4-hydroxybenzoate (**2VP**), vinyl 2,5-dihydroxybenzoate (**3VP**), vinyl vanillate (**4VP**), vinyl syringate (**5VP**), vinyl 3,4-dimethxoybenzoate (**6VP**), vinyl 4-chlorophenylacetate (**7VP**), vinyl 4-hydroxyphenylacetate (**8VP**), vinyl 3-phenylpropanoate (**9VP**), vinyl 4-phenylbutyrate (**10VP**), vinyl 5-phenylvalerate (**11VP**), vinyl 3-(4-hydroxyphenyl) propionate (**12VP**), vinyl 3-(3,4-dihydroxyphenyl) propanate (**13VP**), vinyl cinnamate (**14VP**), vinyl 2-hydroxycinnamate (**15VP**), vinyl 3-hydroxycinnamate (**16VP**), vinyl 4-hydroxycinnamate (**17VP**), vinyl 4-hydroxy-3-methoxycinnamate (**18VP**), vinyl 3,4-dimethoxycinnamte (**19VP**) and vinyl 4-hydroxy-3,5-dimethoxycinnamate (**20VP**) were chemically synthesized and purified as previously described (Wang, Hwang, & Lim, 2015). The ascorbyl phenolates were synthesized via a two-step chemo-enzymatic route, wherein vinyl esters were produced as intermediates, which were subsequently esterified with L-ascorbic acid (AA). The vinyl esters (0.5 mM) and 5 mL of the selected organic solvents were added to 10 mL vials for reaction with AA (2 mM); this reaction was catalyzed by immobilized *Candida antarctica* lipase (Novozyme 435®). The 18 organic solvents screened for testing are listed in Table 1. The solutions were incubated at 55 °C with stirring for 48–168 h. The reaction progress was monitored regularly by TLC with methylene chloride/methanol elution (7:3, v/v). At the end of the reaction, the enzyme was filtered and the solvent was evaporated. The resulting solid residue was purified using silica gel chromatography with methylene chloride/methanol (9.7:0.3–8.5:1.5, v/v) as the eluent, to yield 11 ascorbyl phenolates (**APs**).

Table 1
Effects of solvents on conversion yield of L-ascorbyl cinnamate.

Solvent	LogP[a]	Yield (%)[b]
Methnol	−0.74	ND[c]
Acetonitrile	−0.33	6.49
Ethanol	−0.3	ND
Acetone	0.23	8.17
n-Propanol	0.25	ND
Tetrahydrofuran	0.49	10.21
tert-Butanol	0.6	12.12
Ethyl acetate	0.65	3.67
n-Butanol	0.8	4.93
Diethyl ether	0.89	5.54
4-Methyl-2-pentanone	1.31	6.71
n-Pentanol	1.34	4.74
Chloroform	1.97	3.22
Benzene	2.13	2.39
2-Methyl-butane	3.1	3.55
n-Pentane	3.45	4.63
n-Hexane	3.5	7.86
Isooctane	4.37	9.88

*The reactions were carried out at 50 °C. Vinyl cinnamate (0.5 mM) and L-ascorbic acid (2.0 mM) with 30 mg of *Candida Antarctica* (Novozyme 435®) 1500 Unit (60 mg/mL) in different solvents (5 mL) for 24 h.
[a] LogP, where P is the partition coefficient of a given solvent between water and *n*-octanol, is a parameter to describe solvent hydrophobicity.
[b] The conversion yield is the ratio of the amount of product actually obtained to the maximum amount of product possible.
[c] Not determined.

The percentage yield of the reaction was calculated as the ratio of the obtained amount of product to the maximum theoretical amount, multiplied by 100%. The structures of the isolated **APs** were confirmed by ^{1}H and ^{13}C NMR. ^{1}H and ^{13}C NMR spectra were recorded at 400 MHz in $(CD_3)_2SO$, using a Bruker Avance 400 NMR spectrometer (Karlsrube, Germany) operating at 400 and 100 MHz, respectively. Chemical shifts are expressed in ppm, using tetramethylsilane (TMS) as an internal standard.

2.3. Hemoglobin-δ-gluconolactone assay on the amadori compound formation (early stage)

Evaluation of initial stage of protein glycation was determined by δ-gluconolactone assay (Rahbar, Yernini, Scott, Gonzales, & L1alezari, 1999). Briefly, fresh human blood (50 mg/mL) was incubated with glucose (144 mg/mL) in phosphate buffer (pH 7.4) containing 0.2 g/L NaN_3 under sterile, dark conditions at 37 °C for 7 days. In certain experiments, the indicated sample was added to the model system in the concentration range of 0.01–1 mM. Fluorescence of samples was measured at the excitation and

emission maxima of 355 nm and 460 nm, respectively. AG was tested as a known inhibitor.

2.4. Bovine serum albumin-methylglyoxal assay on AGE formation (middle stage)

Bovine serum albumin (50 mg/mL) was incubated at 37 °C for 24 h with methylglyoxal (100 mM) in sodium phosphate buffer (0.1 M, pH 7.4) in the presence of various concentrations of the compounds (including a control). The dimethylsulfoxide used for dissolving samples was found to have no effect on the reaction. All reagents and samples were sterilized by filtration through 0.2 mm membrane filters. The fluorescent intensity was measured at an excitation wavelength of 355 nm and an emission wavelength of 460 nm with a Luminescence spectrometer LS50B (Perkin-Elmer Ltd., Buckinghamshire, England) (Wu & Yen, 2005). AG was tested as a positive control. The concentration of each test sample exhibiting 50% inhibition of activity (IC_{50}) was estimated from the least squares regression line of the logarithmic concentration plotted against the remaining activity.

2.5. N-acetyl-glycyl-lysine-methyl ester D-ribose assay on the AGEs cross-linking (late stage)

This test was used to evaluate the ability of samples to inhibit the cross-linking of the GK peptide in the presence of D-ribose using the method described by Rahbar et al. (1999). GK peptide (26.7 mg/mL) was incubated with D-ribose (200 mg/mL) in sodium phosphate buffer (0.5 M, pH 7.4) under sterile conditions at 37 °C for 24 h. The synthesized compounds were added to the model system at a final concentration of 1 mM with the exception of AG, which was used at 10 and 50 mM. At the end of the incubation period, the fluorescent intensity was measured at an excitation wavelength of 335 nm and an emission wavelength of 460 nm.

2.6. Statistical analysis

Inhibition rates were calculated as percentages (%) with respect to the control value and IC_{50} value was defined as the concentration at which 50% inhibition occurs. Data are expressed as mean values ± SD of triplicate experiments and comparisons among data were carried out using Student's unpaired *t*-tests ($P < 0.05$).

3. Results and discussion

3.1. Synthesis of vinyl phenolates and ascorbyl phenolates

In the present study, 20 **VPs** were synthesized via a chemical vinyl exchange reaction as previously described (Fig. 1a and b). The synthesized **VPs** were used as intermediates to produce **APs** using Novozyme 435® as catalyst. Enzymatic reaction offers several advantages over other methods, including mild reactions conditions, minimization of side reactions and low environment impact. However, application of this process is limited owing to lengthy reaction times and poor yields. To improve the reaction efficiency of yield in a lipase-catalyzed reaction, vinyl esters were used as acyl donors, owing to their affinity for the lipase active site to form the acyl-enzyme intermediate.

APs were synthesized via *trans*-esterification, which was synthesized by vinyl cinnamate (**14VP**) in *t*-BuOH. The conversion yield of the acylation in other solvents (methanol, ethanol, *n*-propanol, *n*-butanol, *n*-pentanol, acetone, 4-methyl-2-pentanone, tetrahydofuran, 2-methyl-butane, *n*-pentane, hexane, isooctane, benzene, ethyl acetate, diethyl ether, chloroform, acetonitrile) ranged from 3% to 7%, which was lower than that in *t*-BuOH under the same conditions (Table 1). Otto et al. (1998) reported that the esterification yield of cinnamic and phenylbutric acid from AA in a reaction catalyzed by Novozyme 435® lipase in hydrocarbons such as *t*-BuOH. In addition, Yan et al. (1999) **used aliphatic acids to achieve the enzymatic acylation of AA in the presence of Novozyme 435® in *t*-BuOH. Our results were similar to those reported in literature. The conversion yield of ascorbyl cinnamate (**14AP**) was inconsistent at increasing solvent polarity conditions. Log *P*, where *P* is the partition coefficient of a given solvent between water and *n*-octanol, is a widely used parameter to describe solvent hydrophobicity and its effects on lipase activity. Accordingly, solvents with log $P < 2$ are more hydrophilic and tend to strip away the water molecules present on the surface of the lipase, thereby decreasing the catalytic activity. Conversely, solvents with high log *P* values are hydrophobic, stabilize lipases and are the most suitable solvents for biocatalytic processes. However, our results showed different correlations between log *P* and conversion yield from that mentioned in the above theory. The polarity of the organic solvent potentially not only affects the lipase stability but also the substrate solubility. Solvent polarity and substrate solubility decrease at increasing log *P* values. No product could be detected when methanol, ethanol, or propanol were used, most likely owing to lipase damage as well as poor substrate solubility. Hence, *t*-BuOH was chosen for better conversion yield.

A total of 11 **APs** were successfully synthesized and isolated by silica gel column chromatography. The structures of the acylated **APs** were confirmed by ^1H and ^{13}C NMR. A peak corresponding to the new ester at the C-6 position of AA was observed and the signals from vinyl peaks had disappeared. The regioselectivity of the lipase-catalyzed acylation was specific at the C-6 position of 11 with the use of an AA moiety. Negative effects on lipase activity were mediated by different factors, including the presence of hydroxyl groups on the aromatic ring of phenolic acids, 5-heterocyclic structure of AA or a single/double bond and the number of carbons in side chain of **APs**. Furthermore, adsorption of silica gel owing to the hydroxyl groups of AA or **APs** affected the isolation of the products.

Ascorbyl 3,4-dimethoxybenzoate (6AP): 6AP was a white solid powder. R_f 0.23 [methylene chloride:methanol (7:3)]; ESI-MS (*m*/*z*) 341.28 $[M+H]^+$ ($C_{15}H_{16}O_9$). ^1H NMR [400 MHz, CD_3OD]: δ 7.62 (d, 1H, J = 8.33 Hz, H-6′), 7.50 (s, 1H, H-2′), 6.93 (d, 1H, J = 8.51 Hz, H-5′), 4.38 (d, 1H, J = 1.64 Hz, H-4), 3.79 (s, 6H, $-OCH_3$), 3.78 (t, 2H, J = 5.76 Hz, H-6ab), 3.24 (m, 1H, H-5). ^{13}C NMR [100 MHz, CD_3OD]: δ 173.6 (C-1), 168.1 (C-7′), 155.4 (C-3), 154.5 (C-4′), 150.6 (C-3′), 125.5 (C-1′), 123.9 (C-6′), 120.6 (C-2), 113.9 (C-5′), 112.3 (C-2′), 77.8 (C-4), 68.6 (C-5), 67.0 (C-6), 56.9 ($-OCH_3$).

Ascorbyl 4-chlorophenylacetate (7AP): 7AP was a white solid powder. R_f 0.28 [methylene chloride:methanol (7:3)]; ESI-MS (*m*/*z*) 328.70 $[M+H]^+$ ($C_{14}H_{13}O_7Cl$). ^1H NMR [400 MHz, $(CD_3)_2SO$]: δ 7.39-7.27 (m, 4H, H-2′, 4′, 5′, 6′), 4.69 (d, 1H, J = 1.54 Hz, H-4), 4.10 (t, 2H, J = 5.79 Hz, H-6ab), 3.97 (m, 1H, H-5), 3.73 (dd, 2H, H-7′ab). ^{13}C NMR [100 MHz, $(CD_3)_2SO$]: δ 171.1 (C-1), 171.0 (C-8′), 152.5 (C-3), 134.5 (C-4′), 133.7 (C-1′), 131.9 (C-2′, 6′), 128.6 (C-3′, 5′), 118.5 (C-2), 75.3 (C-4), 65.8 (C-5), 65.4 (C-6), 48.9 (C-7′).

Ascorbyl 4-hydroxyphenylacetate (8AP): 8AP was a white solid powder. R_f 0.27 [methylene chloride:methanol (7:3)]; ESI-MS (*m*/*z*) 311.26 $[M+H]^+$ ($C_{14}H_{14}O_8$). ^1H NMR [400 MHz, $(CD_3)_2SO$]: δ 7.07–7.02 (m, 2H, H-2′, 6′), 6.71-6.68 (m, 2H, H-3′, 5′), 4.65 (d, 1H, J = 1.58 Hz, H-4), 4.07 (t, 2H, J = 5.84 Hz, H-6ab), 3.97 (m, 1H, H-5), 3.59 (dd, 2H, H-7′ab). ^{13}C NMR [100 MHz, $(CD_3)_2SO$]: δ 171.8 (C-1), 170.7 (C-8′), 156.5 (C-4′), 155.1 (C-3), 130.7 (C-2′, 6′), 125.5 (C-1′), 118.5 (C-2), 115.5 (C-3′, 5′), 75.3 (C-4), 65.8 (C-6), 65.2 (C-5), 48.9 (C-7′).

Ascorbyl 4-phenylbutyrate (9AP): 9AP was a white solid powder. R_f 0.30 [methylene chloride:methanol (7:3)]; ESI-MS (*m*/*z*) 323.31 $[M+H]^+$ ($C_{16}H_{18}O_7$. ^1H NMR [400 MHz, $(CD_3)_2SO$]: δ

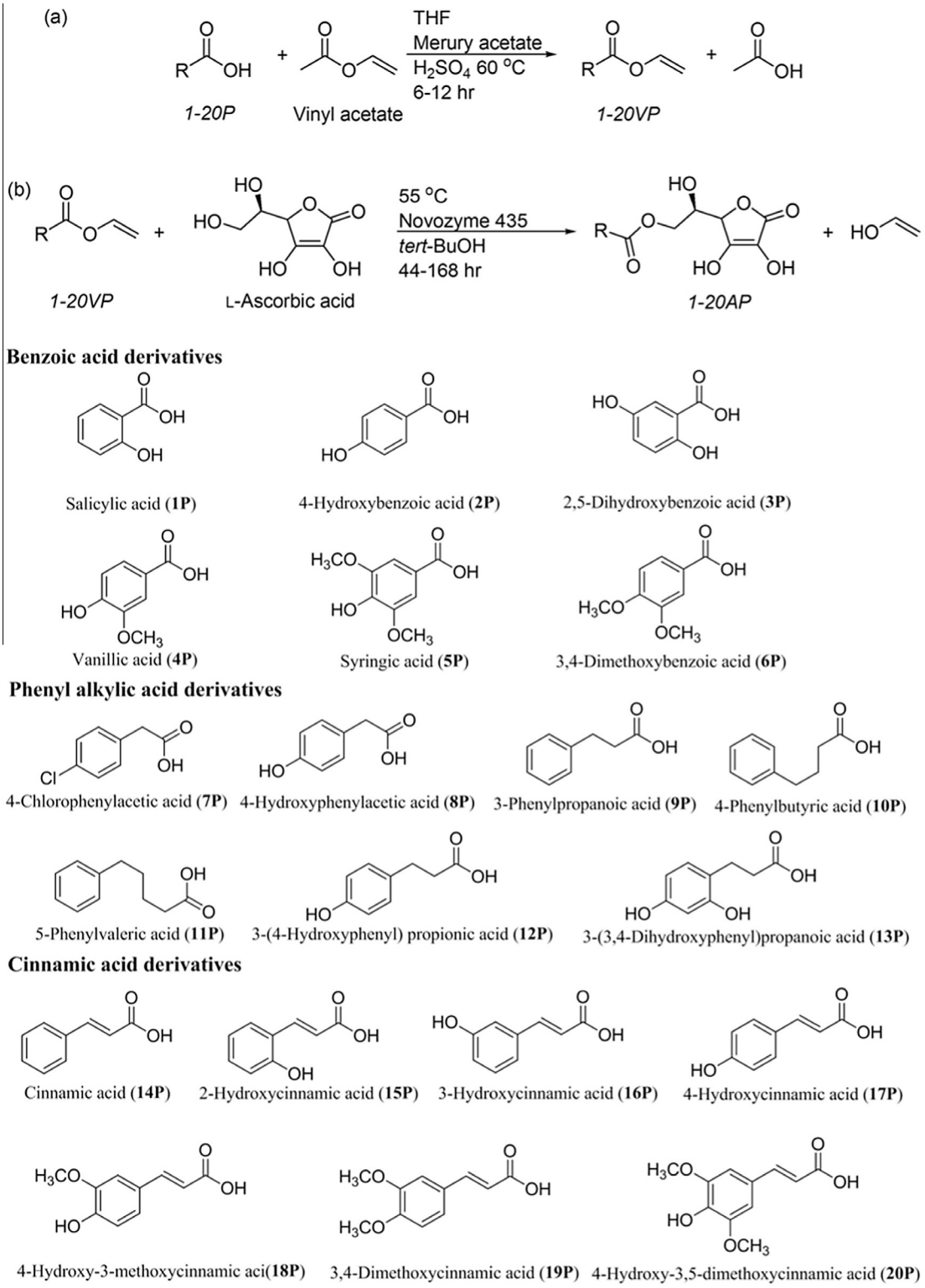

Fig. 1. Scheme for synthesis of vinyl phenolates (a) and ascorbyl phenolates (b) and structures of phenolic acids used in this study.

7.30-7.16 (m, 5H, H-2′, 3′, 4′, 5′, 6′), 4.68 (d, 1H, J = 1.60 Hz, H-4), 4.08 (t, 1H, J = 5.53 Hz, H-6ab), 3.97 (m, 1H, H-5), 2.59 (t, 2H, J = 7.61 Hz, H-7′ab), 2.33 (t, 2H, J = 7.44 Hz, H-9′ab), 1.82 (q, 2H, J = 7.55 Hz, H-8′ab). ^{13}C NMR [100 MHz, $(CD_3)_2SO$]: δ 173.4 (C-1), 171.2 (C-10′), 153.0 (C-3), 142.4 (C-1′), 129.1 (C-2′, 3′, 5′, 6′), 126.7 (C-4′), 119.0 (C-2), 75.9 (C-4), 66.4 (C-6), 65.4 (C-5), 35.2 (C-7′), 33.7 (C-9′), 27.1 (C-8′).

Ascorbyl 5-phenylvalate (11AP): 11AP was a white solid powder. R_f 0.33 [methylene chloride:methanol (7:3)]; ESI-MS (m/z) 353.38 $[M+H]^+$ ($C_{18}H_{24}O_7$). ^{1}H NMR [400 MHz, $(CD_3)_2SO$]: δ 7.28-7.25 (m, 5H, H-2′, 3′, 4′, 5′, 6′), 4.66 (d, 1H, J = 1.57 Hz, H-4), 4.06 (t, 2H, J = 6.14 Hz, H-6ab), 3.96 (m, 1H, H-5), 2.58 (t, 2H, J = 7.10 Hz, H-7′ab), 2.36 (t, 2H, J = 6.93 Hz, H-10′ab), 1.59-1.54 (m, 4H, H-8′ab, 9′ab). ^{13}C NMR [100 MHz, $(CD_3)_2SO$]: δ 173.1

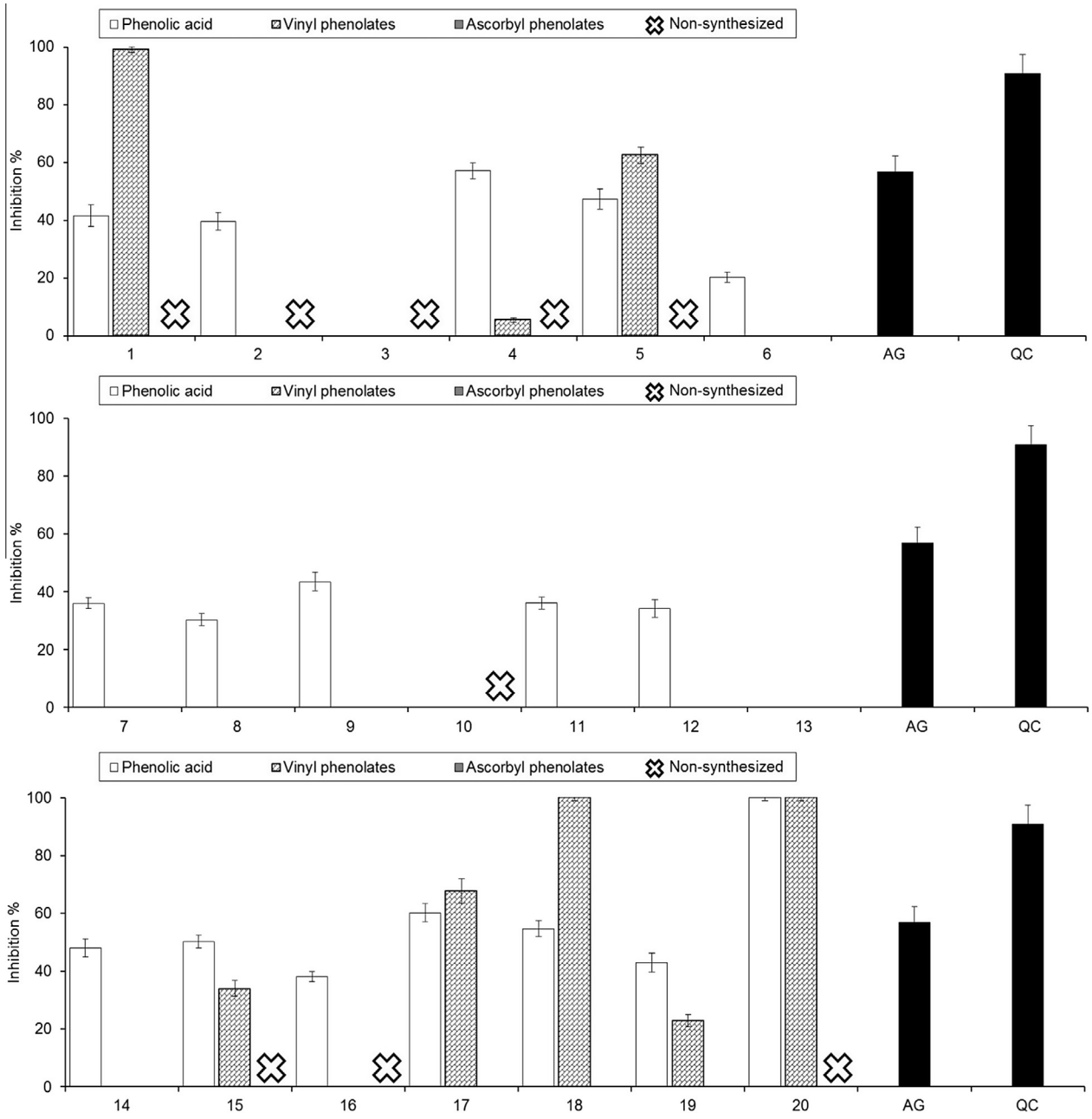

Fig. 2. Inhibition of amadori product formation. (A) Benzoic acid derivatives; (B) phenyl alkylic acid derivatives; (C) cinnamic acid derivatives. Inhibition rates were calculated as percentages (%) with respect to the control value. All values were expressed as mean ± SD of triplicate experiments at the concentration of compound 1 mM. Aminoguanidine (AG) and quercetin (QC) were tested as positive controls at the concentration of 5 mM.

(C-1′), 170.7 (C-11′), 152.5 (C-3′), 142,3 (C-1′), 128.6 (C-2′, 3′, 5′, 6′), 126.0 (C-4′), 118.6 (C-2), 75.3 (C-4), 65.8 (C-6), 64.8 (C-5), 35.1 (C-7′), 33.5 (C-10′), 30.6 (C-8′), 24.3 (C-9′).

Ascorbyl 3-(4-hydroxyphenyl) propionate (12AP): 12AP was a white solid powder. R_f 0.27 [methylene chloride:methanol (7:3)]; ESI-MS (*m*/*z*) 325.28 $[M+H]^+$ ($C_{15}H_{16}O_8$). ^{1}H NMR [400 MHz, $(CD_3)_2SO$]: δ 7.02–6.98 (m, 2H, H-2′,6′), 6.67-6.65 (m, 2H, H-3′, 5′), 4.66 (d, 1H, *J* = 1.76, H-4), 4.05 (t, 2H, *J* = 3.88, H-6ab), 3.96 (m, 1H, H-5), 2.75 (t, 2H, *J* = 7.55, H-7′ab), 2.50 (t, 2H, *J* = 7.52, H-8′ab). ^{13}C NMR [100 MHz, $(CD_3)_2SO$]: δ 174.3 (C-1), 172.5 (C-9′), 155.9 (C-3), 152.5 (C-4′), 133.9 (C-1′), 129.5 (C-2′, 6′), 118.5 (C-2), 115.5 (C-3′, 5′), 75.3 (C-4), 65.8 (C-6), 64.9 (C-5), 35.8 (C-8′), 29.9 (C-7′).

Ascorbyl 3-(3, 4-dihydroxyphenyl) propanate (13AP): 13AP was a white solid powder. R_f 0.21 [methylene chloride:methanol (7:3)]; ESI-MS (*m*/*z*) 341.28 $[M+H]^+$ ($C_{15}H_{16}O_9$). ^{1}H NMR [400 MHz, CD_3OD]: δ 7.06–6.84 (m, 3H, H-2′, 5′, 6′), 4.37 (d, 1H, *J* = 2.03 Hz, H-4), 3.95 (t, 2H, *J* = 5.56 Hz, H-6ab), 3.84 (m, 1H, H-5), 2.73 (t, 2H, *J* = 7.57 Hz, H-7′ab), 2.47 (t, 2H, *J* = 7.57 Hz, H-8′ab). ^{13}C NMR [100 MHz, CD_3OD]: δ 173.3 (C-1), 172.2 (C-9′), 153.3 (C-3), 151.3 (C-3′), 148.8 (C-4′), 133.4 (C-1′), 128.4 (C-6′), 126.3 (C-2′), 119.0 (C-2), 115.9 (C-5′), 76.1 (C-4), 66.9 (C-6), 64.8 (C-5), 35.6 (C-8′), 30.8 (C-7′).

Ascorbyl cinnamate (14AP): 14AP was a white solid powder. R_f 0.28 [methylene chloride:methanol (7:3)]; ESI-MS (*m*/*z*) 307.27 $[M+H]^+$ ($C_{15}H_{14}O_7$). ^{1}H NMR [400 MHz, $(CD_3)_2SO$]: δ 7.74-7.72 (m, 3H, H-2′, 6′, 7′), 7.45-7.41 (m, 2H, H-3′, 4′, 5′), 6.66 (d, 1H, *J* = 16.11 Hz, H-8′), 4.78 (d, 1H, *J* = 1.64 Hz, H-4), 4.20 (t, 2H, *J* = 7.62 Hz, H-6ab), 4.06 (m, 1H, H-5). ^{13}C NMR [100 MHz, $(CD_3)_2SO$]: δ 170.7 (C-1), 166.3 (C-9′), 152.7 (C-3), 145.2 (C-7′), 134.3 (C-1′), 129.3 (C-2′,

Table 2
Inhibitory effects of vinyl and L-ascobyl phenolates on advanced glycation end products.

Entry		Product	Early stage[a]	Middle stage	Last stage
			IC_{50}, mM[b]		
		Aminoguanidine[c]	3.99 ± 0.28	6.95 ± 0.49	4.20 ± 0.31
		Quercetin[d]	0.24 ± 0.02***	1.11 ± 0.09***	0.71 ± 0.03***
17	Ps	4-Hydroxycinnamic acid	–	–	–
	VP	Vinyl 4-hydroxycinnamate	–	1.15 ± 0.11***	–
	AP	Ascorbyl 4-hydroxycinnamate	–	–	–
18	Ps	4-Hydroxy-3-methoxycinnamic acid	3.40 ± 0.31**	–	–
	VP	Vinyl 4-hydroxy-3-methoxycinnamate	0.12 ± 0.01***	0.23 ± 0.01***	1.72 ± 0.13***
	AP	Ascorbyl 4-hydroxy-3-methoxycinnamate	–	2.89 ± 0.29***	3.73 ± 0.36
19	Ps	3,4-Dimethoxycinnamic acid	–	–	–
	VP	Vinyl 3,4-dimethoxycinnamate	–	–	–
	AP	Ascorbyl 3,4-dimethoxycinnamate	–	3.73 ± 0.30***	2.63 ± 0.20***
20	Ps	4-Hydroxy-3,5-dimethoxycinnamic acid	–	2.13 ± 0.24***	4.72 ± 0.42
	VP	Vinyl 4-hydroxy-3,5-dimethoxycinnamate	–	0.34 ± 0.02***	2.00 ± 0.15***
	AP	NS[e]	–	–	–

[a] All values were expressed as mean ± SD of triplicate experiments at the concentration of compound 1 mM.
[b] The IC_{50} (mM) value was defined as the concentration at which 50% inhibition occurs.
[c,d] Aminoguanidine (AG) and quercetin (QC) were tested as positive controls at the concentration of 5 mM.
[e] Non-synthesized.
Statistical significance of differences was calculated between AG and synthetic products. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

6′), 128.5 (C-3′, 5′), 127.9 (C-4′), 118.5 (C-2), 118.2 (C-8′), 75.5 (C-4), 65.9 (C-6), 65.1 (C-5).

Ascorbyl 4-hydroxycinnamate (17AP): 17AP was a white solid powder. R_f 0.25 [methylene chloride:methanol (7:3)]; ESI-MS (*m/z*) 323.27 $[M+H]^+$ ($C_{15}H_{14}O_8$). ^{1}H NMR [400 MHz, $(CD_3)_2SO$]: δ 7.62 (d, 1H, *J* = 16.02 Hz, H-7′), 7.23 (d, 1H, *J* = 7.81 Hz, H-5′), 7.14 (d, 1H, *J* = 7.79 Hz, H-6′), 7.04 (s, 1H, H-2′), 6.84 (d, 1H, *J* = 8.03, H-4′), 6.52 (d, 1H, *J* = 16.01 Hz, H-8′), 4.78 (d, 1H, *J* = 1.59 Hz, H-4), 4.18 (t, 2H, *J* = 5.49 Hz, H-6ab), 4.06 (m, 1H, H-5). ^{13}C NMR [100 MHz, $(CD_3)_2SO$]: δ 171.2 (C-1), 166.8 (C-9′), 158.5 (C-4′), 153.1 (C-3), 145.9 (C-7′), 136.1 (C-2′), 130.8 (C-6′), 127.8 (C-1′), 120.1 (C-2), 118.5 (C-8′), 115.5 (C-5′), 115.5 (C-3′), 75.9 (C-4), 66.4 (C-6), 65.6 (C-5)

Ascorbyl 4-hydroxy-3-methoxycinnamate (18AP): 18AP was a white solid powder. R_f 0.29 [methylene chloride:methanol (7:3)]; ESI-MS (*m/z*) 353.29 $[M+H]^+$ ($C_{16}H_{16}O_9$). ^{1}H NMR [400 MHz, $(CD_3)_2SO$]: δ 7.48 (d, 1H, *J* = 13.88 Hz, H-7′), 7.13 (d, 1H, *J* = 8.23 Hz, H-6′), 7.07 (s, 1H, H-2′), 6.79 (d, 1H, *J* = 8.20 Hz, H-5′), 6.35 (d, 1H, *J* = 15.97 Hz, H-8′), 4.77 (d, 1H, *J* = 1.76 Hz, H-4), 4.19 (t, 2H, *J* = 6.64 Hz, H-6ab), 4.13 (m, 1H, H-5), 3.82 (s, 3H, –OCH_3). ^{13}C NMR [100 MHz, $(CD_3)_2SO$]: δ 170.7 (C-1), 168.3 (C-9′), 152.6 (C-3), 149.4 (C-3′), 148.2 (C-4′), 144.8 (C-7′), 126.1 (C-1′), 123.5 (C-6′), 118.5 (C-2), 115.9 (C-5′), 114.6 (C-2′), 111.6 (C-8′), 75.6 (C-4), 66.0 (C-6), 64.9 (C-5), 56.0 (–OCH_3).

Ascorbyl 3, 4-dimethoxycinnamate (19AP): 19AP was a white solid powder. R_f 0.33 [methylene chloride:methanol (7:3)]; ESI-MS (*m/z*) 367.32 $[M+H]^+$ ($C_{17}H_{18}O_9$). ^{1}H NMR [400 MHz, $(CD_3)_2SO$]: δ 7.64 (d, 1H, *J* = 15.99 Hz, H-7′), 7.36 (s, 1H, H-2′), 6.97 (d, 1H, *J* = 6.06 Hz, H-5′), 6.58 (d, 1H, *J* = 15.97 Hz, H-8′), 6.58 (d, 1H, *J* = 15.97 Hz, H-6′), 4.77 (d, 1H, *J* = 1.57 Hz, H-4), 4.16 (t, 2H, *J* = 5.56 Hz, H-6ab), 4.13 (m, 1H, H-5), 3.80 (s, 6H, –OCH_3). ^{13}C NMR [100 MHz, $(CD_3)_2SO$]: δ 171.2 (C-1), 167.1 (C-9′), 153.1 (C-3), 151.9 (C-3′), 149.8 (C-4′), 145.9 (C-7′), 127.7 (C-1′), 123.9 (C-6′), 119.0 (C-2), 116.1 (C-8′), 112.4 (C-5′), 111.1 (C-2′), 75.9 (C-4), 66.5 (C-6), 65.4 (C-5), 56.3 (–OCH_3).

3.2. Inhibitory effect of vinyl phenolates and ascorbyl phenolates on AGE formation

3.2.1. Inhibitory effects of vinyl phenolates and ascorbyl phenolates on the early stage of protein glycation

Amadori compounds are the early- stage products of glycation. The inhibitory effects of the synthesized vinyl phenolates and ascorbyl phenolates on amadori compound formation were evaluated by a method of Peng et al. (2008) with some modifications. The assay is specific for investigation of early glycation amadori product formation inhibitors (Rahbar et al., 1999). As shown in Fig. 2, the result indicated that vinyl salicylate (**1VP**), vinyl 4-hydroxycinnamate (**17VP**), vinyl 4-hydroxy-3-methoxycinnamate (**18VP**) and vinyl 4-hydroxy-3,5-dimethoxycinnamate (**20VP**) had the most potent inhibitory effects (163.10, 67.67, 164.45 and 132.81%, respectively). Compounds **1VP**, **18VP** and **20VP** were shown to inhibit amadori product formation more effectively than AG. The, IC_{50} values of **18VP** and its precursor were 0.12 and 3.40 mM, respectively, whereas the IC_{50} value of AG was 3.99 mM (Table 2). Some synthetic products AGE-inhibitory activity may be weakened through vinylation. In contrast, the **APs** showed no effect on the early stage of protein glycation.

3.2.2. Inhibitory effects of vinyl phenolates and ascorbyl phenolates against the middle stage of protein glycation

The bovine serum albumin-methylglyoxal assay is used specifically to investigate inhibitors of the middle stage of protein glycation formation and was performed according to the method characterized by Lee et al. (2008a, 2008b). The inhibitory effects of the 20 **VPs** and 11 **APs** against methylglyoxal-mediated protein glycation were evaluated in an albumin-methylglyoxal system. Inhibitory activity of the synthesized compounds against AGE formation was tested using AG as a positive control. Of all the **VP**s, **15VP** (61.84%), **17VP** (87.01%), **18VP** (127.17%), **19VP** (41.31%) and **20VP** (83.46%) showed the highest AGE-inhibitory activity, better than that of AG (29.22%) in Fig. 3. Among them, **17VP**, **18VP** and **20VP** exhibited the most potent inhibitory activity against AGE formation with IC_{50} values of 1.15, 0.23 and 0.34 mM, respectively, compared with AG (IC_{50} 6.95 mM). These compounds showed stronger inhibitory activity than the precursor compounds. However, **3VP** (26.86%) and **15VP** (61.84%) had less AGE-inhibitory activity compared to the precursor compounds. Some **VP** compounds show minimally increased inhibitory activities compared to their precursor molecules. In the case of **APs**, not all compounds showed inhibitory activity against the early stage of glycation. On the other hand, all compounds exhibited at least slight inhibitory activity against middle-stage AGE formation with rates of inhibition ranging from 5.42% to 65.40%. The inhibitory activities of ascorbyl 4-hydroxy-3-methoxycinnamate (**18AP**, IC_{50} 2.89 mM) and 3,4-dimethoxycinnamate (**19AP**, IC_{50} 3.73 mM) were 2.40- and 1.86-fold better than that of AG (IC_{50} 6.95 mM), respectively.

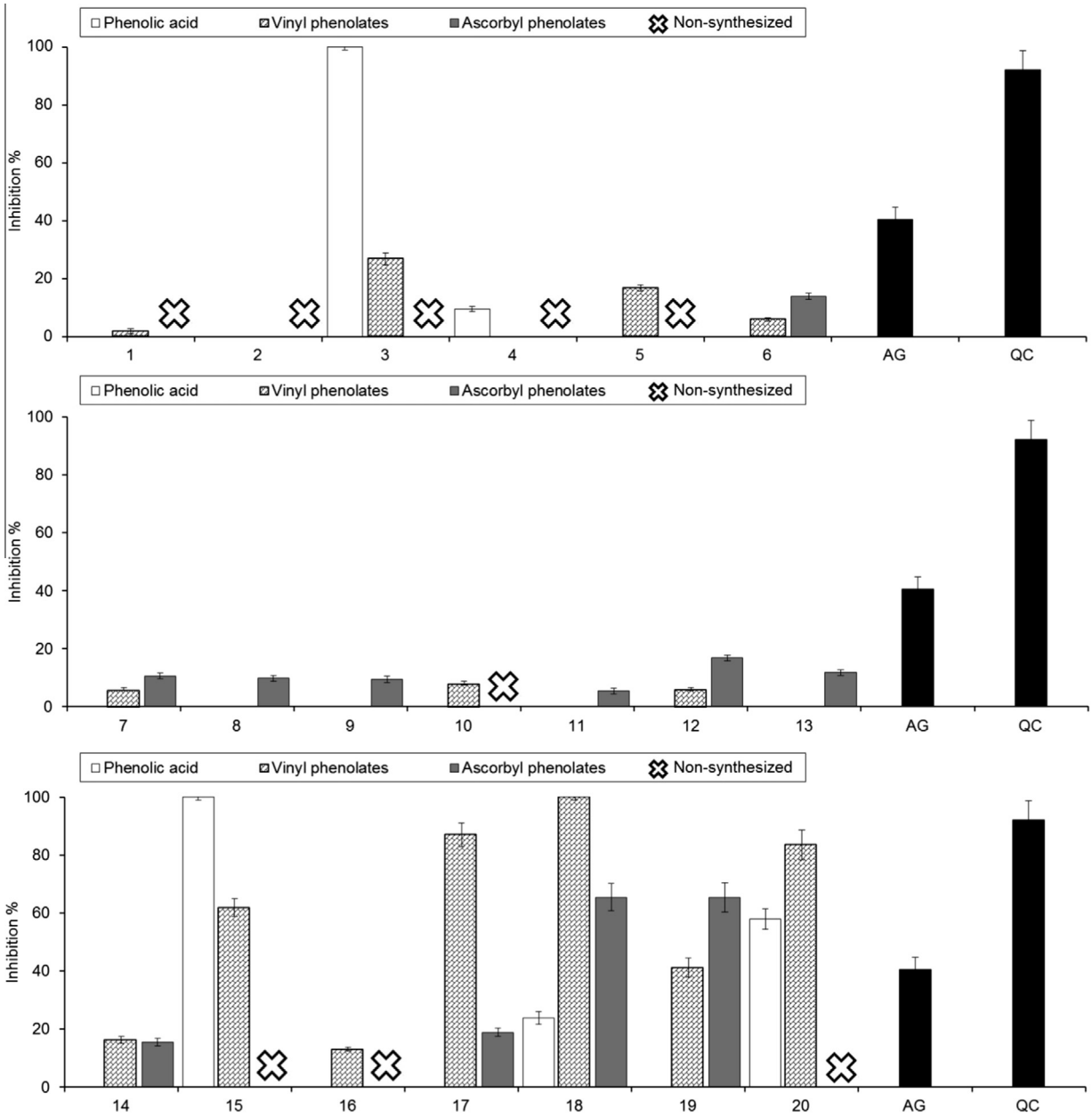

Fig. 3. Inhibition of AGE formation. (A) Benzoic acid derivatives; (B) phenyl alkylic acid derivatives; (C) cinnamic acid derivatives. Inhibition rates were calculated as percentages (%) with respect to the control value. All values were expressed as mean ± SD of triplicate experiments at the concentration of compound 1 mM. Aminoguanidine (AG) and quercetin (QC) were tested as positive controls at the concentration of 5 mM.

3.2.3. Inhibitory effects of vinyl phenolates and ascorbyl phenolates on the last stage of protein glycation

We used a synthetic peptide containing a lysine residue (GK peptide) to evaluate the inhibitory effects of **VPs** and **APs** on protein cross-linking. This test is used to evaluate the ability of synthetic compounds to inhibit the cross-linking of GK peptide (cross-linking of last glycation products) in the presence of ribose, using the method described by Nakagawa, Yokozawa, Terasawa, Shu, and Juneja (2002). The AGE-inhibitory activities of the synthesized compounds were evaluated using AG as a positive control. Compounds **18VP** and **20VP** had the strongest AGE-inhibitory activities of 61.85% and 61.23%, respectively, which were better than that of AG (56.48%) at the same concentration. These compounds also exhibited the most potent inhibitory activity with IC_{50} values of 1.72 and 2.00 mM, respectively. The AGE cross-linking inhibition of the **APs** is shown in Fig. 4; all compounds showed varied inhibitory effects ranging from 37.86% to 67.84%. Among them, **18AP** and **19AP** had IC_{50} values of 3.73 and 2.63 mM, respectively, compared to positive control AG (IC_{50} 4.20 mM, Table 2). In addition, **APs** showed strong inhibitory activity ranging from 37.86% to 67.17% in last stage of glycation.

3.2.4. Structure activity relationship analysis

The anti-AGE behavior of **VP** derivatives and the related SAR were investigated using the AGE assay. AGE-inhibitory effects of **VP** derivatives depend on the number and site of hydroxyl and methyl groups of the aromatic ring in the cinnamic acid structure (including a double bond). Removal of the hydroxyl and methyl substitutions on the aromatic ring conferred stronger AGE inhibitory activity to the compounds. **VPs** containing a single hydroxyl and multiple methyl moieties at 4 positions showed last-stage AGE inhibition that was several fold stronger than that of AG and

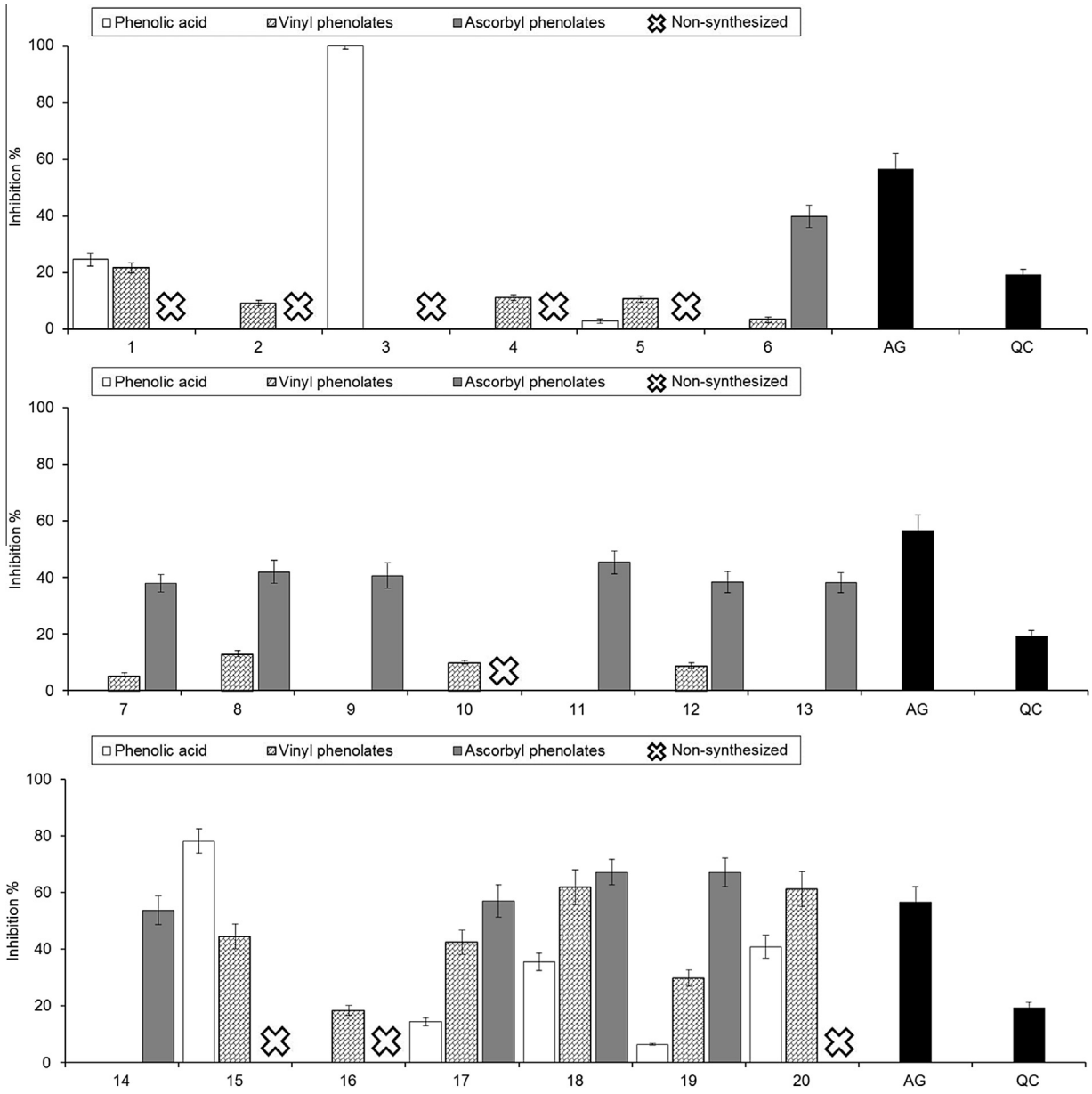

Fig. 4. Inhibition of cross-linking. (A) Benzoic acid derivatives; (B) phenyl alkylic acid derivatives; (C) cinnamic acid derivatives. Inhibition rates were calculated as percentages (%) with respect to the control value. All values were expressed as mean ± SD of triplicate experiments at the concentration of compound 1 mM. Aminoguanidine (AG) and quercetin (QC) were tested as positive controls at the concentration of 5 mM.

its precursor analogue. Hydroxyl substitution at other positions confers no activity. In addition, according to the results for **18VP** and **20VP**, substitution of the methoxy group at the C-3 position and the hydroxyl group at the *para*-position of the ring markedly affected AGE-inhibitory activity. Wu, Huang, Lin, and Yen (2011) reported that phenolic acids, especially methoxyphenolic acid derivatives, were potent inhibitors of multiple stages of glycation. Additionally, vanillic, syringic, ferulic and sinapic acids had methoxyl groups, which significantly inhibited amadori product formation and protein cross-linking. A possible mechanism by which phenolic acids inhibit protein glycation could be related to their antioxidant properties. Many structural properties of B rings of many flavonoids that inhibit AGE formation have been reported. Increasing the number of hydroxyl groups at the 3′ and 4′ positions of the B ring in luteolin increased the inhibitory activities against each stage of protein glycation. In addition, methylation of a 3′ or 4′ hydroxyl group in the B ring of flavones or at the 3′ or 5′ of the B ring of flavonols or flavanones affected their inhibitory activity against glyco-oxidative reaction. This suggested that the hydroxyl and methoxyl groups at the C-3′ and 4′ position of B ring contributes to the AGE inhibitory activity (Jung et al., 2007). However, these flavonoids structure do not have an established SAR to explain the anti-glycation activity demonstrated in the assays above.

4. Conclusion

In the present study, **APs** were bio-catalytically synthesized via a two-step chemo-enzymatic method, in which **VPs** were first synthesized (as the intermediates) by chemical esterification. This demonstrates that it may be feasible to synthesize other similar compounds. In addition, in all stages of protein glycation, **18VP** showed the strongest inhibitory activity among the **VPs**. Some others, including **17VP**, **20VP**, **18AP** and **19AP** had significant

inhibitory effects at each stage. Our results show that generally, AGE-inhibitory activities were 2–10 times stronger for **VPs** than **APs** (**VPs** > **APs** > **AG** or **Ps** > **AA**). These results indicate that synthetic compounds created via a chemo-enzymatic method are effective in either preventing or retarding glycation protein formation.

Conflict of interest

The authors declare that there are no conflicts of interest.

Chemical compounds studies in this article

Vinyl 4-hydroxycinnamate
A white solid powder
MW: 190.20 g/mol
ESI-MS (*m*/*z*) 191.20 $[M+H]^+$
MF: $C_{11}H_{10}O_3$
IUPAC name: (*E*)-vinyl 3-(4-hydroxyphenyl)acrylate
^{1}H NMR [400 MHz, $CDCl_3$]: δ 7.76 (d, 1H, *J* = 15.95 Hz, H-7), 7.46-7.43 (m, H-1′, 2, 6), 6.90-6.89 (m, 2H, H-3, 5), 6.34 (d, 1H, *J* = 15.99 Hz, H-8), 5.01 (d, 1H, *J* = 14.01 Hz, H-2′b), 4.67 (d, 1H, *J* = 6.35 Hz, H-2′a). ^{13}C NMR [100 MHz, $CDCl_3$]: δ 163.8 (C-9), 157.2 (C-4), 144.8 (C-7), 142.4 (C-1′), 134.09 (C-2), 128.7 (C-6), 128.1 (C-1), 117.2 (C-8), 114.2.5 (C-5), 113.7 (C-3), 97.4 (C-2′).

Vinyl 4-hydroxy-3-methoxycinnamate
A yellow liquid
MW: 220.22 g/mol
ESI-MS (*m*/*z*) 221.22 $[M+H]^+$
MF: $C_{12}H_{12}O_4$
IUPAC name: (*E*)-vinyl 3-(4-hydroxy-3-methoxyphenyl) acrylate
^{1}H NMR [400 MHz, $CDCl_3$]: δ 7.65 (d, 1H, *J* = 16.02 Hz, H-7), 7.35 (dd, 1H, *J* = 14.02 Hz, H-1′), 7.03 (d, 1H, *J* = 8.21 Hz, H-5), 6.98 (s, 1H, H-2), 6.86 (d, 1H, *J* = 8.16 Hz, H-6), 6.23 (d, 1H, *J* = 15.95 Hz, H-8), 4.89 (d, 1H, *J* = 14.03 Hz, H-2′b), 4.67 (d, 1H, *J* = 6.43 Hz, H-2′a), 3.86 (s, 3H, –OCH_3). ^{13}C NMR [100 MHz, $CDCl_3$]: δ 163.3 (C-9), 148.3 (C-3), 147.0 (C-4), 142.5 (C-7), 142.4 (C-1′), 125.3 (C-1), 124.1 (C-6), 113.9 (C-5), 112.6 (C-2), 110.6 (C-8), 96.8 (C-2′), 55.6 (–OCH_3).

Vinyl 3, 4-dimethoxycinnamate
A yellow solid powder
MW: 234.25 g/mol
ESI-MS (*m*/*z*) 235.25 $[M+H]^+$
MF: $C_{13}H_{14}O_4$
IUPAC name: (*E*)-vinyl 3-(3,4-dimethoxyphenyl)acrylate
^{1}H NMR [400 MHz, $CDCl_3$]: δ 7.72 (d, 1H, *J* = 15.80 Hz, H-7), 7.41 (dd, 1H, *J* = 14.00 Hz, H-1′), 7.12 (d, 1H, *J* = 8.29 Hz, H-5), 7.08 (s, 1H, H-2), 6.95 (d, 1H, *J* = 8.36 Hz, H-6), 6.31 (d, 1H, *J* = 16.0 Hz, H-8), 4.95 (d, 1H, *J* = 14.04 Hz, H-2′b), 4.60 (d, 1H, *J* = 6.20 Hz, H-2′a), 3.90 (s, 6H, –OCH_3). ^{13}C NMR [100 MHz, $CDCl_3$]: δ 164.1 (C-9), 150.5 (C-3), 147.6 (C-4), 143.5 (C-7), 142.4 (C-1′), 127.2 (C-1), 121.9 (C-6), 114.7 (C-8), 111.5 (C-5), 110.4 (C-2), 96.5 (C-2′), 55.2 (–OCH_3).

Vinyl 4-hydroxy-3, 5-dimethoxycinnamate
A white solid powder
MW: 250.25 g/mol
ESI-MS (*m*/*z*) 251.25 $[M+H]^+$
MF: $C_{13}H_{14}O_5$
IUPAC name: (*E*)-vinyl 3-(4-hydroxy-3,5-dimethoxyphenyl) acrylate
^{1}H NMR [400 MHz, $CDCl_3$]: δ 7.67 (d, 1H, *J* = 15.88 Hz, H-7), 7.39 (dd, 1H, *J* = 13.98 Hz, H-1′), 6.89 (S, 2H, H-2, 6), 6.37 (d, 1H, *J* = 15.17 Hz, H-8), 4.93 (d, 1H, *J* = 14.04 Hz, H-2′b), 4.60 (d, 1H, *J* = 6.45 Hz, H-2′a), 3.85 (s, 6H, –OCH_3). ^{13}C NMR [100 MHz, $CDCl_3$]: δ 167.2 (C-9), 150.5 (C-3), 147.6 (C-5), 143.5 (C-7), 142.4 (C-1′), 136.6 (C-4), 127.2 (C-1), 114.7 (C-8), 105.6 (C-2, 6), 97.1 (C-2′), 55.2 (–OCH_3).

Ascorbyl 4-hydroxycinnamate
A white solid powder
MW: 322.27 g/mol
ESI-MS (*m*/*z*) 323.27 $[M+H]^+$
MF: $C_{15}H_{14}O_8$
IUPAC name: (*E*)-(2*S*)-2-(3,4-dihydroxy-5-oxo-2,5-dihydro furan-2-yl)-2-hydroxyethyl 3-(4-hydroxyphenyl)acrylate
^{1}H NMR [400 MHz, $(CD_3)_2SO$]: δ 7.62 (d, 1H, *J* = 16.02 Hz, H-7′), 7.23 (d, 1H, *J* = 7.81 Hz, H-5′), 7.14 (d, 1H, *J* = 7.79 Hz, H-6′), 7.04 (s, 1H, H-2′), 6.84 (d, 1H, *J* = 8.03, H-4′), 6.52 (d, 1H, *J* = 16.01 Hz, H-8′), 4.78 (d, 1H, *J* = 1.59 Hz, H-4), 4.18 (t, 2H, *J* = 5.49 Hz, H-6ab), 4.06 (m, 1H, H-5). ^{13}C NMR [100 MHz, $(CD_3)_2SO$]: δ 171.2 (C-1), 166.8 (C-9′), 158.5 (C-4′), 153.1 (C-3), 145.9 (C-7′), 136.1 (C-2′), 130.8 (C-6′), 127.8 (C-1′), 120.1 (C-2), 118.5 (C-8′), 115.5 (C-5′), 115.5 (C-3′), 75.9 (C-4), 66.4 (C-6), 65.6 (C-5)

Ascorbyl 4-hydroxy-3-methoxycinnamate
A white solid powder
MW: 352.29 g/mol
ESI-MS (*m*/*z*) 353.29 $[M+H]^+$
MF: $C_{16}H_{16}O_9$
IUPAC name: (*E*)-(2*S*)-2-(3,4-dihydroxy-5-oxo-2,5-dihydro furan-2-yl)-2-hydroxyethyl 3-(4-hydroxy-3-methoxyphenyl) acrylate
^{1}H NMR [400 MHz, $(CD_3)_2SO$]: δ 7.48 (d, 1H, *J* = 13.88 Hz, H-7′), 7.13 (d, 1H, *J* = 8.23 Hz, H-6′), 7.07 (s, 1H, H-2′), 6.79 (d, 1H, *J* = 8.20 Hz, H-5′), 6.35 (d, 1H, *J* = 15.97 Hz, H-8′), 4.77 (d, 1H, *J* = 1.76 Hz, H-4), 4.19 (t, 2H, *J* = 6.64 Hz, H-6ab), 4.13 (m, 1H, H-5), 3.82 (s, 3H, –OCH_3). ^{13}C NMR [100 MHz, $(CD_3)_2SO$]: δ 170.7 (C-1), 168.3 (C-9′), 152.6 (C-3), 149.4 (C-3′), 148.2 (C-4′), 144.8 (C-7′), 126.1 (C-1′), 123.5 (C-6′), 118.5 (C-2), 115.9 (C-5′), 114.6 (C-2′), 111.6 (C-8′), 75.6 (C-4), 66.0 (C-6), 64.9 (C-5), 56.0 (–OCH_3).

Ascorbyl 3, 4-dimethoxycinnamate
A white solid powder
MW: 366.32 g/mol
ESI-MS (*m*/*z*) 367.32 $[M+H]^+$
MF: $C_{17}H_{18}O_9$
IUPAC name: (*E*)-(2*S*)-2-(3,4-dihydroxy-5-oxo-2,5-dihydro furan-2-yl)-2-hydroxyethyl 3-(3,4 dimethoxyphenyl)acrylate
^{1}H NMR [400 MHz, $(CD_3)_2SO$]: δ 7.64 (d, 1H, *J* = 15.99 Hz, H-7′), 7.36 (s, 1H, H-2′), 6.97 (d, 1H, *J* = 6.06 Hz, H-5′), 6.58 (d, 1H, *J* = 15.97 Hz, H-8′), 6.58 (d, 1H, *J* = 15.97 Hz, H-6′), 4.77 (d, 1H, *J* = 1.57 Hz, H-4), 4.16 (t, 2H, *J* = 5.56 Hz, H-6ab), 4.13 (m, 1H, H-5), 3.80 (s, 6H, –OCH_3). ^{13}C NMR [100 MHz, $(CD_3)_2SO$]: δ 171.2 (C-1), 167.1 (C-9′), 153.1 (C-3), 151.9 (C-3′), 149.8 (C-4′), 145.9 (C-7′), 127.7 (C-1′), 123.9 (C-6′), 119.0 (C-2), 116.1 (C-8′), 112.4 (C-5′), 111.1 (C-2′), 75.9 (C-4), 66.5 (C-6), 65.4 (C-5), 56.3 (–OCH_3).

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References

Abraham, E. C., Swamy, M. S., & Perry, R. E. (1989). Non-enzymatic glycosylation (glycation) of lens crystallins in diabetes and aging. *Progress in Clinical and Biological Research, 304*, 123–139.
Bonnefont-Rousselot, D. (2002). Glucose and reactive oxygen species. *Current Opinion in Clinical Nutrition and Metabolic Care, 5*, 561–568.

Claon, P. A., & Akoh, C. C. (1994). Lipase-catalyzed synthesis of terpene esters by transesterification in n-hexane. *Biotechnology Letters, 16*, 235–240.
Dracha, M., Narkiewicz-Michałeka, J., Sienkiewicza, A., Szymulaa, M., & Bravo-Diazb, C. (2011). Antioxidative properties of vitamins C and E in micellar systems and in microemulsions. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 379*, 79–85.
Humeau, C., Girardin, M., Rovel, B., & Miclo, A. (1998). Enzymatic synthesis of fatty acid ascorbyl esters. *Journal of Molecular Catalysis B: Enzymatic, 5*, 19–23.
Jung, S. H., Lee, J. M., Lee, H. J., Kim, C. Y., Lee, E. H., & Um, B. H. (2007). Aldose reductase and advanced glycation end products inhibitory effect of *Phyllostachys nigra*. *Biological and Pharmaceutical Bulletin, 8*, 1569–1572.
Lee, Y. S., Kang, Y. H., Jung, J. Y., Kang, I. J., Han, S. N., Chung, S. J., ... Lim, S. S. (2008). Inhibitory constituents of aldose reductase in the fruiting body of *Phellinus linteus*. *BioMed Research International, 4*, 765–768.
Lee, Y. S., Kang, Y. H., Jung, J. Y., Lee, S. H., Kazuo, O., Shin, K. H., ... Lim, S. S. (2008). Protein glycation inhibitors from the fruiting body of *Phellinus linteus*. *Biological and Pharmaceutical Bulletin, 10*, 1968–1972.
Li, H. M., Hwang, S. H., Kang, B. G., Hong, J. S., & Lim, S. S. (2014). Inhibitory effect of *Colocasia esculenta* (L.) constituents on aldose reductase. *Molecules, 9*, 13212–13224.
Li, H. M., Kim, J. K., Jan, J. M., Kwon, S. O., Cui, C. B., & Lim, S. S. (2012). The inhibitory effect of *Prunella vulagaris* L. on aldose reductase and protein glycation. *BioMed Research, 2012*, 1–7.
Na, N., Delanghe, J. R., Taes, Y. E. C., Torck, M., Baeyens, W. R. G., & Ouyang, J. (2006). Serum vitamin C concentration is influenced by haptoglobin polymorphism and iron status in Chinese. *Clinica Chimica Acta, 365*, 319–324.
Nakagawa, T., Yokozawa, T., Terasawa, K., Shu, S., & Juneja, L. R. (2002). Protective activity of green tea against free radical- and glucose-mediated protein damage. *Journal of Agricultural and Food Chemistry, 50*, 2418–2422.
Otto, R. T., Bornscheuer, U. T., Scheib, H., Pleiss, J., Syldatk, C., & Schmid, R. D. (1998). Lipase-catalyzed esterification of unusual substrates: synthesis of glucuronic acid and ascorbic acid (vitamin C) esters. *Biotechnology Letters, 11*, 1091–1094.
Peng, X., Zheng, Z., Cheng, K. W., Shan, F., Ren, G. X., Chen, F., & Wang, M. (2008). Inhibitory effect of mung bean extract and its constituents vitexin and isovitexin on the formation of advanced glycation end products. *Food Chemistry, 2*, 475–481.
Pietta, P. G., Mauri, P. L., Simonetti, P., & Testolin, G. (1995). HPLC and MEKC determination of major flavonoids in selected food pools. *Fresenius' Journal of Analytical Chemistry, 352*, 788–792.
Rahbar, S., Yernini, K. K., Scott, S., Gonzales, N., & L1alezari, I. (1999). Novel inhibitors of advanced glycation end products. *Biochemical and Biophysical Research Communications, 262*, 651–656.
Seino, K., Uchibori, T., Nishitani, T., & Inamasu, S. (1984). Enzymatic synthesis of carbohydrate esters of fatty acid (I) esterification of sucrose, glucose, fructose, and sorbitol. *Journal of the American Oil Chemists' Society, 61*, 1761–1765.
Tokiwa, Y., Kitagawa, M., Raku, T., Yanagitanib, S., & Yoshino, K. (2007). Enzymatic synthesis of arbutin undecylenic acid ester and its inhibitory effect on melanin synthesis. *Bioorganic and Medicinal Chemistry Letters, 17*, 3105–3108.
Wang, Q. Z., Hwang, S. H., & Lim, S. S. (2015). Lipophilization of phenolic acids with phytosterols by a chemoenzymatic method to improve their antioxidant activities. *European Journal of Lipid Science and Technology, 117*, 1037–1048.
Wu, C. H., Huang, S. M., Lin, J. A., & Yen, G. C. (2011). Inhibition of advanced glycation end product formation by food stuffs. *Food Function, 2*, 224–234.
Wu, C. H., & Yen, G. C. (2005). Inhibitory effect of naturally occurring flavonoids on the formation of advanced glycation end products. *Journal of Agricultural and Food Chemistry, 53*, 3167–3173.
Yan, Y., Bornscheuer, U. T., & Schmid, R. D. (1999). Lipase-catalyzed synthesis of vitamin C fatty acid esters. *Biotechnology Letters, 21*, 1051–1054.