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Synthesis of cinnamoyl and hydroxycinnamoyl amino acid conjugates and evaluation of their antioxidant activity

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Abstract: Fifteen amides of cinnamic, ferulic and sinapic acids with natural and unnatural *C*-protected amino acids have been synthesized. The amides (E)-*N*-(feruloyl)-L-tyrosine methyl ester (10), (E)-*N*-(feruloyl)-L-phenylalanine *t*-butyl ester (11), (E)-*N*-(sinapoyl)-L-tyrosine methyl ester (13) and (E)-*N*-(sinapoyl)-L-phenylalanine *t*-butyl ester (15) with a free carboxyl group of amino acids have been found in nature. The rest of the compounds are unknown. The hydroxycinnamoyl amino acid conjugates have been studied for their antioxidant activity (AOA) in bulk phase lipid autoxidation. The highest AOA has been found for the compounds 11 and 15, which contain the same phenylalanine moiety. Copyright © 2005 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: cinnamic acid amides; hydroxycinnamic acids amides; amino acids; UV; ^{1}H NMR; ESI–MS; antioxidant activity

INTRODUCTION

Cinnamic acid conjugates are commonly isolated from plant sources as the corresponding *N*-substituted amides. These compounds are concentrated mainly in the reproductive organs and are absent in the green parts of the plants [1,2]. They occur in different forms. In the first form (basic, water soluble), only one group of aliphatic di- or polyamine (putrescine, spermine, spermidine) is linked with cinnamic acid derivatives (ferulic, caffeic or *p*-coumaric acid) [3–10]. The other form of cinnamic acid amides (neutral, water-insoluble) can be divided into two classes: in the first class, each terminal group of aliphatic di- and polyamines is symmetrically or asymmetrically substituted with ferulic, caffeic or *p*-coumaric acid [1,3–6]. The plant hydroxycinnamic acid conjugates with polyamines have been proposed to possess a number of biological activities including antiviral to antifungal effects. These plant-derived compounds are similar in their structure with the polyamine toxins from invertebrates. It has been shown that *p*-coumaroylspermidine is active at the glutamate-dependent neuromuscular junction of crustaceans [5]. The second class of these secondary metabolites in plants includes the hydroxycinnamic acid amides conjugated to aromatic amines (tyramine, tryptamine, serotonine) [1,10]. Another form of characteristic secondary metabolites includes the hydroxycinnamic (ferulic, caffeic, *p*-coumaric and sinapic) acid amides conjugated to aromatic amino acid (tyrosine, phenylalanine, tryptophan) [11–14]. Little is known about the biological function of the last class of compounds in nature. Caffeoyltyrosine, caffeoyl DOPA, *p*-coumaroyl DOPA, *p*-coumaroyltyrosine are found to exhibit low antifungal activity [15]. Recently, it has been shown that the 3,4-dihydroxycinnamic acid (L-alanine methyl ester) amide and the 4-dihydroxycinnamic acid (L-alanine methyl ester) amide possess useful biological activities as antiatherosclerotic agents with inhibition of cellular cholesterol storage [16,17]. It is presumed that induction of the accumulation of hydroxycinnamic acid amides is part of the defense system of the plants and that their production is activated in response to various environmental stimuli (wounding, fungal infection, heavy metal ions etc.) [18–20]. These compounds are postulated to contribute to the formation of a phenolic barrier, which makes cell walls more resistant to enzymatic hydrolysis [21].

As the quantity of these amides in nature is very low, we decided to synthesize series of cinnamoyl-, feruloyl- and sinapoyl-amino acid amides in order to define their antioxidant activity (AOA).

MATERIALS AND METHODS

Chemicals

Cinnamic, 3-methoxy-4-hydroxy-cinnamic (*ferulic*) and 3,5-dimethoxy-4-hydroxy-cinnamic (*sinapic*) acids were purchased from Fluka (Buchs, Switzerland) and used without preliminary purification.

The amino acid derivatives were purchased from Sigma, HOBt and 1-[3-(di-methylamino) propyl]-3-ethyl carbodiimide

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hydrochloride were purchased from Merck. *m*-Fluorophenylalanine methyl ester was synthesized according to the method described [22].

Synthetic Procedures of Amides

The phenolic acid (cinnamic, ferulic or sinapic) (1 mM) was dissolved in 2 ml DMF. The solution was cooled in an ice-water bath, and HOBt (1 mM) and 1-[3-(di-methylamino) propyl]-3-ethyl carbodiimide hydrochloride (1 mM) were added. After 8 min, *N*-methylmorpholine (1 mM) and hydrochloride of *C*-protected amino acid ($HCl.NH_2$-CH (R)-COOR′) (1 mM) in 1.5 ml CH_2Cl_2 were added and the resultant reaction mixture was stirred for 18 h at room temperature. The mixture was poured into 5% $NaHCO_3$, extracted with CH_2Cl_2 (five times), washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*.

The residue was purified by TLC on Kieselgel 60 F_{254} (Merck) using a solvent system PE : EtOAc.

Analytical Methods

The NMR spectra were obtained on a Bruker Avance DRX-250 spectrometer operating at 250.13 MHz for ^1H. The measurements in $CDCl_3$ solutions were carried out at ambient temperature (300 K) and tetramethylsilane (TMS) was used as an internal standard. Typical conditions for 1D ^1H spectra were: pulse width 30°, 1 s relaxation delay, 16 K time domain points, zero-filled to 64 K, hard pulse with 90° pulse width of 11.8 μs. The UV spectra EtOH solutions were measured with a Specord UV–VIS spectrophotometer. The ESI mass spectra were obtained on an Esquire3000plus instrument.

Assay of Antioxidative Potential of the Synthesized Amides Against Lipid Peroxidation

Lipid samples: The triacylglycerols of sunflower oil (TGSO) were obtained by removing pre- and antioxidants and trace metals from a commercially available sunflower sample using adsorption chromatography [23] and then storing the triacylglycerols in an inert atmosphere at −20 °C. Samples with 0.1 mM concentration of antioxidants (AH) were prepared by adding aliquots of their solutions in purified acetone to a lipid sample. Acetone was removed in an argon flow (99.99%).

Oxidation at 80 °C (±0.2°) was carried out by blowing air through the samples in the dark at a rate of 100 ml min^{-1}. The process was followed by withdrawing samples at measured time intervals and subjecting them to iodometric determination of the primary products (hydroperoxides, LOOH) concentration, i.e. the peroxide value (PV) [24]. During the initial stage of the process, the rate of peroxide accumulation was equal to the oxidation rate [25]. All kinetic data were the mean results of three independent experiments and were processed using the computer programme Origin 6.

The main kinetic parameters characterizing the AOA are:

- Induction period (IP) – the time in which the concentration of the antioxidants (AH) is fully consumed was determined as a cross point for the tangents to the two parts of the kinetic curves of lipid autoxidation [25–29]. IP_A- stands for IP in the presence of AH and IP_C- for the control lipid sample without AH.
- Initial rate of lipid autoxidation (R_C in the absence and R_A in the presence of AH) was found from the tangent at the initial phase of the kinetic curves of hydroperoxide accumulation [27,30–32].

Statistical analysis of IP determination (10 independent experiments) was carried out in association with previous results on inhibited oxidation [33] according to Doerffel [34]. The standard deviation (SD) for different mean values of IP was (in h) $IP = 2.0$, $SD = 0.2$; $IP = 5.0$, $SD = 0.3$; $IP = 15.0$, $SD = 1.0$. The SD of PV determination (in meq kg^{-1}), according to the modified iodometric method for different mean values of PV, was $PV = 12.0$, $SD = 1.0$; $PV = 30.0$, $SD = 2.0$; $PV = 70.0$, $SD = 5.0$; $PV = 150.0$, $SD = 10$; $PV = 250.0$, $SD = 20$. The R_A and R_C were quite constant, varying by less than 2.0%.

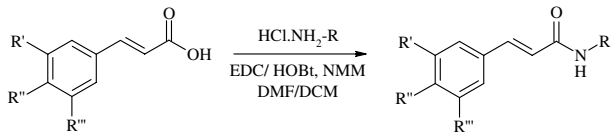
RESULTS AND DISCUSSION

Fifteen amides have been obtained by a standard method of coupling of phenylpropanoic acids with natural and unnatural *C*-protected amino acids (Table 1). The synthesized amides **10**, **11**, **13** and **15** have been found in nature. The rest of cinnamoyl and hydroxycinnamoyl conjugates of amino acids are new.

The structure of amides **1–15** has been characterized using UV, ^1H NMR and ESI-MS spectroscopy. The complete assignment of the ^1H NMR spectra of the compounds **1–15** measured in $CDCl_3$ is reported. A distinction between the cinnamoyl, feruloyl and sinapoyl residues was made using the integral intensities, chemical shifts and multiplicities of the resonance peaks in the aromatic spectral area, as well as the integral intensity of the resonance signals of the protons from OCH_3. The values of the proton–proton vicinal coupling constants ($^3J_{H/H}$ about 15.5 Hz) measured for the olefinic protons of cinnamoyl, feruloyl and sinapoyl residues define *E* configuration of the double bond of all studied compounds. With some exceptions [16,35], *E* configuration of the double bond has been the most frequently found configuration of phenylpropenoates in nature. A complication was observed in the aromatic spectral area of ^1H NMR spectra of compounds **5**, **12** and **14**, which contain *m*-fluorobenzyl residue. The complication was due to the overlap and additional splitting of the resonance signals of the aromatic protons of *m*-fluorobenzyl residue because of the spin–spin coupling ($^3J_{H/F}$ and $^4J_{H/F}$) with the fluorine nucleus. The chemical shift of the proton connected to the nitrogen atom established the amide linkage of the cinnamic and hydroxycinnamic acids and amino acids in each case.

(E) – *N*-(cinnamoyl)-L-valine methyl ester, **1**. UV (EtOH) λ max = 212, 217, 223, 276, 303 nm; ^1H-NMR $\delta = 0.96$ (d, 3H, $J = 6.95$ Hz, C**H**$_3$), 0.99 (d, 3H, $J = 6.95$ Hz, C**H**$_3$), 2.23 (m, 1H, >C**H**), 3.77 (s, 3H, −OC**H**$_3$), 4.74 (dd, 1H, $J = 8.7$, 4.9 Hz, C**H**), 6.12 (d, 1H, $J = 8.7$

Table 1 Synthesized *N*-cinnamoyl- and *N*-hydroxycinnamoyl amino acid amides



Compound	R	R′	R″	R‴	$[\alpha]_D$	Yields (%)
1	$CH(CH(CH_3)_2)-COOCH_3$	H	H	H	(+)4.96, $c = 0.19$	13.00
2	$CH(CH_2-CH(CH_3)_2)-COOCH_3$	H	H	H	(–) 16.35, $c = 0.38$	14.50
3	$CH(CH_2C_6H_5)-COOC(CH_3)_3$	H	H	H	(–) 22.00, $c = 0.25$	71.00
4	$CH(CH_3)-COOC(CH_3)_3$	H	H	H	(–) 3.55, $c = 0.19$	85.00
5	$CH(CH_2C_6H_4-F-m)-COOCH_3$	H	H	H	(–) 44.27, $c = 0.13$	77.00
6	$CH(CH_2C_6H_4-OH-p)-COOCH_3$	H	H	H	(–) 28.19, $c = 0.19$	40.50
7	$CH(CH_3)-COOC(CH_3)_3$	OCH_3	OH	H	(+)41.86, $c = 0.43$	55.00
8	$CH(CH(CH_3)_2)-COOCH_3$	OCH_3	OH	H	(+)10.00, $c = 0.31$	41.00
9	$CH(CH_2-CH(CH_3)_2)-COOCH_3$	OCH_3	OH	H	(–) 5.83, $c = 1.00$	34.00
10	$CH(CH_2C_6H_4-OH-p)-COOCH_3$	OCH_3	OH	H	(–) 28.74, $c = 3.25$	62.00
11	$CH(CH_2C_6H_5)-COOC(CH_3)_3$	OCH_3	OH	H	(–) 20.83, $c = 0.36$	45.00
12	$CH(CH_2C_6H_4-F-m)-COOCH_3$	OCH_3	OH	H	(–) 24.17, $c = 0.20$	49.50
13	$CH(CH_2C_6H_4-OH-p)-COOCH_3$	OCH_3	OH	OCH_3	(–) 36.75, $c = 0.42$	40.50
14	$CH(CH_2C_6H_4-F-m)-COOCH_3$	OCH_3	OH	OCH_3	(–) 91.76, $c = 0.17$	28.90
15	$CH(CH_2C_6H_5)-COOC(CH_3)_3$	OCH_3	OH	OCH_3	(–) 11.36, $c = 2.42$	47.10

Hz, –N**H**), 6.48 (d, 1H, $J = 15.6$ Hz, C**H**=), 7.37 (m, 3H, Ar–**H**), 7.51 (m, 2H, Ar–**H**), 7.65 (d, 1H, $J = 15.6$ Hz, C**H**=); ESI–MS: 261. ($[M]^+$), 284 ($[M + Na]^+$), 545 ($[2M + Na]^+$).

(E) – *N*-(cinnamoyl)-L-leucine methyl ester, **2**. UV (EtOH) λ max = 212, 217, 223, 276, 302 nm; ^{1}H-NMR $\delta = 0.96$ (d, 3H, $J = 6.05$ Hz, C**H**$_3$), 0.98 (d, 3H, $J = 5.65$ Hz, C**H**$_3$), 1.50–1.75 (m, 3H), 3.76 (s, 3H, OC**H**$_3$), 4.82 (m, 1H, C**H**), 6.03 (d, 1H, $J = 8.6$ Hz, –N**H**), 6.43 (d, 1H, $J = 15.6$ Hz, C**H**=), 7.3 (m, 3H, Ar–**H**), 7.50 (m, 2H, Ar–**H**), 7.64 (d, 1H, $J = 15.6$ Hz, C**H**=); ESI–MS: 276. ($[M + H]^+$), 298 ($[M + Na]^+$), 573 ($[2M + Na]^+$).

(E) – *N*-(cinnamoyl)-L-phenylalanine *t*-butyl ester, **3**. UV (EtOH) λ max = 213, 217, 222, 276, 303] nm; ^{1}H-NMR $\delta = 1.43$ (s, 9H, 3 × C**H**$_3$), 3.18 (d, 2H, $J = 5.7$ Hz, >C**H**$_2$), 4.90 (m, 1H, C**H**), 6.12 (d, 1H, $J = 7.4$ Hz, –N**H**), 6.40 (d, 1H, $J = 15.6$ Hz, C**H**=), 7.15–7.52 (10H, Ar–**H**), 7.63 (d, 1H, $J = 15.6$ Hz, C**H**=); ESI–MS: 374 ($[M + Na]^+$), 725 ($[2M + Na]^+$).

(E) – *N*-(cinnamoyl)-L-alanine *t*-butyl ester, **4**. UV (EtOH) λ max = 211, 217, 224, 276, 300 nm; ^{1}H-NMR $\delta = 1.45$ (d, 3H, $J = 7.0$ Hz, CH-C**H**$_3$), 1.49 (s, 9H, 3 × C**H**$_3$), 4.62 (dq, 1H, $J = 7.2$, 7.0 Hz, C**H**), 6.27 (d, 1H, $J = 7.2$ Hz, –N**H**), 6.42 (d, 1H, $J = 15.6$ Hz, C**H**=), 7.35 (m, 3H, Ar–**H**), 7.51 (m, 2H, Ar–**H**), 7.63 (d, 1H, $J = 15.6$ Hz, C**H**=); ESI–MS: 298 ($[M + Na]^+$), 573 ($[2M + Na]^+$).

(E) – *N*-(cinnamoyl)-L-*m*-fluorophenylalanine methyl ester, **5**. UV (EtOH) λ max = 217, 225, 277, 303 nm; ^{1}H-NMR $\delta = 3.18$ (dd, 1H, $J = 13.9$, 5.4 Hz, >C**H**$_2$-a), 3.26 (dd, 1H, $J = 13.9$, 5.6 Hz, >C**H**$_2$-b), 3.77 (s, 3H, OC**H**$_3$), 5.04 (ddd, 1H, $J = 6.7$, 5.6, 5.4 Hz, C**H**), 6.12 (d, 1H, $J = 6.7$ Hz, N**H**), 6.41 (d, 1H, $J = 15.6$ Hz, C**H**=), 6.80–7.05 (m, 3H, Ar–**H**), 7.25 (m, 1H, Ar–**H**), 7.37 (m, 3H, Ar–**H**), 7.50 (m, 2H, Ar–**H**), 7.65 (d, 1H, $J = 15.6$ Hz, C**H**=); ESI–MS: 349 ($[M + Na]^+$), 675 ($[2M + Na]^+$).

(E) – *N*-(cinnamoyl)-L-tyrosine methyl ester, **6**. UV (EtOH) λ max = 217, 225, 279, 301 nm; ^{1}H-NMR $\delta = 3.10$ (dd, 1H, $J = 14.2$, 5.4 Hz, >C**H**$_2$-a), 3.18 (dd, 1H, $J = 14.2$, 5.8 Hz, >C**H**$_2$-b), 3.76 (s, 3H, OC**H**$_3$), 5.0 (ddd, 1H, $J = 7.4$, 5.8, 5.4 Hz, C**H**), 5.10 (br, s, 1H, O**H**), 6.08 (d, 1H, $J = 7.4$ Hz, –N**H**), 6.39 (d, 1H, $J = 15.6$ Hz, C**H**=), 6.74 (d, 2H, $J = 8.5$ Hz, Ar–**H**), 6.98 (d, 2H, $J = 8.5$ Hz, Ar–**H**), 7.37 (m, 3H, Ar–**H**), 7.49 (m, 2H, Ar–**H**), 7.64 (d, 1H, $J = 15.6$ Hz, C**H**=); ESI–MS: 347 ($[M + Na]^+$), 673 ($[2M + Na]^+$).

(E) – *N*-(feruloyl)-L-alanine *t*-butyl ester, **7**. UV (EtOH) λ max = 220, 237, 295, 320 nm; ^{1}H-NMR $\delta = 1.43$ (d, 3H, $J = 7.1$ Hz, C**H**$_3$), 1.49 (s, 9H, 3 × C**H**$_3$), 3.91 (s, 3H, OC**H**$_3$), 4.62 (dq, 1H, $J = 7.2$ Hz, C**H**), 6.29 (d, 1H, $J = 15.6$ Hz, C**H**=), 6.30 (d, 1H, $J = 7.2$ Hz, –N**H**), 6.90 (d, 1H, $J = 8.2$ Hz, Ar–**H**(m)), 6.98 (d, 1H, $J = 1.6$ Hz, Ar–**H**(o), 7.03 (dd, 1H, $J = 8.2$, 1.6 Hz), 7.53 (d, 1H, $J = 15.6$ Hz, C**H**=); ESI–MS: 321. ($[M + H]^+$), 344 ($[M + Na]^+$), 665 ($[2M + Na]^+$).

(E) – *N*-(feruloyl)-L-valine methyl ester, **8**. UV (EtOH) λ max = 218, 237, 295, 322 nm; ^{1}H-NMR $\delta = 0.95$ (d, 3H, $J = 6.9$ Hz, C**H**$_3$), 0.95 (d, 3H, $J = 6.9$ Hz, C**H**$_3$), 2.22 (m, 1H, C**H**), 3.76 (s, 3H, OC**H**$_3$), 3.93 (s, 3H, OC**H**$_3$), 4.73 (dd, 1H, $J = 8.8$, 4.9 Hz, C**H**), 5.80 (br, s, 1H, O**H**), 6.06 (d, 1H, $J = 8.8$ Hz, –N**H**), 6.32 (d, 1H, $J = 15.5$ Hz, C**H**=), 6.89 (d, 1H, $J = 8.1$ Hz, Ar–**H**(m)), 7.01 (d, 1H, $J = 1.8$ Hz, Ar–**H**(o), 7.07 (dd, 1H, $J = 8.1$, 1.8 Hz), 7.56 (d, 1H, $J = 15.5$ Hz, C**H**=); ESI–MS: 308. ($[M + H]^+$), 330 ($[M + Na]^+$), 615 ($[2M + H]^+$), 637 ($[2M + Na]^+$).

(E) – *N*-(feruloyl)-L-leucine methyl ester, **9**. UV (EtOH) λ max = 219, 235, 295, 321 nm; ^{1}H-NMR δ = 0.88 (d, 3H, J = 5.9 Hz, C**H**$_3$), 0.91 (d, 3H, J = 5.8 Hz, C**H**$_3$), 1.40–1.75 (m, 3H, >C**H**$_2$ + C**H**), 3.69 (s, 3H, OC**H**$_3$), 3.85 (s, 3H, OC**H**$_3$), 4.73 (m, 1H, C**H**), 5.97 (d, 1H, J = 8.5 Hz, –N**H**), 6.21 (d, 1H, J = 15.5 Hz, C**H**=), 6.82 (d, 1H, J = 8.1 Hz, Ar–**H**(m)), 6.91 (d, 1H, J = 1.8 Hz, Ar–**H**(o)), 6.97 (dd, 1H, J = 8.1, 1.8 Hz, Ar–**H**(o)), 7.48 (d, 1H, J = 15.5 Hz, C**H**=); ESI–MS: 322. ([M + H]$^+$), 344 ([M + Na]$^+$), 665 ([2M + Na]$^+$).

(E) – *N*-(feruloyl)-L-tyrosine methyl ester, **10**. UV (EtOH) λ max = 294, 320 nm; ^{1}H-NMR δ = 3.09 (dd, 1H, J = 14.0, 5.4 Hz, >C**H**$_2$-a), 3.17 (dd, 1H, J = 14.0, 5.6 Hz, >C**H**$_2$-b), 3.76 (s, 3H, OC**H**$_3$), 3.92 (s, 3H, OC**H**$_3$), 5.0 (ddd, 1H, J = 8.0, 5.6, 5.4 Hz, C**H**), 5.84 (br, s, 1H, OH), 6.05 (d, 1H, J = 8.0 Hz, –N**H**), 6.25 (d, 1H, J = 15.6 Hz, C**H**=), 6.75 (d, 2H, J = 8.3 Hz, Ar–**H** (o-OH)), 6.90 (d, 1H, J = 8.3 Hz, Ar–**H**(m)), 6.97 (d, 2H, J = 8.3 Hz, Ar–**H** (*m*-OH)), 6.99 (s, 1H, Ar–**H**(o)), 7.05 (dd, 1H, J = 8.3, 1.8 Hz, Ar–**H**(o)), 7.55 (d, 1H, J = 15.6 Hz, C**H**=); ESI–MS: 394 ([M + Na]$^+$), 765 ([2M + Na]$^+$).

(E) – *N*-(feruloyl)-L-phenylalanine *t*-butyl ester, **11**. UV (EtOH) λ max = 217, 235, 297, 323 nm; ^{1}H-NMR δ = 1.43 (s, 9H, 3 × C**H**$_3$), 3.18 (d, 2H, J = 5.7 Hz, >C**H**$_2$), 3.92 (s, 3H, OC**H**$_3$), 4.91 (dt, 1H, J = 7.7, 5, 7 Hz, C**H**), 5.82 (s, 1H, O**H**), 6.06 (d, 1H, J = 7.7 Hz, –N**H**), 6.25 (d, 1H, J = 15.6 Hz, C**H**=), 6.90 (d, 1H, J = 8.2 Hz, Ar–**H** (m)), 6.99 (d, 1H, J = 1.8 Hz, Ar–**H** (o)), 7.05 (dd, 1H, J = 8.2, 1.8 Hz, Ar–**H** (o)), 7.19 (m, 2H, Ar–H), 7.27 (m, 3H, Ar–**H**), 7.56 (d, 1H, J = 15.5 Hz, C**H**=); ESI–MS: 398 ([M + H]$^+$), 420 ([M + Na]$^+$), 795 ([2M + H]$^+$), 817 ([2M + Na]$^+$).

(E) – *N*-(feruloyl)-L-*m*-fluorophenylalanine methyl ester, **12**. UV (EtOH) λ max = 232, 267, 295, 320 nm; ^{1}H-NMR δ = 3.17 (dd, 1H, J = 13.8, 5.3 Hz, >C**H**$_2$-a), 3.26 (dd, 1H, J = 13.8, 5.7 Hz, >C**H**$_2$-b), 3.77 (s, 3H, OC**H**$_3$), 3.93 (s, 3H, OC**H**$_3$), 5.04 (ddd, 1H, J = 7.2, 5.7, 5.3 Hz, C**H**), 5.85 (br, s, 1H, OH), 6.06 (d, 1H, J = 7.2 Hz, –N**H**), 6.26 (d, 1H, J = 15.6 Hz, C**H**=), 6.80–6.90 (m, 2H, Ar–H(o-F)), 6.91 (d, 1H, J = 8.2 Hz, Ar–H(m)), 6.97 (m, 1H, Ar–H(p-F)), 7.01 (d, 1H, J = 1.9 Hz, Ar–H(o)), 7.06 (dd, 1H, J = 8.2, 1.9 Hz, Ar–H(o)), 7.57 (d, 1H, J = 15.5 Hz, C**H**=); ESI–MS: 374 [M + H]$^+$.

(E) – *N*-(sinapoyl)-L-tyrosine methyl ester, **13**. UV (EtOH) λ max = 229, 322 nm; ^{1}H-NMR δ = 3.09 (dd, 1H, J = 14.0, 5.4 Hz, >CH$_2$-a), 3.18 (dd, 1H, J = 14.0, 5.6 Hz, >CH$_2$-b), 3.76 (s, 3H, OCH$_3$), 3.90 (s, 6H, 2 × OCH$_3$), 5.00 (ddd, 1H, J = 7.8, 5.6, 5.4 Hz, CH), 5.75 (s, 1H, OH), 6.08 (d, 1H, J = 7.8 Hz, –NH), 6.27 (d, 1H, J = 15.5 Hz, C**H**=), 6.74 (s, 2H), 6.76 (d, 2H, J = 8.1 Hz, Ar–H), 6.97 (d, 2H, J = 8.1 Hz, Ar–H), 7.53 (d, 1H, J = 15.5 Hz, C**H**=); ESI–MS: 401 ([M + H]$^+$), 423 ([M + Na]$^+$), 463 ([M + Na + K]$^+$), 823 ([2M + Na]$^+$).

(E) – *N*-(sinapoyl)-L-*m*-fluorophenylalanine methyl ester, **14**. UV (EtOH) λ max = 240, 323 nm; ^{1}H-NMR δ = 3.17 (dd, 1H, J = 13.9, 5.3 Hz, >C**H**$_2$-a), 3.26 (dd, 1H, J = 13.9, 5.6 Hz, >C**H**$_2$-b), 3.77 (s, 3H, OC**H**$_3$), 3.92 (s, 6H, 2 × OC**H**$_3$), 5.04 (ddd, 1H, J = 7.6, 5.6, 5.3 Hz, C**H**), 5.74 (s, 1H, O**H**), 6.08 (d, 1H, J = 7.6 Hz, –N**H**), 6.28 (d, 1H, J = 15.5 Hz, C**H**=), 6.76 (s, 2H, Ar–H), 6.90 (m, 2H, Ar–**H**), 7.25 (m, 2H, Ar–**H**), 7.55 (d, 1H, J = 15.5 Hz, C**H**=); ESI–MS: 425 ([M + Na]$^+$), 829 ([2M + Na]$^+$).

(E) – *N*-(sinapoyl)-L-phenylalanine *t*-butyl ester, **15**. UV (EtOH) λ max = 240, 324 nm; ^{1}H-NMR δ = 1.42 (s, 9H, 3 × C**H**$_3$), 3.18 (2H, d, J = 5.7 Hz, >C**H**$_2$), 3.92 (s, 6H, 2 × OC**H**$_3$), 4.91 (dt, 1H, J = 7.7, 5.7 Hz, C**H**), 5.30 (s, 1H, O**H**), 6.11 (d, 1H, J = 7.7 Hz, –N**H**), 6.27 (d, 1H, J = 15.5 Hz, C**H**=), 6.74 (s, 2H, Ar–**H**), 7.19–7.29 (5H, Ar–**H**), 7.53 (d, 1H, J = 15.5 Hz, C**H**=); ESI–MS: 427 ([M + H]$^+$), 877 ([2M + Na]$^+$).

The antioxidant properties of five feruloyl- (**8–12**) and three sinapoyl- (**13–15**) amino acid amides tested are shown in Table 2. It has been established that compound **15** exerts the highest AOA in TGSO. All sterically hindered phenols of *N*-sinapoyl amino acids amides (**13–15**) exhibit a higher antioxidant effect than those of *N*-feruloyl amino acids amides (**8–12**). The results obtained have been compared with those of ferulic and sinapic acids (SA). All feruloyl acid amides (**8–12**) have shown a stronger antioxidant effect than ferulic acid (FA).

Comparison of the properties of the antioxidants (**8–15**) under consideration leads to the following conclusions:

1. The antioxidant efficiency determined by relative antioxidant efficiency (RAE) of the tested antioxidants decreased as follows:

$$\text{SA} > \mathbf{15} > \mathbf{14} > \mathbf{11} \geq \mathbf{13} > \mathbf{9} > \mathbf{10} = \mathbf{8} > \mathbf{12} > \text{FA}.$$

Relative antioxidant efficiency, $\text{RAE} = (\Delta IP/IP_C) = (IP_A - IP_C/IP_C) = (IP_A/IP_C) - 1$, measures the relative increase in oxidation stability of the sample in the presence of the antioxidant [26,36]. RAE indicates how many times the oxidation stability of the lipid substrate increases in presence of an inhibitor, taking into account the effect of the control lipid sample.

This sequence describes which antioxidant ensures a longer oxidation stability of lipids (the more effective antioxidants ensure a longer stabilization of the lipids).

The results showed that compound **15** possesses the highest antioxidant efficiency from all tested compounds, similar to those of SA. All feruloyl-amide acid amides (**8–12**) exhibit higher antioxidant efficiency than those of FA.

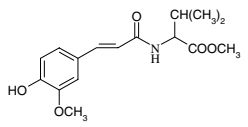
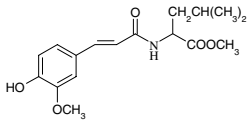
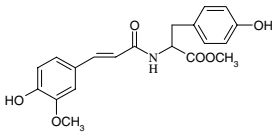
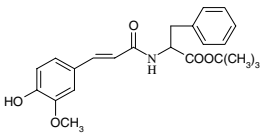
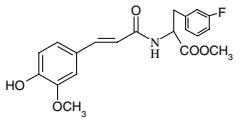
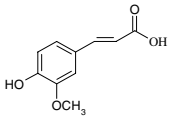
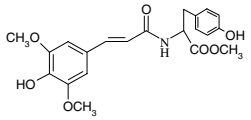
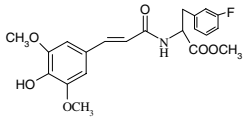
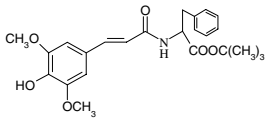
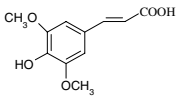
2. Antioxidant strength was determined as inhibition degree (ID)

Inhibition degree, $\text{ID} = R_C/R_A$, shows how many times the antioxidant shortens the oxidation chain length [30–32].

The data in Table 2 showed that antioxidant strength (ID) decreases as follows:

$$\mathbf{9} \geq \mathbf{11} = \mathbf{12} \geq \mathbf{15} > \mathbf{13} > \mathbf{8} > \mathbf{14} > \mathbf{10} > \text{SA} > \text{FA}$$

Table 2 Antioxidant properties of *N*-hydroxycinnamoyl amino acid amides in TGSO autoxidation at 80 °C, $IP_C = (1.3 \pm 0.2)$ h, $R_C = (6.7 \pm 0.5)\ 10^{-6}$ M/s

No	Structures of the tested antioxidants	$RAE = (IP_A - P_C)/IP_C$ [–]	$ID = R_C/R_A$ [–]	$AOA = (IP_A/IP_C)/(R_A/R_C)$ [–]
8		0.8	3.5	5.9
9		1.1	6.8	13.9
10		0.8	3.1	2.5
11		1.3	6.4	15.4
12		0.7	6.4	11.3
FA		0.5	1.2	1.8
13		1.2	4.4	9.7
14		1.34	3.2	7.7
15		2.0	6.0	17.6
SA		2.5	2.6	9.4

The results obtained demonstrated two times higher antioxidant strength of compounds **9**, **11**, **12** and **15** than those of SA. A stronger antioxidant shows a lower hydroperoxide level in the IP time.

3. AOA, the general kinetic parameter, proposed from Yanishlieva and Marinova [24,37,38], unifies the effectiveness of an inhibitor in the termination of the oxidation chain on the one hand and its ability to decrease the oxidation rate during the IP on the other; $AOA = F/ORR$; where $F = IP_A/IP_C$ and $ORR = R_A/R_c$.

The following order of decrease of the general kinetic parameter, AOA, has been found:

15 > **11** > **9** > **12** > **13** > SA > **14** > **8** > **10** > FA.

It is evident that on the basis of kinetic analysis of the inhibited lipid autoxidation in the presence of

hydroxycinnamoyl amides, it is possible to clarify the effect of structural factors on their antioxidant potency. However, the explanation of the mechanism of action of these compounds needs a much more detailed research. The results obtained would be useful for discussing the relationship between the antioxidant action and antiviral, antimicrobial and antifungal activity of the feruloyl- and sinapoyl-amino acid amides. This study is in progress.

CONCLUSIONS

1. For the first time, 15 synthesized amides of phenylpropanoic acids with natural and unnatural *C*-protected amino acids have been studied for their antioxidant efficiency in bulk phase lipid autoxidation. An AOA has been found for sinapoyl- and feruloyl-amino acid amides.
2. The highest AOA has been found for compounds **11** and **15**, containing the same phenylalanine residue.

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