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Chlorogenic Acids Mimics - Synthesis, Structure and Antioxidant Activity

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Abstract

A series of chlorogenic acid analogs involving replacement of quinic moiety by glucuronic acid followed by coupling with ferulic, lipoic or caffeic acid as well as exchange an ester bond between saccharide and natural polyphenol by an amide bond were synthesized. Antioxidant and free radical scavenging properties of such conjugates in comparison to chlorogenic, ferulic, caffeic and lipoic acids were tested by FRAP and ABTS tests, respectively. These results suggest that replacement of quinic by glucuronic moiety yields chlorogenic acid mimics with high antioxidant activity. Experimental evidence was found that antioxidant and scavenging properties are independent properties and are derivative of not only natural type of polyphenol but also sugar moiety. Molecular structure of compounds **10** and stereochemistry of substrate **1** were confirmed on the basis of X-ray analysis of the respective monocrystals.

Keywords: natural polyphenols, chlorogenic acid, oxidative stress, FRAP, ABTS

1. Introduction

Natural polyphenols abundant in fresh and dry fruits, flowers and vegetables are rich source of potential pharmaceuticals. Among these, a large deal of interest is focused on biological activity of chlorogenic acid family (CA), a major polyphenolic components in many plants (e.g. coffee products). This group of compounds involves isomeric quinic and caffeic acid esters: 3-*O*-caffeoylquinic, 4-*O*-caffeoylquinic and 5-*O*-caffeoylquinic acids, and to less extend, various esters of quinic acid and other *trans*-cinnamic acids, e.g. *p*-coumaric and ferulic, etc. acids. Recently discovered their anticancer, anti-Herpes and anti-HIV properties place this group of compounds in the center of numerous clinical studies (Cano-Marquina et al., 2013). Although they are not classic medicines, their broad biological activity involving inhibition of oxidative stress and thus reduction of proteins and phospholipids damage makes them potential nutraceuticals or complementary medicines of chemopreventive value. Among particularly promising developments, is discovery of anti-inflammatory activity of CA that reduces discomforts associated with neuropathic pain (Badgas et al., 2013). Several other recent studies showed involvement of CA in the protective pathways of the central nervous system (CNS) neurons in various oxidative stress models.

Taram et al, showed that CA and caffeic acid but not quinic acid expressed significant protective effect against the nitric oxide donor, sodium nitroprusside. On the other hand, CA and quinic acid had no protective effect against glutamate-induced cell death, whereas caffeic and ferulic acids significantly protected neurons from excitotoxicity. Finally, caffeic acid was the only compound to display significant protective activity against hydrogen peroxide, proteasome inhibition, caspase-dependent intrinsic apoptosis, and endoplasmic reticulum stress (Taram et al., 2016). Fang et al., studied impact of CA on ethanol-induced nerve damage. It has been found that the CA pretreated CNS neurons effectively promoted the proliferation of damaged cells, increased the distribution ratio of the cells at the G2/M and S phases, and enhanced mitochondria transmembrane potential in a concentration dependent manner (Fang et al., 2016).

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In another study Mikami et al., reported the protective effect of CA on glutamate-induced neuronal cell death using primary cultures of mouse cerebral cortex. They found that CA protects neurons from glutamate neurotoxicity by regulating Ca^{2+} entry into neurons (Mikami et al., 2015).

If homeostasis of the nervous system is concerned the only other natural compound with well documented neuroprotective activity against oxidative stress induced by various stressors is *R*-α-lipoic acid that contains the cyclic disulphide bond, and it has been also used in these studies (Muller et al., 1995; Prehn et al., 1992; Youdim et al., 1997; Zhang et al., 2001). Among its several exceptional properties a particularly promising is positive effect on survival of ganglion cells (Osborne, 2008) or retinal neurons (confirmed in *in vitro* and *in vivo studies*) that is essential for prevention of glaucoma (Koriyama et al., 2013). At present, vast majority of studies are focused on antioxidant and radical scavenging properties of polyphenols what is suggested as a predominant factor modulating their biological activity. However, there is growing evidence that their role is much more complex and covers activity as ligands or inhibitors of proteins regulating certain cellular redox processes. From this perspective, studies on retaining in serum appropriate level of antioxidant compounds during the whole day/night cycle is of great importance (Morera-Fumero et al., 2016).

From the accumulated data it is not clear what role in CA family plays quinic acid – is it just a biocompatible carrier or residue that contributes to the biological activity in an individual fashion. In order to evaluate the importance of quinic moiety on antioxidant activity of chlorogenic acids a series of 4-*O*-D-glucuronates of caffeic and ferrulic acid and lipoic acid D-glucuroanamide as well as D-glucuronate-5-carboxy methyl ester of caffeic acid were obtained. Moreover, glucuronic acid and glucuronates are nontoxic compounds abundant in human organism due to metabolic activity of the liver. The new glucuronates were characterized for antioxidant effect measured as reducing power of Fe(III) to Fe(II)against TPTZ complex (FRAP test) and scavenging effect against free cationic radicals (ABTS test) in relation to reference compounds: chlorogenic acid (4-*O*-D-caffeoylquinic acid, CA) and the respective parent polyphenols – ferulic, caffeic and lipoic acids. Among studied analogs of chlorogenic acids the least active in two tests was ester with lipoic acid. It was found that antioxidant and scavenging properties are independent properties and are derivative of not only type of natural polyphenol but also sugar moiety. For example, although 4-*O*-D-caffeoylquinic acid is less active in FRAP test than the caffeic acid alone, it has superior radical scavenging ability.

2. Materials and methods

2.1 General

Chemicals used for the FRAP assay, 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), acetic acid (TCA, ≥99.0%), sodium acetate (≥99.0%), $FeCl_3 \times 6\ H_2O$, $FeSO_4 \times 7\ H_2O$, Trolox® and HCl were purchased from Sigma-Aldrich. Chemicals used for the ABTS assay, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and $K_2S_2O_8$ were purchased from Sigma-Aldrich.

The following polyphenolic standards (purity ≥95%): caffeic acid, ferulic acid, lipoic acid, and chlorogenic acid as well as all solvents were purchased from Sigma-Aldrich.

2.2 Synthesis

General method A. A solution of methyl uronate (**1**, (Chen et al., 2013) 1.00 mmol) and α,β-unsaturated acid (**2** (Hasoda et al., 2001) or **5** (Akita et al., 2000), 1.20 mmol) in dichloromethane (20 mL) was stirred for 20 min at room temperature over molecular sieves (4 Å, 400 mg, finely ground), then cooled to -40 °C and TMSOTf (0.30 mmol) was added. After 2 h the reaction was quenched with Et_3N (1 mL), and the solvents were removed under reduced pressure. Column chromatography of the residue gave the expected product as white foam.

Methyl 1-O-*(4*-O-*acetyl-feruloyl)-2,3,4-tri*-O-*acetyl-β-D-glucopyranuronate* (**3**). Starting from 4-*O*-acetyl-ferulic acid (**2**) protected ester **3** (442 mg, 80%) was obtained. Eluent: hexane–ethyl acetate, 3 : 2. $[\alpha]_D^{20}$ 24.2 (*c* 0.5, dichloromethane). ^{1}H NMR ($CDCl_3$) δ: 7.71 (d, 1 H, *J* 16.0 Hz, =C*H*), 7.06-7.14 (m, 3 H, Ar), 6.35 (d, 1 H, *J* 16.0 Hz, =C*H*), 5.90 (d, 1 H, *J* 7.8, H-1), 5.36 (t, 1 H, *J* 9.2 Hz, H-3), 5.24-5.31 (m, 2 H, H-2, H-4), 4.23 (d, 1 H, *J* 9.6 Hz, H-5), 3.88 (s, 3 H, OC*H*$_3$), 3.74 (s, 3 H, OC*H*$_3$), 2.32 (s, 3 H, OAc), 2.05 (s, 3 H, OAc), 2.04 (s, 3 H, OAc), 2.03 (s, 3 H, OAc). ^{13}C NMR

($CDCl_3$) δ: 169.8 (C=O), 169.4 (C=O), 169.3 (C=O), 168.6 (C=O), 166.8 (C=O), 164.3 (C=O), 151.5 (C), 146.9 (=*C*H), 142.0 (C), 132.7 (C), 123.4 (Ar), 121.8 (Ar), 116.2 (=*C*H), 111.3 (Ar), 91.6 (C-1, $^1J_{C1\text{-}H1}$ 168.4 Hz), 73.1 (C-5), 71.8 (C-3), 70.1 (C-2), 69.0 (C-4), 56.0, 53.0, 20.6, 20.6, 20.5, 20.4. HR-MS (ESI) calc. for $C_{25}H_{28}NaO_{14}$ $[M+Na]^+$: 575.1377. Found: 575.1377.

Methyl 1-O-*(3,4-di*-O-*acetyl-caffeoyl)-2,3,4-tri*-O-*acetyl-β-D-glucopyranuronate* (**6**). Starting from 3,4-di-*O*-acetyl-caffeic acid (**5**) protected ester **6** (291 mg, 50%) was obtained. Eluent: hexane–ethyl acetate, 2 : 3. $[\alpha]_D^{20}$ 22.2 (*c* 0.5, dichloromethane). ^{1}H NMR ($CDCl_3$) δ: 7.70 (d, 1 H, *J* 16.0 Hz, =C*H*), 7.24-7.43 (m, 3 H, Ar), 6.35 (d, 1 H, *J* 16.0 Hz, =C*H*), 5.89 (d, 1 H, *J* 7.8, H-1), 5.36 (t, 1 H, *J* 9.3 Hz, H-3), 5.29 (t, 1 H, *J* 9.3 Hz, H-4), 5.25 (dd, 1 H, *J* 7.8, 9.2 Hz, H-2), 4.24 (d, 1 H, *J* 9.6 Hz, H-5), 3.75 (s, 3 H, OC*H*$_3$), 2.32 (s, 3 H, OAc), 2.31 (s, 3 H, CH_3), 2.06 (s, 3 H, OAc), 2.05 (s, 3 H, OAc), 2.03 (s, 3 H, OAc). ^{13}C NMR ($CDCl_3$) δ: 169.9 (C=O), 169.4 (C=O), 169.2 (C=O), 168.0 (C=O), 167.8 (C=O), 166.7 (C=O), 164.1 (C=O), 145.6 (=*C*H), 144.0 (C), 142.5 (C), 132.6 (C), 126.8 (Ar), 124.1 (Ar), 123.0 (Ar), 117.2 (=CH), 91.6 (C-1, $^1J_{C1\text{-}H1}$ 168.3 Hz), 73.0 (C-5), 71.7 (C-3), 70.0 (C-4), 69.0 (C-2), 53.0, 20.6, 20.6, 20.5, 20.5. HR-MS (ESI) calc. for $C_{26}H_{28}NaO_{15}$ $[M+Na]^+$: 603.1326. Found: 603.1326.

General method B. To a cooled (-30 °C) solution of 0.02 M LiOH in MeOH–THF–H_2O, 5 : 1 : 2 mixture (19 ml) an ester **3** or **6** (0.35 mmol)was added and stirred at the same temperature to complete disappearance of the starting material on TLC. Then, Amberlite IR 120 (0.2 g, acidic form) was added, stirred for additional 10 min, and filtered. Solvents were removed under reduced pressure. Column chromatography (acetonitrile–water, 9 : 1) of the residue gave the expected product as light yellow solid.

1-O-*Feruloyl-β-D-glucopyranuronic acid* (**4**) (Piazzonet al., 2012). Starting from **3** the title compound (39 mg, 30%) was obtained as yellow solid. M.p. 173-174 °C (dec.). $[\alpha]_D^{20}$ –0.8 (*c* 0.1, MeOH). ^{1}H NMR (D_2O) δ: 7.57 (d, 1 H, *J* 15.9 Hz, =C*H*), 7.06 (br s, 1 H, Ar), 6.99-7.01 (m, 1 H, Ar), 6.77-6.79 (m, 1 H, Ar), 6.25 (d, 1 H, *J* 15.9 Hz, =C*H*), 5.56 (d, 1 H, *J* 7.8 Hz, H-1), 3.80 (d, 1 H, *J* 9.5 Hz, H-5), 3.75 (s, 3 H, OCH_3), 3.48-3.56 (m, 3 H, H-2, H-3, H-4). ^{13}C NMR (D_2O) δ: 178.0 (C-6), 170.6 (C=O), 151.0, 150.8, 150.4, 129.5, 126.7, 118.4, 116.0, 114.2, 96.7 (C-1), 79.4, 78.2, 74.8, 74.5, 58.7 (OMe). HR-MS (ESI) calc. for $C_{16}H_{18}NaO_{10}$ $[M+Na]^+$: 393.0798. Found: 393.0798.

1-O-*Caffeoyl-β-D-glucopyranuronic acid* (**7**). Starting from **6** the title compound (58 mg, 46%) was obtained as yellow semisolid. $[\alpha]_D^{20}$ -1.1 (*c* 0.1, MeOH). ^{1}H NMR (CD_3OD) δ: 7.69 (d, 1 H, *J* 15.9 Hz, =C*H*), 7.14 (br s, 1 H, Ar), 7.05-7.07 (m, 1 H, Ar), 6.86-6.87 (m, 1 H, Ar), 6.37 (d, 1 H, *J* 15.9 Hz, =C*H*), 5.59 (d, 1 H, *J* 7.6 Hz, H-1), 3.78-3.80 (m, 1 H, H-5), 3.52-3.58 (m, 3 H, H-2, H-3, H-4). ^{13}C NMR (CD_3OD) δ: 174.9 (C-6), 167.4 (C=O), 148.1, 147.7 (=*C*H), 144.9, 126.4, 122.7, 115.7, 114.6, 113.0, 94.0 (C-1), 76.1, 75.7, 72.1, 71.8. HR-MS (ESI) calc. for $C_{15}H_{16}NaO_{10}$ $[M+Na]^+$: 379.0641. Found: 379.0641.

N-*(Methyl 2,3,4-tri*-O-*acetyl-β-D-glucopyranuronyl)lipoic acid amide* (**10**). The lipoic acid **9**, (400 mg, 1.44 mmol) was dissolved in a dry THF (10 ml) at 0 °C followed by HOBT (195 mg, 1.44 mmol) and DMAP (176 mg, 1.44 mmol) in THF (5 ml), and the mixture was stirred for 10 min. Then, DCC (297 mg, 1.44 mmol) in THF (3 ml) was added. To the above mixture, a solution of amine **8** (Pitt et al., 2004) (400 mg, 1.2 mmol) and TEA (0.5 ml, 3.6 mmol) in THF (5 ml) was added. The stirring was continued for 44 h at room temperature. Solvents were removed under vacuum and the residue was dissolved in EtOAc, washed with $NaHCO_3$ twice (2 × 30 ml), 2% citric acid (2 × 20 ml, twice), H_2O (2 × 20 ml, twice), and dried with $MgSO_4$. Solvents were removed under vacuum and the residue was purified by column chromatography (hexane–ethyl acetate–TEA, 2 : 8 : 0.02) to afford crude title compound (413 mg, 66%) as yellow solid. It was recrystallized from *i*- PrOH to give yellow crystals (282 mg, 45%). M.p. 141-148 °C. $[\alpha]_D^{20}$ 47.6 (*c* 0.5, dichloromethane). ^{1}H NMR ($CDCl_3$) δ: 6.31 (d, 1 H, *J* 9.4 Hz, NH), 5.38 (t, 1 H, *J* 9.5 Hz), 5.30 (t, 1 H, *J* 9.5 Hz), 5.14 (t, 1 H, *J* 9.7 Hz), 4.94 (t, 1 H, *J* 9.6 Hz), 4.16 (d, 1 H, *J* 10.0 Hz, H-5), 3.73 (s, 3 H, OCH_3), 3.52-3.58 (m, 1 H), 3.09-3.20 (m, 2 H), 2.42-2.48 (m, 1 H), 2.16-2.22 (m, 2 H), 2.05 (3 H), 2.03 (6 H), 1.88-1.93 (m, 1H), 1.60-1.71 (m, 4 H), 1.39-1.45 (m, 2 H). ^{13}C NMR ($CDCl_3$) δ: 172.9 (C=O), 170.9 (C=O), 169.6 (C=O), 169.5 (C=O), 167.1 (C=O), 77.9, 74.0, 71.8, 70.3, 69.7, 56.2 (CHS), 52.9 (OMe), 40.2 (douplicated), 38.4, 36.2 (douplicated), 34.5 (douplicated), 28.6 (douplicated), 24.7 (douplicated), 20.6, 20.5, 20.4. HR-MS (ESI) calc. for $C_{21}H_{31}NNaO_{10}S_2$ $[M+Na]^+$: 544.1287. Found: 544.1287.

N-*(β-D-Glucuronopyranosyl)lipoic acid amide* (**11**). Amide **10** (0.4 g, 1.15 mmol) was deprotected as described for the synthesis of **4**. Column chromatography (ethyl acetate–methanol–water, 5 : 2 : 1) gave the title compound (0.25, 85%) as light yellow solid.

^{1}H NMR (CD_3OD) δ: 6.7 (d, 1 H, *J* 9.2 Hz, NH), 5.72 (d, 1 H, *J* 7.8 Hz, H-1), 4.06 (d, 1 H, *J* 9.8 Hz, H-5), 3.4 – 3.7 (m, 3 H, H-2, H-3, H-4), 2.61 (t, 2H, lip CH_2), 2.55 (t, 1H, lip CH), 2.54 (m, 2H, lip CH_2), 2.0 (t, 2H, α-CH_2), 1.47 (m, 2H, β-CH_2), 1.22 (m, 2H, γ-CH_2), 1.5, m, 2H, δ-CH_2);

^{13}C NMR (CD_3OD) δ: 171.2 (C-6), 171.6 (CONH), 88.3 (C-1), 81.5 (C-5), 73.6 (C-3), 72.4 (C-4), 72.0 (C-2), 36.8 (C_α), 34.2 (C_δ), 27.6 (C_γ), 25.2((C_β).

HR-MS (ESI) calc. for $C_{14}H_{22}NO_7S_2$ [M-H]$^-$: 380.0838. Found: 380.0838.

N-*(β-D-Glucuronopyranosyl)caffeic acid amide* (**13**). Peracetylated amide **12** (0.15 g, 0.5 mmol) kept in -20 °C was deprotected gradually by adding 0.5 equiv of LiOH dissolved in 10 ml of MeOH-THF-H_2O, 4 : 1 : 2 mixture in two portions: first 0.1 mmol for 10 min and then the rest for 4 h at room temperature. Purification performed on preparative plate with MeCN-H_2O 8:2 mixture of solvents. Finally, column chromatography (ethyl acetate–methanol–water, 7 : 2 : 1) yielded **13** (0.032, g 21%) as light yellow semisolid.

^{1}H NMR (CD_3OD) δ: 7.47 (d, 1 H, *J* 15.6 Hz, =C*H*), 7.02 (br m, 1 H, Ar), 6.92 (m, 1 H, Ar), 6.76 (m, 1 H, Ar), 6.39 (d, 1 H, *J* 15.6 Hz, =C*H*), 5.05 (d, 1 H, *J* 9.2 Hz, H-1), 3.93 (d, 1 H, *J* 9.1 Hz, H-5), 3.74 (s, 3 H, OCH_3), 3.52 (t, 1 H, H-4), 3.44 (t, 1H, H-2, *J* 8.98 Hz), 3.35 (t, 1H, *J* 8.98 Hz H-1). ^{13}C NMR (CD_3OD) δ: 171.4 (C-6), 170.0 (C=O), 149.2.9, 146.8 (=COH), 128.2, (=CCC), 122.5, 117.9, 116.5, 115.2 (=CH), 81.6, 77.7 (C-1), 78.4 (C-5), 73.7 (C-2), 73.3 (C-4), 72.7 (C-3), 72.0 (C-4), 52.8 (OMe). HR-MS (ESI) calc. for $C_{15}H_{17}NO_9$ [M+Na^+] monoisotopic mass 378.0801, found 378.0801

2.3 X-ray data collection and structure determinations

Single crystals from bulk sample **10** were isolated and mounted immediately into a loop. X-ray measurements were carried out at 150 °K using 0.5° ω scans on the Bruker X8 diffractometer furnished with an APEX II CCD detector using CuKα (λ = 1.54178 Å) radiation.

Integration was done using the SAINT software package that is a part of the APEX II software suite; absorption corrections were conducted using SADABS (Bruker, 2005). The structures were solved by direct methods using SHELXS-2013/1 and refined by full-matrix least squares with SHELXL-2014/7 (Sheldrick, 2008). In each structure, all non-hydrogen atoms were located *via* difference Fourier maps and refined anisotropically. Aromatic, methyl and methine group hydrogen atom were placed at their idealized positions and allowed to ride on the coordinates of the parent atom with isotropic thermal parameters (U_{iso}) fixed at 1.2 Ueq of the carbon atom to which they are attached. Crystallographic data have been deposited at the Cambridge Crystallographic Data Centre, 12 Union Road, 129 Cambridge CB21EZ, UK, and copies can be obtained on request, free of charge, by quoting the publication citation and the deposition number CCDC 1530866.

2.4 FRAP assay

Chemicals: TPTZ (2,4,6-tripyridyl-s-triazine) was dissolved in HCl and left for few hours; $FeSO_4 \cdot 7H_2O$ dissolved in water was used as a standard solution. $FeCl_3 \cdot 6H_2O$ (iron (III) chloride hexahydrate) was dissolved in water one day before analysis.

Assay: Experiments were done according to the method described by Benzie and Strain, (1996) with modifications by Biskup et al., (2013). FRAP working solution was prepared freshly each time: 0.3 M acetate buffer (pH=3.6), 0.01 M TPTZ (2,4,6-tripyridyl-s-triazine) in 0.04 M HCl and 0.02 M $FeCl_3 \cdot 6H_2O$ were mixed in 10:1:1 (v/v/v) and kept away from light. To 2.88 ml FRAP working solution were added 0.24 ml of phenols (final concentration 0.003–0.07 mmol/ml) or FeSO4 · $7H_2O$ (final concentration 0.007 - 0.08 mmol Fe^{2+}/mL) solution. The mixture was incubated at 37 °C for 15 min away from light. Absorbance was measured at 593 nm using an automated UV-VIS Carry 100E spectrophotometer. FRAP working solution with suitable solvent instead of a sample was used as a blank. All determinations were carried out in triplicate.

2.6 ABTS assay

Chemicals: Trolox® (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as an antioxidant standard. Trolox (4.2 mM) was prepared in methanol for use as a stock standard. ABTS, 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) di-ammonium salt was dissolved in potassium persulfate.

Assay: Experiments were performed according to Pellegrini et al., (1999). Potassium persulfate were dissolved in water to a final concentration of 2.45 mM. ABTS was dissolved in the obtained earlier solution of potassium persulfate. The obtained solution 1.8 mM $ABTS^{\bullet+}$ radical cation was incubated for 16 hours at room temperature in the dark before use. For the study of polyphenols the ABTS radical solution was diluted with water to an absorbance of 1.00 at 734 nm.

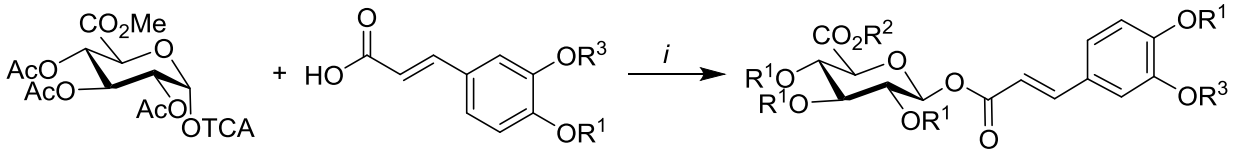
Studied polyphenols (final concentrations 0.007-0.04 mM) or Trolox (final concentration 0-0.03 mM) were added to diluted $ABTS^{\bullet+}$ solution and the absorbance reading was taken 10 min after mixing using an automated UV-VIS Carry 100E spectrophotometer. Results are presented as the ability of phenols to scavenge 50% of free radical $ABTS^{\bullet+}$ (IC_{50}) and TEAC (Trolox equivalent antioxidant capacity).

Parameters IC_{50} (μM) and TEAC (μM) were determined with a relative uncertainty of less than five percent. All analyses were performed in triplicate and the results were expressed as the mean value ± standard deviation.

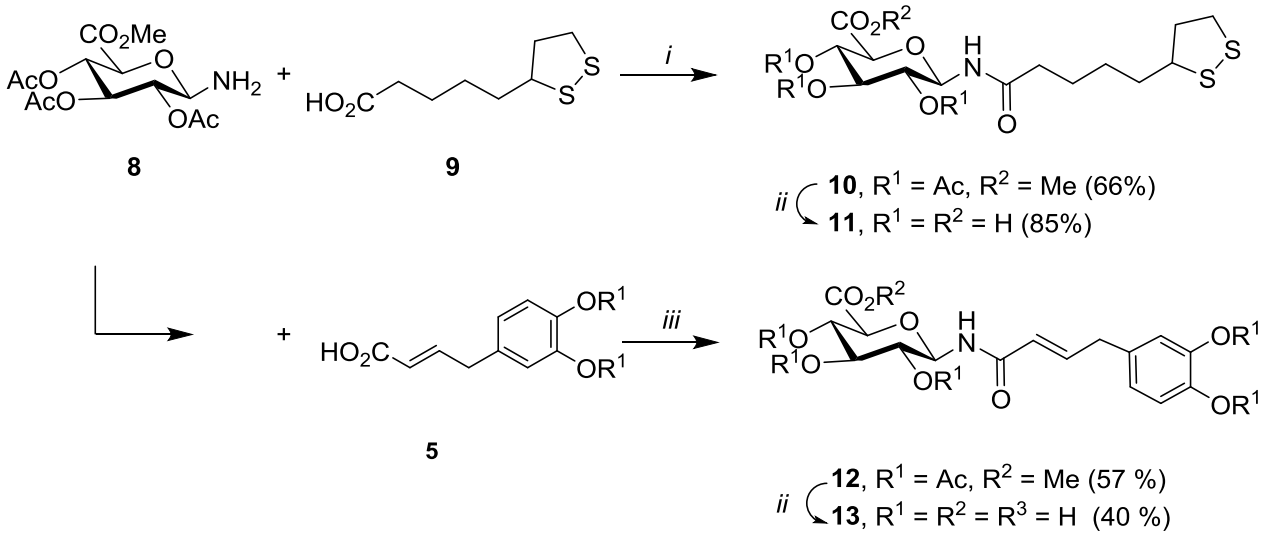
3. Results and discussion

3.1 Synthesis and structure determination

Synthetic procedure leading to the natural polyphenols 4-*O*-D-glucuronates involves coupling of methyl esters of the respective 2,3,4,6-tetra-*O*-acetyl-a-D-glycopyranosyl-1-trifluoroacetamidate or *O*-acetyl-protected glucosamine with protected versions of natural polyphenols, followed by the deprotection procedure. Since hydroxy groups of both acid fragments have different chemical character (carboxyl, glucuronic and phenol hydroxyls), the selective deprotection is a critical step limiting final yield and purity of the glucuronates. In the present case LiOH dissolved in MeOH/MeCN/H_2O of various ratios and different starting and final temperatures and reaction time were used, yielding final products with low to medium yield. Similar problems were reported by Shimotori et al. who used Novozym 435 for deacetylation of glucosylated analogs of *trans*-cinnamic acid series (Shimotori et al., 2012).



Scheme 1. Reagents and conditions: (*i*) dichloromethane, TMSOTf, -40 °C, 2 h; (*ii*) 0.02 M LiOH in MeOH–THF–water, 5 : 1 : 2, -30 °C, 8 h (for 4) or 24 h (for 7).



Scheme 2. Reagents and conditions: (*i*) HOBt, DMAP, DCC, TEA, RT, 44 h; (*ii*) 0.02 M LiOH in MeOH–THF–water, 5 : 1 : 2, -5 °C, 40 min.

The obtained esters are relatively unstable in solution and quality crystals could not be obtained. However, N-(Methyl-2,3,4-tri-O-acetyl-β-D-glucopyranuronyl)lipoic acid amide (**10**) yielded crystals of good quality suitable for X-ray crystal determination. The ^{13}C-NMR spectrum of the amide **10** shows an extra set of peaks - several resonances of lipoic acid fragment and two of carbonyl groups appear as doubled peaks. It is likely that they arise from slow decomposition in $CDCl_3$. Although crystal of compound **10** during X-ray diffraction measurement was cooled down to 150 °K, indeed lipoic acid fragment expressed relatively large thermal motions as evidenced from elongation of thermal ellipsoids in the direction perpendicular to the 5-membered ring (Fig. 1 left). Lipoic acid residue at C1 is located in β-anomeric position. Molecules in the crystal are organized as two-fold symmetry related dimers bonded *via* a pair of NH … O hydrogen bonds (Fig. 1).

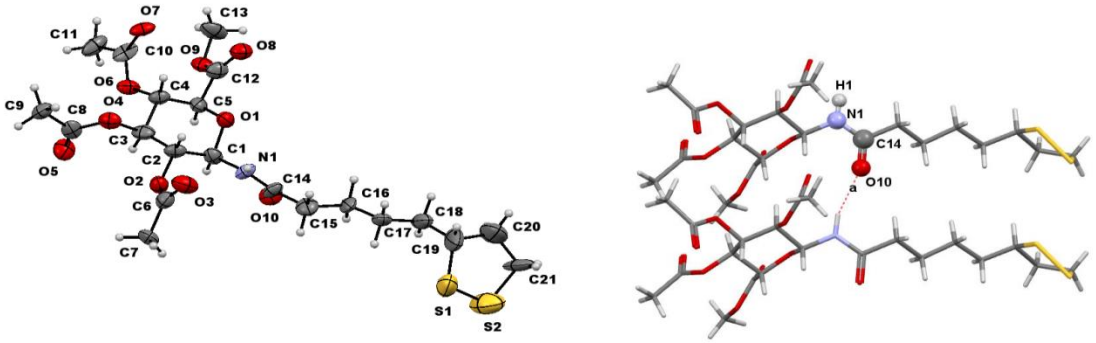


Figure. 1. X-ray structure of compound 10; left) thermal ellipsoids shown at 50 % probability level; right) two neighboring molecules connected by C=O … HN H-bonding.

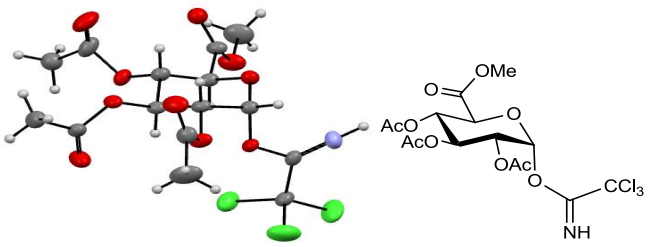


Figure. 2. X-ray structure of the prevailing isomer of trichloroacetamidate1 (crystallizing as second polymorph) confirming its α-structure.

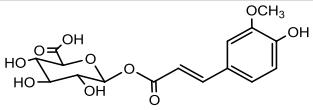
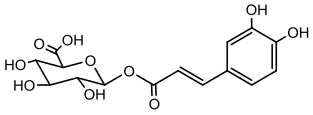
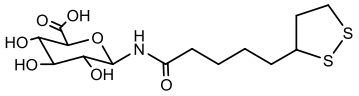
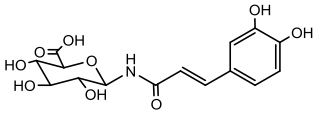
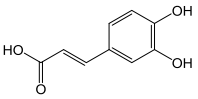
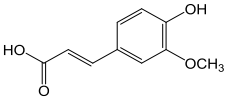
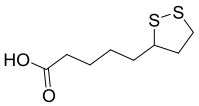
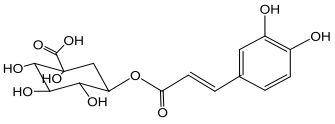
On the other hand, the alpha configuration of the anomeric substituent in the trichloroacetamidate **1**, an active reactant used for glucuronidation of cinnamic acid derivatives was confirmed by analysis of its ^{1}H NMR chemical shifts. X-Ray data also revealed that substrate **1** recrystallized from isopropanol (not tested before) yielded known second polymorphic form (Fig. 2) (Hayes et al., 2013).

3.2 Determinantion of Antioxidant (FRAP, μmol equiv. of Fe(II)/L*)* and Free Radical Scavenging Activity (ABTS)

Natural chlorogenic acids are esters of quinic and caffeic, ferulic, or *p*-coumaric acids, with the caffeic acid derivatives being the most abundant. Since 5'-caffeoylquinic acid is by far the dominant isomer in coffee and also the only chlorogenic acid isomer commercially available, we used the generic name of chlorogenic acid to indicate this isomer in the following chapters of this study. *R*-α- and its D-glucuronamidewere used in this research as reference compounds, since apart from different chemical structures and probably mechanism of action at cellular level they express similar biological activity.

Antioxidant potential (AP) of the obtained compounds was estimated as Ferric Reducing Antioxidant Power (FRAP test), i.e. ability of a compound to reduce Fe(III) complex of ferric 2,4,6-tris(2pyridyl)-*s*-triazine[Fe(III)-TPTZ] to its Fe(II) oxidation state providing a strong absorbance at 593 nm, in relation to two reference antioxidant compounds of a known concentration: $FeSO_4$ and Trolox. Second test, i.e. free radical scavenging ability assay shows capability of a compound to interact with stable radicals prepared from 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS). Both applied tests are presenting different aspects of antioxidative mechanism. While FRAP assay measures the reducing capacity of antioxidants in terms of ability to transfer electron between Fe(III) and Fe(II) ions, ABTS assay determines cationic free radical scavenging activity involving both electron and hydrogen transfer mechanism. These are two independent properties that relay on chemical/electronic structure of the molecule.The results obtained for the tested chlorogenic acid analogs and of the respective parent *trans*-cinnamic acid derivatives are presented in Table 1. Contrary to the antioxidant level of the respective natural *trans*-cinnamic acids, the antioxidant power of caffeic 1-*O*-*β*-D-glucuronide (**7**) measured in FRAP test (as referenced to $FeSO_4$ and Trolox antioxidant power) is lower than that of the ferulic 1-*O*-*β*-D-glucuronide (**4**). Moreover, it is significantly lowered when instead of ester bond between glucuronic residue and caffeic acid an amide linkage is introduced (comp **13**). In all of the above cases FRAP value of glucoronate is lower than FRAP of the corresponding parent polyphenol. According to the present testing, lipoic acid alone is rather weak antioxidant and cationic radical scavenger what suggests that its well-documented neuroprotective properties, for example due to radiation damage (Manda et al., 2007, Manda et al., 2008) are not directly associated with antioxidant or radical scavenging ability. Its 1-*O*-*β*-D-glucuronide **11**, expresses low FRAP value and low scavenging ability in the ABTS test. The highest free cationic radical scavenging ability in ABTS test were found for chlorogenic (9.17) and ferulic (8.46) acids. Within the glucuronates family the highest ability to quench reactive oxidative species possess derivative of ferulic acid [5,3(5) μmolTrx/L]. Studies of metabolic pathway of CA in human dietary tract after consumption of CA alone or CA-rich products revealed that most likely CA is cleaved by esterase contained in colonic flora into quinic and caffeic acid (Plumb et al., 1999). Although no hydrolysis of CA was observed by the intestine, liver or plasma enzymes the extensive sulfation and glucuronidation occurred in the liver. Although both processes yielded mostly phenol *O*-esters, the glucuronic acid *O'*-esters were also identified in urine (Nardini et al., 2002). As authors pointed out the phenol *O'*-glucuronidation significantly diminished antioxidant activity**.** Our studies supported these findings. The postulated mechanism of chlorogenic acids antioxidant activity depends on cleavage of an ester bond and release of polyphenols. Since amide bond is chemically more stable than an ester bond it explains why compound **13** is much less active. Natural polyphenols express broad spectrum of biological activity and it is obvious that even if the antioxidant property is suggested as being responsible for their observed beneficial effects they are likely involved in more than one metabolic pathway. These results are in agreement with the proposed metabolic pathway that CA is undergoing change in gastrointestinal track that postulates cleavage of an ester bond and release of the active caffeic acid. This indicates one of possible pathways occurring in mammals and indeed, such metabolism products were found in body fluids(Mateos et al., 2006; Wong et al., 2010).

Table 1. Redox (FRAP, μmol Fe^{2+}/L and μmolTrx/L)*)and radical scavenging potency (ABTS, μmolTrx/L), of chlorogenic acid analogs and reference polyphenols.

Compounds			FRAP		ABTS
			μmol Fe^{2+}/L	μmolTrx/L	μmolTrx/L
4		$C_{16}H_{18}O_{10}$ M_w = 370.31	10,96 ±0,43	8,02 ±0,31	4,07 ±0,45
7		$C_{15}H_{16}O_{10}$ M_w = 356.28	7,06 ±1,08	5,16 ±0,79	3,45 ±0,43
11		$C_{14}H_{23}NO_7S_2$ M_w = 381.46	0,49± 0,02	0,36 0,02	0,19 ±0,06
13		$C_{15}H_{17}NO_9$ M_w = 355.30	8,78 ±0,47	6,42 ±0,35	2,20 ±0,27
Caffeic acid		$C_9H_8O_4$ M_w = 180.16	14,87 ±0,28	10,88 ±0,20	5,44 ±0,52
Ferulic acid		$C_{10}H_{10}O_4$ M_w = 194.18	11,67 ±0,32	8,54 ±0,24	8,36 ±0,74
Lipoic acid		$C_8H_{14}O_2S_2$ M_w = 206.33	2,57 ±0,31	1,88 ±0,23	0,21 ±0,05
Chlorogenic acid		$C_{16}H_{18}O_9$ M_w = 354.31	12,41 ±0,69	9,08 ±0,51	9,06 ±1,13

*) Trolox

2.3. Conclusions

The present study provided synthetic methods for new chlorogenic acid derivatives linking several common polyphenols with a relatively cheap 4-*O*-D-glucuronic acid moiety. The resulting group of compounds expressed significant antioxidant (FRAP) and cationic radical scavenging properties (ABTS). Experimental evidence was also found that antioxidant and scavenging properties are independent properties that cannot be predicted and depend both on type natural of polyphenol and also sugar moiety.

Although natural polyphenols are highly bioavailable they undergo fast metabolism and excretion and, therefore, their effective concentration in cellular compartments might be too low to exert beneficial therapeutic effect.

This requires chemical modifications of natural polyphenols to assure longer lifetime, resistance to enzymatic processes and therefore, adequate concentration in the tissue. Current results show that there is great potential in designing new and cheaper analogs that are more chemically stable (chlorogenic acid glucuronamides *vs.* esters) and express high activity as radical scavengers and antioxidants.

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References

Cano-Marquina, A.,Tarin, J.J., Cano, A. (2013). The impact of coffee on health. Maturitas, 75, 7-21.

Bagdas, D.,Cinkilic, N.,Ozboluk, H.Y.,Ozyigit, M. O.,Gurun, M. S. (2013). Antihyperalgesic activity of chlorogenic acid in experimental neuropathic pain. Part: A. J. Nat. Medicines*,* 67, 698–704.

Taram, F.,Winter, A.N.,Linseman, D.A.(2016). Neuroprotection comparison of chlorogenic acid and its metabolites against mechanistically distinct cell death-inducing agents in cultured cerebellar granule neurons. Brain Res., 1648, 69-80.

Fang, S.Q.,Wang, Y.T.,Wei, J.X.,Shu, Y.H.,Xiao, L.,Lu, X.M. (2016). Beneficial effects of chlorogenic acid on alcohol-induced damage in PC12 cells. Biomedicine Pharmacother., 79, 254-262.

Mikami, Y.,Yamazawa, T. (2015). Chlorogenic acid, a polyphenol in coffee, protects neurons against glutamate neurotoxicity. Life Sci., 139, 69-74.

Muller, U.,Krieglstein, J. (1995). Prolonged Pretreatment with α-Lipoic Acid Protects Cultured Neurons against Hypoxic, Glutamate-, or Iron-Induced. Injury. J. Cerebral Blood Flow andMetabolism, 15,624-630.

Prehn, J.H.M.,Karkoutly, C.,Nuglisch,J.,Peruche, B.,Krieglstein, J. (1992). Dihydrolipoate Reduces Neuronal Injury after Cerebral Ischemia. J. Cereb. Blood Flow Metab., 12, 78-87.

Youdim, M.B.H., Ben-Shachar, D.,Eshel, G. (1997). Neuroprotection by desferrioxamine and lipoicacid against 6-hydroxydopamine induced dopaminergic neurotoxicity. NeuroscienceLett.*,* Suppl. 48*,* S54-S54.

Zhang, L., Xing, G.Q., Barker, J.L., Chang, Y.,Maric, D.,Ma, W.,Li, B.S.,Rubinow, D. R. (2001). alpha-Lipoicacid protects rat cortical neurons against cell death induced by amyloid and hydrogen peroxide through the Aktsignalling pathway.Neuroscience Lett., 312,125-128.

Osborne, N.N.(2008). Pathogenesis of ganglion "cell death" in glaucoma and neuroprotection: focus on ganglion cell axonal mitochondria. Prog. Brain Res., 173, 339-352.

Koriyama, Y.,Nakayama, Y.,Matsugo, S.,Kato, K. (2013). Protective effect of lipoic acid against oxidative stress is mediated by Keap1/Nrf2-dependent heme oxygenase-1 induction in the RGC-5 cellline. Brain Res., 173, 145-157.

Morera-Fumero, A. L., Abreu-Gonzalez, P., Fernandez-Lopez, L., Díaz-Mesa, E.,Cejas-Mendez, M. R. (2016). Summer Day/Night Gender Differences in Serum TotalAntioxidant Capacity as a Methodological Pitfall in HumanAntioxidant Research. J. Chem. Biochem., 4, 1-13.

Chen, Y., Li, Y., Yu, H.,Sugiarto, G., Thon, V., Hwang, J., Ding, L.,Hie, L., Chen, X. (2013). Tailored Design and Synthesis of Heparan Sulfate Oligosaccharide Analogues Using Sequential One-Pot Multienzyme Systems. Angew. Chem. Int. Ed., 52, 11852-11856.Hosoda, A., Nomura, E.,Mizuno, K., Taniguchi, H. (2001).Preparation of a (+/-)-1,6-di-O-feruloyl-myo-inositol derivative: an efficient method for introduction of ferulic acid to 1,6-vicinal hydroxyl groups of myo-inositol.J. Org. Chem., 66, 7199-7201.

Akita, H.,Nozawa, M.,Mitsuda, A.,Ohsawa, H. (2000). A convenient synthesis of (+)-albicanol based on enzymatic function: total syntheses of (+)-albicanyl acetate, (-)-albicanyl 3,4-dihydroxycinnamate, (-)-drimenol, (-)-drimenin and (-)-ambrox.Tetrahedron: Asymmetry, 11, 1375-1388.

Piazzon, A.,Vrhovsek, U.,Masuero, D.,Mattivi, F.,Mandoj, F., Nardini, M. (2012).Absorption of Chlorogenic Acid and Caffeic Acid in Rats after Oral Administration.Agric. Food Chem., 60, 12312−12323.

Pitt, N., Duane, R. M., O'Brien, A., Bradley, H., Wilson, S. J., O'Boyle, K. M., Murphy, P.V. (2004). Synthesis of a glucuronic acid and glucose conjugate library and evaluation of effects on endothelial cell growth. Carbohydr. Res., 339, 1873-1887.

Bruker **2005**. *APEX2*, *SAINT* and *SADABS* Bruker AXS Inc., Madison, Wisconsin, USA.

Benzie, I.F., Strain J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal. Biochem., 239, 70 – 76.

Biskup, I., Golonka, I.,Gamian, A., Sroka, Z. (2013). Evaluation of antioxidant properties of *Salacia macrosperma* leaf extracts. PostepyHig. Med. Dos., 67, 958 – 963.

Re, R., Pellegrini, N.,Proteggente, A.,Pannala, A.,Yang, M.,Rice-Evans C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med., 26, 1231 – 1237.

Shimotori, Y.,Tsutano, K., Soga, K., Yosuke Osawa, Y., Aoyama, M., Miyakoshi, T. (2012). Synthesis of glycosylferulate derivatives by amine-promoted glycosylation with regioselective hydrolysis using Novozym 435 and evaluation of their antioxidant properties. Carbohydrate Research, 359, 11–17

Hayes, J.A., Eccles, K.S.,Elcoate, C.J.,Daly, C.A.,Lawrence, S.E., Moynihan, H.A. (2013). CrystalPolymorphismofMethyl2,3,4-tri-O-acetyl-1-O-(trichloroacetimidoyl)-alpha-D-glucopyranouronate. J. Chem. Crystallogr., 43, 138–143.

Manda, K.,Ueno, M.,Moritake, T.,Anzai, K. (2007). Radiation-induced cognitive dysfunction and cerebellar oxidative stress in mice: Protective effect of alpha-lipoicacid. Behaviour. Brain Res., 177, 7-14.

Manda, K.,Ueno, M.,Anzai, K. (2008). Memory impairment, oxidative damage and apoptosis induced by space radiation: Ameliorative potential of alpha-lipoicacid. Behaviour. Brain Res., 187,387-395.

Plumb, G.W.,Garcia-Conesa, M.T.,Kroon, P.A.,Rhodes, M.,Ridley, S.,Williamson, G. (1999). Metabolismofchlorogenicacidbyhumanplasma, liver, intestine and gutmicroflora. J. Sci. Food Agric.,79, 390-392.

Nardini, M.,Cirillo E.,Natella F.,Scaccini C. (2002). Absorption of Phenolic Acids in Humans after Coffee Consumption. J. Agric. Food Chem.,50, 5735-5741.

Mateos, R., Goya, L., Bravo, L. (2006). Uptake and metabolism of hydroxycinnamic acids (chlorogenic, caffeic, and ferulic acids) by HepG2 cells as a model of the human liver. J. Agric. Food Chem,54, 8724-8732.

Wong, C.C.,Meinl, W.,Glatt, H.R.,Barron, D.,Stalmach, A.,Steiling, H.,Crozier, A. (2010). Ain vitro and in vivo conjugation of dietary hydroxycinnamic acids by UDP-glucuronosyltransferases and sulfotransferases in humans. J.Nutrit. Biochem.,21, 1060-1068.