European Journal of Medicinal Chemistry 50 (2012) 383–392

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Novel molecular combination deriving from natural aminoacids and polyphenols: Design, synthesis and free-radical scavenging activities

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ARTICLE INFO

Article history:
Received 6 August 2011
Received in revised form
6 February 2012
Accepted 8 February 2012
Available online 17 February 2012

Keywords:
Cysteine
Methionine
Tyrosine
Molecular conjugation
Antioxidant capacity
Phenolic acids

ABSTRACT

Following the recent output of scientific publications in the matter of synergic activity between different antioxidants, we have undertaken the present study with the aim to synthesize new molecules with radical-scavengers activity based on the conjugation of bioactive portions (i.e. phenols, cysteine, methionine or tyrosine), characterized by different structures and mechanisms of action, to promote the simultaneous quenching of different radical species in the site of the oxidative damage. In this context, derivatives of phenolic acid, aminoacids and dopamine have been also prepared. The newly synthesized compounds were evaluated *in vitro* applying specific and complementary antioxidant test such as DPPH assay and ORAC test. As emerged from the evaluation, prerequisites for the activity of the synthesized molecules were: i) the maintenance of at least two hydroxylic groups on the aromatic moiety of phenolic portion, ii) the presence of a spacer between the aromatic moiety and the carbonilic group.

1. Introduction

Several lines of evidence suggest that the damage from free radicals plays a role in the development of a variety of disorders, including atherosclerosis [1], cancer [2], ischemia-riperfusion injury [3], rheumatoid arthritis [4], neurodegeneration and aging [5]. Until the endogenous mechanisms of protection are sufficient to contrast the various reactive oxidative injuries generated both endogenously, metabolic reactions [6,7], or exogenously, xenobiotics [8], severe damages are avoided to the biomolecules. When these defences are overwhelmed, an unbalance occurs, and it becomes necessary to provide, from external sources, the required protection to avoid oxidative stress and related irreversible alterations of biomolecules.

In vitro and *in vivo* studies, have shown the effective role of antioxidants in the prevention or suppression of disorders such as oxidative damage to DNA, proteins, and lipid, or many chronic diseases [9–13]. In consideration of this, it becomes important to balance the normal diet or an eventual therapy with an antioxidant treatment, able to reduce the oxidative damage and to improve therefore the clinical profile of the patient [14].

Various scientific studies suggest that a cocktail of antioxidants, endowed with different mechanism of action and molecular structure, result more effective than a single antioxidant by virtue of the synergic effect among the molecules [15–21]. Several antioxidants, such as α-tocopherol, quercetin, catechins, N-acetylcysteine, and β-carotene play paradoxical roles, acting as "double-edged swords" and inducing pro-oxidant and cytotoxic effects under some circumstances, due to the formation of reactive oxygen species [22] and to the formation of intermediate metabolites [23]. To highlight possible synergic mechanisms and to better understand mechanistic aspects, the design of modified and/or dualistic molecules is a valuable approach.

Great efforts have been done in recent years to design synthetic tocopherol analogues with increased activity and stability and to

Abbreviations: AAPH, 2,2′-azobis(2-amidino-propane) dihydrochloride; AcOEt, ethylacetate; Boc, *tert*-butyloxcarbonyl; ANOVA, analysis of variance; Boc_2O, di-*tert*-butyl dicarbonate; BOP, (Benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate; CH_3COOH, acetic acid; CH_2Cl_2, dichloromethane; CH_3CN, acetonitrile; Cys, cysteine; Cys-trit, trityl-cysteine; DCC, N,N-dicyclohexylcarbodiimide; DCU, dicyclohexyl urea; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; DPPH, 1,1-diphenyl-2-picryl-hydrazyl radical; Et_3Si, triethylsilane; $FeCl_3$, ferric chloride; HBr, Hydrobromic acid; $KMnO_4$, Potassium permanganate; MeOH, methanol; NHS, N-Hydroxysuccinimmide; ORAC, Oxygen Radical Absorbance Capacity; ROS, reactive oxygen species; sat.d aq., satured aqueous; TEA, triethylamine; TFA, trifluoroacetic acid; TLC, thin layer chromatography; Trolox, (±)-6-hydroxy-2,5,7,8-tetramethyl-chromane-2-carboxylic acid.

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doi:10.1016/j.ejmech.2012.02.018

get better insight into structure–activity relationships in this class of molecules [24,25].

In this regards, the molecular combination of tocopherols and other antioxidants, a strategy termed "centaurus tactic" developed by us, has led to obtain new compounds with increasing radical scavenging activity [26]. The combination with the 2,3-dihydroxy-2,3-enono-1,4-lactone ring of ascorbic acid produced a compound displaying potent antioxidant properties when compared to the parent synthetic α-tocopherol analogue or to the natural derivatives (α-tocopherol or ascorbic acid), both used alone or in combination [25]. FeAox-6, which combines, into a single molecule, the chromanyl head of vitamin E and a sequence of four conjugated double bonds in the side chain [27] displayed greater antioxidant efficiency than α-tocopherol in protecting cell membranes from oxidative stress. Again, among several novel tocopherol analogues recently published by our group [28], the compounds bearing 3,4-dihydroxyphenyl (catechol) moieties revealed to possessed high capacity to transfer electrons, to chelate ferrous ions, and to scavenge ROS [29–32].

The results of these studies have confirmed that the molecular combination is useful to investigate structure–activity relationships and that we can improve the antioxidant efficiency of the natural α-tocopherol by designing synthetic α-tocopherol analogues that possess a modified side chain.

On the bases of the literature evaluation on the properties of phenols [33] and sulphur compounds [34], we have selected, in phase of design, molecules containing phenolic hydroxyl functions and aminoacids known to exert in human antioxidant effects such as cysteine, methionine and tyrosine. Here we report the synthesis of a new set of molecular combinations between phenolic acid, aminoacid and dopamine to investigate the influence of the different phenolics (in particular caffeic and 3,4-dihydroxyphenylacetic acid) on the antioxidant activity of the resulting molecules. The synthetic approach here described leads to achieve the desired molecular combination derivatives by easy steps. This strategy allows us to candidate the compounds obtained as a good scaffold for further structure-activity studies.

2. Results and discussion

2.1. Chemistry

We have started the synthesis of the new class of the conjugates from cysteine with the aim of exploit the multiple redox characteristics of this aminoacid [35], known for its antioxidant activity in humans. Firstly cysteine N-acetylated or methyl-ester were used for the coupling reactions with the selected acids or amines. All tried attempts were unsuccessfully due to the interferences of the free thiolic group during the coupling phase. Hence a prior protection of thiolic group was accomplished by treatment of L-cysteine hydrochloride with trityl alcohol in acidic medium. The Cys-tritylated derivative (**2**) [36,37] was then used to prepare the compounds **4** and **5**, the key-intermediates for the preparation of the molecular conjugates (Scheme 1).

Compound **5** [38], leading a free amino group suitable for amidic-type bonds, was used for the coupling [39] with different phenolic acids in particular several derivatives of the hydroxycinnamic, phenylacetic and benzoic acids. Coupled derivatives (**6**, **8**, **10**, **12**, **14**, **16**, **18**, **20** and **22**) were unblocked at thiolic function by treatment with TFA, Et_3Si in CH_2Cl_2 [41] affording compounds **7**, **9**, **11, 13, 15, 17, 19, 21** and **23** all characterized by the availability of all the redox functions responsible of the activity (Scheme 2).

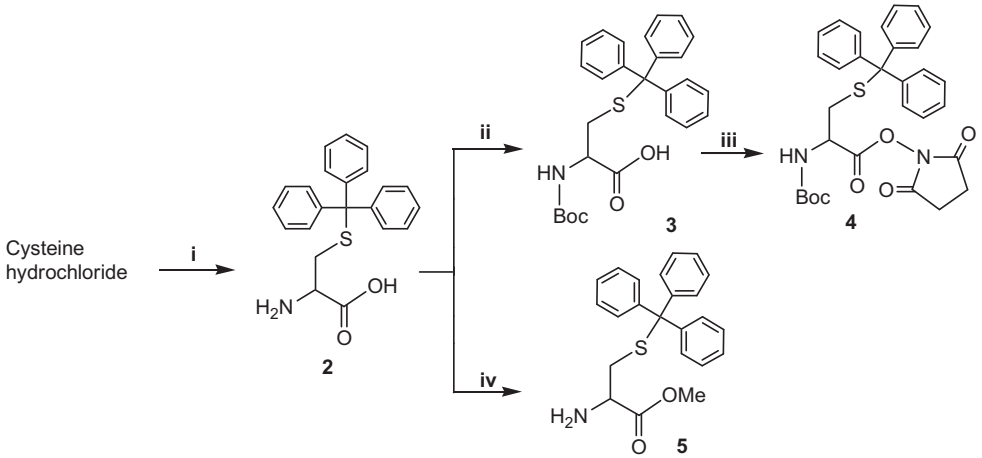
With the aim to better understand the influence of the thiolic group on the antioxidant capacity of the synthesized compounds, we have decided to combine active phenolic acids such as caffeic and 3,4-dihydroxyphenylacetic acid with methionine and tyrosine (**24**–**27**). The selected aminoacids, used as methylesters in the coupling reaction (Scheme 3), lead in the place of the cysteine thiol function, a S-Me or a phenolic moiety respectively.

A second set of compounds was prepared staring from the derivative **4** obtained from **3** after the activation of the carboxylic moiety with N-hydroxysuccinimide (NHS) [40] (Scheme 1).

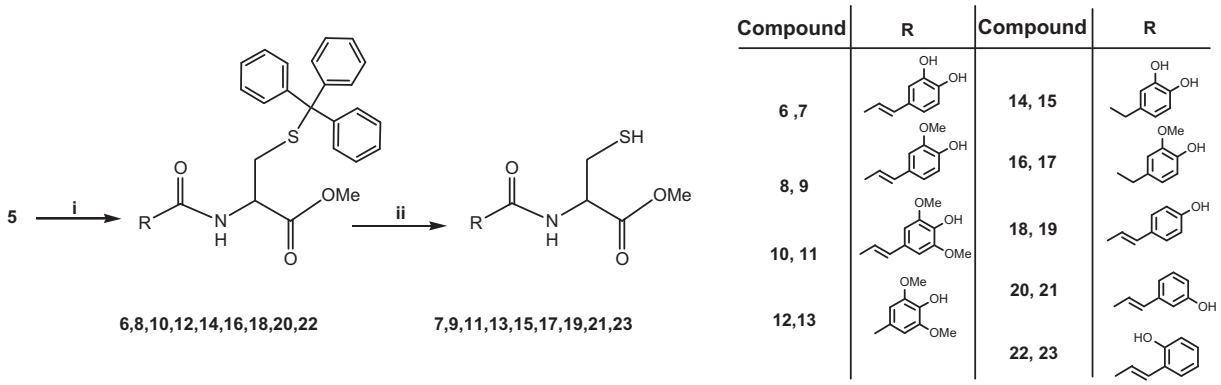
Reaction of compound **4** with the aminic function of dopamine in presence of TEA in dry DMF allowed to obtain the derivative **28** that was next deprotected with aqueous TFA at amino function (**29**) (Scheme 4).

Compound **29** was selected to achieve more complex conjugates by the combination of three elements i) dopamine, ii) aminoacid, iii) phenolic acid with the aim to increase the antioxidant effect of the final products. In this case both the carboxy or the amine function of the aminoacids have been used in order to obtained the desired conjugates. Conjugation with caffeic or 3,4-dihydroxyphenylacetic acid on compound **29** allowed to obtain derivative **30** and **31** whose the thiolic group was then cleaved in standard conditions to give **32** and **33** derivatives(Scheme 4) [41].

Finally we have obtained the methionine- or tyrosine-caffeic acid conjugates (**24**, **25**, **26** and **27**) with the dopamine hydrochloride using BOP as copulating agent [39]. The reaction provided compound **35**, **37, 39** and **41** in a fairly good yield (over two steps) (Scheme 5).



Scheme 1. Synthesis of the key-intermediates **4** and **5**. Reagents and conditions: i) Trityl alcohol, TFA; ii) $(Boc)_2$, NaOH acq. 4N; iii) NHS, DCC, CH_3CN; iv) H_2SO_4, MeOH, reflux.



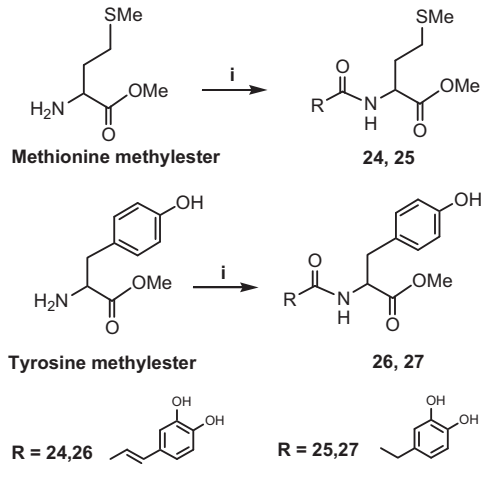
Scheme 2. Synthesis of the Cysteine derivatives **7**, **9**, **11**, **13**, **15**, **17**, **19**, **21**, **23**. Reagents and conditions: i) selected acid, BOP, TEA, DMF an.; ii) TFA, Et_3Si, CH_2Cl_2.

2.2. Radical scavenging activity

Newly synthesized compounds were assayed applying DPPH and ORAC tests to determine their *in vitro* antioxidant activity.

The critical reading of the results (Table 1) consent to draw different considerations. In particular the activity against the nitrogen radicals involved in the DPPH test resulted correlated to the number of hydroxyl groups present in the molecule: compounds **19**, **21**, and **23**, characterized by one only phenolic group, revealed in fact a lower activity.

Differently, the poor antioxidant capacity of derivative **13**, obtained from the conjugation of cysteine and siringic acid, it could be correlated to the presence of a carbonylic group directly linked to the aromatic ring that reduces the capacity of the molecule to transfer hydrogen atoms, as previously described for other benzoic acids and related esters [42]. This hypothesis is confirmed by the result obtained analyzing the compound **11** where the introduction between the 4-hydroxy-3,5-dimetoxyphenolic aromatic ring and the carbonylic group of a spacer −CH═CH−, determined an increase of the antioxidant capacity in respect to **13**. A further element responsible to increase the antioxidant activity is the presence of a second OH group on the aromatic ring. In fact, compounds **7** and **15**, resulting from the molecular conjugation of the cysteine with caffeic acid or 3, 4-dihydroxyphenylacetic acid respectively, show a higher antioxidant activity than **11**. Concerning compound **7**, a significant contribution to the activity is probably assignable also to the presence of the −CH═CH− group able to warrant a major stabilization of the radical form generated after the interaction of the molecule with the oxidant species. Comparing the activity of this derivative (**7**) with that of its single components (cysteine and caffeic acid), it results clear that the molecular conjugation leads to an advantage in terms of antioxidant capacity.



Scheme 3. Synthesis of compounds **24**, **25**, **26** and **27**. Reagents and conditions: i) Caffeic acid or 3,4-dihydroxyphenylacetic acid, BOP, TEA, DMF an.

Finally, appraising the radical-scavenger capacity of the conjugates between the caffeic acid or 3,4-dihydroxyphenylacetic acid with the three selected aminoacids (cysteine, methionine and tyrosine), we can affirm that the presence of the sulphured group, in particular the thiolic group of the cysteine, results the best choice to obtain molecules with a high antioxidant capacity (**7**, **24**, **26**) (Table 1).

ORAC test was performed only on the best radical-scavengers derivatives emerged from DPPH test; these results underlined a high antioxidant activity for the compounds characterized by phenylacetic function. In fact, compounds **15**, **25** and **27** result the more active against the peroxyl radical. This data is consistent with the ORAC results of the pure compounds, where 3, 4-dihydroxyphenylacetic acid has greater activity of caffeic acid.

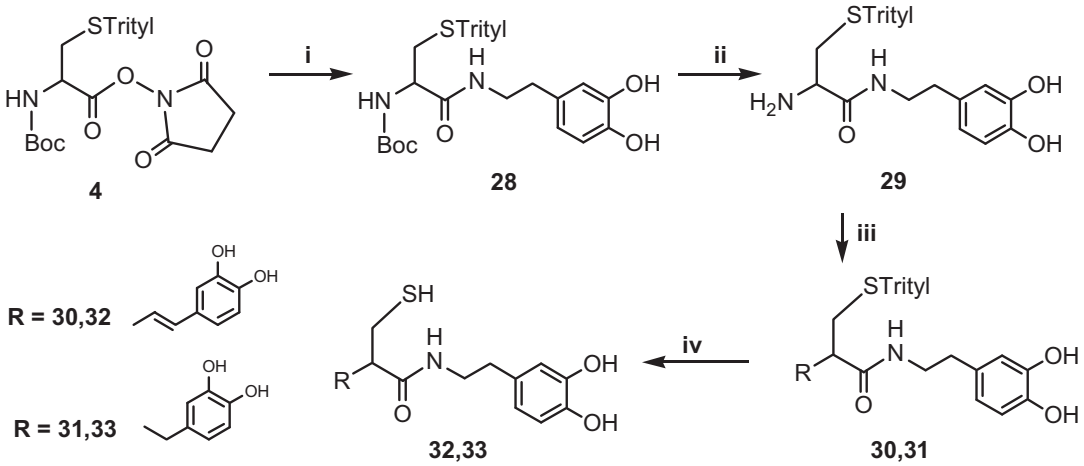
Moreover the derivative obtained from conjugation with the tyrosine showed the higher radical−scavenger activity, probably due to the presence of a further phenolic group. Besides, compound **27**, the best compound of the series, results more powerful of the single components from which it derives (tyrosine and 3, 4-dihydroxyphenylacetic acid).

Regarding phenolic acid-aminoacid-dopamine derivatives, in general we can say that DPPH and ORAC values are good but we can not observe significant improvement of activity with the addition of dopamine compared to the caffeic or 3,4-dihydroxyphenylacetic acid-aminoacid derivatives (Table 2).

The results of both tests are comparable with the results of their conjugates without dopamine.

3. Experimentals protocols

Chemicals and solvents were purchased from Sigma−Aldrich and Carlo Erba reagenti (Italia). Reactions were monitored by TLC (pre-coated plates of silica gel Macherey−Nagel durasil-25). Ninhydrin (1.5% in EtOH), $FeCl_3$ (1% in H_2O) or $KMnO_4$ (1.5% in H_2O) spray reagents were employed to detect the compounds. All moisture−sensitive reactions were performed under an argon atmosphere. The molecular weights of the compounds were determined by matrix assisted laser desorption ionization time-of-flight (MALDI-TOF) (Helwett-Packard HPG2025a), and the values are expressed as $[MH]^+$. ^1H NMR spectra were recorded on a Varian VXR-200 or a Mercury$_{Plus}$ 400 spectrometer.



Scheme 4. Synthesis of the Cysteine derivatives **30**, **31**, **33**, and **33**. Reagents and conditions: i) Dopamine hydrochloride, TEA, DMF an.; ii) TFA, H_2O; iii) Caffeic acid or 3,3-dihydroxyphenilacetic acid, BOP, TEA, DMF an.; iv) TFA, Et_3Si, CH_2Cl_2.

3.1. Synthesis

3.1.1. 2-Amino-3-tritylsulphanyl-propionic acid (S-trityl-L-Cysteine) (**2**)

Triphenylmethanol (3.3 g, 12.7 mmol) was added to a solution of Cysteine chlorydrate (2 g, 12.7 mmol) in TFA (25 mL) and the mixture was stirred for 2 h. After cooling to 0 °C, NaOH 4N and diethyl ether (40 mL) were added until pH 4–5 and then 10% sodium acetate aqueous solution was added until pH 5–6. The precipitated obtained was filtered, washed with fresh Et_2O and finally dried to obtain the desired product (5 g, 98% yield).

^{1}H NMR (DMSO): δ (ppm) 2.35–2.61 (m, 2H, CH_2); 2.85–2.98 (m, 1H, CH); 7.2–7.45 (m, 15H, trityl-H).

MALDI-TOF MS: *m/z*364.7 Da $[M + H]^+$, $C_{22}H_{21}NO_2S$, Mol. Wt.: 363.47.

3.1.2. 2-Ter-butoxycarbonylamino-3-tritylsulphanyl-propionic acid (N-boc-S-trityl-cysteine) (**3**)

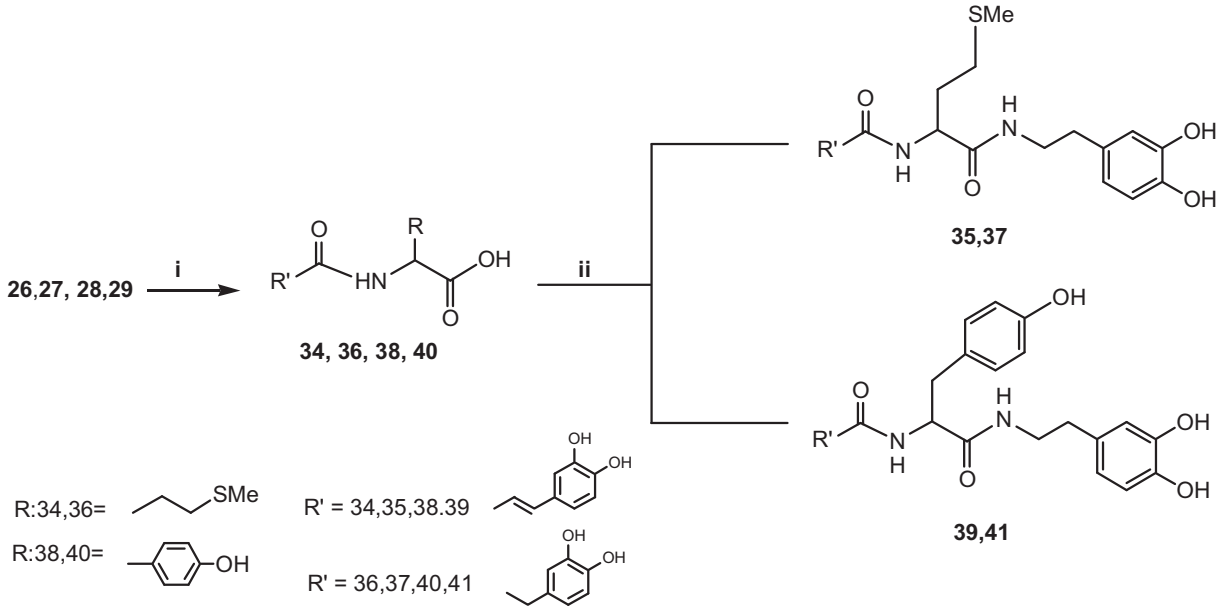
To a solution of **2** [36,37] (5 g, 12.7 mmol) in NaOH 2N (80 mL) was added Boc_2O (4.5 g, 20 mmol) and the reaction mixture was stirred at room temperature for 24 h. The aqueous solution was then acidified with HCl concentrated until pH 2 and extracted with CH_2Cl_2 (2 × 100 mL); the combined organic phases were washed with brine, dried and concentrated under reduce pressure to give without any further purification compound **3** (5.3 g, 90%) as white foam.

^{1}H NMR (DMSO): δ (ppm) 1.37 (s, 9H, 3 × CH_3); 2.29–2.58 (m, 2H, CH_2); 3.76–3.79 (m, 1H, CH); 6.84 (d, J = 7.8 Hz, 1H, NH); 7.14–7.38 (m, 15H, trityl-H).

MALDI-TOF MS: *m/z* 464.8 Da [M + H], $C_{27}H_{29}NO_4S$, Mol. Wt.: 463.59.

3.1.3. 2-tert-Butoxycarbonylamino-3-tritylsulphanyl-propionic acid 2, 5-dioxo-pyrrolidin-1-il ester (**4**)

To a solution of **3** (4 g, 10 mmol) and NHS (2.25 g, 19.5 mmol) in CH_3CN (40 mL) a 0 °C was added DCC (4 g, 19.5 mmol) and the solution was stirred for 2 h at 0 °C and then at room temperature for 12 h under Argon atmosphere. After filtration of the dicyclohexyl urea (DCU), the solvent was evaporated *in vacuo* and the residue obtained was dissolved in Et_2O and then filtered. Finally, the



Scheme 5. Synthesis of compounds **35**, **37**, **39**, **41**. Reagents and conditions: i) NaOH 1N, MeOH; ii) dopamine hydrochloride, BOP, TEA, DMF an.

Table 1
Antioxidant activity of the cysteine, methionine and tyrosine derivatives. Values are expressed as mean ± standard deviation of three replicate assays.

Compounds	DPPH (IC_{50} nmol/mL)	ORAC TE (mmol/mmol)
Trolox®	55 ± 2.5	1
Dihydroxyphenilacetic acid	26 ± 0.9	4.2 ± 0.19
Caffeic acid	41 ± 1.5	2.7 ± 0.11
Cysteine methyl-ester •HCl	123 ± 4	0.3 ± 0.01
7	22 ± 0.5	1.4 ± 0.05
9	84 ± 3.4	1.4 ± 0.04
11	76 ± 2.3	1.3 ± 0.03
13	143 ± 4.9	–[a]
15	29 ± 1.2	3 ± 0.12
17	82 ± 3.1	2.4 ± 0.08
19	148 ± 5.5	–[a]
21	185 ± 6.2	–[a]
23	150 ± 5.9	–[a]
Methionine methyl-ester •HCl	>1250	0.8 ± 0.02
24	25 ± 0.7	2.3 ± 0.09
25	48 ± 1.7	3.6 ± 0.13
Tyrosine methyl-ester •HCl	>1280	3.9 ± 0.15
26	30 ± 1.1	3.4 ± 0.12
27	44 ± 1.3	4.8 ± 0.19

[a] Not determined.

solvent was concentrated to give without any further purification compound **4** (5 g, 90%) as yellow-pale foam.

^{1}H NMR ($CDCl_3$): δ (ppm) 1.43 (s, 9H, 3 × CH_3); 2.65–2.82 (m, 6H, 3 × CH_2); 4.25–4.38 (m, 1H, CH); 5.83 (br s, 1H, NH); 7.15–7.46 (m, 15H, trityl-H).

MALDI-TOF MS: *m/z* 561.8 Da $[M + H]^+$, $C_{31}H_{32}N_2O_6S$, Mol. Wt.: 560.66.

3.1.4. 2-Amino-3-trytilsulphanyl-propionic acid methyl ester (S-trityl-L-cysteine methyl-ester) (**5**)

A solution of **2** [36,37] (590, 1.5 mmol) and H_2SO_4 concentrated (200 μL) in MeOH (5 mL) was refluxed for 12 h. The solvent was then evaporated *in vacuo* and the residue obtained was dissolved in H_2O and extracted with AcOEt. The organic phase was dried, filtered and evaporated to give a crude residue purified by chromatography over silica gel (Eluent: CH_2Cl_2/MeOH = 93:7) to afford product **5** (420 mg, 75%) as white foam.

^{1}H NMR (DMSO): δ (ppm) 2.20–2.41 (m, 2H, CH_2); 3.15–3.20 (m, 1H, CH); 3.57 (s, 3H, OCH_3); 7.24–7.35 (m, 15H, trityl-H).

MALDI-TOF MS: *m/z* 378.7 Da $[M + H]^+$, $C_{23}H_{23}NO_2S$, Mol. Wt.: 377.50.

3.1.5. General procedure to obtain the conjugates cysteine-phenolic acids

To a solution of **5** [38] (370 mg, 1 mmol) and TEA (350 μL, 2 mmol) in dry DMF (10 mL) were added the opportune phenolic acid (caffeic acid, syringic, *p*-coumaric, *o*-coumaric, 3,4-dihydoxyphenil-acetic, sinapic, *m*-coumaric, homovanillic or ferulic respectively) (1 mmol) and BOP (442 mg, 1 mmol). The reaction mixture was stirred at 0 °C for 30 min and then for 12 h at room temperature under argon atmosphere. After evaporation of the solvent, the residue was dissolved in AcOEt and washed with HCl 1N, sat.d aq. $NaHCO_3$ solution and H_2O. The organic phase was dried, filtered and evaporated to give a crude residue purified by chromatography over silica gel to afford the desired products.

Table 2
Antioxidant activity of the phenolic acid-aminoacid-dopamine conjugates. Values are expressed as mean ± standard deviation of three replicate assays.

Compounds	DPPH (IC_{50} nmol/mL)	ORAC TE (mmol/mmol)
32	26 ± 1.2	0.855 ± 0.03
33	20 ± 0.8	1.766 ± 0.07
35	23 ± 1.1	3.385 ± 0.15
37	68 ± 2.5	1.242 ± 0.04
39	32 ± 1.4	2.791 ± 0.09
41	32 ± 1.2	3.82 ± 0.16

3.1.6. 2-[3-(3,4-Dihydroxy-phenyl)-acryloylamino]-3-trytilsulphanyl-propionic acid methyl ester (6)

Grad.: CH_2Cl_2/MeOH, 95:5, 25% yield, pale-yellow foam.

^{1}H NMR (DMSO): δ (ppm) 2.42–2.58 (m, 2H, CH_2); 3.57 (s, 3H, CO_2CH_3); 4.25–4.35 (m, 1H, CH); 6.4 (d, J = 15.6 Hz, 1H, CH = CH_b); 6.75–6.96 (m, 3H, Ar); 7.24 (d, J = 15.6 Hz, 1H, CH_a = CH); 7.28–7.39 (m, 15H, trityl-H); 8.40 (d, J = 7.6, 1H, NH); 9.3 (br s, 2H, OH).

MALDI-TOF MS: *m/z* 540.7 Da $[M + H]^+$, $C_{32}H_{29}NO_5S$, Mol. Wt.: 539.64.

3.1.7. 2-[3-(4-Hydroxy-3-metoxy-phenyl)-acryloylamino]-3-trytilsulphanyl-propionic acid methyl ester (**8**)

Grad.: CH_2Cl_2/MeOH, 100:0 → 98:2, yield 60%, pale-yellow foam.

^{1}H NMR (DMSO): δ (ppm) 2.38–2.61 (m, 2H, CH_2); 3.57 (s, 3H, CO_2CH_3); 3.81 (s, 3H, OCH_3); 4.23–4.38 (m, 1H, CH); 6.53 (d, J = 15.6 Hz, 1H, CH = CH_b); 6.78–6.81 (m, 1H, Ar); 6.99–7.14 (m, 2H, Ar); 7.25–7.39 (m, 16H, trityl-H, CH_a = CH); 8.40 (d, J = 8, 1H, NH); 9.47 (br s, 1H, OH).

MALDI-TOF MS: *m/z* 528.9 Da $[M + H]^+$, $C_{33}H_{31}NO_5S$, Mol. Wt.: 553.67.

Grad.: CH_2Cl_2/MeOH, 100:0 → 98:2, 60% yield, pale-yellow foam.

3.1.8. 2-[3-(4-Hydroxy-3,5-dimethoxy-phenyl)-acryloylamino]-3-trytilsulphanyl-propionic acid methyl ester (**10**)

Grad.: CH_2Cl_2/MeOH, 100:0 → 98:2, 33% yield, pale-yellow foam.

^{1}H NMR (DMSO): δ (ppm) 2.42–2.62 (m, 2H, CH_2); 3.57 (s, 3H, CO_2CH_3); 3.79 (s, 6H, 2 × OCH_3); 4.29–4.34 (m, 1H, CH); 6.57 (d, J = 15.6 Hz, 1H, CH = CH_b); 6.87 (s, 2H, Ar); 7.24–7.37 (m, 16H, trityl-H, CH_a = CH); 8.41 (d, J = 8, 1H, NH); 8.85 (br s, 1H, OH).

MALDI-TOF MS: *m/z* 584.8 Da $[M + H]^+$, $C_{34}H_{33}NO_6S$, Mol. Wt.: 583.69.

3.1.9. 2-(4-Hydroxy-3,5-dimethoxy-benzoylamino)-3-trytilsulphanil-propionic acid methyl ester (**12**)

Grad.: CH_2Cl_2/MeOH, 100:0 → 98:2, v/v, 30% yield, pale-yellow foam.

^{1}H NMR (DMSO): δ (ppm) 2.70–2.82 (m, 2H, CH_2); 3.54 (s, 3H, CO_2CH_3); 3.81 (s, 6H, 2 × OCH_3); 4.28–4.32 (m, 1H, CH); 7.20 (s, 2H, Ar); 7.25–7.37 (m, 15H, trityl-H); 8.72 (d, J = 7.8 Hz, 1H, NH); 9.03 (br s, 1H, OH).

MALDI-TOF MS: *m/z* 558.8 Da $[M + H]^+$, $C_{32}H_{31}NO_6S$, Mol. Wt.: 557.66.

3.1.10. 2-[2-(3,4-Dihydroxy-phenyl)-acetylamino]-3-trytilsulphanyl-propionic acid methyl ester (**14**)

Grad.: CH_2Cl_2/MeOH, 100:0 → 98:2, 55% yield, pale-yellow foam.

^{1}H NMR (DMSO): δ (ppm) 2.31–2.58 (m, 2H, CH_2); 3.23–3.25 (m, 2H, CH_2); 3.53 (s, 3H, CO_2CH_3); 4.05–4.19 (m, 1H, CH); 6.45–6.68 (m, 3H, Ar); 7.24–7.33 (m, 15H, trityl-H); 8.41 (d, J = 8, 1H, NH); 9.70, 8.75 (br s, 2H, 2 × OH).

MALDI-TOF MS: *m/z* 528.9 Da $[M + H]^+$, $C_{31}H_{29}NO_5S$, Mol. Wt.: 527.63.

3.1.11. 2-[2-(4-Hydroxy-3-methoxy-phenyl)-acetylamino]-3-trytilsulphanyl-proprionic acid methyl ester (**16**)

Grad.: CH_2Cl_2/MeOH, 100:0 → 98:2, 34% yield, pale-yellow foam.

^{1}H NMR (DMSO): δ (ppm) 2.33–2.58 (m, 2H, CH_2); 2.28–2.32 (m, 2H, CH_2); 3.53 (s, 3H, OCH_3); 3.67 (s, 3H, CO_2CH_3); 4.11–4.20

(m, 1H, CH); 6.63–6.83 (m, 3H, Ar); 7.23–7.37 (m, 15H, trityl-H); 8.51 (d, J = 8, 1H, NH); 8.78 (br s, 1H, OH).

MALDI-TOF MS: m/z 542.6 Da $[M + H]^+$, $C_{32}H_{31}NO_5S$, Mol. Wt.: 541.66.

*3.1.12. 2-[3-(4-Hydroxy-phenyl)-acryloylamino]-3-trytilsulphanil-propionic acid methyl ester (**18**)*

Grad.: CH_2Cl_2/MeOH, 100:0 → 98:2, 30% yield, pale-yellow foam.

^{1}H NMR (DMSO): δ (ppm) 2.46–2.56 (m, 2H, CH_2); 3.57 (s, 3H, CO_2CH_3); 4.22–4.35 (m, 1H, CH); 6.47 (d, J = 16.2 Hz, 1H, CH = CH_b); 6.79 (d, J = 8.4 Hz, 2H, Ar); 7.25–7.38 (m, 16H, trityl-H, Ar, CH_a = CH); 8.40 (d, J = 8 Hz, 1H, NH); 9.88 (br s, 1H, OH).

MALDI-TOF MS: m/z 524.7 Da $[M + H]^+$, $C_{32}H_{29}NO_4S$, Mol. Wt.: 523.64.

*3.1.13. 2-[3-(3-Hydroxy-phenyl)-acryloylamino]-3-trytilsulphanùyl-propionic acid methyl ester (**20**)*

Grad.: CH_2Cl_2/MeOH, 100:0 → 98:2, 72% yield, pale-yellow foam.

^{1}H NMR (DMSO): δ (ppm) 2.52–2.62 (m, 2H, CH_2); 3.58 (s, 3H, CO_2CH_3); 4.27–4.31 (m, 1H, CH); 6.62 (d, J = 16 Hz, 1H, CH = CH_b); 6.78–7.01 (m, 3H, Ar); 7.21–7.38 (m, 16H, trityl-H, CH_a = CH, Ar); 8.60 (d, J = 8.2, 1H, NH); 9.60 (br s, 1H, OH).

MALDI-TOF MS: m/z 524.8 Da $[M + H]^+$, $C_{32}H_{29}NO4S$, Mol. Wt.: 523.64.

*3.1.14. 2-[3-(2-Hydroxy-phenyl)-acryloylamino]-3-trytilsulphanyl-propionic acid methyl ester (**22**)*

Grad.: CH_2Cl_2/MeOH, 100:0 → 98:2, 34% yield, pale-yellow foam.

^{1}H NMR (DMSO): δ (ppm) 2.42–2.63 (m, 2H, CH_2); 3.57 (s, 3H, CO_2CH_3); 4.25–4.31 (m, 1H, CH); 6.73 (d, J = 16 Hz, 1H, CH = CH_b); 6.81–6.90 (m, 2H, Ar); 7.16–7.21 (m, 1H, Ar); 7.26–7.39 (m, 15H, trityl-H); 7.41–7.45 (m, 1H, Ar); 7.62 (d, J = 16 Hz, 1H, CH_a = CH); 7.56 (d, J = 8, 1H, NH); 10.08 (br s, 1H, OH).

MALDI-TOF MS: m/z 524.5 Da $[M + H]^+$, $C_{32}H_{29}NO_4S$, Mol. Wt.: 523.64.

3.1.15. General procedure: deprotection of thiolic group of the cysteine-phenolic acids conjugates

The opportune trytilated derivative (**6**, **8**, **10**, **12**, **14**, **16**, **18**, **20**, **22**) (1 mmol) was treated with a solution of TFA, Et_3Si and CH_2Cl_2 (4/4/92 v/v). The reaction mixture was stirred 5–6 h and then a sat.d aq. $NaHCO_3$ solution was added; the solution was extracted with CH_2Cl_2. The organic phase was dried, filtered and evaporated to give a crude residue purified by chromatography over silica gel to afford the desired products.

*3.1.16. 2-[3-(3,4-Dihydroxy-phenyl)-acryloylamino]-3-mercapto-propionic acid methyl ester (**7**)*

Grad.: CH_2Cl_2/MeOH, 100:0 → 95:5, 31% yield, pale-yellow foam.

^{1}H NMR (DMSO): δ (ppm) 2.59 (t, J = 8.8 Hz, 1H, SH); 2.84–2.86 (m, 2H, CH_2); 3.66 (s, 3H, CO_2CH_3); 4.45–4.60 (m, 1H, CH); 6.45 (d, J = 15.6 Hz, 1H, CH = CH_b); 6.73–6.97 (m, 3H, Ar); 7.28 (d, J = 15.6 Hz, 1H, CH_a = CH); 8.43 (d, J = 8.2, 1H, NH); 9.18, 9.38 (br s, 2H, OH).

MALDI-TOF MS: m/z298.5 Da $[M + H]^+$, $C_{13}H_{15}NO_5S$, Mol. Wt.: 297.33.

*3.1.17. 2-[3-(4-Hydroxy-3-methoxy-phenyl)-acryloylamino]-3-mercapto-propionic methyl ester (**9**)*

Grad.: CH_2Cl_2/MeOH, 100:0 → 98:2, 46% yield, yellow-pale foam.

^{1}H NMR (DMSO): δ (ppm) 2.59 (t, J = 8.2 Hz, 1H, SH); 2.80–2.95 (m, 2H, CH_2); 3.66 (s, 3H, OCH_3); 3.80 (s, 3H, CO_2CH_3); 4.55–4.62 (m, 1H, CH); 6.58 (d, J = 16 Hz, 1H, CH = CH_b); 6.78–7.15 (m, 3H, Ar); 7.35 (d, J = 16 Hz, 1H, CH_a = CH); 8.40 (d, J = 8 Hz, 1H, NH); 9.49 (br s, 1H, OH).

MALDI-TOF MS: m/z 312.5 Da $[M + H]^+$, $C_{14}H_{17}NO_5S$, Mol. Wt.: 311.35.

*3.1.18. 2-[3-(4-Hydroxy-3,5-dimethoxy-phenyl)-acryloylamino]-3-mercapto-propionic acid methyl ester (**11**)*

Grad.: CH_2Cl_2/MeOH, 100:0 → 98:2, 63% yield, pale-yellow foam.

^{1}H NMR (DMSO): δ (ppm) 2.59 (t, J = 8.4 Hz, 1H, SH); 2.80–2.93 (m, 2H, CH_2); 3.67 (s, 3H, CO_2CH_3); 3.80 (s, 6H, 2 × OCH_3); 4.59–4.62 (m, 1H, CH); 6.62 (d, J = 15.6 Hz, 1H, CH = CH_b); 6.88 (s, 2H, Ar); 7.36 (d, J = 15.6 Hz, 1H, CH_a = CH); 8.38 (d, J = 8 Hz, 1H, NH); 8.85 (br s, 1H, OH).

MALDI-TOF MS: m/z342.5 Da $[M + H]^+$, $C_{15}H_{19}NO_6S$, Mol. Wt.: 341.38.

*3.1.19. 2-[3-(4-Hydroxy-3-methoxy-phenyl)-acryloylamino]-3-mercapto-propionic methyl ester (**13**)*

Grad.: CH_2Cl_2/MeOH, 100:0 → 98:2, 46% yield, pale-yellow foam.

^{1}H NMR (DMSO): δ (ppm) 2.63 (t, J = 8 Hz, 1H, SH); 2.86–3.02 (m, 2H, CH_2); 3.66 (s, 3H, CO_2CH_3); 3.81 (s, 6H, 2 × OCH_3); 4.53–4.57 (m, 1H, CH); 7.22 (s, 2H, Ar); 8.63 (d, J = 7.6, 1H, NH); 9.04 (br s, 1H, OH).

MALDI-TOF MS: m/z312.5 Da $[M + H]^+$, $C_{14}H_{17}NO_5S$, Mol. Wt.: 311.35.

*3.1.20. 2-[2-(3,4-Dihydroxy-phenyl)-acetylamino]-3-mercapto-propionic acid methyl ester (**15**)*

Grad.: CH_2Cl_2/MeOH, 100:0 → 99:1, 55% yield, pale-yellow foam.

^{1}H NMR (DMSO): δ (ppm) 2.47 (t, J = 8.4 Hz, 1H, SH); 2.73–2.81 (m, 2H, CH_2); 3.29 (s, 2H, CH_2); 3.64 (s, 3H, CO_2CH_3); 4.40–4.45 (m, 1H, CH); 6.45–6.66 (m, 3H, Ar); 8.41 (d, J = 8, 1H, NH); 9.69, 8.78 (br s, 2H, 2 × OH).

MALDI-TOF MS: m/z286.4 Da $[M + H]^+$, $C_{12}H_{15}NO_5S$, Mol. Wt.: 285.32.

*3.1.21. 2-[2-(4-hydroxy-3-methoxy-phenyl)-acetylamino]-3-mercapto-propionic acid methyl ester (**17**)*

Grad.: CH_2Cl_2/MeOH, 100:0 → 98:2, 79% yield, pale-yellow foam.

^{1}H NMR (DMSO): δ (ppm) 2.51 (t, J = 8.4 Hz, 1H, SH); 2.74–2.85 (m, 2H, CH_2); 3.38 (s, 2H, Ar-CH_2); 3.63 (s, 3H, OCH_3); 3.74 (s, 3H, CO_2CH_3); 4.42–4.47 (m, 1H, CH); 6.65–6.85 (m, 3H, Ar); 8.44 (d, J = 7.6 Hz, 1H, NH); 8.78 (br s, 1H, OH).

MALDI-TOF MS: m/z 300.5 Da $[M + H]^+$, $C_{13}H_{17}NO_5S$, Mol. Wt.: 299.34.

*3.1.22. 2-[3-(4-hydroxy-phenyl)-acryloylamino]-3-mercapto-propionic acid metyl ester (**19**)*

Grad.: CH_2Cl_2/MeOH, 100:0 → 98:2, 38% yield, pale-yellow foam.

^{1}H NMR (DMSO): δ (ppm) 2.56 (t, J = 8.4 Hz, 1H, SH); 2.81–2.87 (m, 2H, CH_2); 3.66 (s, 3H, CO_2CH_3); 4.57–4.60 (m, 1H, CH); 6.53 (d, J = 15.6 Hz, 1H, CH = CH_b); 6.78–6.70 (m, 2H, Ar); 7.36 (d, J = 15.6 Hz, 1H, CH_a = CH); 7.40–7.43 (m, 2H, Ar); 8.43 (d, J = 7.6, 1H, NH); 9.88 (br s, 1H, OH).

MALDI-TOF MS: m/z282.6 Da $[M + H]^+$, $C_{13}H_{15}NO_4S$, Mol. Wt.: 281.33.

*3.1.23. 2-[3-(3-hydroxy-phenyl)-acryloylamino]-3-mercapto-propionic acid methyl ester (**21**)*

Grad.: CH_2Cl_2/MeOH, 100/0 → 98/2, 42% yield, pale-yellow foam.

^{1}H NMR (DMSO): δ (ppm) 2.60 (t, J = 8.4 Hz, 1H, SH); 2.80–2.91 (m, 2H, CH_2); 3.67 (s, 3H, CO_2CH_3); 4.57–4.61 (m, 1H, CH); 6.67 (d, J = 16 Hz, 1H, CH = CH_b); 6.78–6.81 (m, 1H, Ar); 6.94–7.01 (m, 2H, Ar); 7.19–7.24 (m, 1H, Ar); 7.36 (d, J = 16 Hz, 1H, CH_a = CH); 8.57 (d, J = 8 Hz, 1H, NH); 9.61 (br s, 1H, OH).

MALDI-TOF MS: m/z282.5 Da $[M + H]^+$, $C_{13}H_{15}NO_4S$, Mol. Wt.: 281.33.

*3.1.24. 2-[3-(2-hydroxy-phenyl)-acryloylamino]-3-mercapto-propionic acid metyl ester (**23**)*

Grad.: CH_2Cl_2/MeOH, 100:0→98:2, 36% yield, pale-yellow foam.

^{1}H NMR (DMSO): δ (ppm) 2.59 (t, J = 8.4 Hz, 1H, SH); 2.76–2.90 (m, 2H, CH_2); 3.65 (s, 3H, CO_2CH_3); 4.54–4.59 (m, 1H, CH); 6.77 (d, J = 16 Hz, 1H, CH = CH_b); 6.80–6.90 (m, 2H, Ar); 7.15–7.20 (m, 1H, Ar); 7.41–7.43 (m, 1H, Ar); 7.66 (d, J = 16 Hz, 1H, CH_a = CH); 8.50 (d, J = 7.6, 1H, NH); 10.07 (br s, 1H, OH).

MALDI-TOF MS: *m/z*282.6 Da $[M + H]^+$, $C_{13}H_{15}NO_4S$, Mol. Wt.: 281.33.

3.1.25. General procedure to obtain the methionine-phenolic acids conjugates

To a solution of Methionine methyl ester (400 mg, 2 mmol) and TEA (700 μL, 5 mmol) in dry DMF (15 mL) were added at 0 °C the selected phenolic acid (caffeic acid or 3,4-dihydroxyphenil-acetic acid) (2 mmol) and BOP (1.3 g, 2.5 mmol). The reaction mixture was stirred under argon atmosphere for 30 min at 0 °C and then at room temperature for further 12 h. After the evaporation of the solvents, the residue was diluted with AcOEt and washed with HCl 1N, sat.d aq. $NaHCO_3$ and H_2O. The organic phase was dried, filtered and evaporated to give a crude residue purified by chromatography over silica gel to afford the desired products.

*3.1.26. 2-[3-(3,4-Dihydroxy-phenyl)-acryloylamino]-4-methylsulphanyl-butyric acid methyl ester (**24**)*

Grad.: CH_2Cl_2/MeOH, 100:0→95:5, 40% yield, white foam.

^{1}H NMR (DMSO): δ (ppm) 2.05 (s, 3H, CH_3); 2.49–2.53 (m, 2H, CH_2); 3.64 (s, 3H, CO_2CH_3); 4.45–4.51 (m, 1H, CH); 6.38 (d, J = 15.6 Hz, 1H, CH = CH_b); 6.74–6.95 (m, 3H, Ar); 7.25 (d, J = 15.6 Hz, 1H, CH_a = CH); 8.40 (d, J = 7.6 Hz, 1H, NH); 9.16, 9.41 (br s, 2H, 2 × OH).

MALDI-TOF MS: *m/z* 326.6 Da $[M + H]^+$, $C_{15}H_{19}NO_5S$, Mol. Wt.: 325.38.

*3.1.27. 2-[2-(3,4-Dihydroxy-phenyl)-acetylamino]-4-methylsulphanil-butyric acid methyl ester (**25**)*

Grad.: CH_2Cl_2/MeOH, 100:0→97:3, 26% yield, white foam.

^{1}H NMR (DMSO): δ (ppm) 1.82-1-96 (m, 2H, CH_2); 2.01 (s, 3H, CH_3); 2.40–2.50 (m, 2H, CH_2); 3.25 (s, 2H, Ar-CH_2); 3.62 (s, 3H, CO_2CH_3); 4.29–4.40 (m, 1H, CH); 6.46–6.65 (m, 3H, Ar); 8.36 (d, J = 7.4 Hz, 1H, NH); 8.68, 8.76 (br s, 2H, 2 × OH).

MALDI-TOF MS: *m/z* 314.4 Da $[M + H]^+$, $C_{14}H_{19}NO_5S$, Mol. Wt.: 313.37.

3.1.28. General procedure to obtain the conjugates tyrosine-phenolic acids

To a solution of tyrosine methyl ester (500 mg, 2.6 mmol) and TEA (1.3 mL, 5.1 mmol) in dry DMF (15 mL) were added at 0 °C the selected phenolic acid (caffeic acid or 3,4-dihydroxyphenil-acetic acid) (2.6 mmol) and BOP (1.1 g, 2.6 mmol). The reaction mixture was stirred under argon atmosphere for 30 min at 0 °C and then at room temperature for 12 h. After the evaporation of the solvents, the residue was diluted with AcOEt and washed with HCl 1N, sat.d aq. $NaHCO_3$ and H_2O. The organic phase was dried, filtered and evaporated to give a crude residue purified by chromatography over silica gel to afford the desired products.

*3.1.29. 2-[3-(3, 4-dihydroxy-phenyl)-acryloylamino]-3-(4-hydroxy-phenyl)-propionic acid methyl ester (**26**)*

Eluent: CH_2Cl_2/MeOH, 93:7, 34% yield, white foam

^{1}H NMR (DMSO): δ (ppm) 2.75–2.99 (m, 2H, CH_2); 3.60 (s, 3H, CO_2CH_3); 4.43–4.54 (m, 1H, CH); 6.38 (d, J = 16 Hz, 1H, CH = CH_b); 6.63–7.03 (m, 7H, Ar); 7.20 (d, J = 16 Hz, 1H, CH_a = CH); 8.36 (d, J = 8, 1H, NH); 9.24 (br s, 3H, 3 × OH).

MALDI-TOF MS: *m/z* 358.5 Da $[M + H]^+$, $C_{19}H_{19}NO_6$, Mol. Wt.: 357.3.

*3.1.30. 2-[2-(3, 4-Dihydroxy-phenyl)-acetylamino]-3-(4-hydroxy-phenyl)-propionic acid methyl ester (**27**)*

Grad.: CH_2Cl_2/MeOH, 96:4, 56% yield, white foam

^{1}H NMR (DMSO): δ (ppm) 2.69–2.82 (m, 2H, CH_2); 2.85 (s, 2H, Ar-CH_2); 3.57 (s, 3H, CO_2CH_3); 4.30–4.36 (m, 1H, CH); 6.37–6.42 (m, 1H, Ar); 6.58–6.66 (m, 4H, Ar); 6.92–6.97 (m, 2H, Ar); 8.28 (d, J = 7.8, 1H, NH); 8.66, 8.75, 9.22 (br s, 3H, 3 × OH).

MALDI-TOF MS: *m/z* 346.6 Da $[M + H]^+$, $C_{18}H_{19}NO_6$, Mol. Wt.: 345.35.

3.2. Synthesis of the conjugates derivatives

*3.2.1. {1-[2-(3,4-Dihydroxy-phenyl)-ethyilcarbamoyl]-2-trytilsulphanyl-ethyl}-carbamic acid ter-butyl ester (**28**)*

To a solution of **4** (250 mg, 0.44 mmol) in dry DMF (10 mL) were added dopamine hydrochloride (168 mg, 0.88 mmol) and TEA (230 μL). After 3 h at room temperature the reaction mixture was diluted with AcOEt and washed with H_2O (2 × 20 mL). The organic phase was dried, filtered and evaporated to give a crude residue purified by chromatography over silica gel (grad.: CH_2Cl_2/MeOH, 98:2) to afford the desired products **28** (190 mg, 72% yield) as white foam.

^{1}H NMR (DMSO): δ (ppm) 1.37 (s, 9H, 3x CH_3); 2.18–2.46, 3.02–3.23 (m, 6H, 3 × CH_2), 3.83–3.98 (m, 1H, CH); 6.35–6.33, 6.54–6.62 (m, 3H, Ar); 6.86 (d, J = 7.8 Hz, 1H, NH); 7.24–7.36 (m, 15H, trtyl-H); 7.95 (br s,1H, NH); 8.68, 8.76 (br s, 2H, 2 × OH).

MALDI-TOF MS: *m/z* 599.9 Da $[M + H]^+$, $C_{35}H_{38}N_2O_5S$, Mol.Wt.: 598.75.

*3.2.2. 2-Amino-N-[2-(3,4-dihydroxy-phenyl)-ethyl]-3-trytilsulphanyl-propionamide (**29**)*

The derivative **28** (130 mg, 0.22 mmol) was treated with a mixture of TFA (3 mL) and H_2O (200 μL) for 3 h. The solvents were then evaporated to obtain a crude residue purified by chromatography over silica gel (grad.: CH_2Cl_2/MeOH, 95:5) to afford the desired product **29** (86 mg, 77% yield) as white foam.

^{1}H NMR (DMSO): δ (ppm) 2.29–2.46, 3.02–3.20 (m, 6H, 3 × CH_2), 3.81–3.98 (m, 1H, CH); 6.31–6.39, 6.54–6.59 (m, 3H, Ar); 6.86 (d, J = 8 Hz, 1H, NH); 7.20–7.38 (m, 15H, trityl-H); 7.76 (t, J = 6.4 Hz 1H, NH); 8.64, 8.72 (s, 2H, 2 × OH).

MALDI-TOF MS: *m/z* 499.8 Da $[M + H]^+$, $C_{30}H_{30}N_2O_3S$, Mol. Wt.: 498.64.

3.2.3. General procedure to obtain the conjugates derivatives

To a solution of **29** (383 mg, 0.77 mmol) and TEA (215 μL, 1.5 mmol) in dry DMF (15 mL) were added at 0 °C the opportune phenolic acid (caffeic acid or 3,4-dihydoxyphenilacetic acid respectively (0.77 mmol) and BOP (340 mg, 0.77 mmol). The reaction mixture was stirred under argon atmosphere at 0 °C for 30 min and then at room temperature for further 12 h. After evaporation of the solvent, the residue was dissolved in AcOEt and washed with HCl 1N, sat.d aq. $NaHCO_3$ solution and H_2O. The organic phase was dried, filtered and evaporated to give a crude residue purified by chromatography over silica gel to afford the desired products.

*3.2.4. 3-(3, 4-Dihydroxy-phenyl)-N-{1-[2-(3, 4-dihydroxy-phenyl)-etylcarbamoyl]-2-trytilsulphanyl-ethyl}-acrylamide (**30**)*

Grad.: CH_2Cl_2/MeOH, 95:5→90:10, 70% yield, amber-coloured foam.

^{1}H NMR (DMSO): δ (ppm) 2.17–1.21 (m, 1H, SH); 2.62–2.79 (m, 4H, 2 × CH_2); 3.12–3.33 (m, 4H, 2 × CH_2); 4.39–4.46 (m, 1H, CH); 6.42–7.30 (m, 22H, 6 × Ar, trityl-H, CH = CH_b); 7.25 (d, J = 15.6 Hz,

1H, CH_a = CH); 8.11 (m, 1H, NH); 8.18 (d, J = 8.4 Hz, 1H, NH); 8.64, 8.74, 9.15, 9.38 (br s, 4H, 4 × OH).

MALDI-TOF MS: *m/z* 661.9 Da $[M + H]^+$, $C_{39}H_{36}N_2O_6S$, Mol.Wt.: 660.78.

*3.2.5. 2-[2-(3,4-Dihydroxy-phenyl)-acetylamino]-N-[2-(3,4-dihydroxy-phenyl)-ethyl]-3-trytilsulphanyl-propionamide (**31**). grad. : CH_2Cl_2/MeOH, 95:5, 41% yield, white foam*

^{1}H NMR (DMSO): δ (ppm) 2.29–2.44 (m, 2H, 1 × CH2); 3.11–3.28 (m, 6H, 3 × CH2); 4.28 (m, 1H, CH); 6.37 (dd, 1H, Ar); 6.39 (dd, 1H, Ar); 6.51 (d, 1H, Ar); 6.60 (d, 1H, Ar); 6.62 (d, 1H, Ar); 6.66 (d, 1H, Ar); 7.9 (t, 1H, NH); 8.1 (d, 1H, NH); 8.65, 8.68, 8.74, 8.78 (s, 4H, 4 × OH). ESI + MS: *m/z* 649.8 Da [M + H]+, C38H36N2O6S, Mol. Wt.: 648.77.

3.2.6. General procedure: deprotection of thiolic group of the dimeric conjugates

The opportune trytilated derivative (**30**, **31**) (0.5 mmol) was treated with a solution of TFA, Et_3Si and CH_2Cl_2 (4/4/92 v/v). The reaction mixture was stirred 5–6 h and then a sat.d aq. $NaHCO_3$ solution was added; the solution was extracted with CH_2Cl_2. The organic phase was dried, filtered and evaporated to give a crude residue purified by chromatography over silica gel to afford the desired products.

*3.2.7. 3-(3, 4-Dihydroxy-phenyl)-N-{1-[2-(3, 4-dihydroxy-phenyl)-ethylcarbamoyl]-2-mercapto-ethyl}-acrylamide (**32**)*

Grad.: CH_2Cl_2/MeOH, 93:7 → 90:10, 45% yield, as white foam.

^{1}H NMR (DMSO): δ (ppm) 2.17–1.21 (m, 1H, SH); 2.63–2.81 (m, 4H, 2 × CH_2); 3.10–3.33 (m, 4H, 2 × CH_2); 4.41–4.47 (m, 1H, CH); 6.42–6.96 (m, 7H, 6 × Ar, CH = CH_b); 7.25 (d, J = 15.6 Hz, 1H, CH_a = CH); 8.12 (m, 1H, NH); 8.19 (d, J = 8.4 Hz, 1H, NH); 8.64, 8.74, 9.15, 9.40 (br s, 4H, 4 × OH).

MALDI-TOF MS: *m/z* 419.5 Da $[M + H]^+$, $C_{20}H_{22}N_2O_6S$, Mol. Wt.: 418.46.

*3.2.8. 2-[2-(3,4-Dihydroxy-phenyl)-acetylamino]-N-[2-(3,4-dihydroxy-phenyl)-ethyl]-3-propionamide (**33**)*

Grad.: CH_2Cl_2/MeOH, 90:10, 20% yield, white-yellow foam.

^{1}H NMR (DMSO): δ (ppm) 2.08 (t, 1H, SH); 2.61–2.71 (m, 2H, 1 × CH2); 3.11–3.28 (m, 6H, 3 × CH2); 4.28 (m, 1H, CH); 6.42 (dd, 1H, Ar); 6.50 (dd, 1H, Ar); 6.56 (d, 1H, Ar); 6.60 (d, 1H, Ar); 6.62 (d, 1H, Ar); 6.66 (d, 1H, Ar); 8.01 (t, 1H, NH); 8.07 (d, 1H, NH); 8.65(s, 1H, OH), 8.68(s, 1H, OH), 8.74(s, 1H, OH), 8.78 (s, 1H, OH).

MALDI-TOF MS: *m/z* 407.9 Da [M + H]+, $C_{38}H_{36}N_2O_6S$, Mol. Wt.: 406.45.

3.2.9. General procedures to obtain the coniugates methionine- or tyrosine-caffeic acid or 3,4-diidroxyphenylacetic acid with dopamine

A solution of **24**, **25**, **26**, or **27** (1.3 mmol) in MeOH (20 mL) was treated with NaOH 4N (8 mL, 7.92 mmol) and was stirred at room temperature for 6 h. After the evaporation *under vacuum* of organic solvent the residue was acidified with HCl 2N until pH 2 and the aqueous phase was extracted with AcOEt. The organic phase was dried, filtered and evaporated to give the saponified derivatives **34**, **36**, **38**, **40** that were used for the further reaction without purification.

To a solution of **34**, **36**, **38**, **40** (1.3 mmol)) and TEA (400 μL, 2.6 mmol) in dry DMF (15 mL) were added at 0 °C dopamine hydrochloride (240 mg, 1.3 mmol) and BOP (570 mg, 1.3 mmol). The reaction mixture was stirred under argon atmosphere at 0 °C for 30 min and then at room temperature for further 12 h. After the evaporation of the solvents, the residue was diluted with AcOEt and washed with HCl 1N, sat.d aq. $NaHCO_3$ and H_2O. The organic phase was dried, filtered and evaporated to give a crude residue purified by chromatography over silica gel.

*3.2.10. 3-(3,4-Dihydroxy-phenyl)-N-{1-[2-(3,4-dihydroxy-phenyl)-ethylcarbamoyl]-3-methylsulphanil-propyl}-acrilamide (**35**)*

Grad.: CH_2Cl_2/MeOH, 95:5 → 90:10, 8% yield, white foam.

^{1}H NMR (DMSO): δ (ppm) 1.80–1.99 (m, 2H, CH_2); 2.03 (s, 3H, S-CH_3); 2.38–2.60 (m, 4H, 2 × CH_2); 3.10–3.21 (m, 2H, CH_2); 4.35–4.45 (m, 1H, CH); 6.40–6.96 (m, 7H, 6 × Ar, CH = CH_b); 7.24 (d, J = 6 Hz, 1H, CH_a = CH); 8.02 (m, 1H, NH); 8.15 (d, J = 8 Hz, 1H, NH); 8.64, 8.74, 9.15, 9.38 (br s, 4H, 4 × OH).

MALDI-TOF MS: *m/z* 447.7 Da $[M + H]^+$, $C_{22}H_{26}N_2O_6S$, Mol. Wt.: 446.52.

*3.2.11. 2-[2-(3,4-Dihydrossi-phenyl)-acetylamino]-N-[2-(3,4-dihydroxyi-phenyl)-ethyl]-4-methylsulphanil-butyramide (**37**)*

Grad.: CH_2Cl_2/MeOH, 93:7, 40% yield, white foam.

^{1}H NMR (DMSO): δ (ppm) 1.7–1.8 (m, 2H, 1 × CH2); 1.95 (s, 3H, S-CH3); 2.46 (m, 2H, 1 × CH2); 3.12–3.27 (m, 6H, 3 × CH2); 4.19 (m, 1H, CH); 6.41 (dd, 1H, Ar); 6.49 (dd, 1H, Ar); 6.56 (d, 1H, Ar); 6.60 (dd, 2H, Ar); 6.62 (d, 1H, Ar); 6.65 (d, 1H, Ar); 7.90 (t, 1H, NH); 8.03 (d, 1H, NH); 8.6–8.8 (s broad, 4H, 4 × OH).

MALDI-TOF MS: *m/z* 435.0 Da $[M + H]^+$, $C_{21}H_{26}N_2O_6S$, Mol. Wt.: 434.51.

*3.2.12. 3-(3,4-Dihydroxy-phenyl)-N-{1-[2-(3,4-dihydroxy-phenyl)-ethylcarbamoyl]-2-(4-hydroxy-phenyl)ehtyl}-acrilamide (**39**)*

Grad.: CH_2Cl_2/MeOH, 9:1, 29% yield, white foam

^{1}H NMR (DMSO): δ (ppm) 2.6–2.9 (m, 2H, CH2); 3.10–3.21 (m, 4H, 2 × CH2); 4.44–4.54 (m, 1H, CH); 6.40 (d, J = 16 Hz, 1H, CH = C*Hb*); 6.42–7.02 (m, 10H, Ar); 7.15 (d, J = 16 Hz,1H, C*Ha* = CH); 8.06 (t, 1H, NH); 8.15 (d, J = 8 Hz, 1H, NH); 8.64, 8.75, 9.15, 9.16, 9.37 (br s,5H, 5 × OH).

MALDI-TOF MS: *m/z* 479.6 Da [M + H]+, C26H26N2O7, Mol. Wt.: 478.49.

*3.2.13. 2-[2-(3,4-Dihydroxy-phenyl)-acetylamino]-N-[2-(3,4-dihydroxy-phenyil)-ethyl]-3-(4-idroxy-phenyl)-propionamide (**41**)*

Grad.: CH_2Cl_2/MeOH, 98:2, 71% yield, white foam.

^{1}H NMR (DMSO): δ (ppm) 2.43(t, 2H, CH2); 2.6(m, 1H); 2.78(m, 1H); 3.10–3.19 (m, 4H, 2 × CH2); 4.3 (m, 1H, CH); 6.38 (m, 2H, Ar); 6.59 (m, 6H, Ar); 6.92 (2H, Ar); 7.92 (m 2H, 2 × NH) 8.64, 8.65, 8.72, 8.73, 9.14 (s, 5H, 5 × OH).

MALDI-TOF MS: *m/z* 467.0 Da $[M + H]^+$, $C_{25}H_{26}N_2O_7$, Mol. Wt.: 466.48.

3.3. Antioxidant activity assays

3.3.1. Free radical scavenging activity on DPPH

The DPPH assay, using 1,1-diphenyl-2-picryl-hydrazyl radical (DPPH), is one of the simpler and faster methods for assessing the antioxidant capacity of extracts or pure compounds it is rather unspecific because the radical taken into account is not typically organic and need anyway to be coupled by complementary techniques. Indeed, it is particularly useful when large general screenings need to be conducted. This assay is based on the measurement of the reducing ability of antioxidants toward $DPPH^•$ characterized by a deep purple colour that fades in the presence of an antioxidant agent. This ability can be evaluated by measuring the decrease in absorbance at 517 nm of the solution after the radical reaction with products to be tested and the percentage of the DPPH remaining is calculated as

$$\%DPPH^•_{REM} = 100 \times [DPPH^•]_{REM}/[DPPH^•]_T = 0$$

The percentage of remaining DPPH• (DPPH •$_{REM}$) is proportional to the antioxidant concentration, and the concentration that causes a decrease in the initial DPPH• concentration by 50% is defined as IC_{50}. To a DPPH methanolic solution (1.5 mL) was added 0.750 mL of compound solution at different concentration (1, 0.5, 0.25, 0.125 and 0.0625 mg/mL) and the absorbance was measured by a spectrophotometer UV–VIS (ThermoSpectronic Helios γ, Cambridge, UK) at 517 nm, according to a described procedure [43]. IC_{50} values expressed as nmol/mL were determined by regression analysis of the results obtained at different concentrations of the sample.

3.3.2. Oxygen radical absorbance capacity (ORAC) assay

The ORAC assay was carried out on a Fluoroskan FL® ascent (Thermo Fisher Scientific, Inc. Waltham, MA) with fluorescent filters (excitation wavelength: 485 nm; emission filter: 538 nm). The procedure was based on that given by Hong, Guohua & Ronald (1996) as modified in our previous work [44]. Briefly, in the final assay mixture (0.2 mL total volume), fluorescein sodium salt (85 nM) was used as a target of free radical attack with 2,2′-azo-bis(2-amidino-propane) dihydrochloride (AAPH) as a peroxyl radical generator. Trolox, a water-soluble analogue of vitamin E, was used as a standard control: a calibration curve was carried out with 10, 20, 30, 40, 50 μM solution. The tested compounds were dissolved in PBS and prepared immediately before the experiments. The fluorescence measurements, carried out at 37 °C, were recorded at 5 min intervals up 30 min after the addition of AAPH. The ORAC values, calculated as difference of the areas under the quenching curves of fluoresceine between the blank and the sample, were expressed as Trolox equivalents (TE), pH = 7.4. All the experiments were performed with three replicates.

3.3.3. Data analysis

Relative standard deviations and statistical significance (Student's *t* test; $P \leq 0.05$) were given where appropriate for all data collected. One-way ANOVA and LSD post hoc Tukey's honest significant difference test were used for comparing the antioxidant effects of different compounds. All computations were made using the statistical software STATISTICA 6.0 (StatSoft Italia srl).

4. Conclusion

This work has been conducted in order to obtain new molecules with radical-scavenging activity based on the conjugation of natural bioactive portions (i.e. phenols, cysteine, methionine or tyrosine), characterized by different structures and action mechanisms, to achieve the simultaneous quenching of different radical species in the site of the oxidative damage. To this aim, we have synthesized phenolic acid-aminoacid and phenolic acid-aminoacid-dopamine conjugates to improve the antioxidant activity of natural aminoacids by the presence of the catecholic functions. All the newly obtained molecules were screened by DPPH assay and the compounds with the best radical-scavenging ability were assayed with ORAC test. As a general result, we have demonstrated that at least two hydroxyl moieties on the aromatic ring is necessary as well as a spacer between the aromatic ring and the carboxylic acid. As far as concerned with the trimer compounds phenolic acid-aminoacid-dopamine, no significant improvement of the antioxidant activity was inferred by the presence of two additional OH groups. This latter finding suggest that also the shape and complexity of the molecule may play a role and that there is a "saturation" on the maximum potency achievable by this approach. All compounds here prepared, derive from parent natural products already well known for their properties and application in the nutritional fields. However, once applied *in vivo* in prevention of diseases they show limited efficacy due to the poor distribution to target tissues (i.e. brain and other lypophilic tissues that usually undergo oxidative stress conditions). The design of conjugates that brings to the target tissues mechanistically complementary scavenging moieties at the same time, open new perspectives in exploiting the potential of parent natural molecules. Further studies are currently on-going on the most promising candidates emerging from this study in order to assess potential therapeutic applications.

Acknowledgements

We thank the Ministry of Education and Research (PRIN, Grant 20082L3NFT_003) and Ambrosialab for financial support and Alberto Casolari for NMR analysis.

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