An efficient one-pot synthesis of polyphenolic amino acids and evaluation of their radical-scavenging activity

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\textbf{A B S T R A C T}

A simple and efficient procedure for the synthesis of N-acyl 4-hydroxy, 4-hydroxy-3-methoxy and 3,4-dihydroxy phenylglycine amides by a strategy based on the multicomponent Ugi reaction is proposed. Hydroxybenzaldehyde derivatives were reacted with 4-methoxybenzylamine, cyclohexyl isocyanide and benzoic acid or 2-naphthylacetic acid to give Ugi adducts that were treated with trifluoroacetic acid yielding N-acyl hydroxyphenylglycine amides in good yields. The same procedure using as acid component protocatechuic acid or hydrocaffeic acid gave N-catechol 3,4-dihydroxyphenylglycine amides. The use of N-benzylxycarbonylglycine as acid component allowed the preparation of a 3,4-dihydroxyphenylglycyl dipeptide derivative. Radical-scavenging activity studies of the polyphenolic amino acid derivatives showed a sharp increase in activity with the increase in number of hydroxyl or catechol groups present. Cyclic voltammetry experiments established a correlation between oxidation peak potentials and the radical-scavenging activity.

1. Introduction

Phenolic amino acids represent a wide class of compounds endowed with interesting biological activities. The natural phenolic amino acid, tyrosine displays antioxidant properties [1], performing vital antioxidant functions inside lipid bilayers and protecting cells from oxidative destruction [2]. Non-proteinogenic phenolic amino acids are crucial components of certain peptidic natural products such as the vancomycin group of antibiotics [3] (4-hydroxyphenylglycine) and of cell-penetrating peptides (CPPs) [4]. CPPs are short, nontoxic peptides containing cationic and non-coded hydrophobic amino acids that can carry small molecules. They are able to cross, not only the cellular membrane, but also to target inside specific cellular organelles such as nucleus and mitochondria. Of the CPPs tested as mitochondria targeted antioxidants [5], the Szeto-Schiller (SS) peptides are the most promising [6]. There are a number of surprising features about these peptides. Despite being water soluble, with a net charge of 3+, they are readily taken up by all cells via passive diffusion [7–9]. The presence of a β-amino acid and non-coded amino acids at the tips of the peptide renders them resistant to aminopeptidase action. The mechanism behind their cell permeability is unclear, but the aromatic rings may serve as electron cages to shield the cationic charges via cation-π interaction. These SS peptides may be viewed as “cloaked” or “stealth” as they can evade cellular membranes, even penetrating cell barriers with tight junctions including the blood-brain barrier [7,10]. The antioxidant action of SS peptides in scavenging radical oxygen species and inhibiting low density lipoprotein oxidation, is attributed to the 2,5-dimethyl-L-tyrosine residue.

The \textit{ortho}-dihydroxyaryl function present in catechols gives compounds with a broad scope of properties that confer important biochemical functions [11]. Such properties result from; the ability to establish reversible equilibria at moderate redox potentials and pHs; irreversible cross-linking through complex oxidation mechanisms; excellent chelating properties; interaction of the vicinal hydroxyl groups with surfaces of different nature. Despite these properties, little attention has been given to amino acids with the \textit{ortho}-dihydroxyaryl function, such as 3,4-dihydroxyphenylglycine. This non-natural amino acid has been studied as copper ligand [12] and as a substrate for tyrosinase, being converted to 3,4-dihydroxybenzaldehyde via spontaneous decarboxylation of the enzymatically generated \textit{ortho}-quinone [13].

Amino acids coupled with phenolic or catecholic groups are bioactive substances involved in suppression of deleterious effects of oxidative stress [14–17] and have a wide range of biological activities such as anti-atherogenic [18], anticancer [19] and antimicrobial [20–23]. Studies have confirmed that conjugation between different types of compounds such as amino acids and phenolic acids is useful, not only to investigate structure activity relationships, but can also constitute a strategy to improve antioxidant efficiency and bioactivity [24,25].
Recently, we established an innovative synthetic strategy that allowed the preparation of a library of N-phenolic and N-catecholic dehydroamino acid derivatives [26].

The development of synthetic strategies that allow the synthesis of new amino acids with phenolic or catecholic side chains and conjugates of these amino acid with phenolic or catecholic groups would give rise to a new repertoire of interesting synthetic building blocks. These may have a wide application in the preparation, not only of specific cellular organelle targeted antioxidant peptides [5], but can also have other intrinsic biological activities or broader applications, such as: cross-linkers with proteins or carbohydrates [27]; transition metal ligands [28]; or used in the design of new peptide hydrogels that mimic mussel adhesive proteins [29–32].

2. Results and discussion

A simple approach to obtain new hydroxyphenylglycines and/or conjugates of hydroxyphenylglycines with phenolic or catecholic acids would be a strategy where simple natural phenols are joined together in a one-step economical way. One such multicomponent reaction is the Ugi reaction, first reported by Ivar Ugi [33,34]. It is classified as an isocyanide-based multicomponent reaction, consisting of the simultaneous joining of 4 different classes of compounds: an aldehyde; a carboxylic acid; an amine and an isocyanide.

Lambuschini et al. [35] using the Ugi reaction, were able to prepare a series of complex polyphenols containing two to four hydroxy-substituted aryl groups. This methodology, using hydroxy substituted benzoic acids and benzoaldehydes, gave hydroxylated bis-amides and their effect on quenching radicals and DNA oxidation was estimated [36]. However, derivatives with the ortho-catechol moiety as the amino acid side chain have not been reported.

A strategy based on the modified Ugi reaction, using as amine component 4-methoxybenzylamine and as isocyanide the commercially available and relatively stable cyclohexyl isocyanide, followed by treatment of the adducts with trifluoroacetic acid (TFA) has previously been explored [37,38]. This procedure using as aldehyde component phenolic or catecholic benzyldehydes and as acid component catecholic acids, could be an efficient approach for the synthesis of novel N-(catechoyl)-hydroxy and 3,4-dihydroxy phenylglycines. Thus, a strategy based on the modified Ugi reaction using phenolic and/or catecholic benzaldehydes and carboxylic acid components was explored.

A severe limitation of the application of ortho-catechol moieties in synthesis is their high instability in basic environment. Thus, due to the basic nature of the amine component, protection of the hydroxyl function of the benzaldehyde would be required. O tert-Butyloxycarbonylbenzaldehydes can be easily obtained by reaction of hydroxybenzaldehydes with tert-butyldicarbonate in the presence of dimethylaminopyridine as catalyst [26]. Furthermore, this temporary protection can be removed in the subsequent treatment with TFA, a reagent generally used in peptide synthesis to remove acid-labile protecting groups [39,40].

A preliminary test was carried out using as reactants tert-butyyl (4-formylphenyl) carbonate, 4-methoxybenzylamine, benzoic acid and cyclohexyl isocyanide in a 1/1 solution of ethanol and trifluoroethanol (TFA) to give compound 1a′ in high yield (Scheme 1). The proton NMR spectrum of the compound gave the expected peaks with the diastereotropic CH₂ protons of the benzylamine group appearing as two signals at 4.41 ppm and 4.63 ppm. The Ugi adduct was treated with TFA at 80 °C for 10 min. Removal of TFA gave an oily residue that precipitated in acetone. The procedure was also carried out using as acid component a N-protected amino acid, namely, N-benzoxycarbonylglycine. A Ugi adduct was obtained (compound 6c′, Scheme 2) which, after treatment with TFA gave a 3,4-dihydroxyphenylglycine dipeptide derivative in 74% yield (Scheme 2). Thus, the methodology developed also allows the preparation of hydroxyphenylglycine dipeptide derivatives.

In order to establish the antioxidant capacity of the N-acetyl hydroxyphenylglycines prepared, the radical-scavenging activity of selected compounds was determined using 1,1-diphenyl-2-picyrylhydrazyl (DPPH) as a stable radical [41]. The EC₅₀ values (relative
concentration of antioxidant required to lower the initial DPPH% observed with the monocatecholic derivatives (compounds in activity with the increase in number of hydroxyl or catechol groups is present in the phenylglycine derivatives. A high increase in activity against the DPPH radical and the number of hydroxyl or catechol groups present in the phenylglycine derivatives. A high increase in radical-scavenging activity of compounds (compounds 1c and 3a) caused a further 30% increase in activity. The N-hydrocaffeoyl 3,4-dihydroxyphenylglycine derivative (compound 4c) showed similar activity to the N-protopocatechyl derivative 3c. Thus, the radical-scavenging activities of compounds 3c and 4c were almost double the activities of derivatives with a single catecholic group (compounds 1c or 3a).

These results show a direct correlation between radical-scavenging capacity against the DPPH radical and the number of hydroxyl or catechol groups present in the phenylglycine derivatives. A high increase in activity with the increase in number of hydroxyl or catechol groups is observed with the monocatecholic derivatives (compounds 1c and 3a) having twice the radical-scavenging activity of protocatechuic acid, while the N-catechol derivatives 3c and 4c have almost four times this activity. Thus, the introduction of the catecholic moiety in an amino acid structure induces a significant increase in its intrinsic radical scavenging activity.

Correlation between antioxidant profile and redox properties of low molecular weight antioxidants is well established [42]. Cyclic voltammetry has been used as an important tool for the evaluation of antioxidant capacity of this type of antioxidants. Compounds with lower oxidation potentials are more readily oxidized and usually show better antioxidant activities. Thus, oxidation peak potentials for the hydroxyphenylglycine derivatives were determined by cyclic voltammetry using a glassy carbon working electrode and measured vs a Ag/AgCl electrode (Table 1). Experiments were run using as supporting electrolyte a phosphate buffer at the physiological pH of 7.30.

Compound 1a, which showed very weak radical-scavenging activity, presented the highest oxidation peak potential (0.703 V). A decrease in over 0.2 V in potential occurs when a methoxy group is added in ortho position to the hydroxyl group (compound 1b) in agreement with a significant increase in radical-scavenging activity against the DPPH radical. The substitution of the methoxy group by another hydroxyl group (compound 1c) increases more than ten times the radical-scavenging activity. A slightly higher activity is observed when (2-naphthyl)acetyl is substituted for benzoyl as N-acyl group (compound 2c). The N-protopocatechyl 4-hydroxyphenylglycine derivatives (compound 3a) showed radical-scavenging activity comparable to that of compound 1c, which also has an ortho-dihydroxyl group (0.145 V, data not shown). The potential of the second peak, although this peak was similar to that determined by us for octyl hydrocaffeate (0.233 V), the first peak probably resulted from an oxidation potential (0.228 V), which also agrees with the ten-fold increase observed in radical-scavenging activity. Compounds with a single catecholic group (compounds 2c, 3a and 3b) gave anodic peaks with similar potentials ca. 0.270 V, that corresponded to similar radical-scavenging activities. These potentials were lower than the peak potential for the catecholic reference protocatechuic acid (0.324 V).

Compound 3c bearing two similar catecholic groups gave only one anodic peak at a similar potential to the other catecholic compounds (0.272 V). Thus, the increase in radical-scavenging activity of compound 3c, when compared to the activities of the monocatecholic compounds, must result from an additive effect of the two catechol groups.

On the other hand, two anodic peaks were observed for compound 4c, one at 0.145 V and another at 0.233 V. The first peak probably results from oxidation of the N-hydrocaffeoyl group, since the potential of this peak was similar to that determined by us for octyl hydrocaffeose (0.145 V, data not shown). The potential of the second peak, although slightly lower, is in line with the peak potentials presented by the other glycine derivatives with an ortho-dihydroxyl side chain (compounds 1c, 2c and 3c). Despite the lower oxidation potential of the first peak, compound 4c did not present higher radical-scavenging activity against the DPPH radical than compound 3c, which also has catecholic groups both as N-acyl and as side chain moiety.

These cyclic voltammetry experiments indicate a significant variation in the redox properties of the hydroxyphenylglycine derivatives, which can be attributed to the presence of the catecholic groups and their position with respect to the amino acid side chain.
decrease in oxidation potential with the increase in number of hydroxyl groups. Thus, a correlation between oxidation potentials and radical-scavenging activity can be established, with lower oxidation potentials corresponding to higher radical-scavenging activities.

Despite the increase in the number of hydroxyl groups in these non-coded amino acid, their log $P$ is significantly higher (Table 1) than that for tyrosine. This probably confers these new non-coded amino acids with better cell penetrating capacities.

### 3. Conclusions

A simple and efficient one-pot procedure for the synthesis of N-acyl hydroxyphenylglycine amides has been established. These novel hydroxyphenylglycine derivatives, in particular the polyphenolic amino acids N-protocatechyl and N-hydrocaffeoyl 3,4-dihydroxyphenylglycine amides exhibited high radical-scavenging activity.

The synthesis of the N-benzyloxy carbonyl-3,4-dihydroxyphenylglycyl dipeptide derivative shows that the methodology developed gives rise to a substrate that, after N-acyl deprotection, can be coupled with other amino acid residues to give, among others, cell-penetrating type peptides that can be tested as mitochondria targeted antioxidants.

In addition, these non-coded amino acid derivatives can have other intrinsic biological activities or be applied as: cross-linkers; transition metal ligands; or used in the design of new peptide hydrogels.

### 4. Experimental section

#### 4.1. General methods

Melting points (°C) were determined in a Gallenkamp apparatus and are uncorrected. $^1$H and $^{13}$C NMR spectra were recorded on a Bruker Avance II $^+$ instrument. Spectra were taken at room temperature in CDCl$_3$, CD$_3$COCD$_3$ and CD$_3$OD at 400 MHz ($^1$H) and 100.6 MHz ($^{13}$C) by using TMS as an internal standard (1H NMR in CDCl$_3$: 0.000 ppm). $^1$H-$^1$H spin-spin decoupling, DEPT $\theta$45°, HMQC and HMBC were used to attribute some signals. Chemical shifts are given in ppm and coupling constants in Hz. HRMS data were recorded by the Laboratory for Structural Elucidation of the Materials Centre of the University of Porto on an LTQ Orbitrap™ XL hybrid mass spectrometer (Thermo Fischer Scientific, Bremen, Germany) controlled by LTQ Tune Plus 2.5.5 and Xcalibur 2.1.0. Elemental analysis was performed on a LECO CHNS 932 elemental analyzer. The reactions were monitored by thin layer chromatography (TLC). Column chromatography was performed on Macherey-Nagel silica gel 230–400 mesh. Petroleum ether refers to the boiling range 40–60 °C. Solvents were used without purification except for acetonitrile which was dried using standard procedures.

#### 4.1.1. DPPH $^*$ radical essay

DPPH $^*$ radical-scavenging activity was assessed as described previously [41,43]. Methanolic DPPH $^*$ stock solution (1.93 mM) was diluted to give a 0.10 mM working solution. The reaction between DPPH $^*$ and each antioxidant was monitored at 515 nm by using a Powerwave XS Microplate Reader (Bio-Tek Instruments, Inc) thermostated at $T = 25.0 \pm 0.1$ °C. The wells of a 96-well microplate contained a methanolic solution of the antioxidant (3–200 µM) and 80 µM DPPH $^*$. The absorbance of each well was recorded at 1 min intervals for a 60 min. period. The absorbance of each solution was subtracted from the blank (80 µM DPPH $^*$ without antioxidant). The antiradical activity was defined as the relative concentration of antioxidant required to lower the initial DPPH $^*$ concentration by 50% [EC50 (M phenolic compound per unit DPPH concentration)] obtained at different reaction times.

#### Table 1

DPPH $^*$ Radical-scavenging capacity, EC50, oxidation peak potentials and Log $P$ of N-acyl 4-hydroxy, 4-hydroxy-3-methoxy and 3,4-dihydroxy phenylglycine cyclohexylamides and of protocatechuic acid and tyrosine.

<table>
<thead>
<tr>
<th>Compound</th>
<th>R1</th>
<th>R2</th>
<th>EC50</th>
<th>$E_{p,a}$ (V vs Ag/AgCl)</th>
<th>Log $P$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>5 min</td>
<td>60 min</td>
<td></td>
</tr>
<tr>
<td>1a</td>
<td></td>
<td></td>
<td>24.5 ± 13.8</td>
<td>11.0 ± 11.9</td>
<td>0.703 ± 0.016</td>
</tr>
<tr>
<td>1b</td>
<td></td>
<td></td>
<td>3.76 ± 0.20</td>
<td>2.08 ± 0.60</td>
<td>0.500 ± 0.006</td>
</tr>
<tr>
<td>1c</td>
<td></td>
<td></td>
<td>0.32 ± 0.01</td>
<td>0.22 ± 0.01</td>
<td>0.272 ± 0.006</td>
</tr>
<tr>
<td>2a</td>
<td></td>
<td></td>
<td>0.26 ± 0.01</td>
<td>0.22 ± 0.01</td>
<td>0.266 ± 0.005</td>
</tr>
<tr>
<td>3a</td>
<td></td>
<td></td>
<td>0.37 ± 0.01</td>
<td>0.23 ± 0.01</td>
<td>0.274 ± 0.003</td>
</tr>
<tr>
<td>3b</td>
<td></td>
<td></td>
<td>0.28 ± 0.01</td>
<td>0.19 ± 0.01</td>
<td>0.280 ± 0.006</td>
</tr>
<tr>
<td>3c</td>
<td></td>
<td></td>
<td>0.20 ± 0.01</td>
<td>0.13 ± 0.01</td>
<td>0.272 ± 0.015</td>
</tr>
<tr>
<td>4a</td>
<td></td>
<td></td>
<td>0.19 ± 0.01</td>
<td>0.16 ± 0.01</td>
<td>0.145 ± 0.008</td>
</tr>
<tr>
<td>4b</td>
<td></td>
<td></td>
<td>0.28 ± 0.01</td>
<td>0.19 ± 0.01</td>
<td>0.280 ± 0.006</td>
</tr>
<tr>
<td>4c</td>
<td></td>
<td></td>
<td>0.20 ± 0.01</td>
<td>0.13 ± 0.01</td>
<td>0.272 ± 0.015</td>
</tr>
<tr>
<td>Protocatechuic acid</td>
<td></td>
<td></td>
<td>0.69 ± 0.03</td>
<td>0.31 ± 0.01</td>
<td>0.324 ± 0.005</td>
</tr>
<tr>
<td>Tyrosine</td>
<td></td>
<td></td>
<td>26.5 ± 11.70</td>
<td>nd</td>
<td>0.715 ± 0.018</td>
</tr>
</tbody>
</table>

* The antiradical activity was defined as the relative concentration of antioxidant required to lower the initial DPPH $^*$ concentration by 50% [EC50 (M phenolic compound per unit DPPH concentration)] obtained at different reaction times.

4.1.3. Statistical analysis

The cyclic voltammetry experiments were carried out using a Hi-Tek potentiostat, type DT 2101, and a Hi-Tek wave generator type PPRL, connected to a Philips recorder, type PM 8043, and to a three electrode, home-built glass cell. The working electrode was a vitreous carbon disc (diameter: 3 mm), the counter electrode a platinum spiral and the reference electrode a mercury pool. The supporting electrolyte was a phosphate buffer (0.10 M) with pH adjusted to 7.30. Stock solutions of each compound (0.01 M) were prepared by dissolving an appropriate amount in ethanol. The voltammetric working solutions were prepared in the electrochemical cell by diluting 0.10 mL of the stock solution in 10 mL of supporting electrolyte in order to obtain a final concentration of 0.001 M. At the end of each experiment the potential of the mercury pool was measured vs a Ag/AgCl electrode. Between experiments the working electrode was repolished with alumina powder (∼0.05 μm).

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4.2. Synthesis

Procedure A: Synthesis of O-tert-butyloxycarbonylated benzaldehydes. To a solution of the hydroxybenzaldehyde (5.00 mmol) in dry acetonitrile (100 mL) and washed with KHSO4 (1 M) and brine (3 times 25 mL each). The organic layer was dried with MgSO4 and the solvent evaporated at reduced pressure. The product obtained was then recrystallized or subject to column chromatography.

tert-Butyl (3-formylphenyl) carbonate (a) [44]. Procedure A using 3-hydroxybenzaldehyde (0.691 g, 5.000 mmol) was followed to give a.

Yield: 1.066 g (96%); white solid (from diethyl ether/n-hexane). The spectroscopic properties were in agreement with those previously reported [44].

tert-Butyl (4-formyl-2-methoxyphenyl) carbonate (b) [45]. Procedure A using 4-hydroxy-3-methoxybenzaldehyde (0.761 g, 5.000 mmol) was followed to give b.

Yield: 1.197 g (95%); white solid (from diethyl ether/n-hexane). The spectroscopic properties were in agreement with those previously reported [45].

di-tert-Butyl (4-formyl-1,2-phenylene) dicarbonate (c) [46]. Procedure A using 3,4-dihydroxybenzaldehyde (0.691 g, 5.000 mmol) was followed to give c.

Yield: 1.575 g (93%); light brown oil. The spectroscopic properties were in agreement with those previously reported [46].

di-tert-Butyl (3-formyl-1,2-phenylene) dicarbonate (d). Procedure A using 2,3-dihydroxybenzaldehyde (0.691 g, 5.000 mmol) was followed to give d.

Yield: 1.622 g (96%); light brown solid.

HRMS (ESI): m/z M+ calculated for C14H22O7: 338.1366; found: 338.1597.

Procedure B: Two step synthesis of N-acyl, hydroxyphenylglycine cyclohexylamide. To a solution of the O-tert-butyloxycarbonylated benzaldehyde (1.00 mmol) in ethanol/2,2,2-trifluoroethanol (1/1) (0.17 M) under a stream of nitrogen, 1.10 equiv of 4-methoxybenzylamine was added. After 4 h, 1.10 equiv of the carbolic acid and 1.10 equiv of cyclohexyl isocyandie were added and left to react for 2 days. The reaction mixture was then evaporated at reduced pressure and the residue was dissolved in ethyl acetate (100 mL) and washed with KHSO4 (1 M) and brine (3 times 25 mL each). The organic layer was dried with MgSO4 and the solvent evaporated at reduced pressure to give the corresponding Ugi adduct. Trifluoroacetic acid was added to the adduct (0.25 M) and the solution refluxed at 80 °C for 10 min. TFA was then evaporated at reduced pressure. The product obtained was then recrystallized or subject to column chromatography.

N-Benzoyl, (4-hydroxyphenyl)glycine cyclohexylamide (1a). Procedure B using tert-butyl (4-formylphenyl) carbonate (0.222 g, 1.00 mmol) and benzoic acid was followed to give 1a.

Yield: 0.537 g (94%); white solid (from diethyl ether).

Procedure C using 2,3-dihydroxybenzaldehyde (0.691 g, 5.000 mmol) was followed to give c.

Yield: 0.116 g (17%); light yellow oil.

Trifluoroacetic acid was added to 1a (0.307 g, 0.537 mmol) (0.25 M) and the solution refluxed at 80 °C for 10 min. TFA was then evaporated at reduced pressure to give 1a.

Yield: 0.118 g (63%); white solid (from diethyl ether).

HRMS (ESI): m/z M+ calculated for C24H29NO8: 572.69; found: C 71.31, H 7.04, N 4.89; M.p. 58–59 °C.

N-Benzoyl, (4-hydroxyphenyl)glycine cyclohexylamide (1b). Procedure B using tert-butyl (4-formylphenyl) carbonate (0.222 g, 1.00 mmol) and benzoic acid was followed to give 1b.

Yield: 0.537 g (94%); white solid (from diethyl ether).

Procedure C using 3,4-dihydroxybenzaldehyde (0.691 g, 5.000 mmol) was followed to give c.

Yield: 0.116 g (17%); light yellow oil.

Trifluoroacetic acid was added to 1b (0.307 g, 0.537 mmol) (0.25 M) and the solution refluxed at 80 °C for 10 min. TFA was then evaporated at reduced pressure to give 1b.

Yield: 0.118 g (63%); white solid (from diethyl ether).

HRMS (ESI): m/z M+ calculated for C24H29NO8: 572.69; found: C 71.31, H 7.04, N 4.89; M.p. 141–142 °C.

Tentative synthesis of N-benzoyl, (2,3-dihydroxyphenyl)glycine cyclohexylamide (1d). Procedure B using di-tert-butyl (3-formyl-1,2-phenylene) dicarbonate (0.338 g, 1.000 mmol) and benzoic acid was followed. The residue obtained was chromatographed through silica using as eluent ethyl acetate/petroleum ether 1:3 to give the Ugi adduct 1d in low yield.

Yield: 0.116 g (17%); light yellow oil.

cyclohexyl), 1.46, 1.57, 1.76 [s, 18H, C(CH3)3], 3.79 (br. s, 4H, OCH3 + CH cyclohexyl), 4.25 (d, J = 4.8 Hz, 2H, C6H4CH2), 4.80 (br. s, 1H, αCH), 5.92 (br. d, J = 7.6 Hz, 1H, NH), 6.86 (d, J = 8.8 Hz, 2H, ArH), 6.94 (t, J = 8.0 Hz, 1H, ArH), 7.14 (dd, J = 8.0 Hz, 1H, 1H, ArH), 7.18–7.22 (m, 3H, ArH), 7.47 (t, J = 8.0 Hz, 2H, ArH), 7.59 (t, J = 7.2 Hz, 1H, ArH), 8.01 (dd, J = 8.0 Hz, J = 1.2 Hz, 2H, ArH) ppm.

13C NMR (100.6 MHz, CDCl3): δ = 46.06 (C6H4CH2), 46.79 (C6H4C), 51.11 (OCH3), 60.40 (αCH), 83.89 [2C(CH3)], 113.96 (2CH), 119.46 (CH), 120.20 (CH), 120.56 (C), 121.56 (C), 122.49 (CH2), 124.29 (CH2), 124.99 (2CH), 129.62 (C), 130.51 (C), 130.87 (CH), 130.94 (C), 131.18 (CH), 144.88 (C), 158.89 (2C = O), 168.22 (C = O), 170.22 (C = O) ppm.


Trifluoroacetic acid was added to 5a (0.255 g, 0.500 mmol) and the solution refluxed at 80 °C for 10 min. TFA was then evaporated at reduced pressure to give 5a.

White solid (from acetone).

Rf = 0.78 (petroleum ether/ethyl acetate 1:4).

M.p. 241–242 °C.

1H NMR (400 MHz, DMSO): δ = 1.04–1.24 (m, 5H, CH2 cyclohexyl), 1.26–1.49 (m, 3H, CH3), 1.52–1.73 (m, 5H, CH2 cyclohexyl), 1.82 (s, 3H, CH3CO), 3.40–3.45 (m, 1H, NCH2), 5.31 (d, J = 8.4 Hz, 1H, αCH), 6.66 (d, J = 8.4 Hz, 2H, ArH), 7.15 (t, J = 8.4 Hz, 2H, ArH), 7.99 (d, J = 8.0 Hz, 1H, NH), 8.29 (d, J = 8.4 Hz, 1H, OH), 9.34 (s, 1H, OH) ppm.

13C NMR (100.6 MHz, DMSO): δ = 22.46 (CH3), 24.37 (CH2), 24.49 (CH2), 25.17 (CH2), 32.18 (CH2), 32.30 (CH2), 47.52 (CH2 cyclohexyl), 55.38 (C=O), 114.88 (2CH), 128.11 (2CH), 129.62 (C), 156.59 (C), 166.89 (C = O), 169.29 (C = O) ppm.


N-Benzoxycarbonylglycine, (3,4-dihydroxyphenyl)glycine cyclohexylamide (6c). Procedure B using tert-butyl (4-formyl-1,2-phenyle) dicarbonate (0.338 g, 1.000 mmol) and N-benzoxycarbonylglycine was followed to give 6c.

Yield: 0.731 g (94%); light pink solid (from ethyl acetate/petroleum ether).

Rf = 0.60 (petroleum ether/diethyl ether 1:5).

M.p. 113–114 °C.

1H NMR (400 MHz, CDCl3): δ = 1.10–1.12 (m, 3H, CH2 cyclohexyl), 1.51–1.56 (m, 8H, C(CH3)2), 1.65–1.70 (m, 3H, CH2 cyclohexyl), 1.84–1.88 (m, 2H, CH2 cyclohexyl), 2.37–3.80 (m, 4H, OCH3 + CH cyclohexyl), 3.93 (br. d, J = 16.8 Hz, 1H, αCH), 4.10 (br. d, J = 16.8 Hz, 1H, αCH), 4.43 (br. d, J = 17.2 Hz, 1H, C6H4CH2), 4.60 (br. d, J = 17.2 Hz, 1H, C6H4CH2), 5.10 (s, 2H, CH2 Z), 5.66 (d, J = 7.6 Hz, 1H, αCH), 5.71–5.73 (m, 2H, 2NH), 6.75 (d, J = 8.4 Hz, 2H, ArH), 6.94 (d, J = 8.4 Hz, 2H, ArH), 7.15 (s, 2H, ArH), 7.30–7.35 (m, 6H, ArH) ppm.

13C NMR (100.6 MHz, CDCl3): δ = 24.73 (CH3), 24.81 (CH2), 25.40 (CH2), 27.58 [2C(CH3)], 29.63 (CH2), 31.90 (CH2), 32.65 (CH2), 43.46 (αCH), 48.91 (CH cyclohexyl), 49.27 (C6H4CH2), 55.17 (OCH3), 62.75 (αCH), 66.85 (CH3 Z), 84.03 [2C(CH3)], 114.17 (CH), 123.37 (CH), 125.00 (CH), 127.69 (CH), 127.95 (2CH), 128.03 (2CH), 128.46 (2CH), 133.04 (C), 136.39 (C), 142.59 (C), 142.75 (C), 150.39 (C), 150.43 (C), 156.14 (C = O), 158.91 (2C = O), 167.37 (C = O), 170.12 (C = O) ppm.


Trifluoroacetic acid was added to 6c (0.583 g, 0.750 mmol) and the solution refluxed at 80 °C for 10 min. TFA was then evaporated at reduced pressure to give 6c.

Yield: 0.252 g (74%); white solid (from ethyl acetate/diethyl ether).

Rf = 0.82 (petroleum ether/diethyl ether 1:4).

M.p. 162–163 °C.
αCH2), 5.10 (s, 2H, CH2), 5.32 (d, J = 7.6 Hz, 1H, αCH), 6.70 (br. d, J = 7.6 Hz, 1H, ArH), 6.73 (s, 2H, ArH), 6.91 (s, 1H, ArH), 7.26–7.39 (m, 6H, ArH + NH), 7.66 (d, J = 7.2 Hz, 1H, NH) ppm.

13C NMR (100.6 MHz, CD2COCD3): δ = 25.62 (CH2), 26.27 (CH2), 29.26 (CH2), 33.26 (CH2), 33.38 (CH2), 45.01 (αCH2), 49.20 (CH cyclohexyl), 57.16 (αCH), 66.93 (CH2), 115.45 (CH), 115.90 (CH), 119.80 (CH), 128.63 (2CH), 129.24 (3CH), 131.60 (C), 138.16 (C), 145.66 (C), 145.78 (C), 157.63 (C =O), 169.13 (C =O), 170.00 (C =O) ppm.


N-Protocatechyl (3,4-dihydroxyphenyl)glycine cyclohexylamide (3a). Procedure C using tert-butyl (4-formyl-1,2-phenylene) carbonate (0.222 g, 1.000 mmol) and benzoic acid was followed. The residue obtained after removal of TFA was dissolved in ethyl acetate (100 mL) and washed with KHSO4 (1 M), NaHCO3 (1 M) and brine (3 times 25 mL each). The organic layer was dried with MgSO4 and the solvent evaporated at reduced pressure to give 3a. Yield: 0.283 g (68%); white solid (from ethyl acetate/dichloromethane).

Rf = 0.47 (petroleum ether/ethyl acetate 1:4). M.p. 129–130 °C.

1H NMR (400 MHz, CD2COCD3): δ = 1.17–1.35 (m, 5H, CH2 cyclohexyl), 1.58–1.89 (m, 5H, CH2 cyclohexyl), 3.68–3.73 (m, 1H, CH cyclohexyl), 5.67 (s, 3H, OCH3), 5.67 (s, 1H, αCH), 6.81 (d, J = 8.0 Hz, 1H, ArH), 7.01 (dd, J = 8.0 Hz, J = 2.0 Hz, 1H, ArH), 7.19 (d, J = 2.0 Hz, 1H, ArH), 7.46–7.57 (m, 3H, ArH), 7.94–7.96 (m, 2H, ArH), 8.04 (s, 1H, NH) ppm.

13C NMR (100.6 MHz, CD2COCD3): δ = 25.48 (CH2), 25.56 (CH2), 26.22 (CH2), 33.21 (CH2), 33.29 (CH2), 49.09 (CH cyclohexyl), 56.23 (CH2), 57.60 (αCH), 111.94 (CH), 115.53 (CH), 121.15 (CH), 128.12 (2CH), 129.16 (2CH), 131.49 (C), 132.13 (CH), 135.43 (C), 147.05 (C), 148.17 (C), 166.46 (C =O), 170.07 (C =O) ppm.


N-Protocatechyl (3,4-dihydroxyphenyl)glycine cyclohexylamide (3c). Procedure C using di-tert-butyl (4-formyl-1,2-phenylene) dicarbonate (0.338 g, 1.000 mmol) and protocatechic acid was followed. The residue obtained after removal of TFA was chromatographed through silica using as eluents from ethyl acetate/petroleum ether/ethyl acetate 1:4:1. The residue obtained after removal of TFA was dissolved in ethyl acetate (100 mL) and washed with KHSO4 (1 M), NaHCO3 (1 M) and brine (3 times 25 mL each). The organic layer was dried with MgSO4 and the solvent evaporated at reduced pressure to give 3c.

Yield: 0.283 g (78%); yellow solid (from ethyl acetate/dichloromethane).

Rf = 0.75 (petroleum ether/ethyl acetate 1:4). M.p. 220–221 °C.

1H NMR (400 MHz, CD2COCD3): δ = 1.06–1.33 (m, 5H, CH2 cyclohexyl), 1.63–1.83 (m, 5H, CH2 cyclohexyl), 3.69–3.75 (m, 1H, CH cyclohexyl), 3.81 (d, J = 2.8 Hz, CH2 (2-naphthyl)acetyl), 5.38 (d, J = 8.0 Hz, 1H, αCH), 6.75 (s, 2H, ArH), 6.95 (s, 1H, ArH), 7.17 (d, J = 8.0 Hz, 1H, C6H4CONH), 7.46–7.53 (m, 3H, ArH), 7.70 (d, J = 7.6 Hz, 1H, NH), 7.84–7.89 (m, 6H, ArH + 2OH) ppm.

13C NMR (100.6 MHz, CD2COCD3): δ = 24.52 (CH2), 25.52 (CH2), 26.18 (CH2), 33.19 (CH2), 33.28 (CH2), 43.57 (CH2 (2-naphthyl)acetyl), 49.01 (CH cyclohexyl), 57.28 (αCH), 115.46 (CH), 115.84 (CH), 119.79 (CH), 126.32 (CH), 126.80 (2CH), 128.40 (2CH), 128.54 (CH), 128.59 (CH), 131.80 (C), 133.29 (C), 134.49 (C), 134.85 (C), 145.57 (C), 145.74 (C), 157.04 (C =O), 170.25 (C =O) ppm.

ether 1:3 to neat ethyl acetate to give 3c. 

Yield: 0.292 g (69%); white solid (from ethyl acetate/dichloromethane).

R$_\text{f}$ = 0.41 (petroleum ether/ethyl acetate 1:4).

M.p. 177–178 °C.

1H NMR (400 MHz, CD$_3$OD): $\delta$ = 1.21–1.33 (m, 6H, CH$_2$ cyclohexyl), 1.57–1.71 (m, 4H, CH$_2$ cyclohexyl), 5.55 (d, $J$ = 8.4 Hz, 1H, ArH), 6.85 (dd, $J$ = 8.4 Hz, $J$ = 2.0 Hz, 1H, ArH), 6.90 (d, $J$ = 8.0 Hz, 1H, ArH), 7.03 (d, $J$ = 2.0 Hz, 1H, ArH), 7.48 (d, $J$ = 2.0 Hz, 1H, ArH), 7.72 (d, $J$ = 7.2 Hz, 1H, C$_6$H$_4$CONH), 7.95 (br. s, 1H, OH), 8.08 (br. s, 1H, OH), 8.45 (br. s, 1H, OH), 8.49 (br. s, 1H, OH) ppm.

1C NMR (100.6 MHz, CD$_3$COCD$_3$): $\delta$ = 23.27 (CH$_2$), 26.06 (CH$_2$), 26.12 (CH$_2$), 49.23 (CH cyclohexyl), 56.35 (CH$_2$), 131.47 (C), 131.68 (C), 134.96 (C), 144.61 (C), 146.46 (C), 159.31 (C), 166.17 (C=O), 170.34 (C=O) ppm.

HRMS (ESI): m/z [M+Na]$^+$ calc'd for C$_{24}$H$_{24}$Na$_2$O$_4$: 423.1523; found: 423.1528.

N-Hydroxycinnamoyl, (3,4-dihydroxyphenyl)glycine cyclohexylamide (4c). Procedure C using di-tert-butyl (4-formyl-1,2-phenylene) dicarboxonate (0.338 g, 1.00 mmol) and hydroxycinnamic acid was followed. The residue obtained after removal of TFA was chromatographed through silica using as eluents from ethyl acetate/petroleum ether 1:3 to neat ethyl acetate to give 4c.

Yield: 0.317 g (74%); white solid.

M.p. 100-101 °C.

HRMS (ESI): m/z [M+Na]$^+$ calc'd for C$_{24}$H$_{22}$Na$_2$O$_4$: 423.1523; found: 423.2030.

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Conflict of interest

The authors declare that they have no conflict of interest.

This article does not contain any studies with human participants or animals performed by any of the authors.

References