Research Article

Structure-Activity Relationships of *N*-Cinnamoyl and Hydroxycinnamoyl Amides on α -Glucosidase Inhibition

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Currently, there is an increasing interest towards α -glucosidase inhibition of various diseases including diabetes mellitus type 2, cancer, HIV, and B- and C-type viral hepatitis. Cinnamic acid derivatives have been shown to be potentially valuable as a new group of α -glucosidase inhibitors. Therefore, herein, the α -glucosidase inhibitory activity of *trans*-*N*-cinnamoyl and hydroxycinnamoyl amides was studied in vitro. Results revealed that the tested hydroxycinnamoyl amides (**1–16**) inhibited α -glucosidase with IC_{50} s ranging between 0.76 and 355.1 μ g/ml. Compounds **1**, **2**, **5**, **6**, **9**, **14**, and **15** showed significant inhibition of yeast α -glucosidase, being even more potent ones than the used positive inhibitor acarbose ($IC_{50} = 2.50 \pm 0.21$ μ g/ml).

1. Introduction

Universally it is accepted that overproduction of radicals in biological systems, termed as oxidative and nitrosative stress [1–4], has been implicated in the pathophysiology of a variety of diseases, including cancer, ischemia/reperfusion injury, inflammatory diseases, neurodegenerative disorders, ageing, and diabetes mellitus [5].

Nowadays, diabetes mellitus is known to affect nearly 30 million people worldwide. Being the most serious chronic metabolic disorder, diabetes is associated with high concentration of blood glucose [6, 7]. Glucosidases (EC 3.2.1.20) [8, 9] belong to glycoside hydrolase enzymes which convert complex sugars into their monomeric forms. Therefore, the possible way for reduction of the rate of carbohydrate digestion and hence decrease of the after-meal glucose levels is an inhibition of the activity of α -glucosidase.

The multiple functions of glucosidase inhibitors in the organism comprise the treatment of diabetes mellitus [10–13] and other diseases such as obesity, HIV infections, and

tumors [14–23], which define these agents as potentially beneficial tools in prevention and treatment of such disorders. At the present, three antidiabetic drugs, acarbose [24], miglitol [25], and voglibose [26], have been therapeutically used for non-insulin-dependent diabetes (type 2). As per the World Health Organization (WHO), it is expected that the diabetes will be the 7th leading cause of death in 2030 [27].

Therefore, the search for potential therapeutic glucosidase inhibitors from both synthetic and natural origin is of great demand.

There is a variety amongst the glucosidase inhibitors, whose structures differ from a sugar scaffold. An example of such compounds is hydroxycinnamic acids (*p*-coumaric, caffeic, ferulic, and sinapic) and their derivatives (esters, amides, and glycosides) which possess a pool of biological activities, including and antidiabetic effects [28–32].

In this context, the aim of the current study is the evaluation of α -glucosidase inhibitory potential of cinnamic and hydroxycinnamic acid amides with amino acid.

2. Materials and Methods

2.1. General Reagents and Instrumentation. Cinnamic (CA), 3-methoxy-4-hydroxy-cinnamic (ferulic, FA), 3,5-dimethoxy-4-hydroxy-cinnamic (sinapic, SA), and 3,4-dihydroxycinnamic (caffeic, CafA) acids, as well as amino acid derivatives, HOBt, trifluoroacetic acid (TFA), N,N'-diisopropylcarbodiimide (DIC), acarbose, *p*-nitrophenyl- α -D-glucopyranoside (pNPG), and α -glucosidase [(EC 3.2.1.20)] type I, from *Saccharomyces cerevisiae*, were purchased from Sigma Aldrich (FOT, Bulgaria). 1-[3-(Dimethylamino) propyl]-3-ethylcarbodiimide hydrochloride (EDC) was obtained from Acros Organics. Fmoc-protected amino acids residues and Rink amide resin (0.7 mmol/g, 100–200 mesh) were bought from Novabiochem.

Thin-layer chromatography (TLC) was conducted on pre-coated Kieselgel 60F254 plates (Merck, Germany). Separation of the compounds was accomplished by using preparative thin-layer chromatography with silica gel 60 GF245 (Merck, Bulgaria), and the solvents were used without further purification.

The NMR experiments were recorded on Bruker Avance III 600 or Bruker Avance III 400 spectrometer, operating at 600.13 and 400.15 MHz for protons, respectively. The measurements in CDCl₃ solutions were carried out at ambient temperature (300 K) and tetramethylsilane (TMS) was used as an internal standard. The UV spectra of the compounds were measured with an "Agilent 8453" UV-vis spectrophotometer. Electrospray Ionisation (ESI) mass spectra were recorded on an Esquire 3000.

2.2. Synthesis of *N*-Cinnamoyl and Hydroxycinnamoyl Derivatives

2.2.1. Solution Phase Peptide Synthesis. *N*-Hydroxycinnamoyl amides (**1–14**) were obtained in moderate to excellent yields by solution phase peptide synthesis, according to the previously described procedure [33]. The chemical structures of all known compounds (**1–14**) have been assigned on the basis of their spectral data and compared with published data [33, 34].

The detailed UV, IR, NMR, and ESI-MS spectra data of the new *N*-(sinapoyl)-*L*-valine methyl ester are as follows:

(*E*)-*N*-(sinapoyl)-*L*-valine methyl ester (**10**) 66.2%

UV (C₂H₅OH) $\lambda_{\max}$ = 202, 239, 323 nm; IR (ATR) $\nu_{\max}$: 3352, 2961, 2843, 1734, 1658, 1594, 1529.9, 1514, 1458, 1432, 1325, 1283, 1158, 1108, 977, 826 cm⁻¹; ¹H-NMR (CDCl₃)/600 MHz/ δ = 0.9 (dd, 6H, -CH(CH₃)₂), 3.4 (m, 1H, CH), 3.4 (s, 3H, OCH₃), 3.8 (s, 6H, 2xOCH₃), 4.3 (m, 1H, NH-CH), 6.2 (d, 1H, *J* = 8.8 Hz, -NH), 6.5 (d, 1H, *J* = 15.5 Hz, -CH=CH-), 6.7 (s, 2H, Ar-H), 7.5 (d, 1H, *J* = 15.5 Hz, -CH=CH-); ESI-MS: 338.4 ([M + H]⁺), 360.1 ([M + Na]⁺).

2.2.2. Fmoc Solid Phase Peptide Synthesis. Synthetic procedure for sinapoyl peptides (**10**, **11**) followed the standard SPPS conditions [35].

Briefly, loading of the Fmoc-Gly-OH to Rink amide resin and all acylation reactions were carried out for 1 h using a 3-fold excess of Fmoc-amino acids activated with

DIC (3.5 mmol) in the presence of HOBt (3.5 mmol). The efficiency of coupling was checked by ninhydrin or isatin tests. The sinapoyl-peptide was removed from the resin with acidolysis with 95% TFA/H₂O for 45 min. The crude products were purified by preparative TLC (CHCl₃/CH₃OH = 2 : 1).

SA-Pro-Leu-Gly-NH₂ (**15**). UV (CH₃OH) $\lambda_{\max}$ = 205, 243, 302 nm; IR (ATR) $\nu_{\max}$: 3322, 2933, 2850, 1704, 1625, 1515, 1456, 1110, 1030 cm⁻¹; ESI-MS: 490.9 ([M + H]⁺), 528.9 ([M + K]⁺).

SA-Tyr-Pro-Leu-Gly-NH₂ (**16**). UV (CH₃OH) $\lambda_{\max}$ = 203, 227, 325 nm; IR (ATR) $\nu_{\max}$: 3276, 2926, 1615, 1514, 1448, 1205, 1111 cm⁻¹; ESI-MS: 654.0 ([M + H]⁺), 676.0 ([M + Na]⁺).

α -Glucosidase Inhibitory Assay. The α -glucosidase inhibition activity of tested compounds was determined according to the method reported by Mayur et al. [36] with slight modifications. Briefly, the reaction mixture contained 50 μ l of 0.1 M phosphate buffer (pH 7.0), 25 μ l of 0.5 mM 4-nitrophenyl- α -D-glucopyranoside, 20 μ l of test sample at various concentrations, and 25 μ l of α -glucosidase solution (0.8 Unit/ml). The mixtures were then incubated at 37°C for 10 min, and then 100 μ l of 0.2 M Na₂CO₃ was added to terminate the reaction. Absorbance of *p*-nitrophenol released during the enzymatic hydrolysis of substrate 4-nitrophenyl α -D-glucopyranoside was monitored at 405 nm using microplate reader Stat Fax 303 Plus. Acarbose was used as standard glucosidase inhibitor.

All experiments were carried out in triplicate. The inhibition percentage of α -glucosidase was estimated by the following formula:

% Inhibition

$$= \frac{100 \times (\text{Abs}_{\text{contr}} - (\text{Abs}_{\text{compd}} - \text{Abs}_{\text{referent}}))}{\text{Abs}_{\text{contr}}} \quad (1)$$

where IC₅₀ values were calculated by the graphic method.

3. Results and Discussion

Following the assumption that hyperglycemia causes oxidative stress, herein we investigated if the antioxidant active hydroxycinnamoyl amides might serve as potential α -glucosidase inhibitors.

For this purpose a series of cinnamoyl and hydroxycinnamoyl amides (**1–16**) were synthesized in solution phase by EDC/HOBt-mediated couplings as in previously described procedure [33]. Moreover, for the synthesis of C-terminal peptide amides (compounds **15**, **16**) the standard Fmoc-SPPS was carried out on Rink amide resin. The cleavage (95% TFA/H₂O) from the solid support afforded the corresponding sinapoyl-prolyl-leucyl-glycinamide (**15**) and sinapoyl-tyrosyl-prolyl-leucyl-glycinamide (**16**).

In order to investigate the structure- α -glucosidase inhibitory relationships of hydroxycinnamoyl amides, some of the previous evaluated cinnamoyl compounds were used for comparison (Table 1).

TABLE 1: Glucosidase inhibitory activity of *N*-hydroxycinnamoyl amides. The given values are the means $\pm$ SD of triplicate samples.

Chemical name	R	R'	R''	R'''	IC ₅₀ [μ g/ml]
(1) CafA-Trp-Trp-OMe	-Trp-Trp-OCH ₃	OH	OH	H	0.76 $\pm$ 0.10
(2) SA-Trp-Trp-OMe	-Trp-Trp-OCH ₃	OCH ₃	OH	OCH ₃	0.13 $\pm$ 0.02
(3) CafA-Trp-OMe	-Trp-OCH ₃	OH	OH	H	355.1 $\pm$ 0.2
(4) SA-Trp-OMe	-Trp-OCH ₃	OCH ₃	OH	OCH ₃	233.5 $\pm$ 0.4
(5) CafA-Phe-OBu ^t	-Phe-OC(CH ₃) ₃	OH	OH	H	0.78 $\pm$ 0.04
(6*) SA-Phe-OBu ^t	-Phe-OC(CH ₃) ₃	OCH ₃	OH	OCH ₃	1.82 $\pm$ 0.17
(7) CA-Phe-OBu ^t	-Phe-OC(CH ₃) ₃	H	H	H	3.36 $\pm$ 0.03
(8) CA-Phe(3-F)-OMe	-Phe(3-F)-OCH ₃	H	H	H	>5
(9) CafA-Val-OMe	-Val-OCH ₃	OCH ₃	OH	OCH ₃	2.10 $\pm$ 0.03
(10) SA-Val-OMe	-Val-OCH ₃	OCH ₃	OH	OCH ₃	10.10 $\pm$ 0.05
(11*) FA-Val-OMe	-Val-OCH ₃	OCH ₃	OH	H	2.61 $\pm$ 0.09
(12*) CA-Leu-OMe	-Leu-OCH ₃	H	H	H	>3
(13*) CA-Ala-OBu ^t	-Ala-OC(CH ₃) ₃	H	H	H	>8
(14) FA-Pro-OEt	-Pro-OC ₂ H ₅	OCH ₃	OH	H	1.42 $\pm$ 0.15
(15) SA-Pro-Leu-GlyNH ₂	-Pro-Leu-GlyNH ₂	OCH ₃	OH	OCH ₃	1.44 $\pm$ 0.21
(16) SA-Tyr-Pro-Leu-GlyNH ₂	-Tyr-Pro-Leu-GlyNH ₂	OCH ₃	OH	OCH ₃	2.45 $\pm$ 0.04
Sinapic acid (SA)					1.17 $\pm$ 0.1
Caffeic acid (CafA)					4.67 $\pm$ 0.04
Ferulic acid (FA)					10.5 $\pm$ 0.1
Cinnamic acid (CA)					65.1 $\pm$ 0.1
Acarbose					2.50 $\pm$ 0.21

*These results were previously obtained by us [32] and were used for comparison.

Inhibition of α -Glucosidase Activity by N-Hydroxycinnamoyl Amides and Cinnamic Acids. The inhibitory effects of synthesized *N*-hydroxycinnamoyl amides and substituted cinnamic acids on yeast α -glucosidase were estimated in vitro. For comparison of their inhibitory potencies, the IC_{50} values of the tested compounds and acarbose (a clinically used α -glucosidase inhibitor) were determined ($IC_{50} = 2.5 \pm 0.04 \mu\text{g/ml}$).

The preliminary inhibitory results (Table 1) displayed that the hydroxycinnamoyl amides (**11**, **16**) were similar in potency to acarbose, whereas the lead compounds (**1**, **2**, **5**, **6**, **9**, **14**, and **15** and sinapic acid) were the most potent α -glucosidase inhibitors.

However, our findings revealed that *N*-hydroxycinnamoyl amides with one tryptophan residue (**3**, **4**) were inactive ($IC_{50} > 200 \mu\text{g/ml}$), whereas the introduction of two tryptophan moieties in caffeic and sinapic acid amides (**1**, **2**) pronouncedly exerted a higher glucosidase inhibition. Interestingly, the observed significant differences are due to the binding affinity of hydroxycinnamoyl derivatives with four catalytic regions of the family I α -glucosidases [37]. Our ongoing experiment should be focused on the clarification of molecular mechanisms of hydroxycinnamic acid derivatives against α -glucosidase.

Considering the cinnamoyl and hydroxycinnamoyl amides with the same phenylalanine moiety (**5**–**8**) it was found that the presence of hydroxyl or methoxyl groups at cinnamoyl moiety resulted in an enhancement of glucosidase inhibitory activity. Likewise, amongst the hydroxycinnamic acids, the cinnamic acid displayed the lowest potency, whereas sinapic acid showed the highest inhibition (the IC_{50} value of $1.17 \pm 0.1 \mu\text{g/ml}$) on yeast α -glucosidase.

Moreover, the hydroxycinnamic acid amides containing proline (**14**–**16**) were also found to inhibit significantly the enzyme. This strong inhibition effects of compounds (**14**–**16**) are in accordance with literature data, concerning five-membered iminocyclitol derivatives as potent glycosidase inhibitors [38].

4. Conclusions

In conclusion, sixteen *N*-cinnamoyl and hydroxycinnamoyl amides were synthesized in satisfactory yields using solution or solid phase carbodiimide-mediated couplings.

A preliminary study of synthetically obtained amides on α -glucosidase inhibition indicated that *N*-hydroxycinnamoyl amides **1**, **2**, **5**, **6**, **9**, **14**, and **15** exhibited extremely high *in vitro* inhibitory potency against α -glucosidase and can serve as very promising therapeutic glucosidase inhibitors.

Conflicts of Interest

The authors declare no conflicts of interest.

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