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Research Article

The α-Glucosidase Inhibitory Activities of Phenolic Acid Amides with L-Amino Acid Moiety †‡

Bin Liu,§[a] Ji-Mei Ma,§[a] Hang-Wei Chen,[a] Zi-Long Li,[a] Lin-Hao Sun,[a] Zhen Zeng[a] and Hong Jiang*[a]

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Abstract: α-glucosidase inhibitors can effectively control the postprandial hyperglycemia. In this paper, a series of phenolic acid with L-amino acid moiety were synthesized and their inhibitory activities against α-glucosidase from *Saccharomyces cerevisiae* (EC 3.2.1.20) were evaluated. The results suggested that all these compounds showed strong α-glucosidase inhibitory activities. In particular, the *N*-(4-hydroxyl-phenylpropenoyl)-L-alanine (**c2**) and *N*-(4-hydroxyl-phenylpropenoyl)-L-methionine (**c8**) exhibited much higher potency (IC_{50} values 0.04 mM) than the positive control acarbose (IC_{50} values 1.70 mM). Three-dimensional quantitative structure-activity relationship (3D-QSAR) model for comparative molecular field analysis (CoMFA) was generated and the result showed that bulk groups and high electron density groups on the amino acid residues were benefit to their activities, moreover, the substituents with low electron density and small steric hindrance on the para position of the benzene ring were helpful to improve the activities. Kinetic analysis indicated that compound (**c2**) acted as a mixed-type inhibitor with *Ki* value of 0.0124 mM. Docking analysis revealed that they could bind to α-glucosidase at the catalytic site via hydrogen bonds and a π-π stacking.

1. Introduction

Diabetes mellitus (DM) is a common chronic metabolic diseases characterized by elevating plasma glucose levels.[1,2] The type Ⅱ diabetes mellitus (TIIDM) caused by insulin resistance accounts for more than 90 percent DM,[3] and has become a worldwide health concern due to its constantly growing incidence and serious complications.[4,5] Currently glucose-lowering agents include sulfonylureas, glinides, biguanides and α-glucosidase inhibitors.[6-8] Among them, α-glucosidase inhibitors can effectively control the postprandial hyperglycemia through inhibiting the catalytic activity of α-glucosidase, a key enzyme catalyzes the final step in the digestive process of carbohydrates.[9,10] Sugar analogue α-glucosidase inhibitors such as acarbose, miglitol and voglibose are common used in clinic. However, most of them have gastrointestinal side effects.[11-13] Many natural non-glycosidic compounds present in plants have been reported with good α-glucosidase inhibition.[14-16] This may provide a valuable approach for the development of safe and effective α-glucosidase inhibitors.

Polyphenols are widespread in plants and exhibit various medicinal properties.[17,18] As an important kind of polyphenol, phenolic acid amides with L-amino acid moiety are widely distributed in plants such as cocoa (*Theobroma cacao*),[19,20] *Angelica archanglica root, Sambucus nigra flowers, Coriandrum sativum fruit* etc.[21] Researches demonstrated that the cocoa extract or cocoa products could well control the glucose level in diabetic-induced rats and genetically inherited diabetic rats. [22, 23] Abbe Maleyki et al. (2008) revealed that the polyphenol extract from cocoa had an effect on postprandial glucose control.[24] Apart from polyphenol, are there any other α-glucosidase inhibitors in cocoa products? Exploring this problem would be valuble to develop food-based glucose-lowering agents. Considering the large family of phenolic acid amides with L-amino acid moiety present in cocoa, we speculated that they may act as α-glucosidase inhibitors and cause hypoglycemic effect induced by cocoa. To validate this hypothesis, 42 such phenolic acid amides were synthesized, and some of them have already been isolated from cocoa or other natural products. Their α-glucosidase inhibitory activities *in vitro* were investigated.

2. Results and discussion

2.1. Chemistry

The synthetic method was illustrated in Fig. 1 and ferulic acid derivative was exemplified. 42 kinds of *N*-phenylpropenoyl-L-amino acids were synthesized. The structures were illustrated in Table 1. All the synthesized compounds were confirmed by the ^{1}H NMR and MS techniques. All spectral data were in accordance with the assumed structure.

2.2. The inhibitory activities against α-glucosidase and 3D QSAR model development

All the synthesized compounds were evaluated for their inhibitory activities against α-glucosidase. The IC_{50} values are summarized in table 1. Some compounds had better activities than acarbose (IC_{50} value of 1.70 ㎜). Among them, compound **c2**, **c3**, and **c8** showed much more strong activities than acarbose with their IC_{50} values

[a] Department of Chemistry, College of Science, Huazhong Agricultural University, Wuhan 430070, Hubei, PR China.
Telephone: +86-13554252568; Fax: +86-02787284018;
E-mail: jianghong0066@126.com.
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§ These authors contributed equally to this work.

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0.04, 0.07 and 0.04 mM, respectively, indicating much more active than acarbose. Compounds a9, a13, a14, c9, c13, c14, which were natural present in the cocoa beans, [25] showed similar or better activities than acarbose. This result indicated that the phenolic acid amides in cocoa might play part roles on the hypoglycemic effects of cocoa beans.

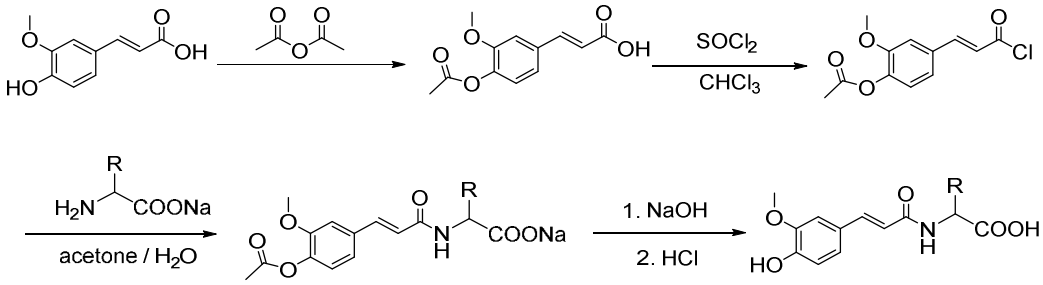
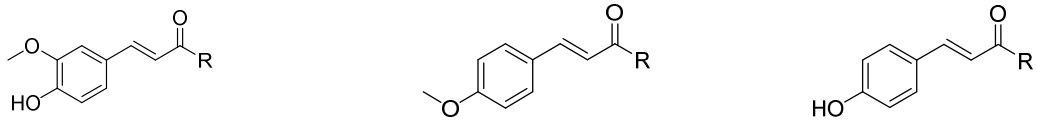


Fig. 1 The synthetic route of *N*-[4-hydroxy-3-methoxyl-(*E*)-cinnamoyl]-L-amino acid

Table.1 The *in vitro* α-glucosidase inhibition activities of compounds



Compound	R	IC_{50} (mM)	Compound	R	IC_{50} (mM)	Compound	R	IC_{50} (mM)
a1	Gly	34.43	b1	Gly	3.53	c1	Gly	0.72
a2	Ala	23.07	b2	Ala	9.63	c2	Ala	0.04
a3	Leu	31.50	b3	Leu	6.43	c3	Leu	0.07
a4	Ile	17.76	b4	Ile	10.60	c4	Ile	0.27
a5	Val	22.16	b5	Val	15.29	c5	Val	0.40
a6	Pro	2.16	b6	Pro	29.42	c6	Pro	0.21
a7	Phe	2.13	b7	Phe	1.17	c7	Phe	0.30
a8	Met	19.27	b8	Met	11.16	c8	Met	0. 04
a9	Trp	1.84	b9	Trp	3.95	c9	Trp	0.68
a10	Ser	1.10	b10	Ser	1.20	c10	Ser	3.42
a11	Gln	4.31	b11	Gln	2.93	c11	Gln	1.42
a12	Thr	3.25	b12	Thr	3.80	c12	Thr	0.23
a13	Glu	2.10	b13	Glu	1.95	c13	Glu	0.12
a14	Asp	1.91	b14	Asp	1.53	c14	Asp	0.13

* IC_{50} (acarbose) = 1.70 mM

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The statistical parameters of CoMFA were showed in Table 2. An optimum number of principal components (ONC=10) was recommended based on the LOO cross-validated run with a q^2 value of 0.561. The subsequent non-cross-validated correlation coefficients r^2 value was 0.977, greater than the value required for a good model. The CoMFA model contour map included 51.2% of the electrostatic field descriptor, and 48.8% of the contributing steric field, indicating a greater influence of the electrostatic field.In order to validate the prediction capabilities of the 3D QSAR model further, predicted activity and the residuals between the experimental and the predicted activity of the training set and test set were measured respectively (see supplement information Table S1). The Linear regression analysis plot bwtween predicted pIC50 agaainst observed pIC50 of traning set (Fig.2(A)) showed that the predicted pIC50 values were almost in accordance with the experimental values with a slop of 0.980. The predicted pIC50 values of test set well suited the experiment data (Figure 2(B)). The result indicated that the CoMFA model had a good predictability.

2.3. Interpretation of the inhibitory activity and 3D QSAR model

The steric and electrostatic contour map obtained *via* CoMFA model was showed in Fig. 3. In the steric field, bulky groups (the green area distributed on the amino acid residual chain, such as aromatic rings in compound **a7**, **a9**, **b7** and **b9**) were responsible for the high inhibitory activities, while compounds with small groups (**a1-a5** and **a8**, **b1-b5** and **b8**) had much lower activities in the **a** and **b** series compounds. But on the para position of benzene rings (the yellow area), bulk groups decreased the inhibitory activities, thus the activities of **b** series compouds with methoxy on the para of benzene rings were weaker than that of **c** series. In the electrostatic fields, groups with high electron density (red areas on the residual part of amino acids) increased the activities. For example, compounds with negative electron groups such as hydroxyl (**a10**, **a12**, **b10** and **b12**), amino (**a11** and **b11**), carboxyl (**a13**, **a14**, **b13** and **b14**) on their amino acid side chains performed better inhibitory activities among **a** and **b** series compounds. Substituents with low electron density (blue) regions on the benzene ring of cinnamic acid were expected to increase activities. Among the three series, the electron density of substituents on the cinnamic acid had the following order: 4-hydroxy-3-methoxy>4-methoxy>4-hydroxy,and the 4-hydroxy substituted compounds (**c** series) exhibited much stronger activities than **a** and **b** series. On the whole, the electrostatic field had more influence than steric field on the activities.

2.4. Kinetic analysis

To further explore the inhibitory characteristics of these compounds, compound **c2** were chosen as a representative to study the inhibition mode. The kinetic analysis was performed using Lineweaver-Burk (Fig. 4(A)) and Dixon plots (Fig. 4(B)). Compounds **c2** showed a mixed inhibitory type against α-glucosidase from *S. cerevisiae*. The inhibitory constant (*Ki*) value was 0.0124 mM. The result indicated that the inhibitory type was different from acarbose, which was reported to be a competitive inhibitor. [26]

Table 2 Statistical results of the CoMFA model (q^2 was leave-one-out cross-validated correlation coefficient; ONC, optimum number of principal component; r^2, non-cross-validated correlation coefficients; SEE, standard error of the estimate; F, the ratio between explained and unexplained variance.)

LOO		NV			Relative contributor	
q^2	ONC	r^2	SEE	F	Steric	Electrostatic
0.561	10	0.977	0.143	101.655	0.488	0.512

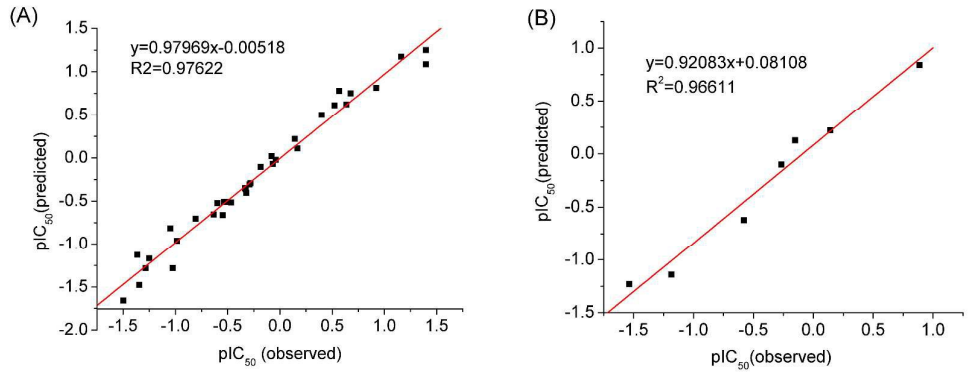

Fig. 2 Predicted versus observed pIC_{50} of the (A) training set and (B) test set

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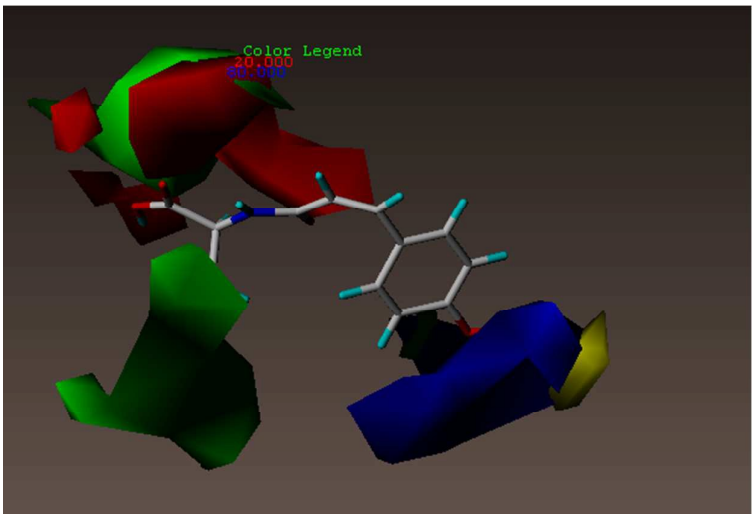

Fig. 3 CoMFA contour map for α-glucosidase. Steric areas: bulk groups increase (green) or decrease (yellow) potency. Electrostatic areas: high electron density (red) and low electron density (blue) regions are exp.ected to increase potency.

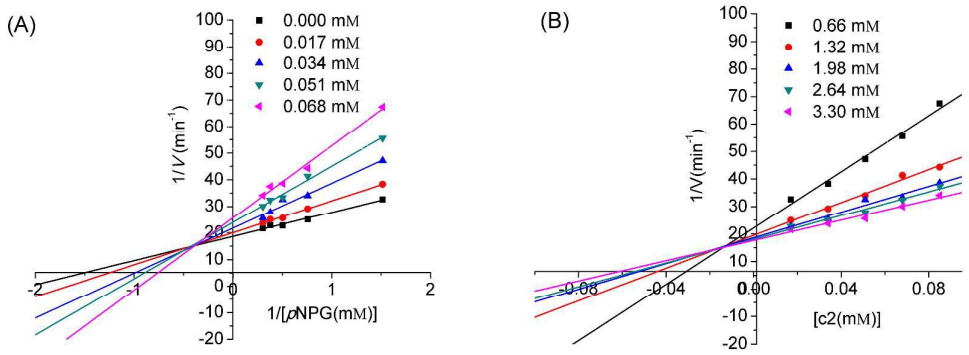

Fig. 4 Lineweaver–Burk (A) and Dixon (B) plots of α-glucosidase inhibition at different concentrations of *p*NPG and compound **c2**

2.5. Docking study

The Ramachandran plot (see supplement information Fig. S1) analysis by PROCHECK confirmed the quality of the modeling 3D structure with 87.6% of residues lied in the most favored regions, 11.6% in the additional allowed regions, 0.2% in the generously allowed regions respectively. Considering the inhibitory type was a mixed one, blind docking was firstly conducted using **c2** as ligand model, interestingly, the binding sites were coincident with the catalytic pocket of α-glucosidase from *Saccharomyces cerevisiae* (Fig. 5(A)).

As showed in Fig. 5 (A), amino acids terminal of the compound **c2** are oriented towards the core of the suit. And **c2** had a close interaction with the active sites Asp214 of the pocket. In the meantime, the hydroxyl group on the benzene ring of **c2** interacted with the residues of Asp349 and Arg 439. Furthermore, a π-π stacking between the benzene ring and Phe157 also contributed to the binding. In all, as shown in Fig. 5(B) **c2** had a very similar locationto the native ligand maltose (Fig. 5(B)). Thus, **c2** could well block the active center and exert its inhibitory actions. However, it was different in the case of compound **a2** (Fig. 5(C)). The introduction of 3-methoxyl to the benzene ring caused the reverse of the binding locations. Moreover the phenyl group entered the pocket in a sideways because of the narrow pore of the pocket. When the hydrophilic property of the amino acid terminal was increased by introduction of a carboxyl to compound **a14** (Fig. 5(D)), it could enter the pocket in a perfect way by binding to Asp214, Glu276, Asp349, Asp68, Arg212, Arg439 *via* hydrogen bond. Therefore, the binding types were not totally the same among these compounds, both the substituents on the phenyl ring and amino acid residues had great influence on the bonding position.

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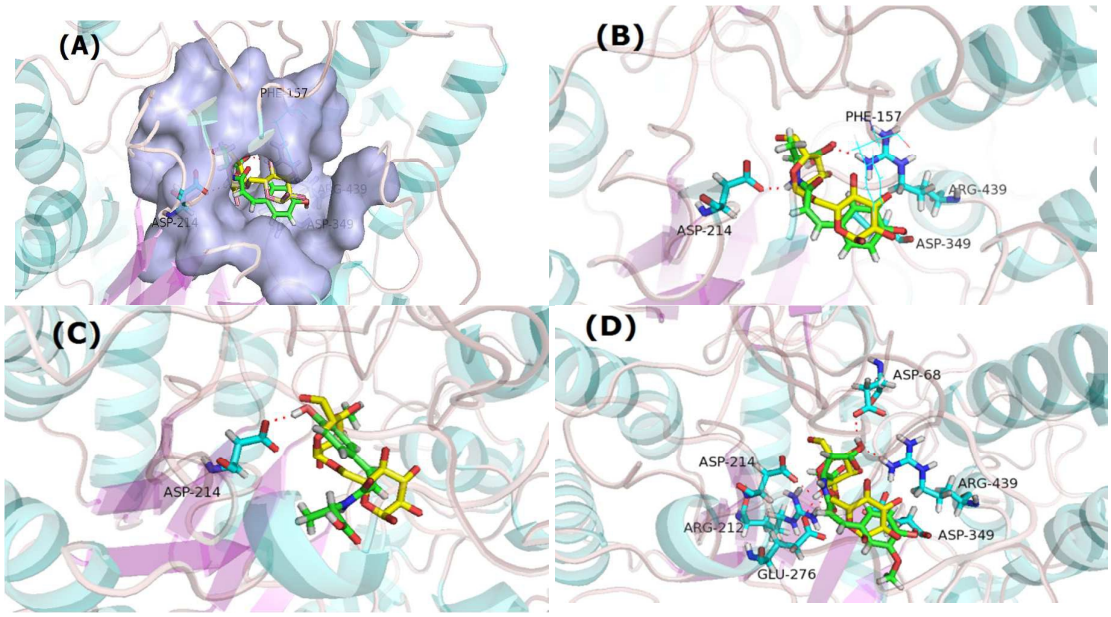

Fig. 5 Docking model predicted structural details of compound **c2** (A and B), **a2**(C), **a14** (D) Green: target inhibitors; Yellow: native ligand maltose; Blue: Bonding residues of inhibitors.

4 Experimental

4.1. General

α-Glucosidase (EC 3.2.1.20) from *Saccharomyces cerevisia*e was purchased from Sigma. The *p*-Nitrophenyl-α-Dglucopyranoside (*p*NPG), phenolic acids and amino acids were purchased from Aladdin Co. Ltd (Shanghai, China) and all the chemicals were of analytical reagent grade. Melting points (m.p) were measured on a Taike digital micro melting point apparatus X-4 (Beijing, China), ^{1}H NMR spectroscopy was obtained using Bruker AM spectrometers on600 MHz or 400Hz in DMSO. MS (ESI) spectra were recorded on Agilent 6100 Single Quad spectrometer.

4.2. Syntheses of *N*-phenylpropenoyl-L-amino acids

The synthetic method was based on the reported method with modifications[27]. The experiment procedure was as following using ferulic acid derivative as example : (*E*)-ferulic acid (10 mmol each) was added to aqueous solution (15 mL) of sodium hydroxide (64 mmol) below 15 ℃. After the solid dissolved completely, acetic anhydride (26 mmol) was added dropwise to the solution in an ice bath. After stirring for 30 min at room temperature, the reaction mixture was acidified to pH 1.0 with hydrochloric acid (2 mol/L) to precipitate. The suspension was filtered. The filtered cake was washed three times with water and dried in vacuum. The solid was used in the next step without further purification. Thionyl chloride (2 mmol) and a catalytic amount of DMF were added to the suspension of the above acetylated phenylpropenoic acid derivative in 20 mL chloroform. The mixture was heated for 7-8 h at 55 ℃. Remaining thionyl chloride and solvent were removed by evaporation to give a pale yellow oily liquid.

A solution of the above acyl chlorides (10 mmol) in 20 mL acetone and 10 mL hydroxide sodium (10 mmol) solution were add dropwise simultaneously to a solution of sodium salts of L-amino acid in 10 mL acetone and water (1:1) in ice bath. Followed by 2 h of stirring, two more equivalent of sodium hydroxide solid (20 mmol) was added to the reaction mixture. The mixture was stirred for an additional 3-4 h at room temperature. The acetone was then evaporated. The residual aqueous phase was adjust to pH 2 with aqueous hydrochloric acid and then extracted with ethyl acetate(20 mL×3). The combined organic phase was washed with water, dried over $MgSO_4$ and concentrated to afford the crude product, which was purified by column chromatography on silica gel (ethyl acetate/petroleum ether 1:2 (v/v)) to get the finnal product. The spectra data and physical properties of the compounds were showed as following:

N-[4-hydroxy-3-methoxy-(*E*)-cinnamoyl]-L-glycine acid **a1**, a light yellow solid (1.63 g, 65% yield); mp 224-226 °C; ^{1}H NMR (400 MHz, d_6-DMSO) δ 12.56 (s, 1H, -COOH), 9.44 (s, 1H, -OH), 8.23 (t, J=5.9Hz, 1H, -NH), 7.34 (d, *J* = 15.7 Hz, 1H, Ph-CH=CH), 7.15 (d, *J* = 1.8 Hz, 1H, Ph-H), 7.01 (dd, *J* = 8.2, 1.8 Hz, 1H, Ph-H), 6.79 (d, *J* = 8.1 Hz, 1H, Ph-H), 6.54 (d, *J* = 15.7 Hz, 1H, -CH=CH-), 3.87 (d, *J* = 5.9 Hz, 2H, -CH_2), 3.81 (s, 3H, -CH_3); MS *m/z* 252.2 $[M+H]^+$.

N-[4-hydroxy-3-methoxy-(*E*)-cinnamoyl]-L-alanine acid **a2**, a yellow amorphous solid (1.59 g, 60% yield); mp 95-97 °C; ^{1}H NMR (400 MHz, d_6-DMSO) δ 12.84 (s, 1H, -COOH), 9.45 (s, 1H, -OH), 8.23 (d, *J* = 7.4 Hz, 1H, -NH), 7.33 (d, *J* = 15.7 Hz, 1H, Ph-CH=CH-), 7.13 (d, *J* = 1.7 Hz, 1H, Ph-H), 7.00 (dd, *J* = 8.2, 1.7 Hz, 1H, Ph-H), 6.79 (d, *J* = 8.1 Hz, 1H, Ph-H), 6.52 (d, *J* = 15.7 Hz, 1H, Ph-CH=CH-), 4.32 (q, *J* = 7.8 Hz, 1H, -CH-), 3.80(s, 3H, -OCH_3), 1.31 (d, *J* = 7.3 Hz, 3H, -CH_3); MS *m/z* 266.2 $[M+H]^+$.

N-[4-hydroxy-3-methoxy-(*E*)-cinnamoyl]-L-leucine acid **a3**, a light yellow amorphous solid (1.68 g, 55% yield); mp 96-98 °C; ^{1}H NMR (400 MHz, d_6-DMSO) δ 12.56 (s, 1H, -COOH), 9.45 (s, 1H, -OH), 8.18 (d, *J* = 8.1 Hz, 1H, -NH), 7.33 (d, *J* = 15.7 Hz, 1H, Ph-CH=CH), 7.13 (d, *J* = 1.6 Hz, 1H, Ph-H), 6.99 (dd, *J* = 8.2, 1.7 Hz, 1H, Ph-H), 6.79 (d, *J* = 8.1 Hz, 1H, Ph-H), 6.53 (d, *J* = 15.7 Hz, 1H, -CH=CH), 4.34 (dt, *J* = 8.9, 5.6 Hz, 1H, -CH-), 3.81 (s, 3H, -OCH_3), 1.75 – 1.61 (m, 1H, -CH-), 1.61

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– 1.48 (m, 2H, -CH_2-), 0.91 (d, *J* = 6.5 Hz, 3H, -CH_3-), 0.86 (d, *J* = 6.5 Hz, 3H, -CH_3-). Ms m/z 308.4 $[M+H]^+$.

N-[4-hydroxy-3-methoxy-(*E*)-cinnamoyl]-L-isoleucine acid **a4**, a white amorphous solid (1.53 g, 50% yield); mp 94-97 °C; ^{1}H NMR (400 MHz, d_6-DMSO) δ 12.58 (s, 1H, -COOH), 9.45 (s, 1H, -OH), 8.05 (d, *J* = 8.5 Hz, 1H, -NH), 7.33 (d, *J* = 15.7 Hz, 1H, Ar-CH=CH), 7.13 (d, *J* = 1.6 Hz, 1H, Ar-H), 6.99 (dd, *J* = 8.2, 1.6 Hz, 1H, Ar-H), 6.81 (t, *J* = 12.4 Hz, 1H, Ar-H), 6.67 (d, *J* = 15.7 Hz, 1H -CH=CH), 4.32 (dd, *J* = 8.4, 5.9 Hz, 1H, -NH-CH-), 3.81 (s, 3H, -OCH_3), 1.82 (dd, *J* = 7.5, 5.6 Hz, 1H, -CH-), 1.44 – 1.16 (m, 2H, -CH_2), 0.92 – 0.85 (m, 6H, -(CH_3)$_2$); MS *m/z* 308.4 $[M+H]^+$.

N-[4-hydroxy-3-methoxy-(*E*)-cinnamoyl]-L-valine acid **a5**, a white amorphous solid (1.61 g, 55% yield); mp 90-92 °C; ^{1}H NMR (400 MHz, d_6-DMSO) δ 12.53 (s, 1H, -COOH), 9.45 (s, 1H, -OH), 8.03 (d, *J* = 8.6 Hz, 1H, -NH), 7.32 (d, *J* = 15.7 Hz, 1H, Ph-CH=CH-), 7.13 (d, *J* = 1.7 Hz, 1H, Ph-H), 6.99 (dd, *J* = 8.2, 1.7 Hz, 1H, Ph-H), 6.79 (d, *J* = 8.1 Hz, 1H, Ph-H), 6.68 (d, *J* = 15.7 Hz, 1H, Ph-CH=CH-), 4.28 (dd, *J* = 8.5, 5.6 Hz, 1H, -NH-CH-), 3.81 (s, 3H, -OCH_3), 2.14 – 2.06 (m, 1H, -CH-), 0.97-0.85 (m, 6H, -CH(CH_3)$_2$); MS *m/z* 294.3 $[M+H]^+$.

N-[4-hydroxy-3-methoxy-(*E*)-cinnamoyl]-L-proline acid **a6**, a white solid (1.90 g, 65% yield); mp 171-173 °C; ^{1}H NMR (400 MHz, d_6-DMSO) δ 12.39 (s, 1H, -COOH), 9.46 (s, 1H, -OH), 7.38 (d, *J* = 15.4 Hz, 1H, Ph-CH=CH-), 7.29 (d, *J* = 1.6 Hz, 1H, Ph-H), 7.10 (d, *J* = 8.2 1H, Ph-H), 6.81 (d, *J* = 15.8 Hz, 1H, Ph-CH=CH-), 6.78 (d, *J* = 8.3 Hz, 1H, Ph-H), 4.33 (dd, *J* = 8.7, 3.9 Hz, 1H, -N-CH-), 3.82 (s, 3H, -OCH_3), 3.77 – 3.61 (m, 1H, -CH_2-), 2.22 – 1.81 (m, 3H, -CH_2-, -CH_2-); MS *m/z* 292.4 $[M+H]^+$.

N-[4-hydroxy-3-methoxy-(*E*)-cinnamoyl]-L-phenylalanine acid **a7**, a white amorphous solid (2.11 g, 62% yield); mp 82-85 °C; ^{1}H NMR (400 MHz, d_6-DMSO) δ 12.67 (s, 1H, -COOH), 9.45 (s, 2H, -OH), 8.23 (d, *J* = 8.1 Hz, 2H, -NH), 7.24 (m, 6H, Ph-H, Ph-CH=CH-), 7.11 (d, *J* = 1.7 Hz, 1H, Ph-H), 6.98 (dd, *J* = 8.2, 1.7 Hz, 1H, Ph-H), 6.78 (d, *J* = 8.1 Hz, 1H, Ph-H), 6.52 (d, *J* = 15.7 Hz, 1H, Ph-CH=CH-), 4.56 (td, *J* = 9.1, 4.8 Hz, 1H), 3.80 (s, 3H, -OCH_3), 3.11 (dd, *J* = 13.9, 4.8 Hz, 1H, -CH_2-), 2.92 (dd, *J* = 13.9, 9.5 Hz, 1H, -CH_2-); MS *m/z* 342.3 $[M+H]^+$.

N-[4-hydroxy-3-methoxy-(*E*)-cinnamoyl]-L-methionine acid **a8**, a yellow amorphous solid (1.85 g, 57% yield); mp 92-94 °C; ^{1}H NMR (400 MHz, d_6-DMSO) δ 9.47 (s, 1H, -OH), 8.27 (d, *J* = 7.9 Hz, 1H, -NH), 7.37 (d, *J* = 15.7 Hz, 1H, Ph-CH=CH-), 7.15 (d, *J* = 1.6 Hz, 1H, Ph-H), 7.02 (dd, *J* = 8.2, 1.6 Hz, 1H, Ph-H), 6.81 (d, *J* = 8.1 Hz, 1H, Ph-H), 6.55 (d, *J* = 15.7 Hz, 1H, Ph-CH=CH-), 4.46 (td, *J* = 8.7, 4.6 Hz, 1H, -CH-), 3.82 (s, 3H, -OCH_3), 2.66 – 2.31 (m, 2H, -CH_2-CH_2-), 2.10 – 1.98 (m, 5H, -CH_2-CH_2-S-CH_3); MS *m/z* 326.3 $[M+H]^+$.

N-[4-hydroxy-3-methoxy-(*E*)-cinnamoyl]-L-tryptophane acid **a9**, a white solid(1.93 g, 51% yield); mp 85-87 °C;^{1}H NMR (400 MHz, d_6-DMSO) δ 10.83(s, 1H, -OH), 9.44 (s, 1H, -NH), 8.18 (d, *J* = 7.9 Hz, 1H, -NH), 7.54 (d, *J* = 7.8 Hz, 1H, Ar-H), 7.32 (dd, *J* = 15.6, 11.9 Hz, 2H, Ph-CH=CH-, Ar-H), 7.16 (d, *J* = 2.1 Hz, 1H, Ph--H), 7.12 (d, J = 1.6 Hz, 1H, Ar-H), 7.07 (t, *J* = 7.2 Hz, 1H, Ar-H), 7.02 – 6.94 (m, 2H, Ar-H, Ph-H), 6.79 (d, *J* = 8.1 Hz, 1H, Ph-H), 6.56 (d, *J* = 15.7 Hz, 1H, Ph-CH=CH-), 4.63 (td, *J* = 8.3, 5.0 Hz, 1H, -CH-), 3.80 (s, 3H, -OCH_3), 3.23 (dd, *J* = 14.7, 4.9 Hz, 1H, -CH_2-), 3.07 (dd, *J* = 14.7, 8.7 Hz, 1H, -CH_2-); MS *m/z* 381.3 $[M+1]^+$.

N-[4-hydroxy-3-methoxy-(*E*)-cinnamoyl]-L-serine acid **a10**, a light yellow solid (1.32 g, 47% yield); mp 184-187 °C; ^{1}H NMR (400 MHz, d_6-DMSO) δ 12.51 (s, 1H, -COOH) 9.44 (s, 1H, Ph-OH), 8.04 (d, *J* = 8.0 Hz, 1H, -NH), 7.32 (d, *J* = 15.7 Hz, 1H, Ph-CH=CH-), 7.15 (d, *J* = 1.7 Hz, 1H, Ph-H), 7.00 (dd, *J* = 8.2, 1.7 Hz, 1H, Ph-H), 6.79 (d, *J* = 8.1 Hz, 1H, Ph-H), 6.69 (d, *J* = 15.7 Hz, 1H, Ph-CH=CH-), 5.06 (s, 1H, -OH), 4.42 – 4.39 (m, 1H, -CH-), 3.75 (dd, *J* = 10.8, 5.0 Hz, 1H, -CH_2), 3.66 (dd, *J* = 10.8, 4.1 Hz, 1H, -CH_2). MS *m/z* 282.3 $[M+H]^+$.

N-[4-hydroxy-3-methoxy-(*E*)-cinnamoyl]-L-glutamine acid **a11**, a white amorphous solid (1.61 g, 50% yield); mp 96-99 °C; ^{1}H NMR (400 MHz, d_6-DMSO) δ 12.40 (s, 1H, -COOH), 9.46 (s, 1H, Ph-OH), 8.21 (d, *J* = 7.9 Hz, 1H, -NH), 7.33 (d, *J* = 15.7 Hz, 1H, Ph-CH=CH-), 7.13 (d, *J* = 1.7 Hz, 1H, Ph-H), 7.00 (dd, *J* = 8.2, 1.7 Hz, 1H, Ph-H), 6.80 (d, *J* = 8.1 Hz, 1H, Ph-H), 6.53 (d, *J* = 15.7 Hz, 1H, Ph-CH=CH-), 4.33 (td, *J* = 8.7, 5.1 Hz, 1H，-CH-), 3.81 (s, 3H, -OCH_3-), 2.41 – 2.20 (m, 2H, -CH_2-), 2.09 – 1.93 (m, 1H, -CH_2-), 1.94 – 1.75 (m, 1H, -CH_2-); MS *m/z* 324.3 $[M+H]^+$.

N-[4-hydroxy-3-methoxy-(*E*)-cinnamoyl]-L-threonine acid **a12**, a white solid (1.36 g, 46% yield); mp 192-194 °C; ^{1}H NMR (400 MHz, d_6-DMSO) δ 12.92 (s, 1H, -COOH), 9.44 (s, 1H, Ph-OH), 7.77 (d, *J* = 8.7 Hz, 1H, -NH), 7.31 (d, *J* = 15.7 Hz, 1H, Ph-CH=CH-), 7.17 (d, *J* = 1.6 Hz, 1H, Ph-H), 7.00 (dd, *J* = 8.2, 1.6 Hz, 1H, Ph-H), 6.81 (d, J=8.1 Hz, 1H, Ph-H), 6.80 (d, 1H, Ph-CH=CH-), 4.33 (dd, *J* = 8.7, 3.1 Hz, 1H, -CH-), 4.16 (dd, *J* = 6.3, 3.2 Hz, 1H, -CH-), 3.81 (s, 4H, -OCH_3), 1.07 (d, *J* = 6.3 Hz, 3H, -CH_3); MS *m/z* 296.3 $[M+H]^+$.

N-[4-hydroxy-3-methoxy-(*E*)-cinnamoyl]-L-glutamic acid **a13**, a light yellow amorphous solid (1.97 g, 61%); mp 108-110 °C; ^{1}H NMR (600 MHz, d_6-DMSO) δ 12.41 (s, 2H, -COOH), 9.46 (s, 1H), 8.22 (d, *J* = 7.9 Hz, 1H, -NH), 7.33 (d, *J* = 15.7 Hz, 1H, Ph-CH=CH-), 7.14 (d, *J* = 1.8 Hz, 1H, Ph-H), 7.00 (dd, *J* = 8.2, 1.8 Hz, 1H, Ph-H), 6.79 (d, *J* = 8.1 Hz, 1H, Ph-H), 6.53 (d, *J* = 15.7 Hz, 1H, Ph-CH=CH-), 4.37 – 4.28 (m, 1H, -CH-), 3.81 (s, 3H, -OCH_3), 2.38 – 2.22 (m, 2H, -CH_2-), 2.06 – 1.97 (m, 1H, -CH_2-), 1.85 – 1.178(m, 1H, -CH_2-).; MS *m/z* 324.2 $[M+H]^+$.

N-[4-hydroxy-3-methoxy-(*E*)-cinnamoyl]-L-aspartic acid **a14**, a yel-low solid (1.76 g, 57% yield); mp 187-189 °C; ^{1}H NMR (400 MHz, d_6-DMSO) δ 12.49 (s, 2H, -COOH), 9.45 (s, 1H, -OH), 8.22 (d, *J* = 8.0 Hz, 1H, -NH), 7.33 (d, *J* = 15.7 Hz, 1H, Ph-CH=CH-), 7.14 (d, *J* = 1.6 Hz, 1H, Ph-H), 6.99 (dd, *J* = 8.2, 1.6 Hz, 1H, Ph-H), 6.79 (d, *J* = 8.1 Hz, 1H,Ph-H), 6.58 (d, *J* = 15.7 Hz, 1H, Ph-CH=CH-), 4.66 (dd, *J* = 13.3, 7.2 Hz, 1H, -CH-), 3.81 (s, 3H, -OCH_3), 2.70 (qd, *J* = 16.6, 6.3 Hz, 2H, -CH_2-); MS *m/z* 310.3 $[M+H]^+$.

N-[4-methoxy-(*E*)-cinnamoyl]-L-glycine acid **b1**, a white solid (1.90 g, 81% yield); mp 165-167 °C; ^{1}H NMR (600 MHz, d_6-DMSO) δ 12.58 (s, 1H, -COOH), 8.32 (t, *J* = 5.9 Hz, 1H, -NH), 7.53 (d, *J* = 8.6 Hz, 2H, Ph-H), 7.40 (d, *J* = 15.8 Hz, 1H, Ph-CH=CH-), 6.98 (d, *J* = 8.6 Hz, 2H, Ph-H), 6.57 (d, *J* =15.8 Hz, 1H, Ph-CH=CH-), 3.88 (d, *J* = 5.9 Hz, 2H, -CH_2-), 3.78 (s, 3H, -OCH_3); MS *m/z* 236.3 $[M+H]^+$.

N-[4-methoxy-(*E*)-cinnamoyl]-L-alanine acid **b2**, a white solid (1.99 g, 80% yield); mp 162-164 °C; ^{1}H NMR (600 MHz, d_6-DMSO) δ 12.57 (s, 1H, -COOH), 8.32 (d, *J* = 7.3 Hz, 1H, -NH), 7.51 (d, *J* = 8.7 Hz, 2H, Ph-H), 7.38 (d, *J* = 15.8 Hz, 1H, Ph-CH=CH-), 6.98 (d, *J* = 8.7 Hz, 2H, Ph-H), 6.56 (d, *J* = 15.8 Hz, 1H, Ph-CH=CH-), 4.32 (t, *J* = 7.3 Hz, 1H, -CH-), 3.78 (s, 3H, -OCH_3), 1.32 (d, *J* = 7.3 Hz, 3H, -CH_3); MS *m/z* 250.1 $[M+H]^+$.

N-[4-methoxy-(*E*)-cinnamoyl]-L-leucine acid **b3**, a white amorphous solid (2.15 g, 74% yield); mp 85-87 °C; ^{1}H NMR (600 MHz, d_6-DMSO) δ 12.57 (s, 1H, -COOH), 8.25 (d, *J* = 8.1 Hz, 1H, -NH), 7.51 (d, *J* = 8.8 Hz, 2H, Ph-H), 7.38 (d, *J* = 15.8 Hz, 1H, Ph-CH=CH-), 7.03 – 6.93 (m, 2H, Ph-H), 6.57 (d, *J* = 15.8 Hz, 1H, Ph-CH=CH-), 4.36 (ddd, *J* = 9.8, 8.1, 5.2 Hz, 1H, NH-CH-), 3.79 (s, 1H, -OCH_3), 1.71 – 1.62 (m, 1H, -CH-), 1.62 – 1.50 (m, 2H, -CH_2-), 0.91 (d, *J* = 6.6 Hz, 3H, -CH_3), 0.87 (d, *J* = 6.5 Hz, 3H, -CH_3); MS *m/z* 292.3 $[M+H]^+$.

N-[4-methoxy-(*E*)-cinnamoyl]-L-isoleucine acid **b**4, a white amorphous solid (2.03g, 70% yield); mp 83-85 °C; ^{1}H NMR (600 MHz, d_6-DMSO) δ 12.61 (s, 1H, -COOH), 8.12 (d, *J* = 8.3 Hz, 1H, -NH), 7.51 (d, *J* = 7.9 Hz, 2H, Ph-H), 7.37 (d, *J* = 15.7 Hz, 1H, Ph-CH=CH-), 6.98 (d, *J* = 8.0 Hz, 2H, Ph-H), 6.70 (d, *J* = 15.7 Hz, 1H, Ph-CH=CH-), 4.32 (t, *J* = 6.9 Hz, 1H, -CH-), 3.79 (s, 3H, -OCH_3), 1.83 (m, 1H, -CH-), 1.45 – 1.42 (m, 1H, -CH_2-), 1.26 – 1.98(m, 1H, -CH_2-), 0.89 – 0.85 (m, 6H, -CH_3); MS *m/z* 292.3 $[M+H]^+$.

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N-[4-methoxy-(*E*)-cinnamoyl]-L-valine acid **b5**, a white amorphous solid (1.52 g, 55% yield); mp 70-73 °C; ^{1}H NMR (600 MHz, d_6-DMSO) δ 12.62 (s, 1H, -COOH), 8.11 (d, *J* = 8.6 Hz, 1H, -NH), 7.51 (d, *J* = 8.3 Hz, 2H, Ph-H), 7.38 (d, *J* = 15.7 Hz, 1H, Ph-CH=CH-), 6.99 (d, *J* = 8.3 Hz, 2H, Ph-H), 6.72 (d, *J* = 15.8 Hz, 1H, Ph-CH=CH-), 4.29 (dd, *J* = 8.3, 5.9 Hz, 1H, -CH-), 3.79 (s, 3H, -OCH_3), 2.10 (dd, *J* = 13.2, 6.6 Hz, 1H, -CH(CH_3)$_2$), 0.92 (dd, *J* = 6.5, 4.0 Hz, 6H, -CH(CH_3)$_2$); MS *m/z* 278.2 $[M+H]^+$.

N-[4-methoxy-(*E*)-cinnamoyl]-L-proline acid **b6,** a white solid (1.81 g, 66% yield); mp 197-199 °C; ^{1}H NMR (600 MHz, d_6-DMSO) δ 12.51 (s, 1H, -COOH), 7.66 (d, *J* = 7.9 Hz, 2H, Ph-H), 7.43 (d, *J* = 15.4 HZ, 1H, Ph-CH=CH-), 6.97 (d, *J* = 8.0 Hz, 2H, Ph-H), 6.87 (d, *J* = 15.5 Hz, 1H, Ph-CH=CH-), 4.34 (dd, *J* = 8.6, 3.7 Hz, 1H, -CH-), 3.79 (s, 3H, -OCH_3), 3.77 – 3.67 (m, 2H, -NH-CH_2-), 2.32 – 2.06 (m, 1H, NH-CH_2-CH_2-), 2.01 – 1.79 (m, 3H, NH-CH_2-CH_2-); MS *m/z* 276.2 $[M+H]^+$.

N-[4-methoxy-(*E*)-cinnamoyl]-L-phenylalanine acid **b7**, a white solid (2.43g, 75% yield); mp 161-165 °C; ^{1}H NMR (600 MHz, d_6-DMSO) δ 12.76 (s, 1H, -COOH), 8.31 (d, *J* = 8.1 Hz, 1H, -NH), 7.49 (d, *J* = 8.4 Hz, 2H, Ph-H), 7.33 (d, *J* = 15.8 Hz, 1H, Ph-CH=CH-), 7.30 – 7.24 (m, 4H, Ph-H), 7.20 (dd, *J* = 10.8, 4.1 Hz, 1H , Ph-H), 6.97 (d, *J* = 8.4 Hz, 2H, Ph-H), 6.55 (d, *J* = 15.8 Hz, 1H, Ph-CH=CH-), 4.57 (td, *J* = 8.7, 5.0 Hz, 1H,-CH-), 3.78 (s, 3H,-OCH_3), 3.12 (dd, *J* = 13.9, 4.7 Hz, 1H, -CH_2), 2.99 – 2.85 (m, 1H, -CH_2-); MS *m/z* 326.3 $[M+H]^+$.

N-[4-methoxy-(*E*)-cinnamoyl]-L-methionine acid **b8**, a white solid (1.91 g, 62% yield); mp 149-152 °C; ^{1}H NMR (600 MHz, d_6-DMSO) δ 12.71 (s, 1H, -COOH), 8.33 (d, *J* = 7.9 Hz, 1H, -NH), 7.52 (d, *J* = 8.7 Hz, 2H, Ph-H), 7.39 (d, *J* = 15.8 Hz, 1H, Ph-CH=CH-), 6.99 (d, *J* = 8.7 Hz, 2H, Ph-H), 6.57 (d, *J* = 15.8 Hz, 1H, Ph-CH=CH-), 4.50 – 4.38 (m, 1H, -CH-), 3.79 (s, 3H, -OCH_3), 2.51 – 2.49 (m, 2H, -CH_2-S-), 2.05 (s, 3H, -CH_3), 2.03 – 1.90 (m, 2H, -CH_2-); MS *m/z* 310.2 $[M+H]^+$.

N-[4-methoxy-(*E*)-cinnamoyl]-L-tryptophane acid **b9**, a light yellow amorphous solid (2.00 g, 55%); mp 70-72°C; ^{1}H NMR (600 MHz, d_6-DMSO) δ 12.71 (s, 1H, -COOH), 10.85 (d, *J* = 1.4 Hz, 1H, -NH -), 8.29 (d, *J* = 7.9 Hz, 1H, -NH-COOH), 7.55 (d, *J* = 7.9 Hz, 1H, Ar-H), 7.49 (d, *J* = 8.8 Hz, 2H, Ph-H), 7.34 (d, *J* = 15.7, 1H, Ph-CH=CH-), 7.32 (d, *J*=8.0 Hz, 1H, Ar-H)7.16 (d, *J* = 2.2 Hz, 1H, Ar-H), 7.10 – 7.02 (m, 1H, Ar-H), 7.01 – 6.93 (m, 3H, Ar-H, 2Ph-H), 6.59 (d, *J* = 15.8 Hz, 1H , Ph-CH=CH-), 4.62 (td, *J* = 8.3, 5.0 Hz, 1H, -CH-), 3.78 (s, 3H, -OCH_3), 3.23 (dd, *J* = 14.7, 4.9 Hz, 1H, -CH_2-), 3.07 (dd, *J* = 14.7, 8.8 Hz, 1H, -CH_2-); MS *m/z* 365.2 $[M+H]^+$.

N-[4-methoxy-(*E*)-cinnamoyl]-L-serine acid **b10**, a white solid (1.59 g, 60% yield); mp 183-184 °C; ^{1}H NMR (600 MHz, d_6-DMSO) δ 12.66 (s, 1H, -COOH), 8.17 (d, *J* = 8.0 Hz, 1H, -NH -), 7.52 (d, *J* = 8.7 Hz, 2H, Ph-H), 7.38 (d, *J* = 15.8 Hz, 1H , Ph-CH=CH-), 6.98 (d, *J* = 8.8 Hz, 2H, Ph-H), 6.73 (d, *J* = 15.8 Hz, 1H, Ph-CH=CH-), 4.50 – 4.36 (m, 1H, -CH-), 3.79 (s, 3H ,-OCH_3), 3.76 (dd, *J* = 10.9, 5.0 Hz, 1H, -CH_2-), 3.67 (dd, *J* = 10.9, 4.0 Hz, 1H, -CH_2-), 2.55 – 2.43 (m, 1H, -OH); MS *m/z* 266.3 $[M+H]^+$.

N-[4-methoxy-(*E*)-cinnamoyl]-L-glutamine acid **b11**, a white solid (1.74 g, 57% yield); mp 165-168 °C; ^{1}H NMR (600 MHz, d_6-DMSO) δ 12.79 (s, 1H,-COOH), 8.28 (d, *J* = 6.6 Hz, 1H, -NH-), 7.51 (d, *J* = 8.6 Hz, 2H, Ph-H), 7.37 (d, *J* = 15.9 Hz, 1H, Ph-CH=CH), 7.35(s, 1H, -NH_2), 6.98 (d, *J* = 8.6 Hz, 2H, Ph-H), 6.79 (s, 1H , -NH_2), 6.65 (d, *J* = 15.8 Hz, 1H, Ph-CH=CH-), 4.27 (m, 1H, -CH-), 3.79 (s, 3H , -OCH_3), 2.23 – 2.11 (m, 2H, -CH_2-), 2.01 (m, 1H, -CH_2-), 1.81 (m, 1H, -CH_2-); MS *m/z* 307.3 $[M+H]^+$.

N-[4-methoxy-(*E*)-cinnamoyl]-L-threonine acid **b12**, a white solid (1.08 g, 43% yield); mp 153-156 °C; ^{1}H NMR (600 MHz, d_6-DMSO) δ 12.57 (s, 1H,-COOH), 7.93 (d, *J* = 8.8 Hz, 1H,-NH -), 7.52 (d, *J* = 8.7 Hz, 2H, Ph-H), 7.37 (d, *J* = 15.7 Hz, 1H, Ph-CH=CH-), 6.98 (d, *J* = 8.7 Hz, 2H, Ph-H), 6.83(d, *J* = 15.8 Hz, 1H, Ph-CH=CH-), 4.99 (s, 1H,-OH), 4.35 (dd, *J* = 8.8, 3.1 Hz, 1H, -NH-CH), 4.23 – 4.12 (m, 1H, -CH), 3.79 (s, 3H, -OCH_3), 1.08 (d, *J* = 6.4 Hz, 3H, -CH_3); MS *m/z* 254.2 $[M+H]^+$.

N-[4-methoxy-(*E*)-cinnamoyl]-L- glutamic acid **b13**, a white amorphous solid (1.68 g, 55% yield); mp 85-87 °C; ^{1}H NMR (600 MHz, DMSO) δ 12.44 (s, 1H), 8.30 (d, *J* = 7.9 Hz, 1H), 8.30 (d, *J* = 7.9 Hz, 1H), 7.58 – 7.47 (m, 1H), 7.39 (d, *J* = 15.8 Hz, 1H), 6.99 (d, *J* = 8.8 Hz, 1H), 6.58 (d, *J* = 15.8 Hz, 1H), 4.35 (td, *J* = 8.9, 5.1 Hz, 1H), 3.79 (s, 2H), 2.42 – 2.22 (m, 1H), 2.11 – 1.99 (m, 1H), 1.83 (dtd, *J* = 14.7, 8.9, 6.0 Hz, 1H); MS *m/z* 308.3 $[M+H]^+$.

N-[4-methoxy-(*E*)-cinnamoyl]-L-aspartic acid **b14**, a white solid (1.81 g, 62% yield); mp 160-163 °C; ^{1}H NMR (600 MHz, d_6-DMSO) δ 12.60 (s, 2H,-COOH), 8.34 (d, *J* = 8.0 Hz, 1H,-NH-), 7.53 (d, *J* = 8.7 Hz, 2H, Ph-H), 7.39 (d, *J* = 15.8 Hz, 1H, Ph-CH=CH-), 6.98 (d, *J* = 8.8 Hz, 2H, Ph-H), 6.60 (d, *J* = 15.8 Hz, 1H, Ph-CH=CH-), 4.66 (m, 1H , -NH-CH), 3.79 (s, 3H, -OCH_3), 2.75 (dd, *J* = 16.6, 5.4 Hz, 1H, -CH_2), 2.66 (dd, *J* = 16.6, 7.2 Hz, 1H,-CH_2); MS *m/z* 294.3 $[M+H]^+$.

N-[4-hydroxy-(*E*)-cinnamoyl]-L-glycine acid **c1**, a white solid (1.65 g, 75% yield); mp 232-233 °C; ^{1}H NMR (600 MHz, d_6-DMSO) δ 12.49 (s, 1H, -COOH), 10.00 (s, 1H, -OH), 8.26 (t, *J* = 5.9 Hz, 1H , -NH), 7.41 (d, *J* = 8.6 Hz, 2H, Ph-H), 7.34 (d, *J* = 15.7 Hz, 1H, Ph-CH=CH-), 6.79 (d, *J* = 8.6 Hz, 2H, Ph-H), 6.50 (d, *J* = 15.8 Hz, 1H, Ph-CH=CH-), 3.86 (d, *J* = 5.9 Hz, 2H, -CH_2-); MS *m/z* 222.3 $[M+H]^+$.

N-[4-hydroxy-(*E*)-cinnamoyl]-L-alanine acid **c2**, a white solid (1.59, 68% yield); mp 205-206 °C; ^{1}H NMR (600 MHz, d_6-DMSO) δ 12.55 (s, 1H, -COOH), 9.85 (s, 1H, -OH), 8.27 (d, *J* = 7.4 Hz, 1H, -NH), 7.39 (d, *J* = 8.6 Hz, 2H, Ph-H), 7.33 (d, *J* = 15.7 Hz, 1H, Ph-CH=CH-), 6.79 (d, *J* = 8.6 Hz, 2H, Ph-H), 6.48 (d, *J* = 15.8 Hz, 1H, Ph-CH=CH-), 4.31 (m, 1H,-CH-), 1.31 (d, *J* = 7.3 Hz, 3H, -CH_3); MS *m/z* 236.1 $[M+H]^+$.

N-[4-hydroxy-(*E*)-cinnamoyl]-L-leucine acid **c3**, a white solid (1.77 g, 64% yield); mp 182-184 °C; ^{1}H NMR (600 MHz, d_6-DMSO) δ 12.54 (s, 1H, -COOH), 9.85 (s, 1H, -OH), 8.21 (d, *J* = 8.1 Hz, 1H, -NH), 7.39 (d, *J* = 8.6 Hz, 2H, Ph-H), 7.33 (d, *J* = 15.7 Hz, 1H, Ph-CH=CH-), 6.80 (d, *J* = 8.6 Hz, 2H, Ph-H), 6.49 (d, *J* = 15.8 Hz, 1H, Ph-CH=CH-), 4.34 (ddd, *J* = 9.8, 8.1, 5.2 Hz, 1H, -NH-CH-), 1.73 – 1.59 (m, 1H, -CH-), 1.62 – 1.44 (m, 2H, -CH_2), 0.91 (d, *J* = 6.6 Hz, 3H, -CH_3), 0.86 (d, *J* = 6.5 Hz, 3H, -CH_3); MS *m/z* 278.3 $[M+H]^+$.

N-[4-hydroxy-(E)-cinnamoyl]-L-isoleucine acid c4, a white amorphous solid (1.66 g, 60% yield); mp 108-110 °C; ^{1}H NMR (600 MHz, d6-DMSO) δ 12.61 (s, 1H, -COOH), 9.85 (s, 1H, -OH), 8.10 (d, *J* = 8.5 Hz, 1H, -NH), 7.39 (d, *J* = 8.6 Hz, 2H, Ph-H), 7.33 (d, *J* = 15.7 Hz, 1H, Ph-CH=CH-), 6.80 (d, *J* = 8.6 Hz, 2H, Ph-H), 6.63 (d, *J* = 15.7 Hz, 1H, Ph-CH=CH-), 4.31 (dd, *J* = 8.5, 6.0 Hz, 1H, NH-CH-), 1.90 – 1.75 (m, 1H, -CH-), 1.46 – 1.40 (m, 1H, -CH2-), 1.25 – 1.15 (m, 1H, -CH2-), 0.89 (d, *J* = 6.84, 3H, -CH3) 0.86 (t, *J* = 7.38, 3H, -CH3); MS m/z 278.2 [M+H]+.

N-[4-hydroxy-(*E*)-cinnamoyl]-L-valine acid **c5**, a white amorphous solid (1.23 g, 47% yield); mp 108-107 °C; ^{1}H NMR (600 MHz, d_6-DMSO) δ 12.61 (s, 1H, -COOH), 9.85 (s, 1H, -OH), 8.08 (d, *J* = 8.6 Hz, 1H, -NH), 7.40 (d, *J* = 8.6 Hz, 2H, Ph-H), 7.33 (d, *J* = 15.7 Hz, 1H, Ph-CH=CH-), 6.80 (d, *J* = 8.6 Hz, 2H, Ph-H), 6.65 (d, *J* = 15.7 Hz, 1H, Ph-CH=CH-), 4.28 (dd, *J* = 8.6, 5.7 Hz, 1H, -NH-CH-), 2.10 (dd, *J* = 12.8, 6.7 Hz, 1H, -CH-), 0.91 (d, *J* = 4.0 Hz, 3H,-CH_3), 0.90 (d, *J* = 3.9 Hz, 3H,-CH_3); MS *m/z* 264.2 $[M+H]^+$.

N-[4-hydroxy-(*E*)-cinnamoyl]-L-proline acid, a white solid (1.59 g, 61% yield); mp 235-236 °C; ^{1}H NMR (600 MHz, d_6-DMSO) δ 12.61 (s, 1H, -COOH), 9.85 (s, 1H, , -OH), 8.08 (d, *J* = 8.6 Hz, 1H, -NH), 7.39 (d, *J* = 8.6 Hz, 2H, Ph-H), 7.33 (d, *J* = 15.7 Hz, 1H, Ph-CH=CH-), 6.80 (d, *J* = 8.6 Hz, 2H, Ph-H), 6.64 (d, *J* = 15.7 Hz, 1H, Ph-CH=CH-), 4.28 (dd, *J* = 8.6, 5.7 Hz, 1H, -N-CH-), 2.09 (dd, *J* = 12.8, 6.7 Hz, 1H, -CH_2-), 0.91 (dd, *J* = 6.8, 4.0 Hz, 5H, -CH_2-); MS *m/z* 262.2 $[M+H]^+$

N-[4-hydroxy-(*E*)-cinnamoyl]-L-phenylalanine acid **c7**, a white solid (2.14, 69% yield); mp 235-236 °C; ^{1}H NMR (600 MHz, d_6-

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DMSO) δ 12.77 (s, 1H, -COOH), 9.87 (s, 1H, -OH), 8.30 (d, *J* = 8.1 Hz, 1H, -NH), 7.38 (d, *J* = 8.6 Hz, 2H, Ph-H), 7.32 – 7.23 (m, 5H, Ph-CH=CH-, Ph-H), 7.21 – 7.18 (m, 1H, Ph-H), 6.80 (d, *J* = 8.6 Hz, 2H, Ph-H), 6.49 (d, *J* = 15.8 Hz, 1H, Ph-CH=CH-), 4.57 (ddd, *J* = 9.5, 8.3, 4.8 Hz, 1H, -CH-), 3.11 (dd, *J* = 13.9, 4.8 Hz, 1H, -CH_2-), 2.92 (dd, *J* = 13.9, 9.6 Hz, 1H, -CH_2-); MS *m/z* 312.3 $[M+H]^+$.

N-[4-hydroxy-(*E*)-cinnamoyl]-L-methionine acid **c8**, a white solid (1.68 g, 57% yield); mp 169-172 °C; ^{1}H NMR (600 MHz, d_6-DMSO) δ 12.66 (s, 1H, -COOH), 9.86 (s, 1H, -OH), 8.28 (d, *J* = 7.9 Hz, 1H, -NH), 7.40 (d, *J* = 8.6 Hz, 2H, Ph-H), 7.34 (d, *J* = 15.7 Hz, 1H, Ph-CH=CH-), 6.80 (d, *J* = 8.6 Hz, 2H, Ph-H), 6.49 (d, *J* = 15.8 Hz, 1H, Ph-CH=CH-), 4.45 – 4.41 (m, 1H, -CH-), 2.54 – 2.50 (m, 2H, -CH_2-), 2.05 (s, 3H, -CH_3), 2.02 – 1.88 (m, 2H, -CH_2-); MS *m/z* 296.2 $[M+H]^+$.

N-[4-hydroxy-(*E*)-cinnamoyl]-L-tryptophane acid **c9**, a white solid (1.75 g, 50% yield); mp 171-173 °C;^{1}H NMR (600 MHz, d_6-DMSO) δ 12.68 (s, 1H, -COOH), 10.85 (d, *J* = 1.4 Hz, 1H, -OH), 9.85 (s, 1H, -NH), 8.25 (d, *J* = 7.9 Hz, 1H, -CO-NH-), 7.56 (d, *J* = 7.9 Hz, 1H, Ar-H), 7.38 (d, *J* = 8.6 Hz, 2H, Ph-H), 7.33 (d, *J* = 8.1 Hz, 1H, Ar-H), 7.30 (d, *J* = 15.7 Hz, 1H, Ph-CH=CH-), 7.16 (d, *J* = 2.2 Hz, 1H, Ar-H), 7.11 – 7.03 (m, 1H, Ar-H), 7.02 – 6.93 (m, 1H, Ar-H), 6.79 (d, *J* = 8.6 Hz, 2H, Ph-H), 6.52 (d, *J* = 15.7 Hz, 1H, Ph-CH=CH-), 4.62 (td, *J* = 8.3, 5.1 Hz, 1H, -CH-), 3.23 (dd, *J* = 14.7, 4.9 Hz, 1H, -CH_2-), 3.07 (dd, *J* = 14.7, 8.8 Hz, 1H, -CH_2-);MS *m/z* 351.2 $[M+H]^+$.

N-[4-hydroxy-(*E*)-cinnamoyl]-L-serine acid **c10,** a light yellow solid (1.12 g, 45% yield); mp 185-189 °C; ^{1}H NMR (600 MHz, d_6-DMSO) δ 12.62 (s, 1H, -COOH), 9.85 (s, 1H, Ph-OH), 8.12 (d, *J* = 8.0 Hz, 1H, NH-), 7.40 (d, *J* = 8.6 Hz, 2H, Ph-H), 7.33 (d, *J* = 15.7 Hz, 1H, Ph-CH=CH-), 6.80 (d, *J* = 8.6 Hz, 2H, Ph-H), 6.64 (d, *J* = 15.7 Hz, 1H, Ph-CH=CH-), 5.04 (s, 1H, -OH), 4.41 (dt, *J* = 8.2, 4.7 Hz, 1H, -CH-), 3.75 (dd, *J* = 10.9, 5.1 Hz, 1H,-CH_2-), 3.66 (dd, *J* = 10.9, 4.2 Hz, 1H, -CH_2-); MS *m/z* 252.3 $[M+H]^+$.

N-[4-hydroxy-(*E*)-cinnamoyl]-L-glutamine acid **c11**, a white solid (1.54 g, 53% yield); mp 196-198 °C; ^{1}H NMR (600 MHz, d_6-DMSO) δ 12.59 (s, 1H, -COOH), 9.89 (s, 1H, Ph-OH), 8.27 (d, *J* = 7.7 Hz, 1H, NH-), 7.39 (d, *J* = 8.6 Hz, 2H, Ph-H), 7.33 (d, *J* = 15.7 Hz, 1H, Ph-CH=CH-), 7.31(s, 1H, -NH_2), 6.79 (d, *J* = 8.1 Hz, 2H, Ph-H), 6.78(s, 1H, -NH_2), 6.50 (d, *J* = 15.8 Hz, 1H, Ph-CH=CH-), 4.28 (td, *J* = 8.8, 5.0 Hz, 1H, -CH-), 2.16 – 2.13 (m, 2H, -CH_2-), 2.10 – 1.90 (m, 1H, -CH_2-), 1.79 (m, 1H, -CH_2-); MS *m/z* 293.2 $[M+H]^+$.

N-[4-hydroxy-(*E*)-cinnamoyl]-L-threonine acid **c12**, a white solid (1.08 g, 41% yield); mp 201-204 °C; ^{1}H NMR (600 MHz, d_6-DMSO) δ 12.54 (s, 1H, -COOH), 9.84 (s, 1H, Ph-OH), 7.89 (d, *J* = 8.8 Hz, 1H,-NH), 7.41 (d, *J* = 8.6 Hz, 2H, Ph-H), 7.33 (d, *J* = 15.7 Hz, 1H, Ph-CH=CH), 6.80 (d, *J* = 8.6 Hz, 2H, Ph-H), 6.75 (d, *J* = 15.7 Hz, 1H, Ph-CH=CH), 4.97 (s, 1H, -OH), 4.35 (dd, *J* = 8.8, 3.2 Hz, 1H, -CH), 4.18 (qd, *J* = 6.3, 3.3 Hz, 1H, -CH-), 1.08 (d, *J* = 6.4 Hz, 3H, -CH_3); MS *m/z* 266.2 $[M+H]^+$.

N-[4-hydroxy-(*E*)-cinnamoyl]-L-glutamate acid **c13**, a white amorphous solid (1.63 g, 56%); mp 201-204 °C; ^{1}H NMR (600 MHz, DMSO) δ 12.41 (s, 1H, -COOH), 9.86 (s, 1H, -OH), 8.24 (d, *J* = 7.9 Hz, 1H, -NH), 7.40 (d, *J* = 8.6 Hz, 1H, Ph-H), 7.34 (d, *J* = 15.7 Hz, 1H, Ph-CH=CH), 6.80 (d, *J* = 8.6 Hz, 1H, Ph-H), 6.49 (d, *J* = 15.8 Hz, 1H, Ph-CH=CH), 4.58 – 4.11 (m, 1H, -CH-), 2.37 – 2.24 (m, 2H, -CH_2-), 2.07 – 1.97 (m, 1H, -CH_2-), 1.82 – 1.184(m, 1H, -CH_2-); MS *m/z* 294.3 $[M+H]^+$

N-[4-hydroxy-(*E*)-cinnamoyl]-L- asparagic acid **c14**, a white solid (1.72 g, 62% yield); mp 214-216 °C; ^{1}H NMR (600 MHz, d_6-DMSO) δ 12.59 (s, 2H, -COOH), 9.87 (s, 1H, -OH), 8.30 (d, *J* = 8.0 Hz, 1H, -NH), 7.41 (d, *J* = 8.6 Hz, 2H, Ph-H), 7.34 (d, *J* = 15.7 Hz, 1H, -Ph-CH_2=CH_2), 6.79 (t, *J* = 5.6 Hz, 2H, Ph-H), 6.52 (d, *J* = 15.7 Hz, 1H, Ph-CH_2=CH_2-), 4.65 (td, *J* = 7.4, 5.6 Hz, 1H, -CH-), 2.74 (dd, *J* = 16.6, 5.5 Hz, 1H, -CH_2-), 2.65 (dd, *J* = 16.6, 7.2 Hz, 1H, -CH_2-); MS *m/z* 280.2 $[M+H]^+$

4.3. The inhibitory activity against α-glucosidase

The α-glucosidase inhibitory activities were evaluated by the reported method.[28] 500 μL aqueous solution of sodium salt of the test sample was incubated at 37 ºC for 15 min with 100 μL α-glucosidase (0.5 U/mL in 100 mM solution of sodium phosphate buffer) in 500 μL phosphate buffer (pH 6.8). 0.5 mL of *p*NPG (2.5 mM) was then added and incubated for another 15 min. The reaction was terminated by the addition of 1 mL of 1M sodium carbonate solution. The inhibitory activities were quantified by measuring the absorbance at 405 nm. Acarbose was used as positive control. The inhibition of the test sample was calculated according to the following equation:

$$a(\%) = \left(1 - \frac{A_{sample} - A_{background}}{A_{blank}}\right) \times 100\%$$

Where *a(%) is the* percentage of inhibition of the tested sample or acarbose, A_{sample} is the tested absorbance after reaction, $A_{background}$ is the initial absorbance of the test sample. A_{blank} is the absorbance of enzyme solution with *p*NPG.

Lineweaver–Burk plot analysis was performed to determine the mode of inhibition of α-glucosidase. The reaction was carried out with an increasing concentration of inhibitors and varying concentrations of *p*NPG as the substrate. All the data were analyzed using a computer program for nonlinear regressions (OriginPro 8.0).

4.4. 3D QSAR model development and validation.

The QSAR study was performed in SYBYL 7.3. In our study, 85% of the synthesized compounds and their pIC_{50} ($-logIC_{50}$) was selected for training set to develop the 3D QSAR model and other 15% for test set to evaluate the model, based on their molecular properties (see supporting information Table S1). Prior to the form of the model, compound **c1** was selected as the template molecule and optimized to get the low energy conformation. The other molecules were optimized and superimposed based on the template using Database Alignment. Least-Squares Analysis (LSA) method was used to obtain the CoMFA model. The model was validated using test set correlation and Leave-one-out (LOO) cross validation.

4.5. Docking studies

The crystal 3D structure of α-glucosidase from *S. Cerevisiae* was predicted by homology modeling. The amino acid sequence and the active sites were retrieved from UniProt protein resource data bank with the accessing code P53341 (http://www.uniprot.org). [29] Similarity search and homology modeling were conducted in SWISS MODEL server (http://www.swissmodel.expasy.org). The crystallographic structure of *S. cerevisiae isomaltase* (PDB code 3AXH) was selected as the template with a 72.51% sequence identity with the target protein. The final structure generated from homology modeling was evaluated using PROCHECK. The docking study was run by Surflex-dock in SYBYL 7.3, and the results were visualized using PyMol.

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Conclusions

The synthesized *N*-phenylpropenoyl-L-amino acids exhibited strong α-glucosidase inhibitory activities and some of them were even more active than the traditional anti-diabetic drug acarbose *in vitro* test. This work provided a good proof for the hypoglycemic activities of cocoa beans and other related plant medicine. They may be further developed as a new kind of potential food-based hypoglycemic agents. Considering the low toxicity of their natural property, these compounds have bright future as medicinal lead compounds. However, *in vivo* studies of their anti-diabetic tests need to be investigated throughly.

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A table of contents entry

The phenolic acid amides with L-Amino acid moiety showed excellent inhibitory activities on α-glucosidase.