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Original article

Glycosides and amino acyl esters of carbohydrates as potent inhibitors of angiotensin converting enzyme

K. Lohith, G.R. Vijayakumar, B.R. Somashekar, R. Sivakumar, S. Divakar *

Fermentation Technology and Bioengineering, Central Food Technological Research Institute, KRS Road, 570020 Mysore, Karnataka, India

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Abstract

About 12 glycosides prepared through amyloglucosidase catalysis and 23 amino acyl esters of carbohydrates prepared through lipase catalysis in organic solvents showed angiotensin converting enzyme (ACE) inhibition activity. Both amino acyl esters of carbohydrates and glycosides exhibited IC_{50} values for ACE inhibition in the 0.5 mM to 15.7 mM range. Eugenyl-D-glucoside (IC_{50}: 0.5 ± 0.04 mM), L-isoleucyl-D-glucose (IC_{50}: 0.7 ± 0.067 mM), vanillyl-D-sorbitol (IC_{50}: 0.8 ± 0.09 mM), L-histidyl-D-fructose (IC_{50}: 0.9 ± 0.087 mM), L-tryptophanyl-D-fructose (IC_{50}: 0.9 ± 0.092 mM), octyl-D-glucoside (IC_{50}: 1.0 ± 0.093 mM), vanillyl-D-mannoside (IC_{50}: 1.0 ± 0.089 mM), L-valyl-D-mannitol (IC_{50}: 1.0 ± 0.092 mM) and L-phenylalanyl-D-glucose (IC_{50}: 1.0 ± 0.089 mM) were the compounds, which showed the best ACE inhibitory activities.

Keywords: Glycosides; Amino acyl esters of carbohydrates; Enzymatic synthesis; ACE inhibition; IC_{50} values

1. Introduction

Angiotensin converting enzyme (dipeptidyl carboxypeptidase, EC 3.4.15.1) is a zinc containing nonspecific dipeptidyl carboxypeptidase widely distributed in mammalian tissues [1]. Angiotensin-converting enzyme (ACE) regulates the blood pressure by modulating renin–angiotensin system [2]. This enzyme increases the blood pressure by converting the decapeptide angiotensin I into the potent vaso-constricting octapeptide, angiotensin II. Angiotensin II brings about several central effects, all leading to a further increase in blood pressure. ACE is a multifunctional enzyme that also catalyses the degradation of bradykinin (blood pressure-lowering nanopeptide) and therefore inhibition of ACE results in an overall antihypertensive effect [1,3].

Several synthetic drugs and bio-molecules are available for ACE inhibition. Captopril is a successful synthetic anti-hypertensive drug and similar such molecules like enalapril, perindopril, ceranopril, ramipril, quinapril and fosinopril also show ACE inhibitory activities [4,5]. Some naturally occurring 'biologically active peptides' also act as ACE inhibitors [6–9].

Glycosides from the leaves of *Abeliophyllum distichum* like acteoside, isoacteoside, rutin, and hirsutrin moderately inhibited the Angiotensin I converting enzyme activity [10]. Glycosides like 3-*O*-methyl crenatoside from *Microtoena prainiana* also showed more than 30% ACE inhibitory activity [11]. Phenyl propanoid glycosides from *Clerodendron trichotomum* such as acteoside, leucosceptoside A, martynoside, aceteoside isomer and isomartynoside also showed ACE inhibitory effect [12].

Certain glycosides and amino acyl esters of carbohydrates are used widely in food and pharmaceutical applications as sweeteners, surfactants, microcapsules in pharmaceutical preparations, in delivery of biologically active agents such as antibiotics, nutraceuticals and antitumor agents [13–17]. However, they have not been shown to exhibit ACE inhibition activity so far. The present work deals with detection of ACE inhibition activities for some enzymatically synthesized glycosides using amyloglucosidase and amino acyl esters of carbohydrates using lipases in organic media. The results are presented here.

2. Results

A total of 35 compounds were tested for the inhibitory activities of ACE isolated from pig lung. General scheme for

* Corresponding author.
E-mail address: divakar643@gmail.com (S. Divakar).

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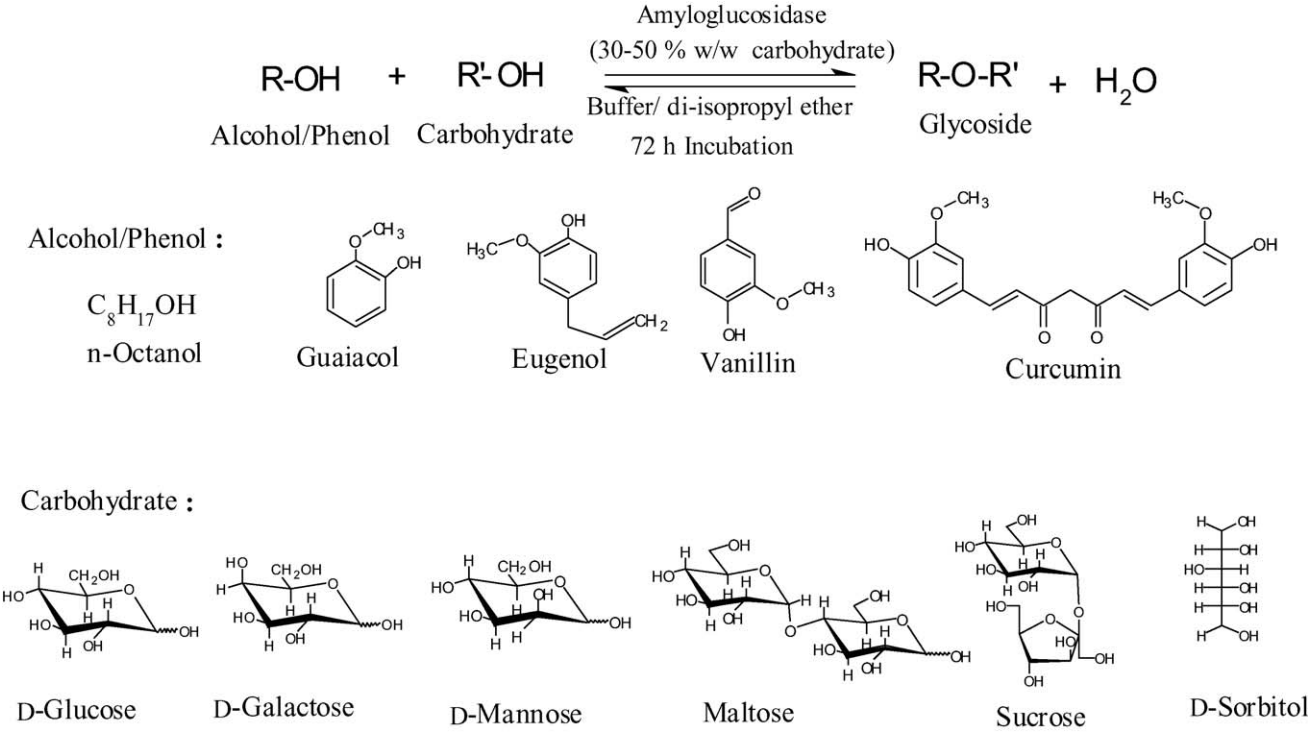
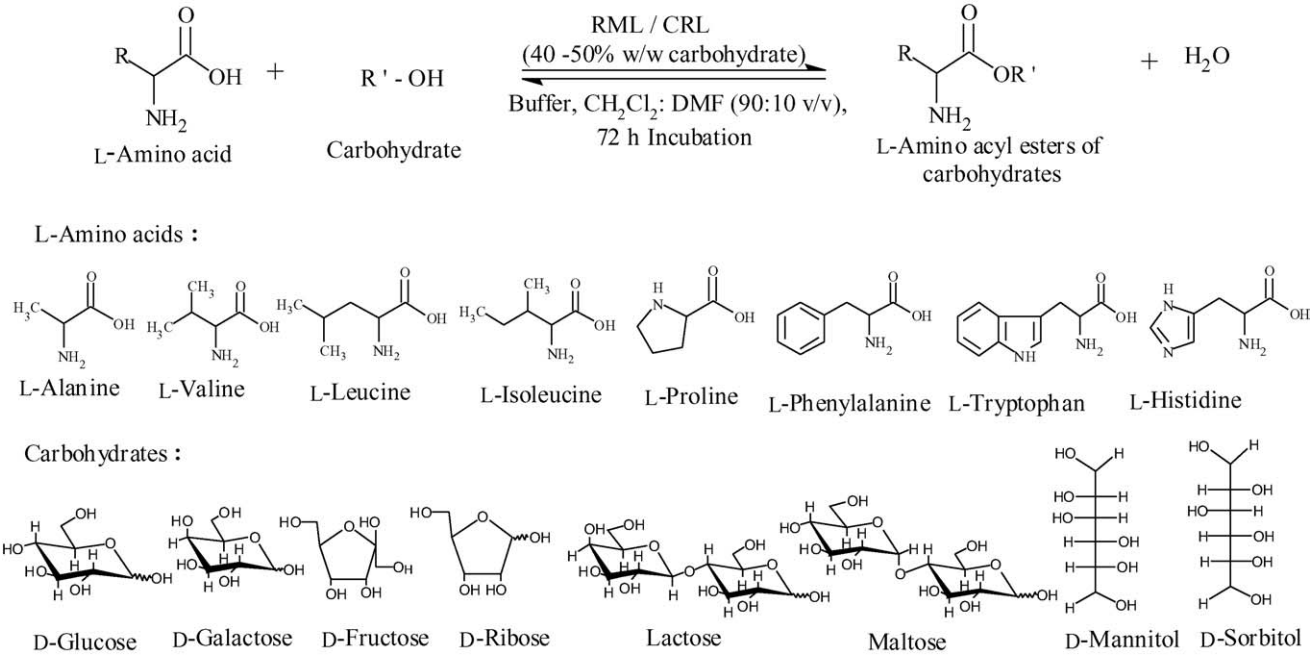
glycosylation and esterification are shown in Schemes 1 and 2. Enzymatic reactions were carried out under optimized conditions worked out for these reactions [18–21]. The enzymatic procedure employed unprotected and unactivated alcohols, phenols, amino acids and carbohydrates.

Typical ACE inhibition plots for eugenyl-D-glucoside, vanillyl-D-glucoside, L-isoleucyl-D-glucose and L-histidyl-D-fructose, are shown in Fig. 1. Tables 1 and 2, respectively, show the glycosides and amino acyl esters tested, their conversion yields from the respective enzymatic reactions, proportions of the products formed and ACE inhibitory activities for these compounds.

n-Octanol, guaiacol, eugenol, vanillin, curcumin and α-tocopherol glycosides of different carbohydrates (D-glucose, D-ga-



Scheme 1. Amyloglucosidase catalyzed synthesis of glycosides.



Scheme 2. Lipases catalyzed synthesis of L-amino acyl esters of carbohydrates.

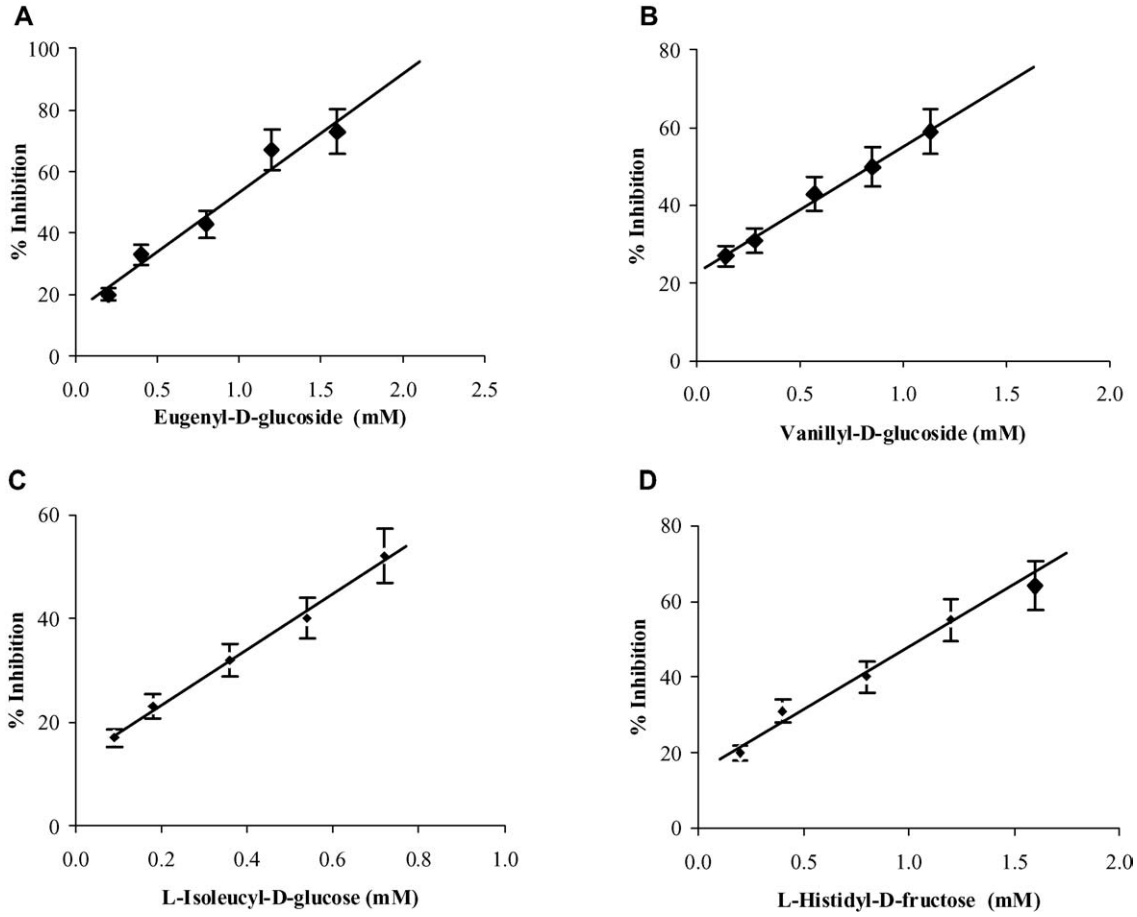


Fig. 1. **A.** Inhibition plot for eugenyl-D-glucoside, ACE: 0.1 ml (1.0 mg in 1.0 ml stock solution), Substrate: 0.1 ml hippuryl-histidyl-leucine (5 mM), Buffer: 100 mM phosphate buffer pH 8.3 contain 300 mM sodium chloride, Incubation period: 30 min, Temperature: 37 °C; **B.** Vanillyl-D-glucoside; **C.** L-Isoleucyl-D-glucose; **D.** L-Histidyl-D-fructose.

lactose, D-mannose, D-fructose, D-ribose, D-arabinose, maltose, sucrose, lactose, D-mannitol and D-sorbitol) were tested for ACE inhibition (Table 1). Among the carbohydrates employed, D-ribose, D-arabinose, D-fructose and lactose did not undergo any glycosylation.

Four amino acids containing alkyl side chains, three amino acids containing aromatic side chains and L-proline were employed for the preparation of esters. In case of aminoacyl esters, L-alanyl, L-valyl, L-leucyl, L-isoleucyl, L-prolyl, L-phenylalanyl, L-tryptophanyl and L-histidyl esters of D-glucose, D-galactose, D-fructose, D-ribose, lactose, maltose, D-sorbitol and D-mannitol were subjected to ACE inhibition activity studies (Table 2). ACE inhibition activity of the above-mentioned glycosides and amino acyl esters of carbohydrates were carried out by the Cushman and Cheung method [22]. Since hippuryl-L-histidyl-L-leucine (HHL) mimics the carboxyl dipeptide of angiotensin I, it has been routinely used as the substrate for screening ACE inhibitors.

Underivatized alcohols, phenols, L-amino acids and carbohydrates individually were also tested for ACE inhibition as controls and they did not show any ACE inhibitory activities. Only glycosides and esters showed activities. Isolated ACE inhibitor tested for lipase and protease activity (Table 3) showed a small extent of protease activity (13.3%) compared to ACE activity but no lipase activity. In presence of glycosides and amino acids esters prepared, the isolated ACE showed 8.2% and 8.9% protease activity, respectively (Table 3) compared to the ACE activity. This confirmed that the ACE inhibition observed in the presence of glycosides and esters prepared is more due to ACE inhibition rather than protease inhibition.

The compounds were characterized by UV, IR, Mass and NMR (two-dimensional heteronuclear single quantum coherence transfer—2D-HSQCT) spectroscopic techniques. In case of glycoside synthesis, the major product was the glycosylated product and relatively lesser amounts of C6-*O*-alkylated or C6-*O*-arylated products were also detected. The reaction was between the alcohol or phenolic OH groups and the anomeric and/or primary C6-OH groups of the carbohydrates.

In case of esters, mono and di esters in different proportions were detected. Individual 1-O, 2-O, 3-O, 5-O, 6-O, and 6″-O monoesters and 1,6-di-O, 2,5-di-O, 2,6-di-O, 3,6-di-O, and 6,6″-di-O esters depending on the carbohydrate employed were found to be formed. It was not possible to separate the individual glycosides and esters from their reaction mixtures even by chromatographic separation on Sephadex G-10. Thus the activities described are for the mixture of these compounds.

Table 1
IC_{50} values for ACE inhibition by glycosides [a]

Glycoside	Conversion Yield [b] (%)	Products formed (% proportions) [c]	IC_{50} value (mM) [d]
n-Octyl-α/β-D-glucoside	46	*n*-Octyl-α-D-glucopyranoside (63) *n*-Octyl-β-D-glucopyranoside (25) C6-*O*-octyl-D-glucose (12)	1.0 ± 0.093
n-Octyl maltoside	22	*n*-Octyl maltoside	1.5 ± 0.13
n-Octyl sucrose	13	C1-*O*-octyl sucrose (44) C6-*O*-octyl sucrose (56)	1.7 ± 0.15
Guaiacyl-α-D-glucoside	52	C1-*O*-guaiacyl-α-D-glucopyranoside (52) C6-*O*-guaiacyl-α-D-glucose (48)	3.7 ± 0.36
Eugenyl-α-D-glucoside	32	C1-*O*-eugenyl-α-D-glucopyranoside (53) C6-*O*-eugenyl-D-glucose (47)	0.5 ± 0.04
Vanillyl-α/β-D-glucoside	53	C1-*O*-vanillyl-α-D-glucopyranoside (52) C1-*O*-vanillyl-β-D-glucopyranoside (17) C6-*O*-vanillyl-D-glucose (31)	1.1 ± 0.10
Vanillyl-α-D-galactoside	18	C1-*O*-vanillyl-α-D-galactopyranoside	1.1 ± 0.13
Vanillyl-α-D-mannoside	13	C1-*O*-vanillyl-α-D-mannopyranoside	1.0 ± 0.089
Vanillyl maltoside	29	C1-*O*-vanillyl-α-maltopyranoside (42) C6-*O*-vanillyl maltose (30) C6″-*O*-vanillyl maltose (28)	1.6 ± 0.14
Vanillyl sucrose	23	C1-*O*-vanillyl sucrose (39) C6″-*O*-vanillyl sucrose (61)	15.7 ± 1.6
Vanillyl-D-sorbitol	13	C1-*O*-vanillyl-D-sorbitol (13) C6-*O*-vanillyl-D-sorbitol (25) C1, C6 Di-*O*-vanillyl-D-sorbitol (62)	0.8 ± 0.09
Curcuminyl-bis-α-D-glucoside	48	C1-*O*-curcuminyl-bis-α-D-glucopyranoside (62) C6-*O*-curcuminyl-bis-α-D-glucose (38)	1.5 ± 0.13

[a] Respective alcohols, phenols and carbohydrates as controls for ACE inhibition activity; non reducing sugar unit carbons of disaccharide are double primed;
[b] Conversion yields were from HPLC with errors in yield measurements ± 10%;
[c] Product proportions determined from ^{13}C 2D-HSQCT NMR C1/C6 peak areas or their cross peaks;
[d] IC_{50} values compared to that of captopril 0.060 ± 0.005 mM determined by Cushman and Cheung method [22].

3. Discussion

Among the glycosides tested, eugenyl-D-glucoside (0.5 ± 0.04 mM), vanillyl-D-sorbitol (0.8 ± 0.09 mM), vanillyl-D-mannoside (1.0 ± 0.089 mM) and octyl-D-glucoside (1.0 ± 0.093 mM) exhibited the best ACE inhibitory activities ($IC_{50} \leq 1.0$ mM; Table 1). Among the carbohydrates employed glucosides showed the best ACE inhibitory activities. Alkyl glycosides showed better inhibitory activities than the phenolic glycosides. The glycosides also showed better ACE inhibitory activities than the amino acyl esters indicating higher potency of glycosides in ACE inhibition.

With increase in alkyl side chain branching, D-glucose esters of L-alanine (3.1 ± 0.30 mM), L-valine (6.0 ± 0.59 mM), L-leucine (2.8 ± 0.27 mM) and L-isoleucine (0.7 ± 0.067 mM) showed better inhibition (lesser IC_{50} values), than the other esters, which could be directly correlated to increase in hydrophobicity (Table 2). IC_{50} values ≤ 1.0 mM were detected for L-isoleucyl-D-glucose (0.7 ± 0.067 mM), L-histidyl-D-fructose (0.9 ± 0.087 mM), L-tryptophanyl-D-fructose (0.9 ± 0.092 mM), L-valyl-D-mannitol (1.0 ± 0.092 mM) and L-phenylalanyl-D-glucose (IC_{50}: 1.0 ± 0.089 mM). Among these, L-isoleucyl-D-glucose (0.7 ± 0.067 mM) was found to exhibit the best inhibitory activity. In general, aromatic amino acid esters showed comparatively higher IC_{50} values than amino acids containing alkyl side chains, emphasizing that the latter were better inhibitors of ACE. Among the carbohydrates employed, D-mannitol esters showed better ACE inhibition (Table 2) than the other carbohydrate esters. In case of L-prolyl esters, IC_{50} values in the 1.6–4.4 mM range matched with those of alkyl side chain containing amino acid esters like L-alanine, L-valine, L-leucine and L-isoleucine (Table 2). Captopril is *N*-[(*S*)-3-mercapto-2-methylpropionyl]-L-proline containing prolyl unit as essential for ACE inhibition [4]. However, mere presence of a prolyl unit in the esters prepared does not give rise to a high level of ACE inhibition. Since, it was difficult to separate the individual esters, the actual potency of the individual esters could not be unequivocally established in the present work.

The present work for the first time has shown the ACE inhibitory potency of the above-mentioned glycosides and esters prepared enzymatically. Since milder reaction conditions were employed, the products formation did not suffer due to side reactions. However, the glycosides and esters tested in the present work, overall, clearly possess groups like alkyl side chains and phenolic groups which can be accommodated in the hydrophobic S_1 and S_2 subsites of angiotensin I converting enzyme [4,23]. The free amino group in the amino acid esters can also serve as good ligands for Zn^{2+} in the ACE active site. Carbohydrates in glycosides and esters could also bind to the hydrophobic and/or hydrophilic subsites of angiotensin I converting enzyme, as they possess both hydrophobic and hydrophilic groups in their structure. Although the ACE preparation of the present work from pig lung is ACE I [24], it showed a low protease inhibitory activity but no lipase activity indicating that the glycosides and esters inhibit ACE rather than the pro-

Table 2
IC_{50} values for ACE inhibition by L-aminoacyl esters of carbohydrates[a]

L-Amino acyl ester of carbohydrates	Conversion Yield (%)[b]	Products (% proportion)[c]	IC_{50} value (mM)[d]
L-Alanyl-β-D-glucose	30	2-*O*-L-alanyl-β-D-glucose (47) 3-*O*-L-alanyl-β-D-glucose (12) 6-*O*-L-alanyl-β-D-glucose (20) 2,6-di-*O*-L-alanyl-β-D-glucose (15) 3,6-di-*O*-L-alanyl-β-D-glucose (6)	3.1 ± 0.30
L-Alanyl-D-ribose	48	3-*O*-L-alanyl-D-ribose (16) 5-*O*-L-alanyl-D-ribose (32) 3,5-di-*O*-L-alanyl-D-ribose (52)	2.7 ± 0.25
L-Alanyl-lactose	20	6-*O*-L-alanyl-lactose (34) 6″-*O*-L-alanyl-lactose (34) 6,6″-di-*O*-L-alanyl-lactose (32)	2.0 ± 0.21
L-Valyl-D-glucose	84	2-*O*-L-valyl-D-glucose (26) 3-*O*-L-valyl-D-glucose (26) 6-*O*-L-valyl-D-glucose (33) 2,6-di-*O*-L-valyl-D-glucose (9) 3,6-di-*O*-L-valyl-D-glucose (6)	6.0 ± 0.59
L-Valyl-D-fructose	34	1-*O*-L-valyl-D-fructose	2.8 ± 0.29
L-Valyl-maltose	42	6-*O*-L-valyl-maltose (35) 6″-*O*-L-valyl-maltose (36) 6,6″-di-*O*-L-valyl-maltose (29)	3.1 ± 0.33
L-Valyl-D-mannitol	56	1-*O*-L-valyl-D-mannitol	1.0 ± 0.092
L-Leucyl-D-glucose	43	2-*O*-L-leucyl-D-glucose (17) 3-*O*-L-leucyl-D-glucose (20) 6-*O*-L-leucyl-D-glucose (48) 2,6-di-*O*-L-leucyl-D-glucose (8) 3,6-di-*O*-L-leucyl-D-glucose (7)	2.8 ± 0.27
L-Isoleucyl-D-glucose	47	3-*O*-L-isoleucyl-D-glucose (42) 6-*O*-L-isoleucyl-D-glucose (58)	0.7 ± 0.067
L-Prolyl-D-glucose	62	2-*O*-L-prolyl-D-glucose (26) 3-*O*-L-prolyl-D-glucose (26) 6-*O*-L-prolyl-D-glucose (48)	1.7 ± 0.19
L-Prolyl-D-fructose	61	1-*O*-L-prolyl-D-fructose (31) 6-*O*-L-prolyl-D-fructose (42) 1,6-di-*O*-L-prolyl-D-fructose (27)	4.4 ± 0.42
L-Prolyl-D-ribose	41	3-*O*-L-prolyl-D-ribose (35) 5-*O*-L-prolyl-D-ribose (65)	2.0 ± 0.18
L-Prolyl-lactose	68	6-*O*-L-prolyl-lactose (58) 6″-*O*-L-prolyl-lactose (42)	1.6 ± 0.15
L-Phenylalanyl-D-glucose	79	2-*O*-L-phenylalanyl-D-glucose (19) 3-*O*-L-phenylalanyl-D-glucose (23) 6-*O*-L-phenylalanyl-D-glucose (25) 2,6-di-*O*-L-phenylalanyl-D-glucose (17) 3,6-di-*O*-L-phenylalanyl-D-glucose (16)	1.0 ± 0.089
L-Phenylalanyl-D-galactose	45	2-*O*-L-phenylalanyl-D-galactose (32) 3-*O*-L-phenylalanyl-D-galactose (20) 6-*O*-L-phenylalanyl-D-galactose (19) 2,6-di-*O*-L-phenylalanyl-D-galactose (16) 3,6-di-*O*-L-phenylalanyl-D-galactose (13)	4.6 ± 0.45
L-Phenylalanyl-D-fructose	50	1-*O*-L-phenylalanyl-D-fructose (72) 6-*O*-L-phenylalanyl-D-fructose (28)	13.6 ± 1.43
L-Phenylalanyl-lactose	61	6-*O*-L-phenylalanyl-lactose (42) 6″-*O*-L-phenylalanyl-lactose (31) 6,6″-di-*O*-L-phenylalanyl-lactose (27)	7.8 ± 0.77
L-Phenylalanyl-D-mannitol	43	1-*O*-L-phenylalanyl-D-mannitol (62) 1,6-di-*O*-L-phenylalanyl-D-mannitol (38)	2.6 ± 0.25
L-Tryptophanyl-D-glucose	42	2-*O*-L-tryptophanyl-D-glucose (22) 3-*O*-L-tryptophanyl-D-glucose (21) 6-*O*-L-tryptophanyl-D-glucose (38) 2,6-di-*O*-L-tryptophanyl-D-glucose (10) 3,6-di-*O*-L-tryptophanyl-D-glucose (9)	7.4±0.73
L-Tryptophanyl-D-fructose	18	1-*O*-L-tryptophanyl-D-fructose (45) 6-*O*-L-tryptophanyl-D-fructose (55)	0.9 ± 0.092

(continued)

Table 2 *(continued)*

L-Amino acyl ester of carbohydrates	Conversion Yield (%)[b]	Products (% proportion)[c]	IC_{50} value (mM)[d]
L-Histidyl-D-glucose	42	2-*O*-L-histidyl-D-glucose (25) 3-*O*-L-histidyl-D-glucose (24) 6-*O*-L-histidyl-D-glucose (28) 2,6-di-*O*-L-histidyl-D-glucose (12) 3,6-di-*O*-L-histidyl-D-glucose (11)	3.5 ± 0.34
L-Histidyl-D-fructose	58	6-*O*-L-histidyl-D-fructose	0.9 ± 0.087
L-Histidyl-D-mannitol	8	1-*O*-L-histidyl-D-mannitol	1.7 ± 0.16

[a] Respective amino acids and carbohydrates as controls for ACE inhibition activity;
[b] Conversion yields were from HPLC with errors in HPLC yield measurements ± 10%;
[c] Product proportions determined from ^{13}C 2D-HSQCT NMR C6 peak areas or their cross peaks, C5 cross peaks in case of ribose;
[d] IC_{50} values compared to that of captopril 0.060 ± 0.006 mM determined by Cushman and Cheung method [22].

Table 3
Protease inhibition assay for eugenyl-D-glucosides and L-isoleucyl-D-glucose[a]

System	Protease activity Unit $min^{-1}mg^{-1}$ enzyme protein[b]	Percentage of protease activity with respect to ACE activity[c]
Control ACE- 0.5 ml + 0.5 ml of 0.6% hemoglobin + 0.5 ml Buffer	0.0436	13.3
Eugenyl-D-glucoside: 0.5 ml glycoside + ACE - 0.5 ml + 0.5 ml of 0.6% hemoglobin	0.0292	8.2
L-Isoleucyl-D-glucose: 0.5 ml ester + ACE -0.5 ml + 0.5 ml of 0.6% hemoglobin	0.0267	8.9

[a] Conditions: ACE: 0.5 ml (0.5 mg), All the solutions were prepared in 0.1 M pH 7.5 Tris–HCl, incubation period: 30 min, temperature: 37 °C, 0.5 ml of 10% trichloroacetic acid added to arrest the reaction; Blank performed without enzyme and glycoside or ester; Absorbance measured at 440 nm; Eugenyl-D-glucoside and isoleucyl-D-glucose: 0.5 ml of 0.8 mM;
[b] Average absorbance values from three individual experiments;
[c] Percentage protease activity with respect to an ACE activity of 0.327 μmol min^{-1} mg^{-1} protein.

tease activity as protease inhibition activity can be construed to be ACE inhibition. The results indicate that both the glycosides and esters bind to these enzymes and hence hold promise as potential inhibitors for the ACE.

4. Experimental section

4.1. Enzymes

Lipozyme IM20 *Rhizomucor miehei* (RML) immobilized on weak anion exchange resin from Novo Nordisk, Denmark, *Candida rugosa* lipase (CRL) from Sigma Chemical Co., USA, for the esterification reactions and amyloglucosidase from *Rhizopus* sp. purchased from M/S Sigma Chemical. Co., MO, USA, for the glycosylation reactions, were used in the present work. RML and CRL employed showed esterification activities of 0.46 and 0.03 μmol min^{-1} mg^{-1} enzyme preparation, respectively [25]. Amyloglucosidase activity determined by the Sumner and Sisler method [26] was found to be 4.7 μmol min^{-1} mg^{-1} protein.

ACE was extracted from pig lung using the method described by Andujar-Sanchez et al. [24]. A 100 g of pig lung was minced and homogenized using a blender with 10 mM, pH 7.0, HEPES buffer containing 0.4 M NaCl at a volume ratio of 5:1 (v/w of pig lung) at 4 °C throughout the procedure. The homogenate was centrifuged at 9000 × *g* for 60 min. The supernatant was discarded and the precipitate was washed twice with 200 ml of 10 mM, pH 7.0, HEPES buffer containing 0.4 M NaCl. The final precipitate was resuspended in 200 ml of 10 mM, pH 7.0, HEPES buffer containing, 0.4 M NaCl, 10 μM $ZnCl_2$, 0.5% (w/v) Triton X100 and stirred over night. The solution was centrifuged to remove the pellets. The supernatant was dialyzed against water and later lyophilized. The protein content of ACE determined by Lowry's method [27] was found to be 8.3%. The remaining is constituted by 17% carbohydrate, lipid and other solid tissue [5,28].

4.2. Chemicals and reagents

L-Amino acids, guaiacol, D-galactose, D-fructose, gum acacia and Tris–HCl from HiMedia (Ind.) Ltd.; D-glucose, sucrose, $ZnCl_2$, trichloroacetic acid, NaOH and NaCl from SD fine chemicals (Ind.) Ltd.; maltose, hippuryl-L-histidyl-L-leucine, bovine hemoglobin and tributyrin from Sigma Chemical Co. USA; D-mannose, D-arabinose, D-ribose, D-mannitol, eugenol, sodium dodecyl sulfate, sodium benzoate, Triton X-100 and hippuric acid from LOBA Chemie Pvt. Ltd. India; curcumin (purity > 95%) from Flavors and Essences Pvt. Ltd. India; HEPES buffer, lactose, *n*-octanol and vanillin (purity > 98%) from Sisco Research Laboratories Pvt. Ltd. India and D-sorbitol from Rolex Laboratory Reagent India Ltd., were employed as such. Sephadex G-10 from Sigma Chemical Co., USA was used. Solvents–CH_2Cl_2, dimethylformamide, di-isopropyl ether, petroleum ether, ethyl acetate and acetonitrile from SD Fine Chemicals (Ind.) Ltd. were employed after distilling once.

4.3. Glycosylation procedure

A general procedure for the glycosylation involved reaction in a 150 ml two necked flat bottom flask by refluxing phenols (1.0 mmol) and carbohydrate (1.0 mmol) in 100 ml di-isopropyl ether in presence of buffer and appropriate quantity of amyloglucosidase (30–50% w/w carbohydrate) for 72 h. In

case of *n*-octanol and guaiacol glycosides, carbohydrate and *n*-octanol/guaiacol were taken in 1: 50 molar ratio. Buffers employed were 0.4 ml to 1.0 ml of sodium acetate for pH 4.0 and 5.0, sodium phosphate for pH 6.0 and 7.0 and sodium borate for pH 8.0. After incubation, the reaction mixture was held in a boiling water bath for 5–10 min to denature the enzyme and 15–20 ml of water was added to dissolve the unreacted carbohydrate and the product glycoside. The unreacted alcohol was separated in a separating funnel with petroleum ether or *n*-hexane and phenols were separated by extracting with chloroform. The aqueous layer was evaporated to dryness to get the unreacted carbohydrate and the product glycoside. The reaction mixtures were analyzed by HPLC using an amino-propyl column (3.9 × 300 mm length) and acetonitrile: water in 80:20 ratio (v/v) as the mobile phase at a flow rate of 1 ml/min with refractive index detector. Conversion yields were determined from HPLC peak areas of the glycoside and free carbohydrate with respect to the free carbohydrate employed. Error in HPLC measurements will be ± 10%. The glycosides formed were separated through size exclusion chromatography using Sephadex G-10 eluting with water.

The isolated glycosides and esters were characterized by UV, IR, MS, 2D-NMR (HSQCT) and optical rotation, which provided good information on the nature and proportions of the products formed. A Shimadzu UV–1601 spectrophotometer was used for recording UV spectra of the isolated glycosides and esters in aqueous solutions at 0.2–1.0 mM concentration. A Nicolet 5700 FTIR instrument was used for recording the IR spectra with 1.0–3.0 mg of glycosides and ester samples as KBr pellet. Specific rotations of the isolated glycosides and esters were measured at 25 °C using Perkin-Elmer 243 polarimeter with 0.5% aqueous solution of the esters. Mass spectra of the isolated glycosides and esters were recorded using a Q-TOF Waters Ultima instrument (No. Q-Tof GAA 082, Waters corporation, Manchester, UK) fitted with an electron spray ionization (ESI) source.

^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX-500 MHz spectrometer (500.13 MHz for ^{1}H and 125 MHz ^{13}C). Proton and carbon 90° pulse widths were 10.5 and 12.25 μs, respectively. About 40 mg of the sample dissolved in DMSO-d_6 and D_2O was used for recording the spectra at 35 °C. Chemical shift values were expressed in ppm relative to internal tetramethylsilane standard to within ± 0.01 ppm. 2D-HSQCT spectra were recorded for the glycosides and esters. In the NMR data, only resolvable signals are shown. Some assignments are interchangeable. *n*-Octanol/phenol signals are primed and signals from non reducing end units in maltose, lactose and sucrose are double primed. Since, the compounds are surfactant molecules, they appear to aggregate in the solvent and usually give broad signals, thus, making it difficult to resolve the coupling constant values accurately.

4.3.1. Glycosides

4.3.1.1. n-Octyl-α/β-D-glucoside. Solid; UV (H_2O, λ_{max}): 206 nm (σ→σ*, ε_{206}–1230 M^{-1}), 278 nm (*n*→π*, ε_{278}–72 M^{-1}). IR (KBr): 1053 cm^{-1} (glycosidic C-O-C symmetrical), 3605 cm^{-1} (OH). Optical rotation (*c* 1, H_2O) $[\alpha]_D$ at 25 °C = +45.8°. MS (*m*/*z*) 294 $[M + 2]^+$, 317 $[M + 2 + Na]^+$. 2D-HSQCT (DMSO-d_6) *n-Octyl-α-D-glucopyranoside:* ^{1}H NMR δ_{ppm}: (500.13 MHz) 4.62 (H-1α), 3.18 (H-2α), 3.42 (H-3α), 3.74 (H-4α), 3.18 (H-5α), 3.42 (H-6α), 3.1 (CH_2-1′), 1.51 (CH_2-2′), 1.23 (CH_2-3′-7′), 0.85 (CH_2-8′); ^{13}C NMR δ_{ppm}: (125 MHz) 98.5 (C1α), 72.0 (C2α), 72.4 (C3α), 70.2 (C4α), 72.1 (C5α), 60.8 (C6 α), 14.0 (C8′), 23.0 (C7′), 31.2 (C6′), 29.8 (C3′), 30.0 (C5′), 70.2 (C1′). *n-Octyl-β-D-glucopyranoside:* ^{1}H NMR δ_{ppm}: 4.17 (H-1β), 2.88 (H-2β), 3.12 (H-5β), 3.60 (H-6β), 3.1 (CH_2-1′), 1.51 (CH_2-2′), 1.23 (CH_2-3′-7′), 0.85 (CH_2-8′); ^{13}C NMR δ_{ppm}: 103.2 (C1β), 74.7 (C2β), 77.1 (C3β), 71.0 (C4β), 77.1 (C5β), 61.5 (C6 β), 14.0 (C8′), 23.0 (C7′), 31.2 (C6′), 30.0 (C5′), 70.5 (C1′). *C6-O-octyl-D-glucose:* ^{1}H NMR δ_{ppm}: 4.90 (H-1α), 3.20 (H-2α), 3.10 (H-5α), 3.64 (H-6α), 3.1 (CH_2-1′), 1.51 (CH_2-2′), 1.21 (CH_2-3′-7′), 0.85 (CH_2-8′); ^{13}C NMR δ_{ppm}: 92.2 (C1α), 72.5 (C2α), 72.1 (C5α), 67.2 (C6 α), 14.0 (C8′), 29.5 (C3′), 30.0 (C5′), 70.5 (C1′).

4.3.1.2. n-Octyl maltoside. Solid; UV (H_2O, λ_{max}): 194 nm (σ→σ*, ε_{194}–1479 M^{-1}), 278.5 nm (*n*→π*, $\varepsilon_{278.5}$–95 M^{-1}). IR (KBr): 1033 cm^{-1} (glycosidic C-O-C symmetrical), 1255 cm^{-1} (glycosidic C-O-C asymmetrical), 3415 cm^{-1} (OH). Optical rotation (c 1, H_2O), $[\alpha]_D$ at 25 °C = +91.1°. MS (*m*/*z*) 455 $[M + 1]^+$. 2D-HSQCT (DMSO-d_6) ^{1}H NMR δ_{ppm}: (500.13 MHz) 4.63 (H-1α), 3.30 (H-5α), 3.66 (H-6α), 4.99 (H-1″α), 3.46 (H-2″), 3.20 (H-3″), 3.08 (H-4″), 3.44 (H-6″), 2.9 (CH_2-1′), 1.11–1.25 (CH_2-2′-7′), 0.85 (CH_2-8′); ^{13}C NMR δ_{ppm}: (125 MHz) 98.8 (C1α), 75.0 (C5α), 60.5 (C6α), 100.8 (C1″α), 71.8 (C2″), 72.1 (C3″), 70.2 (C4″), 61.0 (C6″), 14.1 (C8′), 23.0 (C7′), 31.5 (C6′), 29.8 (C3′), 29.0 (C2′), 70.3 (C1′).

4.3.1.3. n-Octyl sucrose. Solid; UV (H_2O, λ_{max}): 205 nm (σ→σ*, ε_{205}–2570 M^{-1}), 276 nm (*n*→π*, ε_{276}–257 M^{-1}). IR (KBr): 1054 cm^{-1} (glycosidic C-O-C symmetrical), 1259 cm^{-1} (glycosidic C-O-C asymmetrical), 3357 cm^{-1} (OH). Optical rotation (*c* 1, H_2O), $[\alpha]_D$ at 25 °C = +13.3°. MS (*m*/*z*) 455 $[M + 1]^+$. 2D-HSQCT (DMSO-d_6) *C1-O-octyl sucrose*: ^{1}H NMR δ_{ppm} (500.13 MHz): 3.76 (H-1), 3.81 (H-4), 3.79 (H-5), 3.40 (H-6), 5.18 (H-1″α), 3.10 (H-3″), 3.03 (H-4″), 3.54 (H-5″), 3.62 (H-6″), 3.01 (H-1′), 1.01–1.23 (H-2-7′), 0.84 (H-8′); ^{13}C NMR δ_{ppm} (125 MHz): 62.8 (C1), 104.0 (C2), 75.4 (C4), 83.0 (C5), 62.0 (C6), 91.5 (C1″α), 72.2 (C3″), 70.5 (C4″), 72.0 (C5″), 61.0 (C6″), 14.4 (C8′), 23.2 (C7′), 31.5 (C6′), 29.2 (C5′), 29.6 (C2′), 70.2 (C1′). *C6-O-octyl sucrose*: ^{1}H NMR δ_{ppm}: 3.54 (H-1), 3.87 (H-3), 3.72 (H-4), 3.72 (H-5), 3.25 (H-6), 4.90 (H-1″α), 3.17 (H-3″), 3.11 (H-4″), 3.44 (H-5″), 3.48 (H-6″), 3.01 (H-1′), 1.0–1.25 (H-2-7′), 0.85 (H-8′); ^{13}C NMR δ_{ppm}: 61.8 (C1), 104.04 (C2), 77.4 (C3), 76.0 (C4), 82.0 (C5), 63.0 (C6), 92.1 (C1″α), 72.2 (C3″), 70.2 (C4″), 72.0 (C5″), 61.2 (C6″), 14.6 (C8′), 23.3 (C7′), 31.3 (C6′), 29.6 (C4′), 29.7 (C2′), 70.0 (C1′).

4.3.1.4. Guaiacyl-α-D-glucoside. Solid; UV (H_2O, λ_{max}): 210 nm (σ→σ*, ε_{210}–398 M^{-1}), 270 nm (π→π*,

ε_{270}–69 M^{-1}). IR (KBr): 1030 cm^{-1} (glycosidic aryl alkyl C-O-C symmetrical), 1236 cm^{-1} (glycosidic aryl alkyl C-O-C asymmetrical). Optical rotation (*c* 1, H_2O): $[\alpha]_D$ at 25 °C = +92.3°. MS (*m/z*) 286 $[M]^+$. *C1-O-guaiacyl-α-D-glucopyranoside*: ^1H NMR δ_{ppm} (500.13 MHz): 4.65 (H-1α), 3.3 (H-2α), 3.72 (H-3α), 3.75 (H-4α), 3.10 (H-5α), 3.43 (H-6α), 7.08 (H-6′), 6.98 (H-5′), 6.94 (H-4′), 7.09 (H-3′); ^{13}C NMR δ_{ppm} (125 MHz): 98.7 (C1α), 74.9 (C2α), 72.0(C3α), 70.1 (C4α), 74.9 (C5α), 60.9 (C6α), 113.4 (C6′), 118.5 (C5′), 121.9 (C4′), 110.5 (C3′). *C6-O-guaiacyl-α-D-glucose*: ^1H NMR δ_{ppm}: 4.97 (H-1α), 3.06 (H-2α), 3.16 (H-3α), 3.63 (H-4α), 3.14 (H-5α), 3.60 (H-6α), 6.80 (H-5′), 6.84 (H-4′), 6.88 (H-3′); ^{13}C NMR δ_{ppm} 100.0 (C1α), 74.9 (C2α), 74.0(C3α), 70.2 (C4α), 74.0 (C5α), 67.0 (C6α), 119.0(C5′), 121.7 (C4′), 110.0 (C3′).

4.3.1.5. Eugenyl-α-D-glucoside. Solid; UV (H_2O, λ_{max}): 205 nm (σ→σ*, ε_{205}–1792 M^{-1}), 279 nm (π→π*, ε_{279}–396 M^{-1}). IR (KBr): 1653 cm^{-1} (allylic C=C), 1033 cm^{-1} (glycosidic aryl alkyl C-O-C symmetrical), 1268 cm^{-1}, (glycosidic aryl alkyl C-O-C asymmetrical), 3330 cm^{-1} (OH). Optical rotation (*c* 1, H_2O) at 25 °C = +59.6°. MS (*m/z*) 347 $[M\text{-}2+Na]^+$. *C1-O-eugenyl-α-D-glucopyranoside*: ^1H NMR δ_{ppm} (500.13 MHz): 4.65 (H-1α), 3.55 (H-2α), 3.28 (H-3α), 3.73 (H-4α), 3.42 (H-6α). 6.86 (1H, s, H-3′), 6.70 (1H, d, H-5′), 6.85 (1H, d, H-6′), 3.28 (H-7′), 5.92 (1H, m, H-8′), 5.10 (H-9′), 3.40 (OCH_3); ^{13}C NMR δ_{ppm} (125 MHz): 97.0 (C1α), 72.5 (C2α), 74.0 (C3α), 69.0 (C4α), 61.0 (C6α), 52.0 (OCH_3), 113 (C3′), 120 (C5′), 115.3 (C6′), 39.0 (C7′), 137.0 (C8′), 116.0 (C9′). *C6-O-eugenyl-D-glucose*: ^1H NMR δ_{ppm}: 3.6 (H-2α), 3.50 and 3.68 (H-6α a & b), 6.90 (1H, s, H-3′), 6.78 (1H, d, H-5′); ^{13}C NMR δ_{ppm}: 69.0 (C2α), 66.0 (C6α), 52.0 (OCH_3), 112 (C3′), 119 (C5′).

4.3.1.6. Vanillyl-α/β-D-glucoside. Solid; UV (H_2O, λ_{max}): 195.5 nm (σ→σ*, $\varepsilon_{195.5}$–2241 M^{-1}), 279.5 nm (π→π*, $\varepsilon_{279.}$–5291 M^{-1}). IR (KBr): 3358 cm^{-1} (OH), 1260 cm^{-1} (glycosidic aryl alkyl C–O–C asymmetric), 1030 cm^{-1} (glycosidic aryl alkyl C–O–C symmetric), 1408 cm^{-1} (C=C), 1636 cm^{-1} (CO), 2933 cm^{-1} (CH). Optical rotation (*c* 1, H_2O): $[\alpha]_D$ at 25 °C = +62.8. MS (*m/z*) 316 $[M + 2]^+$. 2D-HSQCT (DMSO-d_6) *C1-O-vanillyl-α-D-glucopyranoside*: ^1H NMR δ_{ppm} (500.13 MHz): 4.65 (H-1α, 5.5 Hz), 3.23 (H-2α), 3.42 (H-3α), 3.78 (H-4α), 3.15 (H-5α), 3.60 (H-6α), 6.59 (H-2′), 6.20 (H-5′), 3.73 (OCH_3); ^{13}C NMR δ_{ppm} (125 MHz): 99.2 (C1α), 72.3 (C2α), 73.5 (C3α), 70.2 (C4α), 72.5 (C5α), 60.5 (C6α), 111.4 (C2′), 114.5 (C5′). *C1-O-vanillyl-β-D-glucopyranoside*: ^1H NMR δ_{ppm}: 4.94 (H-1β, 5.3 Hz), 2.98 (H-2β), 3.22 (H-3β), 3.68 (H-6β); ^{13}C NMR δ_{ppm}: 101.5 (C1β), 74.6 (C2β), 76.1 (C3β), 60.8 (C6β). *C6-O-vanillyl D-glucose*: ^1H NMR δ_{ppm}: 4.91 (H-1α), 3.23 (H-2α), 3.20 (H-3α), 3.62 (H-4α), 3.23 (H-5α), 3.55 (H-6α); ^{13}C NMR δ_{ppm}: 92.7 (C1α), 72.3 (C2α), 72.6 (C3α), 70.2 (C4α), 75.2 (C5α), 68.0 (C6α).

4.3.1.7. Vanillyl-α-D-galactoside. Solid; UV (H_2O, λ_{max}): 198.0 nm (σ→σ*, $\varepsilon_{198.0}$–2909 M^{-1}), 281.0 nm (π→π*, $\varepsilon_{281.0}$–519 M^{-1}). IR (KBr): 3271 cm^{-1} (OH), 1261 cm^{-1} (glycosidic aryl alkyl C–O–C asymmetric), 1031 cm^{-1} (glycosidic aryl alkyl C–O–C symmetric), 1406 cm^{-1} (C=C), 1664 cm^{-1} (CO). Optical rotation (*c* 1, H_2O), $[\alpha]_D$ at 25 °C = +8.82. MS (*m/z*) 314 $[M]^+$. 2D-HSQCT (DMSO-d_6): *C1-O-vanillyl-α-D-galactopyranoside*: ^1H NMR δ_{ppm} (500.13 MHz): 4.22 (H-1α), 3.69 (H-2α), 3.52 (H-3α), 3.48 (H-4α), 3.43 (H- 5α), 3.35 (H-6α), 7.38 (H-2′), 6.90 (H-5′), 7.33 (H-6′), 3.86 (OCH_3), 9.74 (CHO); ^{13}C NMR δ_{ppm} (125 MHz): 95.8 (C1α), 69.4 (C2α), 69.9 (C3α), 70.8 (C4α), 71.1 (C5α), 62.0 (C6α), 129.2 (C1′), 111.3 (C2′), 148.6 (C3′), 153.5 (C4′), 115.9 (C5′), 126.4 (C6′), 56.1 (OCH_3), 191.4 (CHO).

4.3.1.8. Vanillyl-α-D-mannoside. Solid; UV (H_2O, λ_{max}): 198.5 nm (σ→σ*, $\varepsilon_{198.5}$–3401 M^{-1}), 278.0 nm (π→π*, $\varepsilon_{278.0}$–284 M^{-1}). IR (KBr): 3365 cm^{-1} (OH), 1249 cm^{-1} (glycosidic aryl alkyl C–O– C asymmetric), 1030 cm^{-1} (glycosidic aryl alkyl C–O–C symmetric), 1406 cm^{-1} (C=C), 1651 cm^{-1} (CO), 2940 cm^{-1} (CH). Optical rotation (*c* 1, H_2O): $[\alpha]_D$ at 25 °C = –3.6. MS (*m/z*) 314 $[M]^+$. *C1-O-vanillyl-α-D-mannopyranoside*: ^{13}C NMR (DMSO-d_6) δ_{ppm} (125 MHz): 100.8 (C1α), 70.5 (C2α), 71.3 (C3α), 67.1 (C4α), 73.8 (C5α), 61.3 (C6α), 109.4 (C2′), 114.72 (C5′), 121.9 (C6′).

4.3.1.9. Vanillyl maltoside. Solid; UV (H_2O, λ_{max}): 194.5 nm (σ→σ*, $\varepsilon_{194.5}$–4782 M^{-1}), 278.5 nm (π→π*, $\varepsilon_{278.5}$–328 M^{-1}). IR (KBr): 3361 cm^{-1} (OH), 1265 cm^{-1} (glycosidic aryl alkyl C–O–C asymmetric), 1024 cm^{-1} (glycosidic aryl alkyl C–O–C symmetric), 1412 cm^{-1} (C=C), 1651 cm^{-1} (CO), 2930 cm^{-1} (CH), 1205 cm^{-1} (OCH_3). Optical rotation (*c* 1, H_2O): $[\alpha]_D$ at 25 °C = +92.0. MS (*m/z*) 478 $[M + 2]^+$. 2D-HSQCT (DMSO-d_6): *C1-O-vanillyl-α-maltoside*: ^1H NMR δ_{ppm} (500.13 MHz): 4.68 (H-1α), 3.10 (H-2α), 3.20 (H-3α), 3.30 (H-4α), 3.72 (H-5α), 3.48 (H-6α), 4.94 (H-1″α, 5.7 Hz), 3.25 (H-2″), 2.88 (H-3″), 3.65 (H-4″), 3.60 (H-6″), 6.26 (H-2′), 6.62 (H-5′), 7.18 (H-6′), 3.73 (OCH_3); ^{13}C NMR δ_{ppm} (125 MHz): 98.2 (C1α), 70.1 (C2α), 75.1 (C3α), 79.1 (C4α), 69.8 (C5α), 60.8 (C6α), 100.3 (C1″α), 73.8 (C2″), 74.5 (C3″), 70.0 (C4″), 60.8 (C6″), 130.0 (C1′), 109.5 (C2′), 114.2 (C5′), 126.8 (C6′). *C6-O-vanillyl maltose*: ^1H NMR δ_{ppm}: 4.88 (H-1α), 3.54 (H-6α), 4.94 (H-1″α); ^{13}C NMR δ_{ppm}: 92.4 (C1α), 67.2 (C6α), 100.3 (C1″α). *C6″-O-vanillyl maltose*: ^1H NMR δ_{ppm}: 4.88 (H-1α), 4.94 (H-1″α), 3.69 (H-6″), ^{13}C NMR δ_{ppm}: 92.4 (C1α), 100.3 (C1″α), 66.1 (C6″).

4.3.1.10. Vanillyl sucrose. Solid; UV (H_2O, λ_{max}): 194.0 nm (σ→σ*, $\varepsilon_{194.0}$–6820 M^{-1}), 278.5 nm (π→π*, $\varepsilon_{278.5}$–423 M^{-1}). IR (KBr): 3374 cm^{-1} (OH), 1254 cm^{-1} (glycosidic aryl alkyl C–O–C asymmetric), 1026 cm^{-1} (glycosidic aryl alkyl C–O–C symmetric), 1412 cm^{-1} (C=C), 1650 cm^{-1} (CO), 2936 cm^{-1} (CH), 1211 cm^{-1} (OCH_3). Optical rotation (*c* 1, H_2O): $[\alpha]_D$ at 25 °C = +48.6. MS (*m/z*) 476 $[M]^+$. 2D-HSQCT (DMSO-d_6): *C1-O-vanillyl sucrose*: ^1H NMR δ_{ppm} (500.13 MHz): 3.49 (H-1), 3.88 (H-3), 3.89 (H-4), 3.86 (H-5), 3.4 (H-6), 4.72 (H-1″α), 3.68 (H-2″), 3.46 (H-3″), 3.62 (H-4″), 3.65 (H-5″), 3.59 (H-6″), 7.22 (H-2′), 6.60 (H-5′), 8.35 (H-6′); ^{13}C NMR δ_{ppm} (125 MHz): 66.0 (C1), 76.8 (C3), 80.9 (C4), 81.5 (C5), 62.2 (C6), 98.5 (C1″α), 71.0 (C2″), 72.2 (C3″), 69.8 (C4″), 72.1 (C5″), 60.5 (C6″), 112.8 (C5′), 126.3 (C6′). *C6″-O-vanillyl*

sucrose: ^{1}H NMR δ_{ppm}: 3.48 (H-1), 3.67 (H-3), 3.57 (H-5), 3.46 (H-6), 4.63 (H-1″α), 3.08(H-2″), 3.42 (H-3″), 3.15 (H-4″), 3.19 (H-5″), 3.72 (H-6″); ^{13}C NMR δ_{ppm}: 62.3 (C1), 76.5 (C3), 82.2 (C5), 60.5 (C6), 98.6 (C1″α), 69.9 (C2″), 72.3 (C3″), 69.8 (C4″), 71.5 (C5″), 66.1 (C6″).

4.3.1.11. Vanillyl-D-sorbitol. Solid; UV (H_2O, λ_{max}): 193.5 nm (σ→σ*, $\varepsilon_{193.5}$–2940 M^{-1}), 273.0 nm (π→π*, $\varepsilon_{273.0}$–290 M^{-1}). IR (KBr): 3386 cm^{-1} (OH), 1260 cm^{-1} (glycosidic aryl alkyl C–O–C asymmetric), 1038 cm^{-1} (glycosidic aryl alkyl C–O–C symmetric), 1409 cm^{-1} (C=C), 2943 cm^{-1} (CH). Optical rotation (*c* 1, H_2O): $[\alpha]_D$ at 25 °C = +13.9. MS (*m/z*) mono-arylated 316 $[M]^+$, di-arylated 451 $[M + 1]^+$. 2D-HSQCT (DMSO-d_6): *C1-O-vanillyl-D-sorbitol*: ^{1}H NMR δ_{ppm} (500.13 MHz): 3.65 (H-1), 3.37 (H-2), 3.48 (H-3), 3.57 (H-4), 3.54 (H-5), 3.58 (H-6), 7.40 (H-2′), 7.20 (H-5′), 7.58 (H-6′), 3.81 (OCH_3), 9.75 (CHO); ^{13}C NMR δ_{ppm} (125 MHz): 67.2 (C1), 70.5 (C2), 74.1 (C3), 71.2 (C4), 69.0 (C5), 62.9 (C6), 130.5 (C1′), 111.2 (C2′), 153.8 (C4′), 111.1 (C5′), 124.5 (C6′), 55.9 (OCH_3), 191.5 (CHO). *C6-O-vanillyl-D-sorbitol*: ^{1}H NMR δ_{ppm}: 3.55 (H-1), 3.54 (H-2), 3.44 (H-3), 3.68 (H-4), 3.46 (H-5), 3.58 (H-6), 6.88 (H-2′), 6.85 (H-5′), 7.39 (H-6′); ^{13}C NMR δ_{ppm}: 63.2 (C1), 70.8 (C2), 72.5 (C3), 73.1 (C4), 68.2 (C5), 66.2 (C6), 129.1 (C1′), 110.8 (C2′), 153.8 (C4′), 111.4 (C5′), 126.1 (C6′). *C1, C6 Di-O-vanillyl-D-sorbitol*: ^{1}H NMR δ_{ppm}: 3.46 (H-1), 3.35 (H-2), 3.36 (H-3), 3.54 (H-4), 3.65 (H-6), 6.65, 6.68 (H-5′), 6.69, 6.84 (H-6′); ^{13}C NMR δ_{ppm}: 66.5 (C1), 67.0 (C2), 73.5 (C3), 76.1 (C4), 65.5 (C6), 129.1, 130.5 (C1′), 111.3,111.7 (C2′), 153.5, 153.5 (C4′), 115.3, 115.9 (C5′), 119.8, 120.3 (C6′).

4.3.1.12. Curcuminyl-bis-α-D-glucoside. Solid; UV (H_2O, λ_{max}): 210 nm (σ→σ*, ε_{210}–1242 M^{-1}), 252 nm (π→π*, ε_{252}–1537 M^{-1}), 430 nm (π→π*extended conjugation, ε_{430} – 42 M^{-1}). IR (KBr): 1664 cm^{-1} (CO), 1027 cm^{-1} (aryl alkyl C-O-C symmetrical), 1254 cm^{-1} (aryl alkyl C-O-C asymmetrical). Optical rotation (C 1, H_2O): $[\alpha]_D$ at 25 °C = +30.3°. MS (*m/z*) 691 $[M-1]^+$. *C1-O-curcuminyl-bis-α-D-glucopyranoside*: ^{1}H NMR δ_{ppm} (500.13 MHz): 4.66 (H-1α), 3.15 (H-2α), 3.73 (H-3α), 3.75 (H-4α), 3.53 (H-6α). 3.85 (6H, s, 2-OCH_3), 6.06 (1H, s, H_1), 6.71 (2H, d, J = 15.8Hz, $H_{3,\ 3'}$), 7.51(2H, d, J = 15.8, $H_{4,\ 4'}$), 7.25 (2H, s, $H_{6,\ 6'}$), 6.81 (2H, d, J = 8.2Hz, $H_{9,\ 9'}$), 7.11 (2 H, dd, J = 1.45 Hz, $H_{10,\ 10'}$); ^{13}C NMR δ_{ppm} (125 MHz): 99.0 (C1α), 72.2 (C2α), 73.6(C3α), 70.6 (C4α), 61.3 (C6α), 56.1 (OCH_3), 101.0 ($C_{1'}$), 183.5 ($C_{2'}C_{2'}$), 121.5 (C_3,$C_{3'}$), 141.0 (C_4,$C_{4'}$), 126.8 (C_5,$C_{5'}$), 111.6 (C_6,$C_{6'}$), 148.4 (C_7,$C_{7'}$), 150.0 (C_8,$C_{8'}$), 116.1 (C_9,$C_{9'}$), 123.4 (C_{10},$C_{10'}$). *C6-O-curcuminyl-bis-α-D-glucose*: ^{1}H NMR δ_{ppm}: 3.25 (H-2α), 3.65 (H-4α), 3.52, 3.70 (H-6α a & b), 6.81 ($H_{3,\ 3'}$), 7.10 ($H_{9,\ 9'}$); ^{13}C NMR δ_{ppm}: 75.0 (C2α), 70.5 (C4α), 66.5 (C6α), 123.0 (C_3, $C_{3'}$), 116 (C_9,$C_{9'}$).

4.4. Esterification procedure

A general procedure employed for the esterification reaction involved reacting 0.001–0.008 mol unprotected L-amino acid (L-alanine, L-valine, L-leucine and L-isoleucine, L-proline, L-phenylalanine, L-tryptophan and L-histidine) and 0.001–0.002 mol of carbohydrate (D-glucose, D-galactose, D-fructose, D-ribose, lactose, maltose, D-sorbitol and D-mannitol) along with 100 ml CH_2Cl_2/DMF (90:10 v/v, 40 °C) or hexane/$CHCl_3$/DMF (45:45:10 v/v, 61 °C) in presence of 0.60–0.180 g of lipases (40–50% w/w carbohydrate employed) under reflux for a period of three days in a flat bottom two necked flask. *Rhizomucor miehei* lipase (RML) in presence of 0.1 mM (0.1 ml), pH 4.0, acetate buffer (L-alanyl carbohydrate esters), *Candida rugosa* lipase (CRL) in presence of 0.1 mM (0.1 ml), pH 7.0, phosphate buffer (L-valyl, L-leucyl, and L-isoleucyl esters of carbohydrates) and CRL in presence of 0.2 mM (0.2 ml), pH 4.0, acetate buffer (L-prolyl, L-phenylalanyl, L-tryptophanyl and L-histidyl esters of carbohydrates) were employed. The condensed vapor of solvents which formed an azeotrope with water was passed through a desiccant before being returned into the reaction mixture, thereby facilitating complete removal of water of reaction [18] and also maintaining a very low water activity of $a_w = 0.0054$ throughout the reaction. After completion of the reaction, the solvent was distilled off, 20–30 ml of warm water was added, stirred and filtered to remove the lipase. The filtrate was evaporated to get a mixture of the unreacted carbohydrate, unreacted L-amino acids and the product esters, which were then analyzed by HPLC. A Shimadzu LC10AT HPLC connected to LiChrosorb RP-18 column (5 μm particle size, 4.6 × 150 mm length) with acetonitrile/water (v/v 20:80) as a mobile phase at a flow rate of 1 ml min^{-1} was employed using an UV detector at 210 nm in case of L-alanyl, L-valyl, L-leucyl, L-isoleucyl and L-prolyl esters and at 254 nm in case of L-phenylalanyl, L-tryptophanyl and L-histidyl esters of carbohydrates. The conversion yields were determined with respect to peak areas of L-amino acids and those of the esters. The esters formed were separated by size exclusion chromatography using Sephadex G-10.

4.4.1. Amino acyl esters of carbohydrates

4.4.1.1. L-Alanyl-β-D-glucose. Solid; UV (H_2O, λ_{max}): 227.0 nm (σ→σ* $\varepsilon_{227.0}$–1151 M^{-1}), 294.0 nm (*n*→π* $\varepsilon_{294.0}$–764 M^{-1}). IR (KBr): 3371 cm^{-1} (NH), 3410 cm^{-1} (OH), 2297 cm^{-1} (CH), 1653 cm^{-1} (CO). Optical rotation (*c* 0.5, H_2O): $[\alpha]_D$ at 25 °C = -38.1°. MS (*m/z*) 274 $[M + Na]^+$. 2D-HSQCT (DMSO-d_6) *2-O-ester*: ^{1}H NMR δ_{ppm} (500.13 MHz): 2.95 (αCH), 1.07 (βCH_3), 3.62 (H-2β), 3.83 (H-3β), 3.67 (H-4β), 3.44 (H-6β); ^{13}C NMR δ_{ppm} (125 MHz): 52.1 (αCH), 15.7 (βCH_3), 102.8 (C1β), 82.6 (C2β), 77.9 (C3β), 68.8 (C4β), 60.5 (C6β). *3-O-ester*: ^{1}H NMR δ_{ppm}: 2.87 (αCH), 3.93 (H-3β), 3.58 (H-4β), 3.36 (H-6β); ^{13}C NMR δ_{ppm}: 51.4 (αCH), 83.3 (C3β), 69.3 (C4β), 57.3 (C6β). *6-O- ester*: ^{1}H NMR δ_{ppm}: 2.95 (αCH), 1.30 (βCH_3), 3.86 (H-2β), 3.76 (H-5β), 3.82 (H-6β); ^{13}C NMR δ_{ppm}: 50.2 (αCH), 15.1 (βCH_3), 171.4 (CO), 101.8 (C1β), 75.0 (C2β), 70.1 (C5β), 63.5 (C6β). *2,6-di-O-ester*: ^{1}H NMR δ_{ppm}: 3.36 (αCH), 1.30 (βCH_3), 3.78 (H-2β), 3.47 (H-6β); ^{13}C NMR δ_{ppm}: 49.5 (αCH), 16.4 (βCH_3), 100.8 (C1β), 76.5 (C2β), 62.7 (C6β). *3,6-di-O- ester*: ^{1}H NMR δ_{ppm}:

1.30 (βCH$_3$), 3.78 (H-3β), 3.82 (H-6β); ^{13}C NMR δ_{ppm}: 51.4 (αCH), 16.7 (βCH$_3$), 81.6 (C3β), 63.1 (C6β).

4.4.1.2. L-Alanyl-D-ribose. Solid; UV (H_2O, λ_{max}): 224.0 nm (σ→σ* $\varepsilon_{224.0}$–3802 M^{-1}), 294.0 nm (*n*→π* $\varepsilon_{294.0}$–1288 M^{-1}). IR (KBr): 3402 cm^{-1} (NH), 3242 cm^{-1} (OH), 2887 cm^{-1} (CH), 1625 cm^{-1} (CO). Optical rotation (*c* 0.5, H_2O): $[\alpha]_D$ at 25 °C = +22.0°. MS (*m*/*z*) 221 $[M]^+$; 2D-HSQCT (DMSO-*d*$_6$) *2-O-ester*: ^{1}H NMR δ_{ppm} (500.13 MHz): 1.25 (αCH), 3.12 (βCH$_3$), 3.67 (H-2α), 3.50 (H-3α), 3.63 (H-4α), 3.64 (H-5α); ^{13}C NMR δ_{ppm} (125 MHz): 48.2 (αCH), 15.9 (βCH$_3$), 75.7 (C2α), 67.2 (C3α), 68.1 (C4α), 60.6 (C5α). *5-O- ester*: ^{1}H NMR δ_{ppm}: 3.39 (αCH), 1.25 (βCH$_3$), 4.95 (H-1α), 4.20 (H-1β), 3.27 (H-3α), 3.88 (H-4α), 3.61(H-5α); ^{13}C NMR δ_{ppm}: 53.0 (αCH), 18.5 (βCH$_3$), 173.5 (CO), 101.6 (C1α), 103.9 (C1β), 75.0 (C3α), 71.0 (C4α), 63.4 (C5α). *2,5-di-O-ester*: ^{1}H NMR δ_{ppm}: 1.20 (βCH$_3$), 3.45 (H-2α), 3.79 (H-4α), 3.52 (H-5α); ^{13}C NMR δ_{ppm}: 18.5 (βCH$_3$), 74.9 (C2α), 71.0 (C3α), 63.4 (C5α).

4.4.1.3. L-Alanyl lactose. Solid; UV (H_2O, λ_{max}): 220.0 nm (σ→σ* $\varepsilon_{220.0}$–436 M^{-1}), 294.0 nm (*n*→π* $\varepsilon_{294.0}$–240 M^{-1}). IR (KBr): 3378 cm^{-1} (NH), 3378 cm^{-1} (OH), 2946 cm^{-1} (CH), 1624 cm^{-1} (CO). Optical rotation (*c* 0.5, H_2O): $[\alpha]_D$ at 25 °C = + 7.4°. MS (*m*/*z*) 436 $[M + Na]^+$. 2D-HSQCT (DMSO-*d*$_6$) *6-O-ester*: ^{1}H NMR δ_{ppm} (500.13 MHz): 3.55 (αCH), 1.25 (βCH$_3$), 4.78 (H-1α), 4.82 (H-1β), 2.95 (H-2α), 3.25 (H-2β), 2.95 (H-3α), 4.05 (H-4α,β), 3.15 (H-5α), 3.35 (H-5β), 3.80 (H-6α,β), 4.90 (H-1″β), 3.90 (H-2″), 2.85 (H-3″), 3.70 (H-4″), 3.60 (H-5″), 3.40 (H-6″); ^{13}C NMR δ_{ppm} (125 MHz): 51.0 (αCH), 15.5 (βCH$_3$), 173.0 (CO), 98.0 (C1α), 100.2 (C1β), 70.3 (C2α), 72.4 (C2β), 74.3 (C3α), 81.0 (C4α,β), 73.3 (C5α), 73.4 (C5β), 61.2 (C6α,β), 100.2 (C1″β), 76.5 (C2″), 75.1 (C3″), 68.5 (C4″), 78.5 (C5″), 60.6 (C6″). *6″-O-ester*: ^{1}H NMR δ_{ppm}: 3.35 (αCH), 3.85 (H-4α), 3.70 (H-6″); ^{13}C NMR δ_{ppm}: 53.5 (αCH), 81.5 (C4α), 64.0 (C6″). *6,6″-di-O- ester*: ^{1}H NMR δ_{ppm} (500.13 MHz): 3.85 (H-6″), ^{13}C NMR δ_{ppm} (125 MHz): 67.5 (C6″).

4.4.1.4. L-Valyl-D-glucose. Solid; UV (H_2O, λ_{max}): 200.0 nm (σ→σ* $\varepsilon_{200.0}$–2630 M^{-1}), 295.0 nm (*n*→π* $\varepsilon_{295.0}$–2089 M^{-1}). IR (KBr): cm^{-1} 2889 (NH), 3407 cm^{-1} (OH), 2950 cm^{-1} (CH), 1622 cm^{-1}(CO). Optical rotation (*c* 0.5, H_2O): $[\alpha]_D$ at 25 °C = +8.8°. MS (*m*/*z*) 302 $[M + Na]^+$. 2D-HSQCT (DMSO-*d*$_6$) *2-O-ester*: ^{1}H NMR δ_{ppm} (500.13 MHz): 3.30 (αCH), 1.90 (βCH), 0.98 (γCH$_3$), 0.98 (δCH$_3$), 3.83 (H-2α), 3.65 (H-2β); ^{13}C NMR δ_{ppm} (125 MHz): 53.2 (αCH), 29.2 (βCH), 18.1 (γCH$_3$), 18.1 (δCH$_3$), 76.1 (C2α), 60.0 (C6α). *3-O- ester*: ^{1}H NMR δ_{ppm}: 3.10 (αCH), 0.94 (γCH$_3$), 3.89 (H-3α), 4.01 (H-3β), 3.33 (H-6α,β); ^{13}C NMR δ_{ppm}: 52.4 (αCH), 9.39 (γCH$_3$), 82.9 (C3α), 83.4 (C3β), 60.3 (C6α,β). *6-O-ester*: ^{1}H NMR δ_{ppm}: 3.20 (αCH), 2.01 (βCH), 0.90 (γ CH$_3$), 4.95 (H-1α), 4.22 (H-1β), 3.17 (H-4α), 3.0 (H-4β), 3.86 (H-6α); ^{13}C NMR δ_{ppm}: 51.9 (αCH), 21.0 (βCH), 8.94 (γ CH$_3$), 95.2 (C1α), 104.5(C1β), 69.5 (C4α), 69.8 (C4β), 63.4 (C6α). *2,6-di-O-ester*: ^{1}H NMR δ_{ppm}: 3.15 (αCH), 3.75 (H-2α), 3.64 (H-6β); ^{13}C NMR δ_{ppm}: 51.7 (αCH), 78.7 (C2α), 61.6 (C6α). *3,6-di-O-ester*: ^{1}H NMR δ_{ppm}: 3.21 (αCH), 1.55 (γ CH$_3$), 3.67 (H-3β), 3.15 (H-6α,β); ^{13}C NMR δ_{ppm}: 49.4 (αCH), 78.6 (C3β), 61.3 (C6α,β).

4.4.1.5. .L-Valyl-D-fructose. Solid; UV (H_2O, λ_{max}): 223.0 nm (σ→σ* $\varepsilon_{223.0}$–53 M^{-1}), 288.0 nm (*n*→π* $\varepsilon_{288.0}$–23 M^{-1}). IR (KBr): 3352 cm^{-1}(NH), 3290 cm^{-1}(OH), 2946 cm^{-1}(CH), 1623 cm^{-1}(CO). Optical rotation (*c* 0.5, H_2O): $[\alpha]_D$ at 25 °C = -6.7°. MS (*m*/*z*) 304 $[M + 2 + Na]^+$. 2D-HSQCT (DMSO-*d*$_6$) *1-O-ester*: ^{1}H NMR δ_{ppm} (500.13 MHz): 3.30 (αCH), 1.90 (βCH), 0.98 (γCH$_3$), 0.98 (δCH$_3$), 3.80 (H-1α), 4.85 (H-2β), 3.85 (H-3α), 3.30 (H-6α); ^{13}C NMR δ_{ppm} (125 MHz): 3.30 (αCH), 1.90 (βCH), 0.98 (γCH$_3$), 0.98 (δCH$_3$), 175.0 (CO), 63.5 (C1α), 102.4 (C2β), 70.8 (C3α), 62.8 (C6α).

4.4.1.6. L-Valyl-maltose. Solid; UV (H_2O, λ_{max}): 221.0 nm (σ→σ* $\varepsilon_{221.0}$–1622 M^{-1}), 291.0 nm (*n*→π* $\varepsilon_{291.0}$–776 M^{-1}). IR (stretching frequency): 3419 cm^{-1} (NH), 3267 cm^{-1} (OH), 2936 cm^{-1} (CH), 1634 cm^{-1} (CO). Optical rotation (*c* 0.5, H_2O): $[\alpha]_D$ at 25 °C = +341.7°. MS (*m*/*z*) 464 $[M + Na]^+$. 2D-HSQCT (DMSO-*d*$_6$) *6-O-ester*: ^{1}H NMR δ_{ppm} (500.13 MHz): 3.05 (αCH), 2.25 (βCH), 0.99 (γ,δCH$_3$), 4.80 (H-1α), 4.20 (H-1β), 4.05 (H-2α,β), 3.30 (H-3α), 3.85 (H-4α,β), 3.65 (H-5α,β), 3.94 (H-6α,β), 4.90 (H-1″α), 2.95 (H-2″), 3.10 (H-3″), 3.50 (H-4″), 3.60 (H-5″), 3.60 (H-6″); ^{13}C NMR δ_{ppm} (125 MHz): 51.0 (αCH), 28.9 (βCH), 18.9 (γ,δCH$_3$), 175.5 (CO), 97.0 (C1α), 100.5 (C1β), 79.7 (C2α,β), 76.4 (C3α), 81.5 (C4α,β), 77.2 (C5α,β), 67.2 (C6α,β), 100.7 (C1″α), 70.3 (C2″), 71.8 (C3″), 69.9 (C4″), 72.4 (C5″), 60.6 (C6″); *6″-O-ester*: ^{1}H NMR δ_{ppm}: 2.99 (αCH), 3.81 (H-6″); ^{13}C NMR δ_{ppm} (125 MHz): 52.5 (αCH), 68.0 (C6″). *6,6″-di-O- ester*: ^{1}H NMR δ_{ppm} (500.13 MHz): 3.81 (H-6″); ^{13}C NMR δ_{ppm} (125 MHz): 64.0 (C6″).

4.4.1.7. L-Valyl-D-mannitol. Solid; UV (H_2O, λ_{max}): 225.0 nm (σ→σ* $\varepsilon_{225.0}$–372 M^{-1}), 270.0 nm (*n*→π* $\varepsilon_{270.0}$–178 M^{-1}). IR (KBr): 3294 cm^{-1} (OH), 2957 cm^{-1} (CH), 1630 cm^{-1} (CO). Optical rotation (*c* 0.5, H_2O): $[\alpha]_D$ at 25 °C = +6.6°. MS (*m*/*z*) 464 $[M + Na]^+$. 2D-HSQCT (DMSO-d_6): *1-O-ester*: ^{1}H NMR δ_{ppm} (500.13 MHz): 3.42 (αCH), 2.05 (βCH), 0.85 (γCH$_3$), 3.52 (H-1), 3.46(H-2), 3.53 (H-3), 3.56 (H-4), 3.41 (H-5), 3.46(H-6); ^{13}C NMR δ_{ppm} (125 MHz): 55.8(αCH), 29.8(βCH$_2$), 19.8 (γCH$_3$), 60.6(C1), 69.6(C2), 68.2(C3), 71.3 (C4), 71.2(C5) 63.6(C6).

4.4.1.8. L-Leucyl-D-glucose. Solid; UV (H_2O, λ_{max}): 221.0 nm (σ→σ* $\varepsilon_{221.0}$–1622 M^{-1}), 291.0 nm (*n*→π* $\varepsilon_{291.0}$–776.2 M^{-1}). IR (KBr): 3419 cm^{-1} (NH), 3267 cm^{-1} (OH), 2936 cm^{-1} (CH), 1634 cm^{-1} (CO). Optical rotation (*c* 0.5, H_2O): $[\alpha]_D$ at 25 °C = +34.7°, MS (*m*/*z*) 464 $[M + Na]^+$. 2D-HSQCT (DMSO-d_6): *2-O-ester*: ^{1}H δ_{ppm} (500.13 MHz): 2.8 (αCH), 0.75 (δ, ε CH$_3$), 3.79 (H-2α); ^{13}C δ_{ppm} (125 MHz): 46.1 (αCH), 23.4 (δCH$_3$), 23.9 (εCH$_3$), 76.9 (C2α). *3-O-ester*: ^{1}H δ_{ppm}: 3.15 (αCH), 1.5 (γCH), 0.77 (δ,εCH$_3$), 3.86 (H-2β), 3.97 (H-3α), 3.88 (H-3β); ^{13}C δ_{ppm}: 50.0 (αCH), 25.0 (γCH), 23.3 (δCH3), 23.1 (εCH3), 84.0 (C3α), 83.2 (C3β). *6-O-ester*: ^{1}H δ_{ppm}: 3.08 (αCH), 2.51 (βCH$_2$), 1.57 (γCH), 0.81 (δ, ε CH$_3$), 3.86 (H-6α); ^{13}C δ_{ppm}:

53.5 (αCH), 36.0 (βCH_2), 25.3 (γCH), 22.5 (δ-CH_3), 23.0 (εCH_3), 173.6 (CO), 102.5 (C1α), 65.0 (C6α). *2,6-di-O-ester*: ^{1}H δ_{ppm}: 3.45 (H-6α), 3.44 (H-6α); ^{13}C δ_{ppm}: 75.5 (C2α), 62.8 (C6α). *3,6-di-O-ester*: ^{1}H δ_{ppm}: 3.68 (H- 3α), 3.45 (H-6α); ^{13}C δ_{ppm}: 82.5 (C3α), 63.1 (C6α).

4.4.1.9. L-Isoleucyl-D-glucose. Solid; UV (H_2O, λ_{max}): 230.0 nm (σ→σ* $\varepsilon_{230.0}$–724 M^{-1}), 297.0 nm (*n*→π* $\varepsilon_{297.0}$–363 M^{-1}). IR (KBr): 3383 cm^{-1} (NH), 3360 cm^{-1} (OH), 2240 cm^{-1} (CH), 1657 cm^{-1} (CO). Optical rotation (*c* 0.5, H_2O): $[\alpha]_D$ at 25 °C = –3.1°. MS (*m*/*z*) 316 $[M + Na]^+$. 2D-HSQCT (DMSO-d_6): *3-O-ester*: ^{1}H δ_{ppm}: 3.13 (αCH), 1.61 (βCH), 1.08 (γCH_2), 0.59 (δ CH_3), 0.61 (ε CH_3), 5.0 (H-1α), 4.4 (H-1β), 4.01 (H-3α), 3.88 (H-3β), 3.46 (H-4α), 3.58(H-6α,β); ^{13}C δ_{ppm}: 51.0 (αCH), 35.4 (βCH), 25.6 (γCH_2), 11.2 (δ CH_3), 14.0 (ε CH_3), 171.4 (CO), 91.8 (C1α), 95.8 (C1β), 82.0 (C3α), 81.9 (C3β), 67.5 (C4α), 63.0 (C6α,β). *6-O-ester*: ^{1}H δ_{ppm}: 3.11 (αCH), 1.61 (βCH), 1.08 (γCH_2), 0.59 (δ CH_3), 0.61 (ε CH_3), 3.59 (H-2α), 3.48 (H-3α), 3.64 (H-4α), 3.63 (H-5α), 3.82 (H-6α,β); ^{13}C δ_{ppm}: 53.1 (αCH), 35.3 (βCH), 25.2 (γCH_2), 11.2 (δ CH_3), 14.0 (ε CH_3), 70.0 (C2α), 72.2 (C3α), 69.0 (C4α), 69.2 (C5α), 63.6 (C6α,β).

4.4.1.10. L-Prolyl-D-glucose. Solid; UV(H_2O, λ_{max}): 200 nm (σ→σ* ε_{200}–1862 M^{-1}), 285 nm (*n*→π* ε_{285}–512 M^{-1}). IR (KBr): 3261 cm^{-1} (OH), 1631 cm^{-1} (CO), 1384 cm^{-1} (CN). Optical rotation (*c* 0.6, H_2O): $[\alpha]_D$ at 25 °C = 19.6°. MS (*m*/*z*) 302 $[M + 2 + Na]^+$. 2D-HSQCT (DMSO-d_6): *2-O-ester*: ^{1}H NMR δ_{ppm} (500.13 MHz): 3.85 (αCH), 2.84 (βCH_2), 3.75 (H-2α), 3.63 (H-2β), 3.55 (H-6α,β); ^{13}C NMR δ_{ppm} (125 MHz): 58.0 (αCH), 32.0 (βCH_2), 75.0 (C2α), 80.0 (C2β), 61.5 (C6α,β). *3-O-ester*: ^{1}H NMR δ_{ppm}: 3.46 (αCH), 2.84 (βCH_2), 2.20 (δCH_2), 3.84 (H-3α), 3.93 (H-3β), 3.46 (H-6α,β); ^{13}C NMR δ_{ppm}: 53.0 (αCH), 32.0 (β-CH_2), 28.0 (δ-CH_2), 98.5 (C1α), 82.4 (C3α), 84.0 (C3β), 61.0 (C6α,β). *6-O-ester*: ^{1}H NMR δ_{ppm}: 3.75 (αCH), 2.85 (βCH_2), 1.94 (γCH_2), 3.15 (δCH_2), 4.38 (H-1α), 4.20 (H-1β), 3.38 (H-3α), 4.20 (H-4α), 3.90 (H-4β), 3.82 (H-6α,β); ^{13}C NMR δ_{ppm}: 56.0 (αCH), 35.0 (βCH_2), 28.0 (γCH_2), 46.0 (δCH_2), 171.6 (CO), 95.2 (C1α), 101.4 (C1β), 72.0 (C3α), 71.0 (C4αβ), 63.6 (C6α,β).

4.4.1.11. L-Prolyl-D-fructose:. Solid; UV (H_2O, λ_{max}): 213 nm (σ→σ* ε_{213}–1479 M^{-1}), 280 nm (*n*→π* ε_{280}–145 M^{-1}). IR (KBr): 3070 cm^{-1} (OH), 1604 cm^{-1} (CO), 1402 cm^{-1} (CN). Optical rotation (*c* 0.5, H_2O): $[\alpha]_D$ at 25 °C = –44.0°. MS (*m*/*z*) 300 $[M + Na]^+$. 2D-HSQCT (DMSO-d_6): *1-O-ester*: ^{1}H NMR δ_{ppm} (500.13 MHz): 3.15 (αCH), 2.72 (βCH_2), 2.08 (γCH_2), 3.12 (δCH_2), 4.13 (H-1α), 3.13 (H-3α), 3.40 (H-3β), 3.80 (H-4α), 3.62 (H-4β), 3.30 (H-5α), 3.93 (H-5β), 3.23 (H-6α,β); ^{13}C NMR δ_{ppm} (125 MHz): 60.0 (αCH), 31.0 (βCH_2), 24.0 (γCH_2), 48.2 (δCH_2), 170.8 (CO), 66.1 (C1αβ), 104.2 (C1α), 71.4 (C3α), 82.1 (C3β), 69.9 (C4α), 78.2 (C4β), 74.0 (C5α), 82.9 (C5β), 63.7 (C6α,β). *6-O- ester*: ^{1}H NMR δ_{ppm}: 3.32 (αCH), 3.33 (δCH_2), 3.78 (H-1α), 3.78 (H-3α), 3.29 (H-3β), 3.38 (H-5α), 4.12 (H-5β), 4.02 (H-6α,β); ^{13}C NMR δ_{ppm}: 59.2 (αCH), 49.3 (δCH_2), 64.4 (C1α), 99.1 (C2α), 71.9 (C3α), 70.7 (C4α), 75.2 (C5α), 81.9 (C5β), 65.8 (C6α,β). *1,6-di-O-ester*: ^{1}H NMR δ_{ppm}: 2.91 (αCH), 4.37 (H-1α), 3.42 (H-3α), 3.54 (H-5α), 4.28 (H-6α,β); ^{13}C NMR δ_{ppm}: 60.0 (αCH), 66.5 (C1αβ), 102.0 (C2α), 70.1 (C3α), 75.6 (C5α), 66.2 (C6α,β).

4.4.1.12. L-Prolyl-D-ribose. Solid; UV (H_2O, λ_{max}): 210 nm (σ→σ* ε_{210}–1820 M^{-1}) and 280 nm (*n*→π* ε_{280}–575 M^{-1}). IR (KBr): 3307 cm^{-1} (OH), 1621 cm^{-1} (CO), 1403 cm^{-1} (CN). Optical rotation (*c* 0.5, H_2O): $[\alpha]_D$ at 25 °C = –45.1°. MS (*m*/*z*) 272 $[M + Na]^+$. 2D-HSQCT (DMSO-d_6): *2-O-ester*: ^{1}H NMR δ_{ppm} (500.13 MHz): 3.52 (αCH), 2.53 (βCH_2), 1.78 (γCH_2), 3.30 (δCH_2), 4.20 (H-1α), 3.55 (H-2α), 3.45 (H-4α), 3.45 (H-5α,β); ^{13}C NMR δ_{ppm} (125 MHz): 61.0 (αCH), 34.5 (βCH_2), 23.7 (γCH_2), 54.0 (δCH_2), 170.9 (CO), 103.8 (C1α), 71.0 (C2α), 73.0 (C4β), 63.0 (C5α,β). *5-O- ester*: ^{1}H NMR δ_{ppm}: 3.62 (αCH), 2.88 (βCH_{2a}), 1.90 (γCH_2), 3.08 (δCH_2), 3.32 (H-2α), 3.42 (H-3α), 3.45 (H-4α), 3.20 (H-5α,β); ^{13}C NMR δ_{ppm}: 61.2 (αCH), 35.8 (βCH_2), 172.0 (CO), 97.0 (C1α), 73.2 (C2α), 66.5 (C3α), 75.0 (C4α), 65.8 (C5α,β).

4.4.1.13. L-Prolyl-lactose. Solid; UV (H_2O, λ_{max}): 201 nm (σ→σ* ε_{201}–2239 M^{-1}) and 278 nm (*n*→π* ε_{278}–447 M^{-1}). IR (KBr): 3084 cm^{-1} (OH), 1609 cm^{-1} (CO), 1419 cm^{-1} (CN). Optical rotation (*c* 0.5, H_2O): $[\alpha]_D$ at 25 °C = –11.4°. MS (*m*/*z*) 462 $[M + Na]^+$. 2D-HSQCT (DMSO-d_6): *6-O-ester*: ^{1}H NMR δ_{ppm} (500.13 MHz): 3.06 (αCH), 2.72 (βCH_2), 1.73 (γCH_2), 3.01 (δCH_2), 4.12 (H-1β), 3.29 (H-2α), 3.36 (H-3α), 4.10 (H-4α), 3.84 (H-5α), 3.76 (H-5β), 3.85 (H-6α,β), 4.01 (H-1″β), 3.24 (H-2″), 3.33 (H-3″), 3.41 (H-4″), 3.36 (H-5″), 3.44 (H-6″); ^{13}C NMR δ_{ppm} (125 MHz): 59.6 (αCH), 30.4 (βCH_2), 23.8 (γCH_2), 44.5 (δCH_2), 101.4 (C1β), 70.6 (C2α), 73.8 (C3α), 81.6 (C4α), 77.1 (C5α,β), 64.8 (C6α,β), 102.9 (C1″β), 70.0 (C2″), 71.6 (C3″), 68.2 (C4″), 74.4 (C5″), 60.5 (C6″). *6″-O- ester*: ^{1}H NMR δ_{ppm}: 2.86 (αCH), 2.48 (βCH_2), 3.99 (H-4α), 3.78 (H-5α), 3.75 (H-6α,β), 3.27 (H-2″), 3.58 (H-4″), 3.38 (H-5″), 3.45 (H-6″); ^{13}C NMR δ_{ppm} (125 MHz): 60.0 (αCH), 33.2 (βCH_2), 82.4 (C4α), 77.3 (C5α,β), 60.8 (C6α,β), 70.2 (C2″), 68.9 (C4″), 75.0 (C5″), 62.8 (C6″).

4.4.1.14. L-Phenylalanyl-D-glucose. Solid; UV (H_2O, λ_{max}): 237.0 nm (σ→σ* $\varepsilon_{237.0}$–1318 M^{-1}), 257.0 nm (π→π* $\varepsilon_{257.0}$ 1259 M^{-1}) and 308.0 nm (*n*→π* $\varepsilon_{308.0}$ – 616 M^{-1}). IR (KBr): 3186 cm^{-1} (OH), 1722 cm^{-1} (CO), 1582 cm^{-1} (aromatic –C=C–). Optical rotation (*c* 0.6, H_2O): $[\alpha]_D$ at 25 °C = –24.2°. MS (*m*/*z*) 350 $[M + Na]^+$. 2D-HSQCT (DMSO-d_6): *2-O-ester*: ^{1}H NMR δ_{ppm} (500.13 MHz): 2.92 (αCH), 2.51 (βCH_{2a}), 4.6 (H-1α), 3.79 (H-2α), 3.80 (H-2β), 3.4 (H-6α); ^{13}C NMR δ_{ppm} (125 MHz): 52.0 (αCH), 35.8 (βCH_2), aromatic- 136.5 (C1), 96.3 (C1α), 75.1 (C2α), 77.3 (C2β), 62.0 (C6α). *3-O- ester*: ^{1}H NMR δ_{ppm}: 3.01 (αCH), 3.11 (βCH_{2a}), 2.96 (βCH_{2b}), 4.4 (H-1α), 3.61 (H-2α), 3.66 (H-2β), 3.82 (H-3α), 3.91 (H-3β), 3.40 (H-6α); ^{13}C NMR δ_{ppm}: 53.0 (αCH), 36.8 (βCH_2), aromatic-136.4 (C1), 97.3 (C1α), 83.4 (C3α), 83.9 (C3β), 61.9 (C6α). *6-O-ester*: ^{1}H NMR δ_{ppm}: 3.07 (αCH), 3.18 (βCH_{2a}), 3.06 (βCH_{2b}), aromatic- 7.18 (H2, H6), 7.26 (H3, H5), 7.16 (H4), 3.16 (H-5α), 3.78 (H-6α), 3.66 (H-6β); ^{13}C NMR δ_{ppm}: 54.2 (αCH), 36.7 (βCH_2), aromatic- 136.3 (C1), 128.9 (C2, C6), 130.7 (C3, C5), 130.3 (C4), 172.5 (CO), 102.2 (C1α),

70.5 (C5α), 65.0 (C6αβ). *2,6-di-O-ester*: ^{1}H NMR δ_{ppm}: 3.51 (H-6α), 3.61 (H-6β), 3.67 (H-2α); ^{13}C NMR δ_{ppm}: 77.0 (C2α), 79.0 (C2β), 62.1 (C6β). *3,6-di-O-ester*: ^{1}H NMR δ_{ppm}: 3.61 (H-3α), 3.66 (H-3β), 3.50 (H-6α); ^{13}C NMR δ_{ppm}: 82.3 (C3α), 83.4 (C3β), 64.8 (C6α).

4.4.1.15. L-Phenylalanyl-D-galactose. UV (H_2O, λ_{max}): 222.0 nm ($\sigma\rightarrow\sigma^*$ $\varepsilon_{222.0}$–871 M^{-1}), 257.5 nm ($\pi\rightarrow\pi^*$ $\varepsilon_{257.5}$ 437 M^{-1}) and 299.0 nm ($n\rightarrow\pi^*$ $\varepsilon_{299.0}$ – 331 M^{-1}). IR (KBr): 3379 cm^{-1} (OH), 1761 cm^{-1} (C=O), 1603 cm^{-1} (aromatic, –C=C–). Optical rotation (*c* 0.5, H_2O): $[\alpha]_D$ at 25 °C = +31.1°. MS (*m/z*) diester – 512[M + K]$^+$; ^{13}C NMR DMSO-d_6 δ_{ppm} (125 MHz,): *2-O-ester:* 55.7 (αCH), 36.7 (βCH_2), aromatic-136.5 (C1), 127.4 (C2, C6), 129.6 (C3, C5), 129.1 (C4), 171.4 (CO), 97.2 (C1α) 76.3 (C2α), 75.1 (C2β), 60.8 (C6α). *3-O-ester*: ^{13}C NMR δ_{ppm}: 97.2 (C1α), 82.4 (C3α), 81.5 (C3β), 61.0 (C6α). *6-O-ester*: ^{13}C NMR δ_{ppm}: 97.2 (C1α), 70.6 (C2α), 72.2 (C3α), 73.1 (C4α), 63.0 (C6α,β). *2,6-di-O-ester*: ^{13}C NMR δ_{ppm}: 77.2 (C2αβ), 63.1 (C6β). *3,6-di-O-ester*: ^{13}C NMR δ_{ppm}: 81.8 (C3α), 62.7 (C6α,β).

4.4.1.16. L-Phenylalanyl-D-fructose. Solid; UV (H_2O, λ_{max}): 198.0 nm ($\sigma\rightarrow\sigma^*$ $\varepsilon_{198.0}$–4467 M^{-1}) and 257.5 nm ($\pi\rightarrow\pi^*$ $\varepsilon_{257.5}$ 776 M^{-1}). IR: 3380 cm^{-1} (OH), 1630 cm^{-1} (CO), 1598 cm^{-1} (aromatic, –C=C–). Optical rotation (*c* 0.4, H_2O): $[\alpha]_D$ at 25 °C = –14.3°. MS (*m/z*). 365 [M – 1 + K]$^+$. ^{13}C NMR DMSO-d_6 δ_{ppm} (125 MHz): *1-O-ester*: 55.1 (αCH), aromatic-138.4 (C1), 64.9 (C1α,β), 102.4 (C2β), 76.2 (C4β), 69.6 (C5β), 62.3 (C6α,β). *6-O-ester*: ^{13}C NMR δ_{ppm}: 55.0 (αCH), 63.4 (C1α,β), 98.1 (C2β), 75.8 (C4β), 81.4 (C5β), 64.2 (C6α,β).

4.4.1.17. L-Phenylalanyl-lactose. Solid; UV (H_2O, λ_{max}): 214.0 nm ($\sigma\rightarrow\sigma^*$ $\varepsilon_{214.0}$–6026 M^{-1}), 257.5 nm ($\pi\rightarrow\pi^*$ $\varepsilon_{257.5}$ 562 M^{-1}) and 290.0 nm ($n\rightarrow\pi^*$ $\varepsilon_{290.0}$ – 302 M^{-1}). IR (KBr): 3378 cm^{-1} (OH), 1632 cm^{-1} (CO), 1556 cm^{-1} (aromatic, –C=C–). Optical rotation (*c* 0.2, H_2O): $[\alpha]_D$ at 25 °C = +31.3°. MS (*m/z*) 512 [M + Na]$^+$. 2D-HSQCT (DMSO-d_6): *6-O-ester*: ^{1}H NMR δ_{ppm} (500.13 MHz): 2.67 (αCH), 2.89 (βCH_{2a}), aromatic- 7.25 (H-2,H-6), 7.28 (H-3,H-5), 7.20 (H-4), 4.32 (H-1α), 4.23 (H-1β), 3.36 (H-2α), 3.49 (H-2β), 3.32 (H-3α), 3.80 (H-3β), 3.56 (H-4α), 3.72 (H-4β), 3.90 (H-5α,β), 3.47 (H-6α,β), 4.16 (H-1″β), 3.14 (H-2″), 3.42 (H-3″), 3.62 (H-4″), 3.39 (H-5″), 3.54 (H-6″); ^{13}C NMR δ_{ppm} (125 MHz): 51.9 (αCH), 38.2 (βCH_2) aromatic- 138.0 (C1), 128.3 (C2, C6), 129.1 (C3, C5), 126.1 (C4), 172.5 (CO), 96.6 (C1α), 101.2 (C1β), 70.5 (C2α), 73.0 (C2β), 74.0 (C3β), 82.5 (C4α), 83.3 (C4β), 77.2 (C5α,β), 62.1 (C6αβ), 103.2 (C1″β), 69.9 (C2″), 71.6 (C3″), 68.2 (C4″), 74.4 (C5″), 60.5 (C6″). *6″-O-ester*: ^{1}H NMR δ_{ppm}: 2.72 (αCH), 3.00 (βCH_{2a}), 5.16 (H-1α), 4.32 (H-1β), 3.16 (H-2α), 3.45 (H-2β), 3.43 (H-3α), 3.50 (H-3β), 3.32 (H-4α), 3.80 (H-5α,β), 3.49 (H-6α,β), 4.15 (H-1″β), 3.36 (H-2″), 3.89 (H-4″), 3.89 (H-5″), 3.45 (H-6″); ^{13}C NMR δ_{ppm}: 52.5 (αCH), 37.1 (βCH_2) Aromatic- 137.6 (C1), 92.0 (C1α), 97.1 (C1β), 69.7 (C2α), 72.7 (C2β), 72.1 (C3α), 74.5 (C3β), 79.9 (C4α), 80.7 (C4β), 76.0 (C5α,β), 61.0 (C6α,β), 104.0 (C1″β), 69.3 (C2″), 72.1 (C3″), 66.3 (C4″), 75.0 (C5″), 63.1 (C6″). *6,6″-di-O-ester*: ^{1}H NMR δ_{ppm}: 2.55 (αCH), 2.89 (βCH_{2a}), 4.32 (H-1α), 3.23 (H-2α), 3.68 (H-2β), 3.33 (H-3α), 4.02 (H-4α), 4.01 (H-5α,β), 3.45 (H-6α,β), 4.21 (H-1″β), 3.14 (H-2″), 3.58 (H-3″), 3.74 (H-4″), 3.46 (H-6″); ^{13}C NMR δ_{ppm}: 51.7 (αCH), 37.9 (βCH_2), aromatic- 137.8 (C1), 95.6 (C1α), 101.9 (C1β), 71.3 (C2α), 73.2 (C2β), 73.3 (C3α), 74.7 (C3β), 82.2 (C4α), 84.2 (C4β), 77.7 (C5α,β), 62.4 (C6α,β), 103.4 (C1″β), 69.9 (C2″), 72.4 (C3″), 67.4 (C4″), 74.7 (C5″), 62.7 (C6″).

4.4.1.18. L-Phenylalanyl-D-mannitol. Solid; UV (H_2O, λ_{max}): 215.0 nm ($\sigma\rightarrow\sigma^*$ $\varepsilon_{215.0}$–631 M^{-1}), 257.5 nm ($\pi\rightarrow\pi^*$ $\varepsilon_{257.5}$ 170 M^{-1}). IR (KBr): 3290 cm^{-1} (OH), 1637 cm^{-1} (CO), 1532 cm^{-1} (aromatic, –C=C–). Optical rotation (*c* 0.6, H_2O): $[\alpha]_D$ at 25 °C = +1.6°. MS (*m/z*) 352 [M + Na]$^+$. 2D-HSQCT (DMSO-d_6): *1-O-ester*: ^{1}H NMR δ_{ppm} (500.13 MHz): 3.48 (αCH), 2.88 (βCH_{2a}), aromatic- 7.26 (H-2, H-6), 7.26 (H-3, H-5), 7.18 (H-4), 3.38 (H-1), 3.46 (H-2), 3.70 (H-3), 3.84 (H-4), 3.52 (H-5), 3.36 (H-6); ^{13}C NMR δ_{ppm} (125 MHz): 55.8 (αCH), 37.1 (βCH_2), aromatic- 137.6 (C_1), 131.2 (C_2, C_6), 126.7 (C_4), 171.0 (CO), 66.0 (C1, C6), 75.0 (C2), 70.0 (C3), 70.2 (C4), 77.8 (C5). *1,6-di-O-ester*: ^{1}H NMR δ_{ppm}: 3.39 (αCH), 3.46 (H-1), 3.45 (H-2, H-6), 3.11 (H-3, H-4), 3.46 (H-6); ^{13}C NMR δ_{ppm}: 55.3 (αCH), 66.8 (C1), 77.0 (C2, C5), 70.4 (C3, C4), 66.8 (C_6).

4.4.1.19. L-Tryptophanyl-D-glucose. Solid; UV (H_2O, λ_{max}): 213.0 nm ($\sigma\rightarrow\sigma^*$ $\varepsilon_{213.0}$–1479 M^{-1}), 276 nm ($\pi\rightarrow\pi^*$ ε_{276} 389 M^{-1}), 315 nm ($n\rightarrow\pi^*$ ε_{315} – 118 M^{-1}). IR (KBr): 3523 cm^{-1} (NH), 3336 cm^{-1} (OH), 1633 cm^{-1} (C=O), 1524 cm^{-1} (aromatic –C=C–). Optical rotation (*c* 0.6, H_2O): $[\alpha]_D$ at 25 °C = –21.7°. MS (*m/z*) 366.1 [M]$^+$. 2D-HSQCT (DMSO-d_6): *2-O-ester*: ^{1}H NMR δ_{ppm} (500.13 MHz): 2.92 (αCH), 3.06 (βCH_{2a}), Aromatic - 6.96–7.59, 4.6 (H-1α), 3.69 (H-2α), 3.72 (H-2β), 3.68 (H-3α), 3.54 (H-6α,β); ^{13}C NMR δ_{ppm} (125 MHz): 53.2 (αCH), 35.0 (βCH_2), aromatic-109.4 (C_1), 124.2 (C_2), 136.0 (C_4), 114.6 (C_6), 120.8 (C_7), 121.1 (C_8), 100.8 (C1α), 75.9 (C2α), 76.2 (C2β) 72.9 (C3α), 62.8 (C6α,β). *3-O-ester*: 1H NMR δ_{ppm}: 2.87 (αCH), 2.84 (βCH_2), 3.82 (H-2α), 3.92 (H-3α), 3.59 (H-3β), 3.58 (H-6α,β); ^{13}C NMR δ_{ppm}: 52.3 (αCH), 35.9 (βCH_2), aromatic-109.3 (C_1), 124.3 (C_2C_3), 136.3 (C_4), 121.0 (C_5), 113.0 (C_6), 116.5 (C_7), 120.0 (C_8), 100.1 (C1α), 101.8 (C1β), 74.2 (C2α), 71.2 (C2β), 82.0 (C3α,β), 62.5 (C6α,β). *6-O-ester*: ^{1}H NMR δ_{ppm}: 2.81 (αCH), 2.70 (βCH_2), 3.41 (H-2α), 3.51 (H-2β), 3.68 (H-3α), 3.52 (H-4α), 3.64 (H-5α), 3.70 (H-5β), 3.65 (H-6α,β); ^{13}C NMR δ_{ppm}: 51.5 (αCH), 31.0 (βCH_2), aromatic- 109.2 (C_1),124.4 (C_2,C_3), 136.4 (C_4), 121.0 (C_5), 111.5 (C_6), 118.2 (C_7), 118.5 (C_8), 172.0 (CO), 96.9 (C1α), 101.9 (C1β), 71.8 (C2α), 69.5 (C2β), 72.6 (C3α), 69.6 (C4α), 68.9 (C5α), 70.2 (C5β), 63.0 (C6α,β). *2,6-di-O-ester*: ^{1}H NMR δppm: 2.78 (βCH_2), 3.92 (H-2α), 3.82 (H-2β), 3.54 (H-6α,β). ^{13}C NMR δ_{ppm}: 31.0 (βCH_2), 77.0 (C2α), 77.3 (C2β), 63.2 (C6α,β). *3,6-di-O-ester*: ^{1}H NMR δ_{ppm} 2.82 (αCH), 2.61 (βCH_2), 3.74 (H-3α), 3.44 (H-6α,β); ^{13}C NMR δ_{ppm}: 51.8 (αCH), 31.0 (βCH_2), 81.2 (C3α), 63.9 (C6α,β).

4.4.1.20. L-Tryptophanyl-D-fructose. Solid; UV (H_2O, λ_{max}): 212.0 nm ($\sigma\rightarrow\sigma^*$ $\varepsilon_{212.0}$–5495 M^{-1}), 265.0 nm ($\pi\rightarrow\pi^*$ $\varepsilon_{265.0}$ 1862 M^{-1}), 308 nm ($n\rightarrow\pi^*$ ε_{308} – 1175 M^{-1}). IR (KBr): 3284 cm^{-1} (OH), 1631 cm^{-1} (C=O), 1364 cm^{-1} (CN) and 1492 cm^{-1} (aromatic, –C=C–). Optical rotation (*c* 0.7, H_2O): $[\alpha]_D$ at 25 °C = –10.8°. MS (*m/z*) 165 $[M - 1]^+$. 2D-HSQCT (DMSO-d_6): *1-O-ester*: ^{1}H NMR δ_{ppm} (500.13 MHz): 3.44 (αCH), 2.59 (βCH_{2a}), aromatic - 7.1 (H-2), 8.18 (H-3), 7.32 (H-7), 7.74 (H-8), 4.32 (H-1α), 3.78 (H-3α), 3.48 (H-4α), 3.42 (H-6α,β); ^{13}C NMR δ_{ppm} (125 MHz): 58.2 (αCH), 30.2 (βCH_2), aromatic-109.5 (C_1), 124.0 (C_2), 122.0 (C_3), 111.5 (C_6), 120.6 (C_7), 119.0 (C_8), 66.4 (C1α), 104.2 (C2α), 71.6 (C3α), 70.6 (C4α), 63.1 (C6α,β). *6-O-ester*: ^{1}H NMR δ_{ppm}: 3.32 (αCH), 2.50 (βCH_2), 3.27 (H-1α), 3.78 (H-4α), 4.38 (H-6α,β); ^{13}C NMR δ_{ppm}: 59.0 (αCH), 29.8 (βCH_2), 63.9 (C1α), 102.0 (C2α), 72.5 (C4α,β), 66.4 (C6α,β).

4.4.1.21. L-Histidyl-D-glucose. Solid; UV (H_2O, λ_{max}): 210.0 nm ($\sigma\rightarrow\sigma^*$ $\varepsilon_{210.0}$–1072 M^{-1}), 264.0 nm ($\pi\rightarrow\pi^*$ $\varepsilon_{264.0}$ 933 M^{-1}). IR (KBr): 3126 cm^{-1} (OH), 1720 cm^{-1} (C=O), 1343 cm^{-1} (NH), 1588 cm^{-1} (aromatic, –C=C–). Optical rotation (*c* 1.0, H_2O): $[\alpha]_D$ at 25 °C = –33.3°. MS (*m/z*) 318 $[M + 1]^+$. 2D-HSQCT (DMSO-d_6): *2-O-ester*: ^{1}H NMR δ_{ppm} (500.13 MHz): 3.08 (αCH), 2.70 (βCH_2), Aromatic - 6.93 (H2), 7.69 (H3), 4.78 (H-1α), 3.86 (H-2α), 3.76 (H-2β), 3.19 (H-3α), 3.12 (H-3β), 3.62 (H-4α), 3.58 (H-6α,β); ^{13}C NMR δ_{ppm} (125 MHz): 52.0 (αCH), 30.8 (βCH_2), aromatic-115.6 (C1), 134.2 (C2), 134.8 (C3), 171.5 (CO), 96.2 (C1α), 75.0 (C2α), 76.7 (C2β), 70.0 (C3α), 80.0 (C3β), 69.3 (C4α), 62.2 (C6α,β). *3-O-ester*: ^{1}H NMR δ_{ppm}: 3.00 (αCH), 2.82 (βCH_2), aromatic- 6.93 (H2), 7.71 (H3), 4.75 (H-1α), 3.60 (H-2α), 3.32 (H-2β), 3.77 (H-3α), 3.93 (H-3β), 3.67 (H-4α), 3.42 (H-6α,β); ^{13}C NMR δ_{ppm}: 52.8 (αCH), 27.7 (βCH_2), aromatic-116.2 (C1), 134.4 (C2), 134.8 (C3), 171.0 (CO), 95.5 (C1α), 72.2 (C2α), 74.9 (C2β), 81.7 (C3α), 83.2 (C3β), 68.9 (C4α), 61.3 (C6α,β). *6-O-ester*: ^{1}H NMR δ_{ppm}: 2.84 (αCH), 3.06 (βCH_2), aromatic – 6.93 (H2), 7.72 (H3), 4.72 (H1-α), 3.59 (H2-α), 3.12 (H3-α), 3.80 (H-4α), 2.90 (H-5α), 3.80 (H-6α,β); ^{13}C NMR δ_{ppm}: 51.2 (αCH), 26.8 (βCH_2), aromatic-115.6 (C1), 134.4 (C2), 134.9 (C3), 170.3 (CO), 95.0 (C1α), 100.2 (C1β), 72.5 (C2α), 73.1 (C3α), 70.6 (C4α), 75.0 (C5α), 63.6 (C6α,β). *2,6 -di-O-ester*: ^{1}H NMR δppm 2.90 (αCH), 2.78 (βCH_2), 3.73 (H-2α), 3.75 (H-2β), 3.47 (H-6α,β); ^{13}C NMR δ_{ppm}: 52.5 (αCH), 30.8 (βCH_2), 102.1 (C1β), 76.7 (C2α), 78.0 (C2β), 70.5 (C4α), 62.7 (C6α,β). *3,6-di-O-ester*: ^{1}H NMR δ_{ppm}: 2.80 (βCH_2), 3.73 (H-3α), 3.60 (H-3β), 3.26 (H-6α,β); ^{13}C NMR δ_{ppm}: 30.0 (βCH_2), 81.7 (C3α), 82.3 (C3β), 70.2 (C4α), 62.4 (C6α,β).

4.4.1.22. L-Histidyl-D-fructose. Solid; UV (H_2O, λ_{max}): 210.0 nm ($\sigma\rightarrow\sigma^*$ $\varepsilon_{210.0}$–617 M^{-1}), 267.0 nm ($\pi\rightarrow\pi^*$ $\varepsilon_{267.0}$ 240 M^{-1}), 321.0 nm ($n\rightarrow\pi^*$ $\varepsilon_{321.0}$–170 M^{-1}). IR (KBr): 3136 cm^{-1} (OH), 1605 cm^{-1} (C=O), 1393 cm^{-1} (CN), 1592 cm^{-1} (aromatic, –C=C–). Optical rotation (*c* 0.6, H_2O): $[\alpha]_D$ at 25 °C = –20.0°. MS (*m/z*) 340 $[M + Na]^+$. 2D-HSQCT NMR: *6-O-ester*: ^{1}H NMR δ_{ppm}(500.13 MHz): 3.45 (αCH), 3.21 (βCH_2), aromatic – 6.85 (H2), 7.42 (H3), 3.67 (H-3α), 3.52 (H-4α), 3.24 (H-6α,β); ^{13}C NMR δ_{ppm} (125 MHz): aromatic-116.5 (C1), 134.2 (C2), 124.5 (C3), 170.5 (CO), 62.8 (C1α), 102.1 (C2α), 69.2 (C3α), 70.0 (C4α) 70.6 (C4α), 64.2 (C6α,β).

4.4.1.23. L-Histidyl-D-mannitol. Solid; UV (H_2O, λ_{max}): 210.0 nm ($\sigma\rightarrow\sigma^*$ $\varepsilon_{210.0}$–3802 M^{-1}), 267.0 nm ($\pi\rightarrow\pi^*$ $\varepsilon_{267.0}$ 1349 M^{-1}), 324.0 nm ($n\rightarrow\pi^*$ $\varepsilon_{324.0}$–776 M^{-1}). IR (KBr): 3344 cm^{-1} (OH), 1631 cm^{-1} (C=O), 1319 cm^{-1} (CN), 1511 cm^{-1} (aromatic). Optical rotation (*c* 0.2, H_2O): $[\alpha]_D$ at 25 °C = +17.4°. 2D-HSQCT NMR: *1-O- ester*: ^{1}H NMR δ_{ppm}(500.13 MHz): 3.46 (αCH), 3.32 (βCH_2), aromatic 7.38 (H3), 3.44 (H-1), 3.39 (H-2), 3.47 (H-3), 3.54 (H-4), 3.57 (H-5), 3.38 (H-6); ^{13}C NMR δ_{ppm} (125 MHz): 54.3 (αCH), aromatic- 128.2 (C2), 123.4(C3), 63.8 (C1, C6), 71.4 (C2, C5), 69.8 (C3, C4).

4.5. Angiotensin converting enzyme (ACE) inhibition assay

ACE inhibition assay for the esters prepared were performed by the Cushman and Cheung method [23]. Aliquots of glycoside or ester solutions in the concentration range 0.12 to 1.60 mM (0.1–0.8 ml of 2.0 mM stock solution) were taken and to this 0.1 ml of ACE solution (0.1% in 0.1 M phosphate buffer, pH 8.3 containing 300 mM NaCl) was added. To this solution, 0.1 ml of 5.0 mM hippuryl-L-histidyl-L-leucine (HHL) was also added and the total volume made up to 1.25 ml by adding phosphate buffer (0.95–0.25 ml of 0.1 M pH 8.3 containing 300 mM NaCl). The solution was incubated on a Heto-Holten shaking water bath for 30 min at 37 °C. Blanks were performed without the enzyme by taking only the glycoside or ester solutions (0.1 to 0.8 ml) along with 0.1 ml of 5.0 mM HHL. The total volume was made up to 1.25 ml by adding same buffer (1.05–0.35 ml). The reaction was terminated by adding 0.25 ml of 1 M HCl. Hippuric acid formed in the reaction was extracted with 1.5 ml of ethyl acetate. One ml of the ethyl acetate layer was evaporated to dryness and treated with equal amount of distilled water and absorbance was measured at 228 nm for hippuric acid. The hippuric acid formed in 1.5 ml of ethyl acetate was determined from a calibration plot prepared by using a standard hippuric acid in 1 ml of distilled water in the concentration range 0–400 nmol and measuring its absorbance at 228 nm.

Specific activity was expressed as hippuric acid formed (mM) per min, per mg of enzyme protein.

$$\text{Specific activity} = \frac{A_{ts} - A_{blank}}{T \times S \times E}$$

A_{ts} = absorbance of test solution, A_{blank} = absorbance of blank solution, T = incubation period in min, S = slope value of the calibration plot ($1.00{\cdot}6 \times 10^{-2}$ Abs units/nmol of hippuric acid), E = amount of the enzyme in mg protein. The specific activity value for each glycoside and ester is an average from two independent measurements. Percentage inhibition was expressed as the ratio of the specific activity of ACE in the pre-

sence of the inhibitor to that in the absence of the inhibitor, the latter being considered as 100%. IC_{50} value was expressed as the concentration of the inhibitor required for 50% reduction in ACE specific activity. Molecular weights of the glycosides and esters employed in the calculations are weighted averages of molecular weights of glycosides and esters detected by NMR and Mass spectroscopy. In case of samples where NMR spectra were not recorded, molecular weights of the mono glycosides and mono esters were considered for the calculations.

4.6. Protease and lipase assay

Protease activity for the ACE inhibitor was determined by the method described by Dubey and Jagannadham [29] and lipase activity by the tributyrin method [30] in presence of eugenyl-D-glucoside (0.8 mM in 0.1 M, pH 7.5, Tris–HCl buffer) and L-isoleucyl-D-glucose (0.8 mM in the same buffer) individually. Specific protease activity was expressed as the increase in absorbance at 440 nm min^{-1} mg^{-1} of the protein employed. Similarly specific lipase activity was determined as μmol of butyric acid formed per min per mg of the protein employed.

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