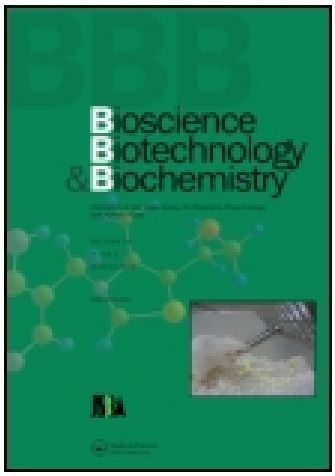

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Isolation and Activity of *N-p*-Coumaroyltyramine, an α-Glucosidase Inhibitor in Welsh Onion (*Allium fistulosum*)

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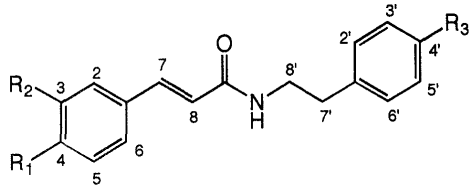
A phenolic amide, *N-p*-coumaroyltyramine (1), was isolated as an α-glucosidase inhibitor from methanol extracts of Welsh onion (*Allium fistulosum*). The inhibitory activity of 1 against a yeast enzyme was as high as K_i 8.4×10^{-7} M. From a structure-activity relationship study of 1 and its related compounds, the occurrence of α-glucosidase inhibitory activity required a *p*-coumaramide structure, with an amide hydrogen and alkyl or aralkyl substituent on the amide part.

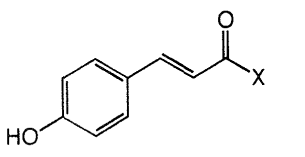
Key words: α-glucosidase inhibitor; *N-p*-coumaroyltyramine; *Allium fistulosum*

The importance of biologically active substances contained in foods has been noticed in recent years. It could be expected that regulating the enzyme activities responsible for specific biochemical reactions in living systems would result in favorable effects on health. Glycosidases play a physiologically important role which includes the digestive process for carbohydrates and a processing step for antigenic sugar chains expressed on the cell surface.[1] We have searched for glycosidase inhibitors in food ingredients, and sulfolipids and quercetin were isolated from hijiki[2] and tochu-cha,[3] respectively, as α-glucosidase inhibitors. A further survey of such inhibitors in vegetables yielded the isolation of a phenolic amide from Welsh onion (*Allium fistulosum*) as a new type of inhibitor against yeast α-glucosidase.

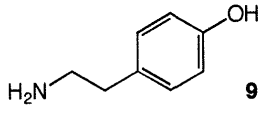
In the screening experiments for α-glucosidase inhibitors in plant foodstuffs,[3] methanol extracts of the edible parts of Welsh onion showed high activity. The ethyl acetate-soluble active fraction was chromatographed twice in a silica gel column and further purified by preparative HPLC to yield an active principle, **1**, as a white amorphous powder. The EI mass spectrum showed a molecular ion at *m/z* 283, and a high-resolution analysis disclosed its molecular formula to be $C_{17}H_{17}NO_3$. The IR spectrum suggested the presence of hydroxyl (3430 cm^{-1}) and amide (1660 and 1450 cm^{-1}) functions. In the ^{1}H-NMR spectrum of **1**, signals assignable to two 1,4-disubstituted benzenes (δ_H 6.75 and 7.05, each 2H, d, J=8.4 Hz, and δ_H 6.84 and 7.41, each 2H, d, J=8.6 Hz) as well as *trans*-olefinic protons (δ_H 6.46 and 7.45, each 1H, d, J=15.6 Hz) were found in the low-field region. On the other hand, two methylenes, δ_H 2.73 (2H, t, J=7.3 Hz) and 3.47 (2H, dt, J=7.3(t) and 6.1(d) Hz) coupled with each other appeared in the high-field region, and the latter methylene was further coupled with a broad signal at δ_H 7.19 that was assigned to an amide proton. The structure of **1** was therefore deduced to be an amide of 4-hydroxycinnamic acid and 2-(4-hydroxyphenyl)ethylamine. The conspicuous fragment ion at *m/z* 147 ($[HOC_6H_4CH=CHCO]^+$) strongly supports the validity of the proposed structure. To confirm the structure of **1**, condensation of *p*-coumaric acid and tyramine in the presence of dicyclohexylcarbodiimide was performed. The physicochemical



	R_1	R_2	R_3
1	OH	H	OH
2	OH	H	H
3	H	H	OH
4	H	H	H
5	OH	OH	OH
6	OH	OH	H
7	OMe	H	OH



	X
8	OH
10	OMe
11	NMe_2
12	NH_2



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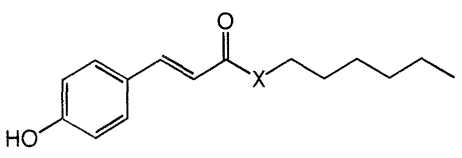

	X
13	NH
14	O
15	NMe

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properties of the resulting amide matched well with those of **1**. Thus, the structure of **1** was concluded to be *N*-*p*-coumaroyltyramine.[4,5] The inhibitory activity of **1** against yeast α-glucosidase was 85% in 2.4×10^{-6} M when compared with the positive control (100%) of 1-deoxynojirimycin (2×10^{-8} M), and the K_i value of **1** was determined to be 8.4×10^{-7} M. This activity is ten times higher than that previously reported for quercetin, a major active principle in tochu-cha.[3] The isolated yield of **1** from fresh Welsh onion was as low as 10^{-5}%, 2.9×10^{-6} mol/kg fr. wt. Since the crude methanol extract with five parts of the solvent showed 86% inhibition against the enzyme,[3] a quarter of the activity of the fresh material would have been derived from **1**. Considering that no other conspicuous activity existed in the fractions throughout the isolation and the isolated insufficiency of the compound, **1** could be concluded as the only principal active substance in Welsh onion. Compound **1** has been isolated from eggplant[4] and Chinese *Allium* species.[5,6] It is interesting that **1** has reportedly inhibited human platelet aggregation,[5] and prostaglandin and thromboxane synthetase,[6] whereas there has been no report on α-glucosidase inhibition by the compound.

Insight from the structure of **1** prompted us to study the structure-activity relationship. Ten structurally related amides, *N*-*p*-coumaroyl-(2-phenylethyl)amine (**2**), *N*-cinnamoyltyramine (**3**), *N*-cinnamoyl-(2-phenylethyl)-amine (**4**), *N*-caffeoyltyramine (**5**), *N*-caffeoyl-(2-phenylethyl)amine (**6**), *N*-(4-methoxycinnamoyl)tyramine (**7**), *N*,*N*-dimethyl-*p*-coumaramide (**11**), *p*-coumaramide (**12**), *N*-*p*-coumaroylhexylamine (**13**), and *N*-*p*-coumaroyl-*N*-methylhexylamine (**15**), and two esters, methyl *p*-coumarate (**10**) and hexyl *p*-coumarate (**14**), were synthesized by condensation of the appropriate components for a structure-activity relationship study. These compounds together with *p*-coumaric acid (**8**) and tyramine (**9**) were measured for their inhibitory activity at a concentration of 4.0×10^{-5} M (Fig.).

A comparison of the activity of hydroxylated *N*-cinnamoyl-(2-phenylethyl)amines indicated that *p*-coumaroylamide with a hydroxyl group at the 4-position of the acid residue, **2**, had moderate activity, although it was weaker than that of **1** with an additional hydroxyl group on the amine part, whereas two cinnamoylamides which lacked the *p*-hydroxyl substituent on the acid residue, **3** and **4**, had no activity regardless of the presence of a hydroxyl group on the amine residue. Furthermore, two caffeoylamides carrying a 3,4-dihydroxyphenyl group at the acid residue, **5** and **6**, showed as strong activity as that of **2**. On the contrary, a *p*-methoxycinnamoylamide, **7**, showed only weak activity compared with its hydroxyl counterpart, **1**. Among the other related *p*-coumaroylamides, the relatively simple amides, **11** and **12**, showed weak activity, while *p*-coumaric acid (**8**), its methyl ester (**10**) and tyramine (**9**) had no activity. Finally, it was unexpected that *N*-*p*-coumaroylhexylamine, **13**, had considerable activity comparable to **2**, although the activity was less in the corresponding *N*-methylamide, **15**, while the corresponding ester analog, **14**, showed only weak inhibition against the enzyme. These results imply the following structural requirements for α-glucosidase inhibition of **1**: a *p*-coumaric amide structure, an amide hydrogen, and an alkyl or aralkyl chain on the amine part. A hydroxyl group at the 4′-position on the amine part acted advantageously, whereas an additional hydroxyl at the 3-position on the acid part weakened the activity. Although there is little information on the interaction between **1** and the enzyme so far, recognition of phenolic OH on the coumaroyl residue and the amide proton by a specific site of the enzyme, together with the contribution of a hydrophobic *N*-alkyl chain might have been responsible for the inhibitory activity.

It would be interesting to discover if *N*-*p*-coumaroyltyramine (**1**) shows inhibitory activity against mammalian intestinal enzymes in respect of sugar uptake suppression. However, it was unexpected that an inhibitory test of **1** against rat intestinal α-glucosidase showed almost no activity, which might have arisen from the considerable structural difference between yeast and animal enzymes. Further inhibitory tests using a human tumor cell line, Caco-2, which expresses brush-border enzymes, are now in progress.

Experimental

Instrumental analyses. ^{1}H- and ^{13}C-NMR spectra were measured with a Bruker AM-500 spectrometer, using tetramethylsilane as an internal stan-

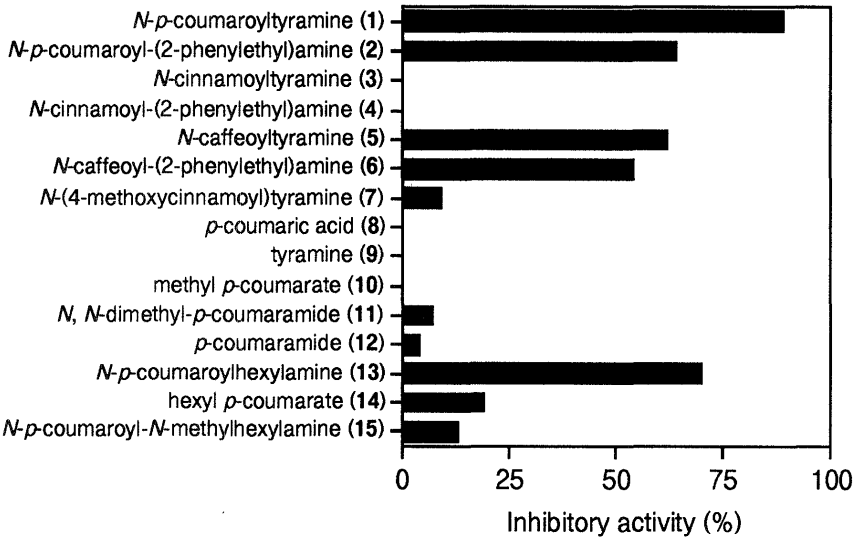

Fig. α-Glucosidase Inhibitory Activity of *N*-*p*-Coumaroyltyramine (**1**) and its Related Compounds.

dard. EI-MS and EI-HR-MS data were obtained with JEOL AX500 and SX110 instruments, respectively, and IR spectra were determined with a Perkin Elmer 2000 instrument.

Isolation of N-p-coumaroyltyramine (**1**). The edible parts of fresh *A. fistulosum* (5.9 kg) were extracted twice with MeOH (10 liter) at room temperature. The combined extract was concentrated under reduced pressure and partitioned between EtOAc–H_2O. The EtOAc layer was washed with 5% $NaHCO_3$, and the resulting EtOAc-soluble fraction (6.53 g) was chromatographed on silica gel with a hexane–EtOAc gradient. The activity was monitored by an inhibitory test against yeast α-glucosidase. The active fractions eluted with hexane–EtOAc (2 : 3 and 1 : 4) were combined and rechromatographed on silica gel with a $CHCl_3$–MeOH gradient. The active $CHCl_3$–MeOH (9 : 1) fractions were subjected to HPLC (column, Inertsil PREP-ODS, 6.0 × 250 mm; mobile phase, MeOH–H_2O (65 : 35); flow rate, 1.5 ml/min; detection, UV 254 nm) to give *N-p*-coumaroyltyramine (**1**, 4.7 mg) as the principal active substance, which was identified by comparing its physicochemical data with those in the literature[4,5] and those of the synthetic material described next.

Preparation of N-p-coumaroyltyramine (**1**). To a solution of *p*-coumaric acid (165 mg, 1.0 mmol), tyramine (140 mg, 1.0 mmol) and *N*-hydroxysuccinimide (170 mg, 1.5 mmol) in dimethylformamide (DMF, 10 ml) was added dicyclohexylcarbodiimide (DCC, 410 mg, 2 mmol) in DMF (1 ml), the mixture being stirred for 13 h at room temperature.[5] The reaction mixture was then poured into H_2O and extracted with EtOAc. The resulting extract was washed with 5% $NaHCO_3$ and evaporated to give a crude amide as pale yellow crystals, which were purified by silica gel column chromatography ($CHCl_3$–MeOH = 9 : 1) and silica gel prep. TLC ($CHCl_3$–MeOH = 9 : 1) to give colorless crystals (36 mg). **1**: mp 255–257°C; EI-HR-MS *m/z* (M^+): calcd. for $C_{17}H_{17}NO_3$, 283.1196; found, 283.1186; EI-MS *m/z* (%): 283 (M^+, 9), 164 (72), 147 (100), 120 (58), 119 (21), 107 (21), 91 (17); ^{1}H-NMR δ (acetone-d_6): 2.73 (2H, t, *J* = 7.3 Hz, 7′-H_2), 3.47 (2H, dt, *J* = 7.3 (t) and 6.1 (d) Hz, 8′-H_2), 6.46 (1H, d, *J* = 15.6 Hz, 8-H), 6.75 (2H, d, *J* = 8.4 Hz, 3′, 5′-H), 6.84 (2H, d, *J* = 8.6 Hz, 3, 5-H), 7.05 (2H, d, *J* = 8.4 Hz, 2′, 6′-H), 7.19 (1H, br. s, NH), 7.41 (2H, d, *J* = 8.6 Hz, 2, 6-H), 7.45 (1H, d, *J* = 15.6 Hz, 7-H), 8.42 (2H, br. s, OH); IR ν_{max} (KBr) cm^{-1}: 3430, 1660, 1450, 980.

Preparation of the structural analogs of **1**. Ten amides and two esters were prepared by conventional condensation methods from the appropriate acid and amine or alcohol. The physicochemical properties of each of these prepared compounds were consistent with their structures.

Amide analogs **2**–**7**, **13**, *and* **15**. Structurally related *N*-cinnamoyl amide derivatives **2**–**7**, **13**, and **15** were prepared by condensing the corresponding acid and amine as for **1**. **2**: Yellow oil; EI-MS *m/z* (%): 267 (M^+, 29), 162 (36), 147 (100), 119 (32), 91 (80), 65 (29); ^{1}H-NMR δ (acetone-d_6): 2.85 (2H, t, *J* = 7.3 Hz, 7′-H_2), 3.54 (2H, dt, *J* = 7.3 (t) and 6.1 (d) Hz, 8′-H_2), 6.48 (1H, d, *J* = 15.7 Hz, 8-H), 6.85 (2H, d, *J* = 8.6 Hz, 3, 5-H), 7.18 (1H, br. t, *J* = 7.0 Hz, 4′-H), 7.23–7.29 (4H, m, 2′, 3′, 5′, 6′-H), 7.34 (br. s, NH), 7.41 (2H, d, *J* = 8.6 Hz, 2, 6-H), 7.48 (1H, d, *J* = 15.7 Hz, 7-H), 8.87 (1H, br. s, OH); IR ν_{max} (NaCl) cm^{-1}: 3270, 1600, 1220. **3**: Colorless crystals; EI-MS *m/z* (%): 267 (M^+, 5), 148 (60), 131 (97), 120 (100), 103 (36), 77 (22); ^{1}H-NMR δ (acetone-d_6): 2.75 (2H, t, *J* = 7.3 Hz, 7′-H_2), 3.50 (2H, dt, *J* = 7.3 (t) and 6.1 (d) Hz. 8′-H_2), 6.67 (1H, d, *J* = 15.7 Hz, 8-H), 6.76 (2H, d, *J* = 8.5 Hz, 3′, 5′-H), 7.06 (2H, d, *J* = 8.4 Hz, 2′, 6′-H), 7.34–7.39 (4H, m, 3, 4, 5-H, NH), 7.53 (1H, d, *J* = 15.7 Hz, 7-H), 7.55 (2H, m, 2, 6-H), 8.21 (1H, br. s, OH); IR ν_{max} (KBr) cm^{-1}: 3280, 1670, 1230. **4**: Colorless crystals; EI-MS *m/z* (%): 251 (M^+, 15), 131 (100), 104 (24), 103 (44), 91 (32); ^{1}H-NMR δ ($CDCl_3$): 2.88 (2H, t, *J* = 6.8 Hz, 7′-H_2), 3.65 (2H, dt, *J* = 6.8 (t) and 6.2 (d) Hz, 8′-H_2), 5.60 (1H, br. s, NH), 6.30 (1H, d, *J* = 15.5 Hz, 8-H), 7.19–7.35 (8H, m, 3, 4, 5, 2′, 3′, 4′, 5′, 6′-H), 7.46 (2H, dd, *J* = 7.8 and 2.3 Hz, 2, 6-H), 7.60 (1H, d, *J* = 15.5 Hz, 7-H); IR ν_{max} (KBr) cm^{-1}: 3320, 1700, 1230. **5**: Yellow oil; EI-MS *m/z* (%): 299 (M^+, 5), 180 (24), 163 (49), 120 (70), 107 (100), 77 (16); ^{1}H-NMR δ (acetone-d_6): 2.72 (2H, t, *J* = 7.2 Hz, 7′-H_2), 3.47 (2H, dt, *J* = 7.2 (t) and 6.2 (d) Hz, 8′-H_2), 6.42 (1H, d, *J* = 15.6 Hz, 8-H), 6.74 (2H, d, *J* = 8.5 Hz, 3′, 5′-H), 6.81 (1H, d, *J* = 8.1 Hz, 5-H), 6.90 (1H, dd, *J* = 8.1 and 2.0 Hz, 6-H), 7.04 (2H, d, *J* = 8.5 Hz, 2′, 6′-H), 7.05 (1H, d, *J* = 2.0 Hz, 2-H), 7.26 (1H, br. s, NH), 7.40 (1H, d, *J* = 15.6 Hz, 7-H), 8.28 (3H, br. s, OH); IR ν_{max} (NaCl) cm^{-1}: 3340, 1650, 1260. **6**: Yellow crystals; EI-MS *m/z* (%): 283 (M^+, 36), 179 (11), 178 (35), 163 (100), 160 (12); ^{1}H-NMR δ (acetone-d_6): 2.84 (2H, t, *J* = 7.1 Hz, 7′-H_2), 3.53 (2H, dt, *J* = 7.1 (t) and 6.1 (d) Hz, 8′-H_2), 6.42 (1H, d, *J* = 15.6 Hz, 8-H), 6.82 (1H, d, *J* = 8.1 Hz, 5-H), 6.92 (1H, dd, *J* = 8.1 and 1.9 Hz, 6-H), 7.06 (1H, d, *J* = 1.9 Hz, 2-H), 7.18 (1H, br. t, *J* = 7.1 Hz, 4′-H), 7.22–7.29 (5H, m, 2′, 3′, 5′, 6′-H, NH), 7.40 (1H, d, *J* = 15.6 Hz, 7-H), 8.23 (2H, br. s, OH); IR ν_{max} (NaCl) cm^{-1}: 3350, 1610, 1220. **7**: White amorphous powder; EI-MS *m/z* (%): 297 (M^+, 14), 178 (55), 161 (100), 133 (23), 120 (27); ^{1}H-NMR δ (acetone-d_6): 2.74 (2H, t, *J* = 7.4 Hz, 7′-H_2), 3.48 (2H, dt, *J* = 7.4 (t) and 6.2 (d) Hz, 8′-H_2), 6.52 (1H, d, *J* = 15.8 Hz, 8-H), 6.75 (2H, d, *J* = 8.4 Hz, 3′, 5′-H), 6.93 (2H, d, *J* = 8.7 Hz, 3, 5-H), 7.05 (2H, d, *J* = 8.4 Hz, 2′, 6′-H), 7.25 (1H, br. s, NH), 7.48 (1H, d, *J* = 15.8 Hz, 7-H), 7.49 (2H, d, *J* = 8.7 Hz, 2, 6-H), 8.19 (1H, br. s, OH); IR ν_{max} (NaCl) cm^{-1}: 3210, 2900, 1700. **13**: Colorless oil; EI-MS *m/z* (%): 247 (M^+, 51), 204 (23), 177 (26), 162 (59), 147 (100), 119 (40), 91 (28); ^{1}H-NMR δ (acetone-d_6): 0.87 (3H, t, *J* = 7.0 Hz, 6′-H_3), 1.26–1.35 (6H, m, 3′, 4′, 5′-H_2), 1.52 (2H, quint, *J* = 7.0 Hz, 2′-H_2), 3.29 (2H, q, *J* = 7.0 Hz, 1′-H_2), 6.48 (1H, d, *J* = 15.7 Hz, 8-H), 6.85 (2H, d, *J* = 8.6 Hz, 3, 5-H), 7.25 (1H, br. s, NH), 7.41 (2H, d, *J* = 8.6 Hz, 2, 6-H), 7.45 (1H, d, *J* = 15.7 Hz, 7-H), 8.83 (1H, br. s, OH); IR ν_{max} (NaCl) cm^{-1}: 3270, 2930, 1650. **15** (56 : 44 mixture of stereoisomers): Colorless oil; EI-MS *m/z* (%): 261 (M^+, 53), 190 (34), 176 (38), 147 (100), 119 (45), 91 (28); ^{1}H-NMR δ (acetone-d_6): 0.87 (3H, t, *J* = 6.6 Hz, 6′-H_3), 1.30 (6H, m, 3′, 4′, 5′-H_2), 1.54 (minor) and 1.63 (major) (total 2H, each m, 2′-H_2), 2.89 (minor) and 2.96 (major) (total 3H, each s, NCH_3), 3.43 (minor) and 3.52 (major) (total 2H, each br. t, *J* = 6.9 Hz, 1′-H_2), 6.85 (2H, d, *J* = 8.7 Hz, 3, 5-H), 6.95 (1H, d, *J* = 15.3 Hz, 8-H), 7.49–7.53 (3H, m, 2, 6, 7-H), 8.82 (1H, br. s, OH); IR ν_{max} (NaCl) cm^{-1}: 3200, 2930, 1740.

Methyl p-coumarate (**10**), *N,N-dimethyl-p-coumaramide* (**11**), *and p-coumaramide* (**12**). Methyl *p*-coumarate (**10**) was prepared by DCC-mediated condensation of *p*-coumaric acid and methanol. The condensation reaction, using DMF as a solvent, produced an unexpected by-product, *N,N*-dimethyl-*p*-coumaramide (**11**) in a 7% yield. *p*-Coumaramide (**12**) was prepared by ammonolysis of **10**.[7] **10**: White amorphous powder; mp 134–138°C; EI-MS *m/z* (%): 178 (M^+, 87), 147 (100), 119 (26), 91 (13); ^{1}H-NMR δ (acetone-d_6): 3.70 (3H, s, OMe), 6.33 (1H, d, *J* = 16.1 Hz, 8-H), 6.87 (2H, d, *J* = 8.7 Hz, 3, 5-H), 7.53 (2H, d, *J* = 8.7 Hz, 2, 6-H), 7.58 (1H, d, *J* = 16.1 Hz, 7-H), 8.90 (1H, br. s, OH); IR ν_{max} (KBr) cm^{-1}: 3380, 1690. **11**: White amorphous powder; mp 197–200°C; EI-MS *m/z* (%): 191 (M^+, 82), 147 (100), 119 (37), 91 (14); ^{1}H-NMR δ (acetone-d_6): 2.95 and 3.17 (each 3H, s, NMe_2), 6.85 (2H, d, *J* = 8.7 Hz, 3, 5-H), 6.97 (1H, d, *J* = 15.3 Hz, 8-H), 7.48 (1H, d, *J* = 15.3 Hz, 7-H), 7.50 (2H, d, *J* = 8.7 Hz, 2, 6-H), 8.70 (1H, br. s, OH); IR ν_{max} (KBr) cm^{-1}: 3130, 1640. **12**: White amorphous powder; mp 192–194°C; EI-MS *m/z* (%): 163 (M^+, 100), 147 (69), 119 (38), 91 (22); ^{1}H-NMR δ (acetone-d_6): 6.51 (1H, d, *J* = 15.7 Hz, 8-H), 6.85 (2H, d, *J* = 8.6 Hz, 3, 5-H), 6.34 and 6.91 (each 1H, s, NH_2), 7.43 (2H, d, *J* = 8.6 Hz, 2, 6-H), 7.46 (1H, d, *J* = 15.7 Hz, 7-H), 8.84 (1H, br. s, OH); IR ν_{max} cm^{-1}: 3330, 1670.

Hexyl p-coumarate (**14**). To a solution of *p*-coumaric acid (820 mg, 5 mmol) and diisopropyl azodicarboxylate (1.01 g, 5 mmol) in DMF (5 ml) was added a mixture of 1-hexanol (765 mg, 7.5 mmol) and triphenylphosphine (1.31 g, 5 mmol) in DMF (5 ml).[8] After stirring for 12 h at room temperature, the reaction mixture was poured into H_2O and extracted with EtOAc. The EtOAc extract was washed with 5% $NaHCO_3$ and evaporated to give a crude oil, which was purified by silica gel prep. TLC ($CHCl_3$–MeOH = 9 : 1) to yield **14** as a colorless oil (15 mg). **14**: Colorless oil; EI-MS *m/z* (%): 248 (M^+, 25), 164 (100), 147 (59), 119 (13), 91 (9); ^{1}H-NMR δ (acetone-d_6): 0.89 (3H, t, *J* = 6.9 Hz, 6′-H_3), 1.28–1.43 (6H, m, 3′, 4′, 5′-H_2), 1.66 (2H, quint, *J* = 6.9 Hz, 2′-H_2), 4.13 (2H, t, *J* = 6.9 Hz, 1′-H_2), 6.34 (1H, d, *J* = 16.0 Hz, 8-H), 6.88 (2H, d, *J* = 8.6 Hz, 3, 5-H), 7.54 (2H, d, *J* = 8.6 Hz, 2, 6-H), 7.59 (1H, d, *J* = 16.0 Hz, 7-H), 8.89 (1H, br. s, OH); IR ν_{max} (NaCl) cm^{-1}: 3350, 2930, 1680.

α-Glucosidase inhibitory assay. The inhibitory activity of the isolated and prepared materials against yeast α-glucosidase was measured by the microplate method described previously.[3] The sample and enzyme solutions used were 4.0×10^{-5} M and 4 μg/ml, respectively.

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