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Structure–activity relationships of *trans*-cinnamic acid derivatives on α-glucosidase inhibition

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Abstract—*trans*-Cinnamic acid and its derivatives were investigated for the α-glucosidase inhibitory activity. 4-Methoxy-*trans*-cinnamic acid and 4-methoxy-*trans*-cinnamic acid ethyl ester showed the highest potent inhibitory activity among those of *trans*-cinnamic acid derivatives. The presence of substituents at 4-position in *trans*-cinnamic acid altered the α-glucosidase inhibitory activity. Increasing of bulkiness and the chain length of 4-alkoxy substituents as well as the increasing of the electron withdrawing group have been shown to decrease the inhibitory activity. 4-Methoxy-*trans*-cinnamic acid was a noncompetitive inhibitor for α-glucosidase, whereas, 4-methoxy-*trans*-cinnamic acid ethyl ester was a competitive inhibitor. These results indicated that *trans*-cinnamic acid derivatives could be classified as a new group of α-glucosidase inhibitors.

α-Glucosidase inhibitors have been shown to be potentially valuable for treatment of various diseases. Inhibition of α-glucosidase decreases the blood glucose levels via delaying digestion of poly- and oligosaccharides to absorbable monosaccharides.[1] This leads to a reduction in glucose absorption and, subsequently, the rise of postprandial hyperglycemia is attenuated. α-Glucosidase inhibitors are also known to be promising as anti-viral, anti-HIV agents, which alter glycosidation of envelope glycoprotein through interference with biosynthesis of N-linked oligosaccharides.[2,3] In addition, they have recently been used for treatment of B- and C-type viral hepatitis.[4] Recent studies have shown that tetrachlorophthalimide[5] and 3-*O*-acyl mesquitol analogues were new examples class of α-glucosidase inhibitors.[6]

trans-Cinnamic acids, originally isolated from plant sources,[7,8] have been reported to possess a variety of biological properties including hepatoprotective,[9] antimalarial[10] and antioxidant activities.[11] For example, *trans*-cinnamic acid induces cytostasis and a reversal of malignant properties of human tumour cells in vitro. Furthermore molecular analysis have been shown that the anti-tumour activity of cinnamic acid may be due in part to the inhibition of protein isoprenylation in mitogenic signal transduction.[12] *p*-Coumaric acid or 4-hydroxy-*trans*-cinnamic acid has shown to possess anti-oxidant activity. It minimized the oxidation of low-density lipoprotein (LDL) involving direct scavenger of reactive oxygen species (ROS).[13] Moreover, the dehydrogenated polymers of *p*-coumaric acid inhibited HIV-1 protease activity.[14] 4-Methoxy-*trans*-cinnamic acid exhibited a potent hepatoprotective activity in rat hepatocytes from toxicity induced by carbon tetrachloride (CCl_4).[15] Consequently, a broad range of biological activities of cinnamic acids have been reported, this leads us to investigate a new pharmacological activity of *trans*-cinnamic acid and its derivatives.

In this study, we studied the α-glucosidase inhibitory activity of *trans*-cinnamic acid and its derivatives, which were obtained from a natural source, synthesis and

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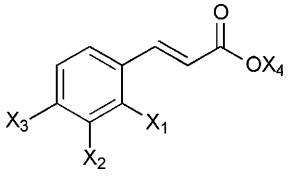
commercially available compounds. We also discuss their structure–activity relationship and kinetics of inhibitory activity.

Compounds **8** and **9** were isolated from the rhizomes of *Kaempferia galanga*.[16] The other *trans*-cinnamic acid derivatives (**1**, **2**, **6**, **7**, **10**–**18**) were synthesized by the Perkin reaction between aromatic aldehydes and aliphatic carboxylic acids following the procedure of Chiriac et al.[17] Compounds **3**–**5** were purchased from Fluka Co. Ltd. 1-Deoxynorjirimycin and α-glucosidase from baker's yeast (EC.3.2.1.20) were purchased from Sigma Chemical Co. Ltd (St. Louis, MO). Structure of isolated and synthesized compounds were confirmed by spectroscopic data (NMR, MS) and all other chemicals used were of analytical grade. The inhibitory effect of each compound on α-glucosidase activity was measured according to the literature procedure.[18] Briefly, α-glucosidase from baker's yeast was assayed using 0.1 M phosphate buffer at pH 6.9, and 1 mM *p*-nitrophenyl-α-D-glucopyranoside (PNP-G) was used as a substrate. The concentration of the enzymes was 1 U/mL in each experiment. α-Glucosidase (40 μL) was incubated in the absence or presence of various concentrations of *trans*-cinnamic acid derivatives (10 μL) at 37 °C. The pre-incubation time was specified at 10 min and PNP-G solution (950 μL) was added to the mixture. The reaction was carried out at 37 °C for 20 min, and then 1 mL of 1 M Na_2CO_3 was added to terminate the reaction. Enzymatic activity was quantified by measuring the absorbance at 405 nm. One unit of α-glucosidase is defined as the amount of enzyme liberating 1.0 μmol of PNP per minute under the conditions specified. 1-Deoxynorjirimycin was used as the positive control in this study (Table 1). The IC_{50} values were expressed as mean ± SE, ($n = 3$). In order to evaluate the type of inhibition using the Lineweaver–Burk plot, the enzyme reaction was performed according to the above reaction with various concentrations of *trans*-cinnamic acid derivatives (**8** and **9**).

As the results, compounds **4**–**10**, **15** and **16** inhibited α-glucosidase activity in dose-dependent manner. Table 1 shows that the compounds **4**–**10**, **15** and **16** had more potent α-glucosidase inhibiting activity than that of 1-deoxynorjirimycin ($IC_{50} = 5.60 \pm 0.42$ mM), which was used as the positive inhibitor in yeast α-glucosidase. [Note, the IC_{50} of 1-deoxynorjirimycin against α-glucosidase type IV (Sigma G6136) was reported to be 330 μM.][19] *trans*-Cinnamic acid (**1**), its ethyl ester (**2**) and the 2-hydroxy-*trans*-cinnamic acid (**3**) were found to be inactive ($IC_{50} > 5$ mM).

4-Hydroxy-*trans*-cinnamic acid (**5**) had very potent inhibitory activity ($IC_{50} = 0.20 \pm 0.06$ mM). 4-Methoxy-*trans*-cinnamic acid (**8**) was the most active compound ($IC_{50} = 0.044 \pm 0.006$ mM), while 2-, and 3-methoxy-*trans*-cinnamic acid (**6** and **7**) were less potent ($IC_{50} = 4.34 \pm 0.78$ mM, $IC_{50} = 0.58 \pm 0.15$ mM, respectively). These results suggested that the presence of hydroxy or methoxy group at 4-position on *trans*-cinnamic acid moiety is necessary to enhance α-glucosidase inhibitory activity. When the α-glucosidase inhibitory activities of compounds **1**, **5** and **8** were compared, it was found that the potency increased in the order of **8** > **5** > **1**. The observation revealed that replacement of the 4-hydroxy substituted in the *trans*-cinnamic acid by a methoxy residue increased α-glucosidase inhibitory activity by 10-fold. Introduction of a methoxy group at *para*-position on *trans*-cinnamate acid ethyl ester (**9**), the

Table 1. IC_{50} values of *trans*-cinnamic acid and its derivatives for inhibition of α-glucosidase



Compounds	X_1	X_2	X_3	X_4	IC_{50} (mM)
1	H	H	H	H	>5
2	H	H	H	C_2H_5	>5
3	OH	H	H	H	>5
4	H	OH	H	H	1.27 ± 0.51
5	H	H	OH	H	0.20 ± 0.06
6	OCH_3	H	H	H	4.34 ± 0.78
7	H	OCH_3	H	H	0.58 ± 0.15
8	H	H	OCH_3	H	0.04 ± 0.01
9	H	H	OCH_3	C_2H_5	0.05 ± 0.03
10	H	H	OPh	H	0.44 ± 0.37
11	H	H	OCH_2Ph	H	>5
12	H	H	OC_4H_9	H	>5
13	H	H	OC_6H_{13}	H	>5
14	H	H	NO_2	H	>5
15	H	H	F	H	0.27 ± 0.06
16	H	H	Cl	H	0.39 ± 0.14
17	H	H	Br	H	>5
1-Deoxynojirimycin					5.60 ± 0.42

IC_{50} values was 0.05 ± 0.03 mM, which was in the same order to that of 4-methoxy-*trans*-cinnamic acid. The evidence supported the previous result (**1** and **2**), that neither the acid group nor the ethyl ester played any important role on α-glucosidase inhibition.

The introduction of 4-phenoxy residue to *trans*-cinnamic acid (**10**) decreased the α-glucosidase inhibitory activity ($IC_{50} = 0.44 \pm 0.37$ mM). The compounds having larger alkoxy substituent (**11**–**13**) were found to have no effect on α-glucosidase inhibition ($IC_{50} > 5$ mM). These results suggested that increasing of the bulkiness, or the chain length of the alkoxy substituent at 4-position may decrease the α-glucosidase inhibitory activity.

While the presence of NO_2 group at 4-position of *trans*-cinnamic acid (**14**) showed no activity ($IC_{50} > 5$ mM), the *trans*-cinnamic acid derivatives having F (**15**) and Cl (**16**) substituent at 4-position gave moderate activity (the IC_{50} values of 0.27 ± 0.06, 0.39 ± 0.14 mM, respectively). This observation supported the notion that a decrease in electron diversity of *trans*-cinnamic acid moiety would result in the decrease of α-glucosidase inhibitory activity. On the other hand, 4-bromo-*trans*-cinnamic acid (**17**) had no effect on α-glucosidase inhibiting activity.

Lineweaver–Burk plot of α-glucosidase kinetics is shown in Figure 1. The kinetic result demonstrated that the mechanism of α-glucosidase inhibition of compound **8** was noncompetitive with K_i value of 0.06 ± 0.01 mM. In contrast, 4-methoxy-*trans*-cinnamic acid ethyl ester (**9**) was a competitive inhibitor with K_i value of 0.02 ± 0.01 mM. At this point, K_i value was calculated using the values of V_{max} obtained at 0 and 55.6 μM for compound **8**, and the values of K_{max} obtained at 0 and 48.1 μM for compound **9**, respectively. To date, the microbial α-glucosidase is known to be structurally different to those of mammalial origins. The microbial α-glucosidase inhibitors are not necessarily the mammalial α-glucosidase inhibitors. For example, (+)-catechin, a natural inhibitor of yeast α-glucosidase does not show any inhibitory activity on mammalial α-glucosidase. On the other hand, acarbose and voglibose show very high inhibitory activity on porcine small intestine α-glucosidase, but both of them show very low inhibitory activity on microbial α-glucosidase,[20] suggesting that ongoing experiments should be focused on the inhibitory activity of these compounds against mammalian intestinal α-glucosidases. Nevertheless, the inhibition of yeast α-glucosidase by *trans*-cinnamic acid derivatives served as an interesting structural activity relationship of this group of inhibitors.

In conclusion, 4-methoxy-*trans*-cinnamic acid (**8**) and 4-methoxy-*trans*-cinnamic acid ethyl ester (**9**) showed the highest activity on microbial α-glucosidase inhibition among the *trans*-cinnamic acid derivatives. Additional studies on α-glucosidase inhibitory effects of *trans*-cinnamic acid derivatives using X-ray crystallography to evaluate the binding activity as well as inhibitory activity of these compounds on α-glucosidase from mammalial sources and in vivo experiments are in progress. In addition, further studies on the elucidation of molecular mechanisms of the *trans* cinnamic acid derivatives against α-glucosidase could also be rewarding.

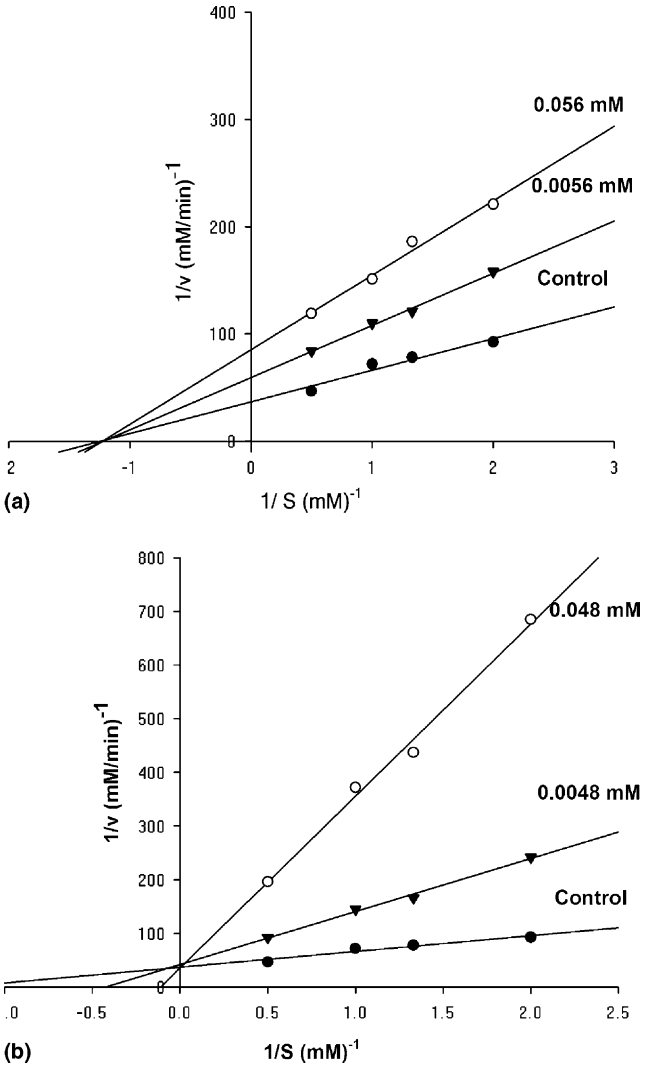


Figure 1. Lineweaver–Burk plot analysis of the inhibition kinetics of α-glucosidase inhibitory effects by (a) 4-methoxy-*trans*-cinnamic acid (**8**), and (b) 4-methoxy-*trans*-cinnamic acid ethyl ester (**9**).

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