Med Chem Res
DOI 10.1007/s00044-013-0659-4

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Effect on α-glucosidase inhibition and antioxidant activities of butyrolactone derivatives from *Aspergillus terreus* MC751

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Received: 25 December 2012 / Accepted: 29 May 2013

Abstract Butyrolactone I and II from *Aspergillus terreus* MC751 as well as three synthetic butyrolactone I derivatives were assessed for α-glucosidase inhibitory and antioxidant activities. Butyrolactone I (**1**), which has a prenyl side chain and an alpha hydroxy-lactone group, was the most potent α-glucosidase inhibitor and also had antioxidant activities with IC_{50} values of 52.17 ± 5.68 and 51.39 ± 3.68 μM, respectively. In contrast, butyrolactone II (**2**) lacking a prenyl side chain was the most potent antioxidant with an IC_{50} of 17.64 ± 6.41 μM, but was less active against α-glucosidase. Acetylation of all hydroxyl groups of butyrolactone I significantly decreased both the α-glucosidase inhibitory and antioxidant activity. The prenyl and alpha hydroxy-lactone groups seem to have a synergic effect on the inhibitory activity but not antioxidant activity. This is the first structure–activity relationship report on the α-glucosidase inhibition and antioxidant activity by butyrolactone derivatives.

Keywords *Aspergillus terreus* MC75 · Butyrolactones derivatives · α-Glucosidase inhibitory activity · Antioxidant

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Introduction

Type 2 diabetes, the most prevalent and serious metabolic disease in the world today (Kaneto *et al.*, 2010; Shaw *et al.*, 2010), is characterized by hyperglycemia (Baynes and Thorpe, 1999; Ceriello, 2012). Intracellular hyperglycemia induces overproduction of free radicals at the mitochondrial level. Hence, overproduction of free radicals is a key event in the activation of all other pathways involved in the pathogenesis of diabetic complications (Ceriello, 2012). It has been shown that antioxidant therapy may help prevent illnesses caused by oxidative stress by quenching free radicals, thereby protecting cells and tissues from oxidative damage (Jung *et al.*, 2008). Inhibition of α-glucosidase is one approach to suppressing postprandial hyperglycemia, thereby delaying glucose absorption and controlling the hyperglycemia in diabetic patients (Gao *et al.*, 2004; Dewi *et al.*, 2007) and reducing chronic vascular complications (Meng and Zhou, 2012). Therefore, the ideal antidiabetic compound should possess both hypoglycemic and antioxidant properties (Mayur *et al.*, 2010; Sancheti *et al.*, 2011).

As part of our efforts to isolate potential α-glucosidase inhibitors and antioxidant compounds from fungi, the terrestrial strains, *Aspergillus terreus* was investigated (Dewi *et al.*, 2007, 2012a, b). *A. terreus* has been recovered both marine and terrestrial sources and is well known for the production of lovastatin, a cholesterol-lowering agent (Parvatkar *et al.*, 2009; Wang *et al.*, 2011). A previous study of *A. terreus* MC751 resulted in the isolation of butyrolactone I as an antidiabetic and antioxidant (Dewi *et al.*, 2012b). Here, we report the isolation, derivatization, and structural elucidation of butyrolactone derivatives from *A. terreus* MC751. The α-glucosidase inhibitory and antioxidant activities, as well as structure–activity relationship (SAR) studies of butyrolactone I derivatives are also described.

Published online: 05 June 2013

Materials and methods

General instrument and chemicals

Melting points were measured on a Yanaco micro melting point apparatus (Yanaco Co., Ltd., Kyoto, Japan) and were uncorrected. Optical rotation values were measured with a Jasco P-2100 polarimeter. UV–Vis absorption spectra of the active compounds in methanol were recorded on a Hitachi U-1600 spectrophotometer. HPLC was conducted with Waters (USA), UV–Vis spectrophotometric detector, and reversed-phase C18 column (Type UG 120, 5 μm, size 4.6 mm ID × 250 mm). The mass spectra of the compound were measured with high-resolution FAB-MS. The nuclear magnetic resonance (NMR) spectra were recorded at 500 MHz for ^{1}H and 125 MHz for ^{13}C on a JEOL JNM-ECA 500 with TMS as an internal standard. HMQC and HMBC techniques were used to assign correlations between ^{1}H and ^{13}C signals. The chemical shift values (δ) are given in parts per million (ppm), and coupling constant (J) in Hz. TLC was run on silica gel 60 F_{254} pre-coated plates (Merck 5554) and spots were detected by UV light.

α-Glucosidase [(EC 3.2.1.20)] type I: from *Saccharomyces cereviceae*, *p*-nitrophenyl α-D-glucopyranoside (*p*-NPG), DMSO, 1,1-diphenyl-2-picrylhydrazyl (DPPH), quercetin dihydrate, and Silica gel (60–200 mesh Wako gel) were purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan). All the solvents used in this study were purchased from Wako Pure Chemicals and distilled prior to use.

Microorganisms and culture conditions

A. terreus MC751 was obtained from the Research Center for Chemistry-Indonesian Institute of Sciences. The stock culture of *A. terreus* MC751 was grown on PDA at 25 °C for 7 days. Optimization of the culture medium was conducted by culturing the *A. terreus* MC751 in five different media: PMP (2.4 % potato dextrose broth, 1 % malt extract, and 0.1 % peptone), PDB (2.4 % potato and 2 % dextrose), and ME (1 % glucose, 0.1 % peptone, and 1.5 % malt extract), each culture broth was incubated at 25 °C with shaking at 100 rpm for 7 days. Czapek-dox (Cz) (3 % sucrose, 0.2 % sodium nitrate ($NaNO_3$), 0.1 % K_2HPO_4, 0.05 $MgSO_4{\cdot}7H_2O$, 0.05 % KCl, and 0.001 % ferrous sulfate), incubated for 10 days under static conditions at 25 °C. Medium Cz, with addition of 0.5 % yeast extract (CzY) incubated for 15 days under static conditions. The culture broth was prepared by 3 rounds of extraction for each broth (200 ml) with EtOAc (100 ml), dried by rotary evaporator, and assessed for α-glucosidase inhibitory and DPPH radical scavenging activities. The butyrolactone I content of the extract was investigated by analytical reversed-phase HPLC, using the isocratic mobile phase of MeOH/water (30:70) at a flow rate of 1 ml min^{-1} with 300 nm as the detection wavelength.

Fermentation on CzY, extraction, and isolation

Five disks (5 mm) of fungal mycelia were used to inoculate 150 ml of seed medium in an Erlenmeyer flask (500 ml) in CzY. Each culture was maintained at 25 °C under static conditions for 15 days. 15-liter cultures were separated from mycelia by filtration. The filtrate was extracted with EtOAc (5 × 1 l), and the extract was dried under reduced pressure to obtain a brown solid (2.18 g). The filtrate extract was fractioned by column chromatography (CC) on silica gel using a stepwise gradient from 100 % *n*-hexane, to 100 % EtOAc, to 50 % EtOAc in MeOH to obtain nine fractions (F1–F9). Fractions 4 and 5 were combined and rechromatographed on a silica gel column eluted using a stepwise gradient from 80 % *n*-hexane in EtOAc, to 100 % EtOAc to give compound **1** (500 mg). F7 (280 mg) was subjected to CC on silica gel (EtOAc/*n*-hexane, step gradient elution from 20:80 to 0:100) followed by preparative TLC ($CHCl_3$:Acetone, 5:1) to give compound **2** (60 mg).

Compound **1**: Yellowish gum, UV (MeOH) λ_{max} nm (log ε): 307.5 (4.44). $[\alpha]_D^{22.5}$ +68.333 ($c = 0.3$, MeOH). ^{1}H NMR and ^{13}C NMR data are presented in Table 2. HRFABMS: $[M+H]^+$ m/z 425.1607, calculated for $C_{24}H_{25}O_7$.

Compound **2**: Colorless gum, UV (MeOH) λ_{max} nm (log ε): 307.5 (4.17). $[\alpha]_D^{28.7}$ +4.78 ($c = 0.45$, Acetone). ^{1}H NMR (Acetone-d_6) δ 3.4 (2H, d, $J = 14.8$, H-6), 3.76 (3H, s, 5-OCH_3), 6.59 (2H, d, $J = 8.0$, H-3″,5″), 6.69 (2H, d, $J = 8.0$, H-2′,6′), 6.98 (2H, d, $J = 8$, H-3′,5′), 7.66 (2H, d, $J = 8.0$, H-2″,6″). ^{13}C NMR (Acetone-d_6) δ 170.94 (C-5), 168.83 (C-1), 158.91 (C-4′), 157.38 (C-4″), 139.28 (C-2), 132.33 (C-2′ and C-6′) 130.12 (C-2″ and C-6″), 128.06 (C-1″), 124.89 (C-1′), 122.78 (C-3), 115.47 (C-3′ and C-5′), 116.66 (C-3″ and C-5″), 85.97 (C-4), 53.79 (OCH_3), 39.18 (C-6). FAB-MS: $[M+H]^+$ m/z 357 for $C_{19}H_{17}O_7$.

Preparation of butyrolactone I derivatives

Derivatization of butyrolactone I (**1**) was conducted by cyclization and acetylation. Cyclization of **1** was carried out according to the method of Parvatkar et al. (2009). Compound **1** (90 mg) was dissolved in MeOH (10 ml) containing conc. HCl (0.2 ml). The mixture was stirred at room temperature for 2 h until complete conversion of compound **1** as indicated by TLC. The solvent was removed under vacuum and the resulting residue was extracted with $CHCl_3$. The $CHCl_3$ soluble fraction was separated by preparative TLC elution with *n*-hexane:EtOAC (2:3), to yield compound **3** (60 mg, 67 %). The butyrolactone I acetate was obtained

treating 212 mg of compound **1** with acetic anhydride in pyridine. After the usual work-up, compounds **4** (90 mg, 32 %) and **5** (60 mg, 42 %) were obtained.

Compound **3**: Yellowish solid crystal, mp: 93–95 °C, UV (MeOH) λ_{max} nm (log ε): 307.5 (4.92); 226.5 (4.85); $[\alpha]_D^{28.7}$ +88.73 ($c = 0.58$, $CHCl_3$). ^{1}H NMR (Acetone-d_6) δ 1.16 (6H, s, H10″,11″), 1.63 (2H, br, $J = 6.5$, H8″), 2.50 (2H, m, H7″), 3.36 (2H, $J = 15$, H6), 3.68 (3H, s, 5-OMe), 6.38 (1H, s, H2″), 6.50 (1H, s, H5″), 6.56 (1H, d, $J = 3.4$, H6″), 6.86 (2H, d, $J = 9.0$, H3′, 5′), 7.65 (2H, d, $J = 9.0$, H2′, 6′). ^{13}C NMR (Acetone-d_6) δ 173.52 (C-5), 172.50 (C-1), 157.98 (C-4′), 154.25 (C-4″), 144.91 (C-2), 74.95 (C-9″), 126.37 (C-2″), 129.61 (C-6′ and C-2′), 125.47 (C-6″), 132.99 (C-1′), 117.48 (C-3″), 130.55 (C-1″), 33.75 (C-8″), 121.19 (C-3), 116.63 (C-5′ and C-3′), 123.74 (C-5″), 86.75 (C-4), 53.77 (OCH_3), 40.01 (C-6), 23.34 (C-7″), 27.41 (C-10″), 27.62 (C-11″). FAB-MS: $[M+H]^+$ *m/z* 425 for $C_{24}H_{25}O_7$.

Compound **4**: Colorless gum, UV ($CHCl_3$) λ_{max} nm (log ε): 281 (3.74). $[\alpha]_D^{20}$ +7.154 ($c = 1.3$, $CHCl_3$). FAB-MS: $[M+H]^+$ *m/z* 551 for $C_{30}H_{31}O_{10}$. ^{1}H NMR and ^{13}C NMR are presented in Table 2.

Compound **5**: Colorless gum, UV ($CHCl_3$) λ_{max} nm (log ε):294 (3.84).$[\alpha]_D^{20}$ +39.957 ($c = 1.15$, $CHCl_3$). FAB-MS: $[M+H]^+$ *m/z* 509, for $C_{28}H_{29}O_9$. ^{1}H NMR and ^{13}C NMR are presented in Table 2.

Biological activities

α-Glucosidase inhibitory activity

The inhibitory activity for α-glucosidase was assessed as reported by Kim *et al.* (2004). Briefly, *p*-NPG (250 µl, 3 mM) and 495 µl of 100 mM phosphate buffer (pH 7.0) were added to a tube containing 5 µl of sample dissolved in DMSO at various concentrations. The reaction mixture was pre-incubated for 5 min at 37 °C, the reaction was started by adding 250 µl of α-glucosidase (0.065 U/ml), and the incubation was continued for 15 min. The reaction was stopped by adding 1 ml of 0.2 M Na_2CO_3. The inhibitory effect on α-glucosidase activity was determined by measuring the amount of *p*-nitrophenol released at λ 400 nm. Individual blanks for test samples were prepared to correct background absorbance where the enzyme was replaced with 250 µl of phosphate buffer. The percent inhibition of α-glucosidase inhibitory and antioxidant activity was assessed using the following formula: % Inhibition = (A − B)/A × 100, where A was the absorbance of the control reaction and B was the absorbance in the presence of the sample. The IC_{50} were calculated from the main inhibitory values by applying logarithmic regression analysis. The assays were carried out in triplicate and the results were expressed as mean values ± SDs. Quercetin was used as reference standard.

DPPH free radicals scavenging activity

The antioxidant activities of compounds **1**–**5** were evaluated according to the method of Yen and Chen (1995). Aliquots of samples in MeOH (2 ml) at various concentrations were each mixed with 0.5 ml of DPPH 1 mM in MeOH. All the mixtures were shaken vigorously and left to stand at room temperature for 30 min in the dark. The absorbance of the reaction solutions were measured spectrophotometrically at 517 nm. IC_{50} value was the effective concentration in which DPPH 50 % of radicals were scavenged and was obtained by interpolation with linear regression analysis. A lower IC_{50} value indicated a greater antioxidant activity.

Results and discussion

Isolation and characterization of active compounds

In the previous study it was reported that butyrolactone I from *A. terreus* MC751 cultured in Cz broth exhibited potential activity as an antidiabetic and antioxidant (Dewi *et al.*, 2012b). In order to enhance the α-glucosidase inhibitory and antioxidant activities and to increase the production of the secondary metabolites we initially examined EtOAc extracts from *A. terreus* MC751 cultured in PDB, PMP, ME, Cz, and CzY media as shown in Table 1. The results revealed that butyrolactone was efficiently produced by the fungal strains when yeast extract was added to the medium. The increase in butyrolactone I led to enhanced activities against both α-glucosidase and DPPH free radicals by the EtOAc extract of CzY compared to other extracts. In an attempt to isolate and characterize the constituent compounds, the culture strain was scaled up to 15 l using CzY under static conditions for 15 days at 25 °C. Purification of the culture filtrate extract using CC

Table 1 Preliminary experiment on ethyl acetate extracts of *A. terreus* MC751

Sample	IC_{50} (µg/ml)[a]		Butyrolactone I (% w/w)[a]
	DPPH	α-GIs	
PMP	40.13 ± 3.23	50.32 ± 2.42	21.83
PDB	48.39 ± 2.21	40.39 ± 3.14	22.05
ME	69.80 ± 5.30	>100	13.64
Cz	24.05 ± 1.56	6.45 ± 1.71	34.66
CzY	17.47 ± 2.63	4.32 ± 0.64	54.31

[a] Data are shown as mean ± SD from three independent experiments

and preparative TLC on silica gel led to compound **1** as a yellowish gum and compound **2** as a colorless gum. Characterization of the active compounds by spectrometric methods revealed compound **1** to be fully consistent with butyrolactone I [(methyl-4-hydroxy-2-(4-hydroxy-3-(3-methylbut-2-enyl)benzyl)-3-(4-hydroxyphenyl)-5-oxo-2,5-dihyrofuran-2-carboxylate] (Dewi *et al.*, 2012b), whereas compound **2** coincided with butyrolactone II [methyl-4-hydroxy-2-(4-hydroxybenzyl)-3-(4-hydroxyphenyl)-5-oxo-2,5-dihydrofuran-2-carboxylate] (Nitta *et al.*, 1983; Rao *et al.*, 2000). In order to verify the structure, compound **1** was treated with alcoholic hydrochloric acid at room temperature, which afforded compound **3**. The molecular formula of compound **3** was found to be identical with that of **1** based on FAB-MS ($C_{24}H_{25}O_7$). The MS and NMR spectral data of compound **3** were fully consistent with those for Aspernolide A [methyl 2-((2,2-dimethylchroman-6-yl)methyl)-4-hydroxy-3-(4-hydroxyphenyl)-5-oxo-2,5-dihydrofuran-2-carboxylate] reported in the literature (Parvatkar *et al.*, 2009). Compound **1** was treated with acetic anhydride in pyridine to give butyrolactone I 2,4′,4″-triacetate (**4**) [methyl-4-acetoxy-2-(4-acetoxy-3-(3-methylbut-2-enyl)benzyl)-3-(4-acetoxyphenyl)-5-oxo-2,5-dihydrofuran-2-carboxylate] and butyrolactone I 4′,4″-diacetate (**5**) [methyl 2-(4-acetoxy-3-(3-methylbut-2-enyl)benzyl)-3-(4-acetoxyphenyl)-4-hydroxy-5-oxo-2,5-dihydrofuran-2-carboxylate]. The structures of compound **4** and **5** were determined from MS and NMR (1 and 2D) data. A comparison of the ^{1}H NMR spectra of compounds **1**, **4**, and **5** showed a similar pattern. Compounds **1**, **4**, and **5** showed only minor differences. 1,4-Disubstituted and 1,2,4-trisubstituted phenols in compound **1** were shifted to low field signals in compounds **4** and **5** (Table 2), indicating that acetylation occurred. All isolated and derivative compounds from *A. terreus* MC751 in CzY are shown in Fig. 1. The compounds were examined for α-glucosidase inhibitory and antioxidant activities.

Table 2 ^{13}C and ^{1}H data of butyrolactone 1 (**1**) and acetylation derivatives 4 and 5 (500 and 125 Hz, respectively, δH and δC in ppm, J in Hz)

	1 (Acetone-d_6)		**4** ($CDCl_3$)		**5** (Acetone-d_6)	
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	169.14	–	169.46	–	168.69	–
2	137.14	–	136.83	–	139.22	–
3	127.88	–	126.25	–	127.43	–
4	86.07	–	86.62	–	85.82	–
5	169.75	–	169.38	–	169.64	–
6	38.58	3.52 d (14.8)	39.29	3.68 d (14.8)	38.94	3.54 d (14.5)
1′	122.28	–	144.67	–	126.64	–
2′,6′	129.59	7.12 d (9.0)	123.79	7.35 d (8.8)	122.38	7.20 d (8.8)
3′,5′	116.00	6.92 d (9.0)	130.35	7.58 d (8.8)	129.03	7.74 d (8.8)
4′	156.53	–	153.73	–	151.13	–
1″	124.63	–	131.24	–	130.68	–
2″	134.51	6.53 dd (3.4)	132.60	6.73 dd (3.4)	132.22	6.78 dd (3.4)
3″	129.25	–	133.51	–	129.15	–
4″	153.26	–	149.43	–	148.23	–
5″	115.18	6.61 d (8.0)	123.00	6.85 d (8.0)	121.96	6.67 d (8.0)
6″	126.48	6.65 d (8.0)	129.73	6.79 dd (8.0)	129.16	6.74 d (8.0)
7″	29.20	3.14 d (6.8)	29.08	3.09 d (6.9)	28.62	3.05 d (6.9)
8″	121.50	5.10 b (7.3)	122.49	5.02 br (7.0)	121.37	5.00 br (7.0)
9″	131.87	–	134.09	–	133.40	–
10″	17.75	1.66 s	17.88	1.61 s	17.86	1.58 s
11″	25.70	1.70 s	25.88	1.67 s	25.78	1.67 s
5-OCH_3	53.58	3.78 s	54.42	3.86 s	54.84	3.74 s
4′-OAc			20.23	2.24 s	21.34	2.33 s
C=O			167.05	–	169.28	–
4″-OAc			20.81	2.24 s	20.99	2.25 s
C=O			165.56	–	169.60	
2-OAc			21.07	2.30 s		
C=O			169.09	–		

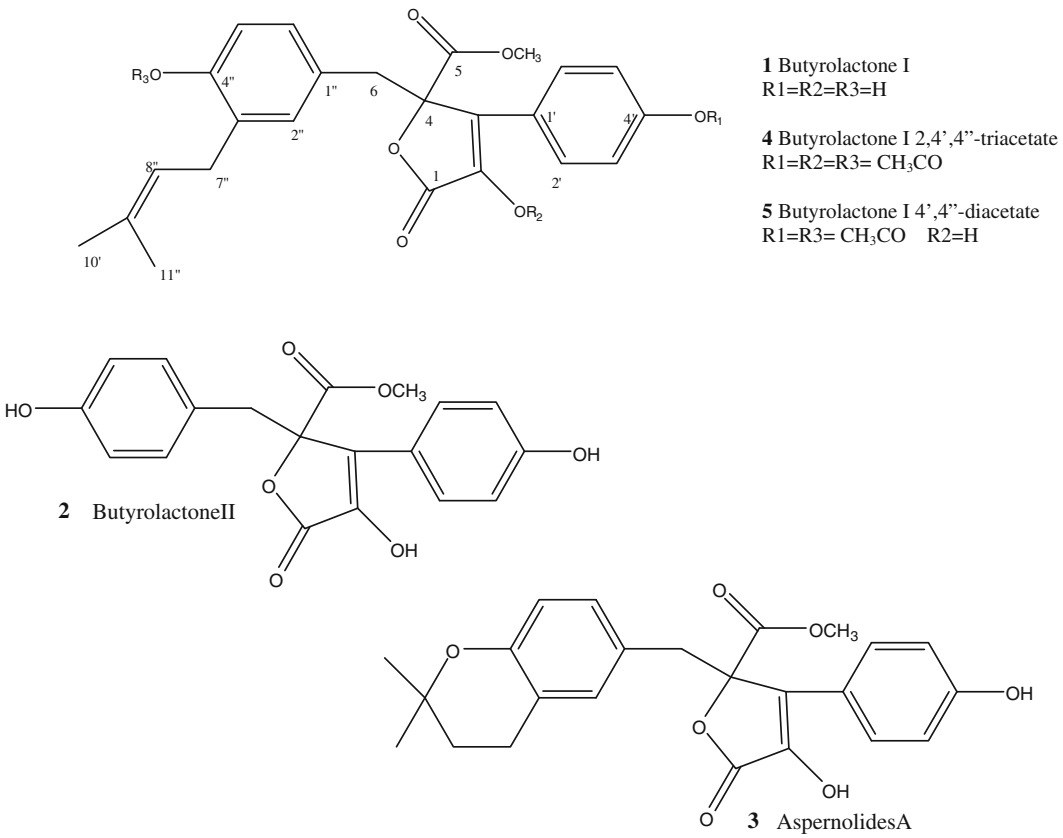

Fig. 1 Structure of butyrolactone I derivatives

Table 3 α-Glucosidase inhibitory and antioxidant activity of compounds **1–5** from *A. terreus* MC751

Compound	IC_{50} (μM)[a]	
	α-Glucosidase	DPPH
1	52.17 ± 5.68	51.39 ± 3.68
2	96.01 ± 3.70	17.64 ± 6.41
3	175.18 ± 5.95	47.55 ± 3.08
4	>300	ND
5	84.18 ± 8.98	ND
Quercetin	14.6 ± 3.72	39.63 ± 5.21

ND not detected

[a] IC_{50} value are shown as mean ± SD from three independent experiments

Table 4 Inhibition constant (K_i value) and mode of compounds **1**, **2**, **3**, and **5** from *A. terreus* MC751 against *S. cereviceae* α-glucosidase

Compound	Inhibition mode	K_i (μM)
1	Mix inhibition	70.51
2	Noncompetitive	152.64
3	Uncompetitive	235.80
5	Uncompetitive	127.88
Quercetin	Mix inhibition	27.13

α-Glucosidase inhibitory activity

We investigated the inhibitory activities of compounds **1–5** against α-glucosidase from *S. cereviceae*. Quercetin was used as a positive control based on the reports that it is a phenolic compound with a stronger inhibitory effect on α-glucosidase from *S. cereviceae* than acarbose (Tadera *et al.*, 2006; Li *et al.*, 2009; Jo *et al.*, 2009). The α-glucosidase inhibitory activities of butyrolactone derivatives (**1–5**) and quercetin are tabulated in Table 3. As previously reported, compound **1** was a potent inhibitor of the α-glucosidase with an IC_{50} of 52.17 μM (Dewi *et al.*, 2012b). In contrast, compound **2**, which lacks a prenyl side chain, exhibited less inhibitory activity. Converting the prenyl side chain to a dihydropyran ring in compound **3** caused a significant decrease in the inhibitory activity (Table 3). Hence, it was assumed that the prenyl side chain of compound **1** contributed to the inhibitory effect. However, the substitution of any hydroxyl group with an acetyl group in butyrolactone I led to a dramatic reduction in inhibitory activity in compound **4**, a finding consistent with Gao *et al.* (2004) which reported that the removal of hydroxyls in flavonoids decreased α-glucosidase inhibitory activity.

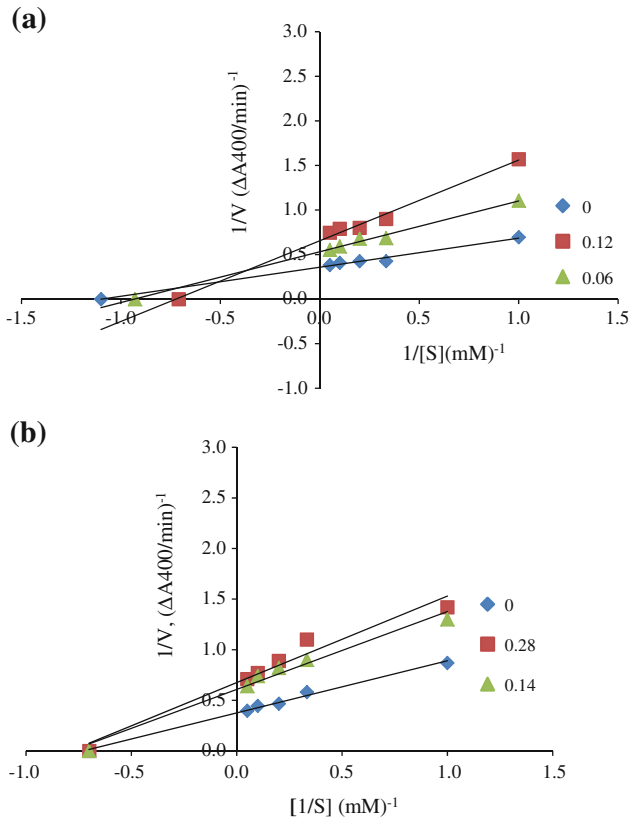

Fig. 2 Lineweaver–Burk plots of compounds **1** (**a**) and **2** (**b**) against *S. cereviceseae* α-glucosidase at different concentrations of pNPG

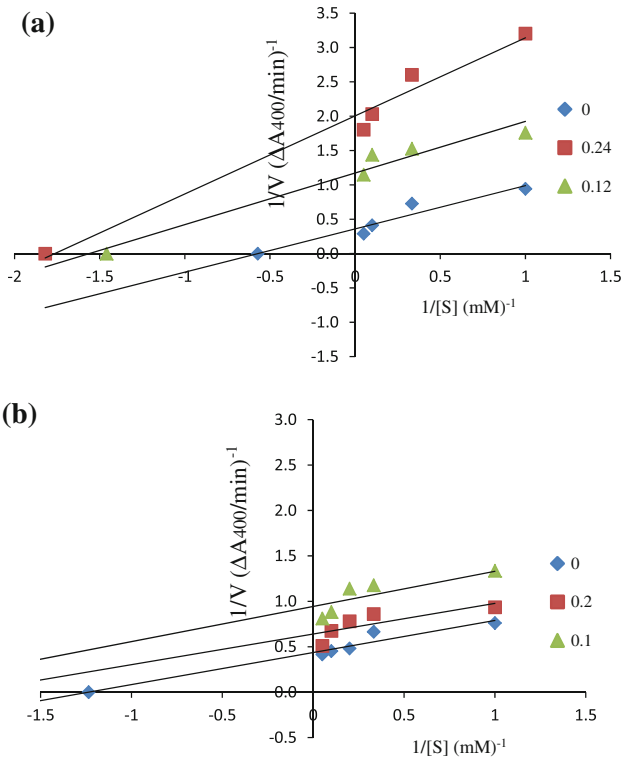

Fig. 3 Lineweaver–Burk plots of compounds **3** (**a**) and **5** (**b**) against *S. cereviceseae*

Compound **5**, which retained one OH-bond as an alpha hydroxy-lactone, showed significantly higher activity against α-glucosidase than compound **4** (Table 3). This is consistent with our suggestion that the hydroxy-lactone moiety in butyrolactone I contributed to the inhibitory effect (Dewi *et al.*, 2012b). Based on the α-glucosidase inhibitory activities of compounds **1**–**5**, SAR inference could be made. Compounds **1** and **5** showed stronger activity than the others, which suggested that the inhibitory effect of these butyrolactones was influenced by both the prenyl side chain and alpha hydroxy-lactone group. The influence of the prenyl side chain in butyrolactones was reported previously (Parvatkar *et al.*, 2009; Cazar *et al.*, 2005); however, no SAR study of the α-glucosidase inhibitory activity of butyrolactone I derivatives has been reported before.

The inhibitory mechanisms of all the active compounds were analyzed using Lineweaver–Burk plots. α-Glucosidase solution (0.065 U/ml) was incubated with increasingly higher concentrations of substrate (pNPG) with and without an inhibitor. The results showed various mechanisms of action (Table 4). Compound **1** exhibited mixed-inhibition close to non-competitive type (Fig. 2a) consistent with our previous report (Dewi *et al.*, 2012b). The mechanism action of compound **2** is shown in Fig. 2b. The 1/V increased with the concentration of compound **2**, but the K_m remained constant. The results suggest that compound **2** displayed non-competitive inhibition which indicated that it binds to a site other than the active site of the α-glucosidase enzyme (Bu *et al.*, 2010). The inhibitory mechanisms of compounds **3** and **5** are presented in Fig. 3a, b. The results showed a straight line parallel to the plot of 1/V versus 1/[S] indicating uncompetitive inhibition (Takahasi and Miyazawa, 2012). Based on the analysis, an inhibition constant (K_i) value was obtained (Table 4). This result indicated that the side chain group in the aromatic ring of butyrolactone influenced the mechanism of inhibition. Compound **4** was not examined due to its poor inhibitory activity against α-glucosidase.

DPPH free radicals scavenging activity

A critical feature of antioxidant is the ability to scavenge free radicals (Jung *et al.*, 2008). Hence, antioxidant activities of compounds **1**–**5** were evaluated in the DPPH free radical scavenging assay. Compounds **1**–**3** showed potential scavenging activity while compounds **4** and **5** did not (Table 3). The antioxidant activity may originate from the phenolic groups. Compound **2**, which lacks a prenyl side chain, was the most powerful antioxidant. This result was consistent with the previous reports (Boiko *et al.*, 2006); Osorio *et al.*,

2012) that a prenylated phenolic group in compound **1** decreased antioxidant activity. Cyclization of the prenyl group in compound **3** also decreased the antioxidant activity. In the case of compounds **4** and **5**, replacement of an OH group with an acetyl group dramatically decreased the antioxidant activity (Boiko *et al.*, 2006; Osorio *et al.*, 2012). Hence, it was concluded that the absence of a prenyl side chain increased the activity and acetylation appeared to be detrimental to the antioxidant activity.

Conclusion

In conclusion, two butyrolactone compounds (**1** and **2**), and three butyrolactone I derivatives (**3**, **4**, and **5**) were studied for α-glucosidase inhibitory and antioxidant activities. Butyrolactone I, which has a prenyl side chain and alpha hydroxy-lactone, was found to be the most potent antidiabetic. In contrast, butyrolactone II (**2**) lacking a prenyl side chain was the most potent antioxidant with an IC_{50} of 17.64 ± 6.41 μM, but was less active against the α-glucosidase enzyme. Acetylation of all hydroxyl groups of butyrolactone I (**1**) significantly decreased both the activity against α-glucosidase and the antioxidant activity. The prenyl and alpha hydroxy-lactone groups appear to have a synergic effect on the α-glucosidase inhibitory activity but not the antioxidant activity. This is the first report on the SAR of butyrolactone derivatives against α-glucosidase inhibitory and DPPH radical scavenging activities.

Acknowledgments The authors are grateful to Sofa Fajriah M.Sc. and Megawati S.Si. from the Research Center for Chemistry-Indonesian Institute of Sciences, for NMR spectral measurements. We also thank Dr. Satoshi Yamauchi of the Faculty of Agriculture, Ehime University for measurements of optical rotation and Dr. Kazutaka Itoh for support and assistance with this research.

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