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α-Glucosidase inhibitor compounds from *Aspergillus terreus* RCC1 and their antioxidant activity

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Abstract As part of the search for naturally derived α-glucosidase inhibitors, the chemical components isolated from *Aspergillus terreus* RCC1 were evaluated. Three butenolides compounds were isolated and their structures were identified as isoaspulvinone E (**1**), aspulvinone E (**2**), and butyrolactone I (**3**). Compounds **1** and **2** exhibited high activity on α-glucosidase inhibitory with IC_{50} values of 8.92 and 2.70 μM, respectively, lower than quercetin (IC_{50} = 10.92 μM). However, these compounds exhibited moderate antioxidant activity with IC_{50} values of 167.82 and 114.86 μM, respectively. To the best of our knowledge, this is the first report of both the α-glucosidase inhibitory and antioxidant activities of aspulvinone compounds. In particular, both the aspulvinone compounds could be employed as a lead compound for a new potential antidiabetic derived from terrestrial fungi.

Keywords *Aspergillus terreus* RCC1 · Isoaspulvinone E · α-Glucosidase inhibitor · Antioxidant

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Introduction

Diabetes mellitus (DM) is now becoming a common metabolic disorder resulting from the inability of our body's response to high blood glucose levels. There were approximately 382 million adult aged 20–79 years with diagnosed DM in 2013 (Ramachandran *et al.,* 2013). Type II diabetes is estimated to account for 90–95 % of all diabetes and is characterized by insulin resistance, a relative insulin deficiency, and hyperglycemia. There is considerable evidence that hyperglycemia results from the generation of reactive oxygen species (ROS) production, ultimately leading to increased oxidative stress in a variety of tissues such as nephropathy, retinopathy, neuropathy, and macro-and micro vascular damage (Evans *et al.,* 2002). Therefore, the controlling postprandial hyperglycemia is critical in the early treatment of diabetes mellitus and in reducing chronic vascular complications (Shihabudeen *et al.,* 2011). One of the therapeutic approaches used to decrease postprandial hyperglycemia is the retardation of glucose absorption by inhibiting carbohydrate hydrolysing enzymes, such as α-glucosidase, in the digestive organs and consequently blunting the postprandial plasma glucose rise (Kim *et al.,* 2005).

Glycosidases are well-known targets in the design and development of antidiabetic, antiviral, antibacterial, and anticancer agents (Du *et al*., 2006; Kim *et al.,* 2008). α-Glucosidase inhibitors competitively bind to the carbohydrate-binding region of α-glucosidase enzymes, and thereby compete with oligosaccharides to prevent their cleavage to absorbable monosaccharides (Baron AD, 1998; Cheng and Josse, 2004). These inhibitors could retard the uptake of dietary carbohydrate to suppress postprandial hyperglycemia. The combination of α-glucosidase inhibitors and antioxidants was recently shown to be effective on

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treating diabetes mellitus and preventing its development (Shibano *et al.*, 2008; Takahashi and Miyazawa, 2012). Furthermore, the use of antioxidant may be very important not only for preventing or delaying the progression of late diabetic complications but also for maintaining the antioxidant level in diabetes patients (Mayur *et al.*, 2010).

Acarbose, a pseudo tetrasaccharide isolated from the fermentation broth of Actinoplanes spp. SE-50, has been utilized as medicine for treatment of type 2 diabetes mellitus (Zhu *et al.*, 2008). Interest in the isolation of α-glucosidase inhibitors from certain microorganisms has increased due to fast growing characteristic of microorganisms. For example, validamycin A was isolated from *Streptomyces hygroscopicus* var. *limoneus,* broth of *Bacillus subtilis* B2 also possessed strong α-glucosidase activity (Zhu *et al.*, 2008), the new *N*-containing maltooligosaccharide GIB-638 was isolated from a culture filtrate of *Streptomyces fradiae* PWH638 (Meng and Zhou, 2012) and Aspergillusol A was isolated from marine-derived fungus *Aspergillus aculeatus* (Ingavat *et al.*, 2009).

As a continuing part of our screening for α-glucosidase inhibitors, prospecting fungi *Aspergillus terreus* were investigated to determine the antihyperglycemic effect. The EtOAc extract of *A. terreus* which cultured in solid state fermentation could suppressed postprandial hyperglycemia in mice (Dewi *et al.,* 2007) and the isolation of EtOAc extract of *A. terreus* MC751 led to butyrolactone I as potential α-glucosidase inhibitors and antioxidant (Dewi *et al.,* 2012; Dewi *et al.,* 2014). In the present study, the isolation and biological activities as well as the structure elucidation of the isolated active compounds from *A. terreus* RCC1 were described.

Materials and methods

General instrumentation and reagents

Optical rotation values were measured with a Jasco P-2100 polarimeter. UV–Vis absorption spectra of the active compound in methanol were recorded on a Hitachi U-1600 spectrophotometer. The mass spectra of this compound were measured with high-resolution FAB-MS. 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 the internal standard. HMQC and HMBC techniques were used to assign correlations between ^{1}H and ^{13}C signals and the chemical shift in the quartenary carbon position. Chemical shift values (δ) were 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 using UV light.

α-Glucosidase [(EC 3.2.1.20)] Type I: from *Saccharomyces cerevisiae*, *p*-nitrophenyl α-$_{D}$-glucopyranoside (*p*NPG), DMSO, 1,1-diphenyl-2-picrylhydrazyl (DPPH), quercetin dehydrate, and silica gel (60–200 mesh Wako gel) were purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan). Bacto peptone was obtained from Difco. Co (Detroit, MI). Malt extract and Yeast extract were obtained from Becton–Dickinson Company (Sparks, MD, USA). All the solvents used in this study were purchased from Wako Pure Chemicals and were distilled prior to use.

Fungal material

Aspergillus terreus RCC1, a mutant developed from ATCC 20542, was obtained from the Research Center for Chemistry, Indonesian Institute of Sciences (RCC-LIPI), Indonesia. The voucher specimen is deposited in RCC-LIPI at −20 °C, whereas working stocks were prepared on MY agar (malt extract 1 %, yeast extract 0.4 %, dextrose 0.4 %, and agar 2 %) stored at 4 °C.

Fermentation, extraction, and isolation

Fermentation was carried out using potato malt peptone broth (potato slices 250 g, malt extract 10 g, peptone 1 g, glucose 20 g, and distilled water 1,000 ml) in the Erlenmeyer flask, previously sterilized at 121 °C for 15 min at 15 lb pressure. Two disks (8 mm) of fungal mycelia were used to inoculate on to potato malt peptone broth. The inoculated broth was kept on rotary shaker incubator operated at 60 rpm at 25 °C for 15 days. After 15 days fermentation, the broth (5 l) was extracted with ethyl acetate (5 × 1 l) and concentrated under reduced pressure to yield a brown dark residue (8 g).

The EtOAc extract (6 g) was chromatographed on silica gel (35 × 30 mm i.d) using a stepwise gradient from hexane (90 %) in EtOAc, to EtOAc (100 %) and then EtOAc (50 %) in methanol (MeOH) to obtain eight fractions (At1-At8) based on TLC analysis. Fraction At4 (1.6 g) was separated by silica gel column chromatography with hexan-EtOAc (2:8) repeatedly, to afford 4 fractions (At4.1-At4.4). Compound **3** (980 mg) was isolated as a white solid from fraction At4.2 (1.3 g) by Sephadex LH20 column with $CHCl_3$:MeOH (1:1) as an eluent and then crystallization with $CHCl_3$ in a yield of 16.33 % relative to EtOAc extract. Fraction At6 (700 mg) was separated by chromatography column on silica gel using hexan: EtOAc, step gradient elution from 20:80 to 100:0, to obtain 6 pooled fractions At6.1-At6.6. Compound **1** (20 mg) was

isolated as yellow crystal from At6.2 (110 mg) by preparative thin layer chromatography (PTLC) eluted with $CHCl_3$–MeOH (4:1) and followed by recrystallization from $CHCl_3$ and MeOH in a yield of 0.33 % relative to EtOAc extract. Compound **2** (15 mg) was isolated as a yellow solid from fraction At6.5 (200 mg) by PTLC eluted with $CHCl_3$–MeOH (4:1) in a yield 0.25 % relative to EtOAc extract.

Compound (**1**): Yellow crystalline (Acetone); m.p. >250 °C; $[\alpha]_D^{22.6}$ −5.14 ($c = 0.35$, MeOH); UV (MeOH) λ_{max} (log ε) 314.5 (4.63) nm; ^{1}H NMR (acetone-*d6,* 500 MHz): $\delta = 8.35$ (2H, d, $J = 9.0$ Hz, H-13, H-17), 8.23 (2H, d, $J = 9.0$ Hz, H-7, H-11), 6.82 (2H, d, $J = 8.4$ Hz, H-8, H-10), 6.68 (2H, d, $J = 8.4$ Hz, H-14, H-16), 6.02 (1H, s, H-5); ^{13}C NMR (acetone-*d6,* 125 MHz): $\delta = 178.8$ (C, C-3), 173.9 (C, C-1), 157.0 (C, C-9), 153.5 (C, C-9), 149.2 (C, C-4), 132.8 (2CH, C-7, C-11), 128.4 (2C, C-6, C-12), 126.2 (2CH, C-13, C-17), 116.1 (2CH, C-8, C-10), 114.8 (2CH, C-14, C-16), 114.9 (CH, C-5), 104.9 (C, C-2); HRFABMS$[M + H]^+$: *m/z* 297.0748 (Calcd. for $C_{17}H_{13}O_5$, 297.0759). Crystal data: $C_{17}H_{22}NaO_{11}$, Mr = 425.34, a yellow needle, triclinic, crystal was obtained by recrystallization from MeOH, crystal dimensions 0.170 × 0.040 × 0.020 mm^3. Space group P-1, $a = 7.193(2)$ Å, $b = 10.5922(3)$ Å, $c = 13.0890(3)$ Å, $\alpha = 75.512(6)°$, $\beta = 78.719(6)°$, $\gamma = 87.867(7)°$, $V = 946.90(5)$ Å^3, $Z = 2$, $T = 100$ K, $R1 = 0.0495$(for $I > 2\sigma(I)$), $R\omega$ $wR2 = 0.1411$.

Compound (**2**): Yellow solid (Acetone); m.p. >250 °C; $[\alpha]_D^{22.6}$ +4.00 ($c = 0.2$, MeOH); UV (MeOH) λ_{max} (log ε) 324.5 (4.61) nm; ^{1}H NMR (Acetone-*d6,* 500 MHz): $\delta = 8.16$ (2H, d, $J = 9.0$ Hz, H-13, H-17), 8.09 (1H, d, $J = 9.0$ Hz, H-7, H-11), 6.82 (2H, d, $J = 8.4$ Hz, H-14, H-16), 6.75 (1H, d, $J = 9.0$ Hz, H-8, H-10), 6.18 (1H, s, H-5); ^{13}C NMR (acetone-*d6,* 125 MHz): $\delta = 171.5$ (C, C-1), 158.1 (C, C-9), 156.6 (C, C-3), 155.6 (C, C-15), 149.3 (C, C-4), 132.5 (CH, C-7, C-11), 128.4 (CH, C-13, C-17), 127.1 (2C, C-6, C-12), 116.3 (2CH, C-14, C-16), 115.5 (2CH, C-8, C-10), 110.8 (CH, C-5), 99.2 (C, C-2). HRFABMS $[M + H]^+$: *m/z* 297.0762 (Calcd. for $C_{17}H_{13}O_5$, 297.0759).

Compound (**3**): White solid (Acetone); m.p. 89–90 °C (deqc); $[\alpha]_D^{22.5}$ +68.33 ($c = 0.3$, MeOH); UV (MeOH) λ_{max} (log ε) 307.5 (4.44) nm; ^{1}H NMR (acetone-*d6,* 500 MHz): $\delta = 7.59$ (2H, d, $J = 7.1$ Hz, H-2′,H-6′), 6.87 (2H, d, $J = 7.1$ Hz, H-3′, H-5′), 6.59 (1H, dd, $J = 7.8$ Hz, H-6″), 6.49 (1H, d, $J = 8.4$ Hz, H-5″), 6.42 (1H, d, $J = 1.9$ Hz, H-2″), 5.06 (1H, t, $J = 3.7$ Hz, H-8″), 3.77 (3H, s, 5-OCH_3), 3.42 (2H, d, $J = 3.9$ Hz, H-6), 3.07 (2H, d, $J = 6.5$ Hz, H-7″), 1.66 (3H, s, H-10″), 1.57 (3H, s, H-11″); ^{13}C NMR (acetone-*d6,* 125 MHz): $\delta = 170.9$ (C, C-5), 168.7 (C, C-1), 158.9 (C, C-4′), 154.8 (C, C-4″), 139.0 (C, C-2), 132.5 (C, C-9″), 132.4 (CH, C-2″), 130.2 (2CH, C-2′,C-6′), 129.6 (CH, C-6″), 128.2 (C, C-3″), 127.9 (C, C-3), 124.9 (C, C-1″), 123.4 (CH, C-8″), 122.9 (C,-1′), 116.6 (2CH, C-3′, C-5′), 114.9 (CH, C-5″), 86.0 (C, C-4), 53.8 (OCH_3, C-5), 39.3 (CH_2, C-6), 29.4 (CH_2, C-7), 25.1 (CH_3, C-10″), 17.8 (CH_3, C-11″); HRFABMS $[M + H]^+$: *m/z* 425.1607 (calcd. for $C_{24}H_{25}O_7$, 425.1593).

Single crystal analysis of compound **1**

The crystal was mounted on a glass fiber. The data were collected at temperature of −173 ± 1 °C to a maximum 2θ value of 136.5°. Total of 96 oscillation images were collected. All measurements were made on Rigaku R-AXIS RAPID diffractometer using multi-layer mirror monochromated Cu Kα radiation. The data were corrected for Lorentz and polarization effects. The structure was solved by direct methods (Burla *et al.,* 2007) and expanded using Fourier techniques. Neutral atom scattering factors were taken from Cromer and Waber (Cromer and Waber, 1974). The values used for the mass attenuation coefficients were those of Creagh and Hubbell (Creagh DC and Hubbell JH, 1992). All calculations were performed using the Crystal Structure crystallographic software package except for refinement, which was performed using SHELXL-97 program.

α-Glucosidase inhibitory assay

α-Glucosidase inhibitory activity was evaluated according to the method previously reported by Kim *et al.,* (2004), with minor modifications. α-Glucosidase (250 µl, 0.065 U/mL), 495 µl of 0.1 M phosphate buffer (pH 7.0), and 5 µl of various concentrations of sample in DMSO were pre-incubated at 37 °C for 5 min. The reaction was started by the addition of 250 µl of 3 mM *p*NPG. The reaction was incubated at 37 °C for 15 min and stopped by adding 1 mL of 0.1 M Na_2CO_3. α-Glucosidase activity was determined by measuring release of *p*NPG at 410 nm.

Kinetics of inhibition against α-glucosidase

The inhibition type of active compounds against α-glucosidase activity was measured with increasing concentrations of *p*NPG as a substrate in the absence or presence of active compounds at different concentrations. The type of inhibition was determined by Lineweaver–Burk plot analysis.

DPPH free radicals scavenging assay

The antioxidant activities of the isolated compounds were evaluated according to the method of Yen and Chen (1995), with minor modification. Aliquots of samples in

MeOH (2 ml) at various concentrations (10–200 μg/ml) were each mixed with 0.5 ml of 1 mM DPPH in MeOH. All mixtures were shaken vigorously and left to stand at room temperature for 30 min in the dark. The change in absorbance was measured at 517 nm.

Results and discussion

Many efforts have been made in order to search effective and safe α-glucosidase inhibitor from natural materials for developing physiological functional food or lead compounds for antidiabetic. In this study, the crude EtOAc extract of *A. terreus* showed strong inhibitory activity against α-glucosidase and DPPH free radical with the IC_{50} were 0.96 and 93.60 μg/ml, respectively. Separations of the crude extract using various chromatographic methods resulted in the isolation of three butenolides compounds those are isoaspulvinone E (**1**), aspulvinone E (**2**), and butyrolactone I (**3**).

Compound **1** was obtained as yellow crystals. The HRFABMS of compound **1** showed a molecular $[M + H]^+$ ion at *m/z* 297.0748, corresponding to the molecular formula $C_{17}H_{12}O_5$ (Calcd. *m/z* 297.0759). The ^1H NMR spectrum of compound **1** showed eight aromatic protons as four pairs with ortho coupling ($J = 8.4$ Hz), indicating two A2B2 system and one olefinic proton at δ_H 6.02 (s, H-5). The ^{13}C NMR spectrum showed eight quartenery carbons including a lactone group at 173.9 and three oxygen-bearing olefinic carbons at 178.8, 157.0, and 153.5. The four aromatic carbons with at a δc range of 114.8–132.8 showed very high intensities, which indicated the occurrence of eight overlapping carbon signals (A2B2 system) characteristic of aspulvinone (Ojima *et al.,* 1973).

Compound **2** was obtained as yellow powder. Its molecular formula corresponded to $C_{17}H_{12}O_5$ based on the HRFABMS molecular ion peak $[M + H]^+$ at *m/z* 297.0762 (Calcd. *m/z* 297.0759). The ^1H and ^{13}C NMR spectra of this compound were very similar to those of compound **1**. Moreover, the planar structure of compound **1** was shown to be the same as compound **2** according to a comprehensive analysis of 2D NMR spectra. Therefore, we assumed these compounds to be stereoisomer of aspulvinone. The difference of these compounds was at chemical shift on C-2 and C-5 [δ_C 104.9, δ_C 114.2 for compound **1**; δ_C 99.2, δ_C 110.8 for **2**], respectively. There was reported that for the exocyclic double bond of E-isomer of synthetically analogous pulvinones, the Δδ of carbon C-2 and C-5 range δ_C 102.2–104.9 and δ_C 102.0–114.9, respectively, while for Z-isomer for C-2 range δ_C 99.0–100.4 and for C-5 range δ_C 97.0–107.4 (Gao *et al.,* 2013; Campbell *et al.,* 1985). Hence, the configuration of compound **1** could be assigned as E and Z for compound **2**.

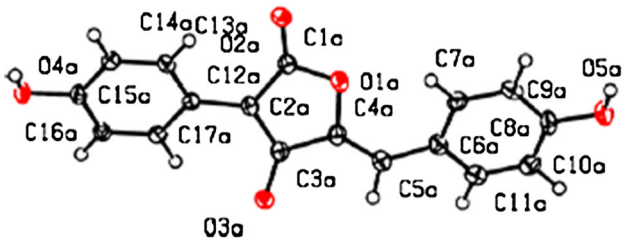


Fig. 1 Crystal structure of compound **1**

Finally, the absolute chemical structure of compound **1** came from X-ray diffraction analysis. The ORTEP representation of the molecular structure of **1** is shown in Fig. 1. Unfortunately, compound **2** did not give suitable crystal for X-ray diffraction analysis. Based on X-ray data, the geometric configuration of compound **1** is determined as E conformation. Therefore, compound **1** was identified as aspulvinone E (E), called Isoaspulvinone E (Gao *et al.,* 2013). Hence, compound **2** was determined geometrically as the Z and identified as aspulvinone E in accordance with the previously reported (Gao *et al.,* 2013; Campbell *et al.,* 1985; Vertesy *et al.*, 2000). The structure of compound **1** was deduced from NMR (1D and 2D), EIMS, and X-ray data, which established its structure as 3-(p-hydroxyphenyl)-4-hydroxy-5-(p-hydroxybenzylidene)-2(5H)-furanone; isoaspulvinone E (Gao *et al.,* 2013) and aspulvinone E (Z) for compound **2** (Ojima *et al.,* 1973; Campbell *et al.,* 1985; Vertesy *et al.*, 2000; Nagia *et al.,* 2012).

Isoaspulvinone E was assumed to be formed from the photoreaction of aspulvinone E during fermentation or subsequent isolation steps (Gao *et al.,* 2013). Aspulvinone E, which contains a butenolide unit and substituted by a phenyl group at C-3 and benzyl group at C-5, is the simplest form of aspulvinones (Aspulvinone A–G) (Ojima *et al.,* 1973), was generally produced as pigments by fungi belonging to the *Aspergillus* family. This class of natural products from *A. terreus* was first reported in 1973 (Ojima *et al.,* 1973). Aspulvinone E was generally produced by *A. terreus*, while isoaspulvinone E was assumed to be formed from the photoreaction of aspulvinone E during fermentation or subsequent isolation steps (Gao *et al.,* 2013). Since compounds **1** and **2** are stereoisomers, they can be changed into each other with light, which indicates that compounds **1** and **2** are photointerconvertible (Gao *et al.,* 2013). Aspulvinones have been shown to exhibit moderate biological activities, including antibacterial activities (Nagia *et al.,* 2012), and slightly inhibit the glucose-6-phosphatase activity of untreated microsomes (Vertesy *et al.,* 2000). Isoaspulvinone E significantly inhibited H1N1 viral neuraminidase (NA), whereas aspulvinone E did not active and was confirmed by structure activity relationship (SAR) that

Table 1 α-Glucosidase inhibitory and antioxidant activities of isolated compounds

Compound	IC_{50} (μM)[a]	
	Inhibition of α-glucosidase	DPPH
1	8.92 ± 3.51	167.82 ± 5.29
2	2.70 ± 2.73	114.86 ± 3.27
3	52.17 ± 5.68	51.39 ± 3.68
Quercetin	10.92 ± 3.72	39.63 ± 5.21

n.d: not detected

[a] IC_{50} values are shown as the mean ± S.D from three independent experiments

the E conformation was shown to be essential for NA inhibitory activity (Gao *et al.,* 2013).

Compound **3** was obtained as a white powder. The molecular formula $C_{24}H_{24}O_7$ of **3** was determined by EIMS, which showed pseudomolecular ion peaks $[M + H]^+$ at 425.1607 (calcd. 425.1593 for $C_{24}H_{25}O_7$). The physical and spectral (one and two dimensional NMR and FABMS) characteristics of the isolated compound was consistent with those of butyrolactone I (α-oxo-β-(p-hydroxyphenyl)-γ-(p-hydroxy-m-3.3-dimethylallylbenzyl)-γ-methoxycarbonyl-γ-butyrlactone I), as previous isolated from MC751 (Dewi *et al.,* 2012). The chemical structures of isolated compounds from *A. terreus* RCC1 are shown in Fig. 2.

Biological activities of the isolated compounds

A yeast α-glucosidase enzyme from *S. cereviseae* which categorized as α-glucosidase type I was selected for general screening of α-glucosidase inhibitors. The isolated compounds were evaluated for their α-glucosidase inhibitory activity compared with quercetin . In this study, we used quercetin as standard due to several reports that quercetin a phenolic compound have stronger inhibitory activity on α-glucosidase from yeast *S. cerevisiae* than acarbose (Tadera *et al.,* 2006; Li *et al.,* 2009; Jo *et al.,* 2009). As reported previously, α-glucosidase broadly consists of type I from yeast *S. cerevisiae* and type II from the mammalian species, and there are structural differences between these types (Kim *et al.,* 2005). There are reports that voglibose and acarbose have high inhibitory effects on mammalian α-glucosidase, but no inhibitory activity for yeast *S. cerevisiae* (Kim *et al.,* 2004). These different inhibitory activities may be caused by structural differences due to the origin of the enzyme.

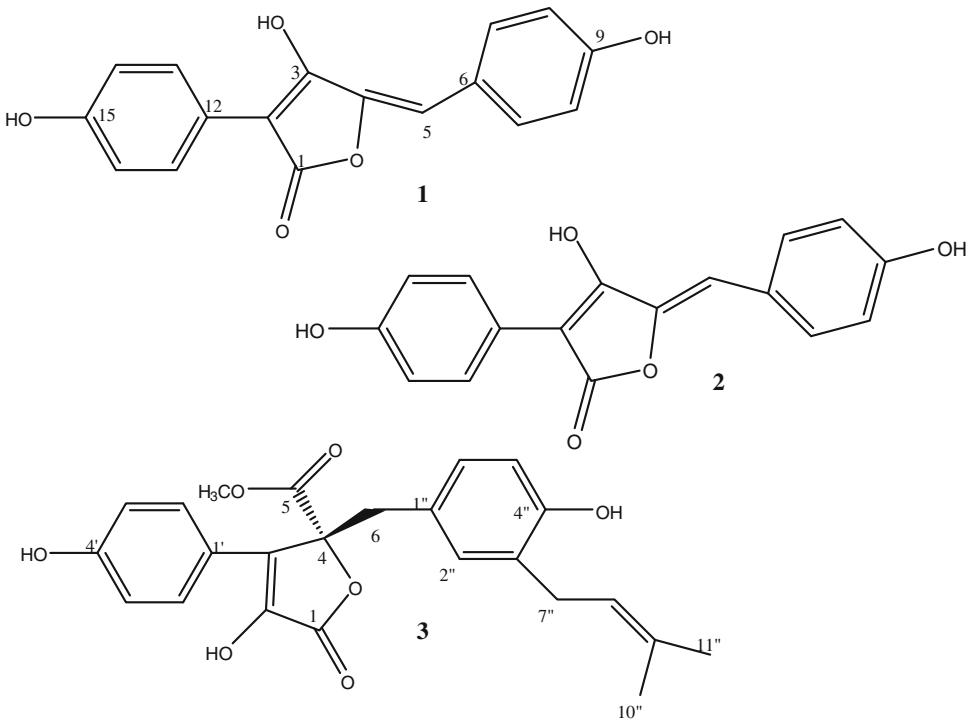
Three isolated compounds (**1**, **2**, and **3**) exhibited strong α-glucosidase inhibitory activity. Compounds **1** and **2** particularly exhibited the higher inhibitory activity against α-glucosidase. The IC_{50} values of compounds **1** and **2** against *S. cereviseae* α-glucosidase were 8.92 and 2.70 μM, respectively, which were lower than quercetin as the reference compound (IC_{50} 10.92 μM) and butyrolactone I (**3**) with an IC_{50} values of 52.17 μM(Table 1). Butyrolactone I (**3**) demonstrated inhibitory activity against α-glucosidase consistent with previous findings report (Dewi *et al.,* 2012; Dewi *et al.,* 2014). Therefore, we considered the pulvinone group which is the generic name used to describe the substituted benzyllidene-4-hydroxy-3-phenyl-5-furan-2(5H)-one (Campbell *et al*., 1985) is a crucial factor for α-glucosidase inhibitory activity in compound **1**, **2**, and **3**(Fig. 2). These results were in accordance with previous report that α-glucosidase was inhibited to a great extent when the compound contained a fused-aromatic ring and heteroaromatic component (Takahashi and Miyazawa, 2012). Furthermore, olefin group in compounds **1** and **2** was also essential for α-glucosidase inhibitory activity. These results supported our previous result that phenolic group and prenyl chain in butyrolactone have synergic effect for α-glucosidase inhibitory activity (Dewi *et al.,* 2014).

To the best of our knowledge, this is the first report of the inhibitory activity of aspulvinone compounds against α-glucosidase. Although compounds **1** and **2** have identical structures, their activities were slightly different; therefore, we assumed that these differences were due to stereochemistry affecting the inhibition activity or recognition of the active site in α-glucosidase.

Kinetic studies of compounds **1** and **2** were performed to determine the mode of inhibition by Lineweaver–Burk plot analysis against α-glucosidase with and without an inhibitor (Fig. 3a, b). The value of 1/V increased with the increasing concentration of inhibitors (compounds **1** and **2**), but *Km* remained constant at 2.36 and 2.66 μM, respectively. This analysis showed a noncompetitive inhibition of compounds **1** and **2**, which indicated that these compounds bind to a site other than the active site of the enzyme. The *Ki* (inhibition constant) values of the compounds were determined to be 37.16 and 10.11 μM, respectively. While compound **3** showed mixed inhibitions with *Ki* value 70.51 μM, this result consistent with the previous report (Dewi *et al.,* 2012).

Antioxidants are substances that can prevent, stop, or reduce oxidative damage; therefore, they are able to protect the human body from several diseases, such diabetes and the complications associated with this disease (Sancheti *et al.,* 2011). An ideal anti-diabetes compound should exhibit the activities of α-glucosidase inhibitors and properties of antioxidants (Shibano *et al.,* 2008). Therefore, the antioxidant activities of isolated compounds from *A. terreus* RCC1 were examined by measuring their DPPH free radical scavenger activity (Table 1).

Fig. 2 Structures of the isolated compounds from *A. terreus* RCC1



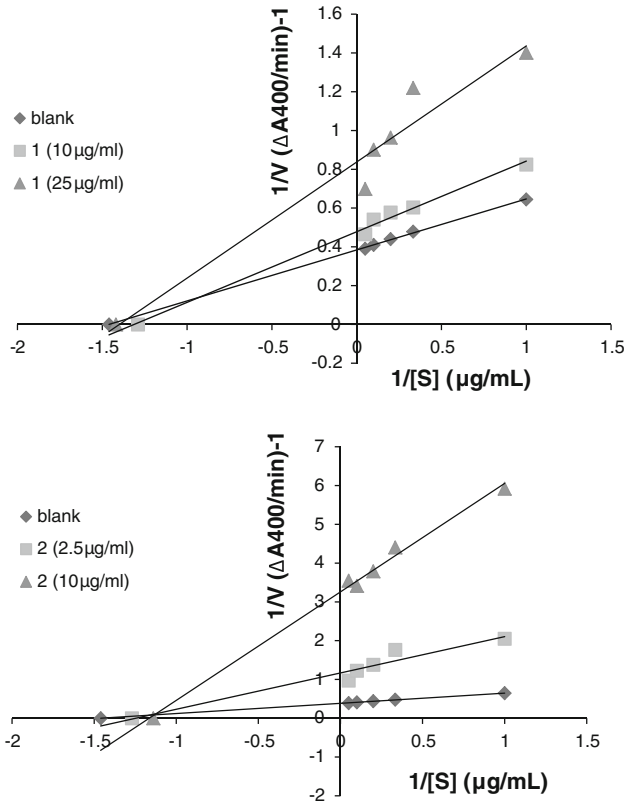


Fig. 3 Lineweaver-Burk plots of α-glucosidase with p-nitrophenyl-α-D-glucopyranoside in the presence of compounds **1** (**a**) and **2** (**b**). The data were obtained from triplicate individual tests

All compounds (**1**–**3**) exhibited significant scavenging activity against DPPH free radicals. The antioxidant activity was attributed to the phenolic group on those compounds. However, the IC_{50} values of **1** and **2** were lower than that of quercetin (Table 1). The antioxidant activities of **1** and **2** have similar values with 6-(4′hydroxy-2′-methyl phenoxy)-(-)(3*R*)-mellein which was isolated from *A. terreus* (BDKU 1164) with IC_{50} 159 ± 0.098 µM (Choudary *et al.,* 2004). In particular, compound **3** showed consistent antioxidant activity with our previous report (Dewi *et al.,* 2012; Dewi *et al.,* 2014). The scavenging activities of compounds **1** and **2** against DPPH free radicals have not been previously reported.

In conclusion, the α-glucosidase inhibition and antioxidant activities of the compounds isolated from EtOAC extract of *A. terreus* RCC1 were investigated. Isoaspulvinone E (**1**) and aspulvinone E (**2**) showed good α-glucosidase inhibitory activity; moreover, the existence of the pulvinone and olefin moiety is essential for α-glucosidase inhibitory activity compared to butyrolactone I (**3**). Butyrolactone I (**3**) was good for both α-glucosidase inhibitory and antioxidant activities. These compounds could be used as a lead compound for new potential antidiabetic derived from fungi.

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