SUPPLEMENTARY MATERIAL

α-Glucosidase inhibitors and phytotoxins from *Streptomyces xanthophaeus*

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Twenty-four metabolites **1**−**24**, were isolated from the fermentation broth of *Streptomyces* sp. caa 01. Their structures were elucidated on the basis of spectroscopic analysis and by comparison of their NMR data with literature data reported. Daidzein **(1)**, genistein **(2)** and gliricidin **(3)** inhibited α-glucosidase with IC_{50} values of 174.2, 36.1, and 47.4 μM, respectively, more potent than the positive control, acarbose. Docking study revealed that the amino acid residue Thr 215 is the essential binding site for active ligands **2**. In addition, the phytotoxic effects of all compounds were assayed on radish seedlings, five of which, **3, 8, 13, 15** and **18**, inhibited the growth of radish (*Raphanus sativus*) seedlings with inhibitory rates of >60% at a concentration of 100 ppm, which was comparable or superior to the positive control glyphosate. This is the first report of the phytotoxicity of the compounds.

Keywords: *Streptomyces* sp.; α-glucosidase inhibitors; phytotoxins; herbicide; diketopiperazine; cyclodipeptide

Experimental

General Experimental Procedures

Optical rotations were recorded on an Autopol III automatic polarimeter (Rudolph Research Analytical). Ultraviolet (UV) spectra were obtained on a UV−vis Evolution 300 spectrometer (Thermo Scientific, USA). NMR spectra were obtained on BrukerAvance III 500 spectrometers with tetramethylsilane as an internal standard at room temperature. ESI-MS were recorded on a Thermo Fisher LTQ Fleet mass spectrometer. Silica gel (200−300 mesh, Qingdao Marine Chemical Ltd., People's Republic of China) and RP- 18 gel (20−45 μm, Fuji Silysia Chemical Ltd., Japan) were used for column chromatography (CC). Fractions were monitored by TLC, and spots were visualized by spraying with 5% H_2SO_4 in ethanol, followed by heating. All other chemicals used in this study are of analytical grade.

Microbial materials

The *Streptomyces* strain caa01 was isolated from soil of the Taibai Mountain (33°57′-34°58′N 107°45′-107°53′E, About 800-3670 meters of elevations). A specimen (No. caa01) was deposited at the College of Science, Northwest A&F University, Shaanxi, China. The total genomic DNA preparation of the strain was carried out following the procedure in the literature (Sambrook et al. 1989). The strain was identified as *Streptomyces xanthophaeus* by complete 16S rRNA gene sequence analysis (Altschul et al. 1997). The strain displayed 99.7% similarity with *Streptomyces xanthophaeus* with the accession number of AB184177, and its sequence has been deposited in GenBank with the accession number: KF317981.

Cultivation

The producing strain was cultured on a plate of Gause's Agar Medium (starch 20.0 g, KNO_3 1.0 g, K_2HPO_4 0.5 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, NaCl 0.5 g, $FeSO_4 \cdot 7H_2O$ 0.01 g, Agar 20 g, H_2O 1000ml, Ph=7.2) at 28 ± 0.5 °C for 7 days. Then one piece (size7 mm^2) of mycelium was inoculated aseptically to 100 mL Erlenmeyer flasks each containing 30 mL of Soybean powder liquid medium (soybean powder 20.0 g， starch 10.0 g，sucrose 3.0 g，peptone 2.0 g，yeast extract 2.0 g，K_2HPO_4 1.0 g，$MgSO_4 \cdot 7H_2O$

0.5 g，$CaCO_3$ 2.0 g，$ZnSO_4$ 0.01 g，$FeSO_4 \cdot 7H_2O$ 0.01 g，NaCl 2.0 g, H_2O 1000 ml, with pH 7.2.), and the seed liquids were incubated at 28 ± 0.5 °C for 3 days on a rotary shaker at 120 rpm. A suspension (200 μL) of the strain was inoculated aseptically to 500 mL Erlenmeyer flasks each containing 200 mL of Soybean powder liquid medium. Fermentation was carried out on a shaker at 130 rpm for 9 days at 28 ± 0.5 °C.

Extraction, Isolation, and Identification of Metabolites

The culture broth (40 L) was filtered, and the filtrate was concentrated to 5 L, then extracted with ethyl acetate (5 L × 3), while the mycelium was extracted three times with $CHCl_3$−MeOH (1:1). The EtOAc layer together with the mycelium extract was concentrated under reduced pressure to give a crude extract (19.5 g), and the latter was applied to a silica gel column eluted with a gradient of $CHCl_3$−MeOH (100:1 100 mL, 50:1 200 mL, 25:1, 120 mL, 15:1 100 mL, 10:1 100 mL,5:1 100 mL, and MeOH 150 mL) to give seven fractions, 1–7.

Fraction 1 ($CHCl_3$/MeOH 100:1) was separated by RP-18 (MeOH/H_2O 10−100%) and repeatedly purified by Sephadex LH-20 ($CHCl_3$/MeOH, 1:1, or MeOH) to afford compounds **23** (20 mg), **20** (6 mg), **6** (6 mg), **7** (7 mg) **11** (22 mg), **17** (18 mg). Fraction 2 ($CHCl_3$/MeOH, 50:1) was separated by RP-18 (MeOH/H_2O 10−100%) and silica gel (petroleum /acetone 3:1) to give compounds**19** (9 mg), **13** (18 mg), **18** (6 mg), **15** (8 mg), **16** (7 mg). Fraction 3 ($CHCl_3$/MeOH 25:1) was subjected to repeated CC on silica gel ($CHCl_3$/acetone 10:1−0:1) and Sephadex LH-20 (CHCl3/MeOH 1:1, MeOH) to give compounds **3** (20 mg), **9** (10 mg),**8** (42 mg), **1** (5 mg) and **2** (10 mg). Fraction 4 ($CHCl_3$/MeOH 15:1) was subjected to CC on RP-18 silica gel (MeOH/H_2O 5:6) and Sephadex LH-20 ($CHCl_3$/MeOH 1:1, MeOH), followed by purification with PTLC ($CHCl_3$/acetone 5:1; $CHCl_3$/MeOH, 20:1) to yield compounds **12** (4 mg), **5** (15 mg), **22**(17 mg). Fraction 5 ($CHCl_3$/MeOH10:1) was subjected to repeated CC on silica gel ($CHCl_3$/acetone, 10:1−0:1) and Sephadex LH-20 ($CHCl_3$/MeOH 1:1, or MeOH) to give compounds **21** (19 mg), **10** (7 mg)and **14** (6.5 mg). Fraction 6 ($CHCl_3$/MeOH, 5:1) was purified by RP-18 (MeOH/H_2O, 3:7−6:4), PTLC (petroleum ether/acetone 2:1) and repeated CC on silica gel

($CHCl_3$/MeOH 4:1) to give compounds **24** (6 mg), **4** (6 mg).

Compound **1.** Colorless crystals, $C_{15}H_{10}O_4$, ESI-MS (negative) *m/z*: 252.98 $[M-H]^-$. ^{1}H-NMR (500MHz, CD_3OD) δ: 8.12 (1H, s, H-2), 8.03 (1H, d, J = 8.8 Hz,H-5), 7.36 (2H, d, J = 8.5 Hz,H-2', 6'), 6.93 (1H, dd, J = 8.8, 2.3 Hz,,H-6), 6.85 (2H, d, J = 8.5 Hz, H-3', 5'), 6.83(1H, m, H-8). ^{13}C-NMR(125 MHz, CD_3OD) δ: 178.2(C-4), 165.1(C-7), 159.8(C-4'), 158.6(C-9), 154.5(C-2), 131.4(C-2', 6'), 128.4(C-5) 125.9(C-1') 124.4(C-3), 118.0(C-10), 116.7(C-6), 116.2(C-3', 5'), 103.3(C-8). It was identified by comparing its NMR data with those of daidzein (Galal et al. 2005).

Compound **2.** Colorless crystals, $C_{15}H_{10}O_5$, ESI-MS (negative) *m/z*: 269.00 $[M-H]^-$. ^{1}H-NMR (500MHz, CD_3OD) δ: 8.02 (1H, s, H-2), 7.36 (2H, d, J = 8.7 Hz,H-2', 6'), 6.85 (2H, d, J = 8.7 Hz, H-3', 5'), 6.32 (1H, d, J = 2.2 Hz,H-8), 6.21 (1H, d, J = 2.2 Hz, H-6). ^{13}C-NMR(125 MHz, CD_3OD) δ: 180.9(C-4), 165.1(C-7), 162.5(C-5), 158.5(C-9), 157.5(C-4'), 153.4(C-2), 130.1(C-2', 6'), 123.5(C-1'), 122.1(C-3), 115.0(C-3', 5'), 104.9(C-10), 99.0(C-6), 93.7(C-8). It was identified by comparing its NMR data with those of genistein (Mazurek et al. 1998, Bai et al. 2009).

Compound **3.** Green prisms, $C_{16}H_{12}O_5$, ESI-MS (positive) *m/z*: 285.24 $[M+H]^+$. ^{1}H-NMR(500MHz, DMSO-d_6) δ: 8.07 (1H, s, H-2), 7.88 (1H, d, J = 8.8 Hz, H-5), 7.11 (1H, d, J = 2.0 Hz, H-2'), 6.92 (1H, dd, J = 8.8, 2.0 Hz, H-6), 6.82 (2H, m, H-5', 6'), 6.68 (1H, d, J = 2.0 Hz, H-8), 3.79 (3H, s, H-7').^{13}C-NMR(125MHz, DMSO-d_6) δ: 174.7(C-4), 165.7(C-7), 157.8 (C-9), 152.0 (C-2), 147.0 (C-4'), 146.3 (C-3'), 126.6 (C-5), 123.4 (C-1'), 123.2 (C-3), 121.2 (C-6'), 116.2 (C-2'), 115.1 (C-10), 115.0 (C-6), 112.9 (C-5'), 101.9 (C-8), 55.5 (C-7'). It was identified by comparing its NMR data with those of gliricidin (Kamnaing et al. 1999, Du et al. 2006).

Compound **4.** Crystalline powder, $C_{12}H_{16}O_2$, ESI-MS (positive) *m/z*: 289.41 $[M+H]^+$. ^{1}H-NMR(500MHz, CD_3OD) δ: 7.65(1H, d, J = 2.0 Hz, H-6), 6.77(1H, d, J = 2.0 Hz, H-5), 5.57 (1H, d, J = 3.5 Hz, H-1'), 3.88-3.41 (6H, m, H-2', 3', 4', 5', 6'), 2.48 (3H, s, H-1). ^{13}C-NMR(125 MHz, CD_3OD) δ: 187.5(C-2), 154.3(C-4),

148.5(C-6), 139.3(C-3), 105.8(C-5), 101.3(C-1'), 75.4(C-5'), 74.7(C-3') 72.9(C-2') 71.1(C-4'), 62.2(C-6') 27.3(C-1). It was identified by comparing its NMR data with those of 2-acetylfuran-3-glucopyranoside (Xue et al. 2013).

Compound **5.** White solid, $C_{11}H_{15}NO_2$, ESI-MS (positive) *m/z*: 216.26 $[M+Na]^+$. ^{1}H-NMR(500MHz, CD_3OD) δ: 7.23(5H, m, H-5, 6, 7, 8, 9), 4.09(1H, m, H-2), 3.52 (2H, m, H-1), 2.90(1H, dd, *J* = 13.8, 6.3 Hz, H-3a), 2.71(1H, dd, *J* = 13.8, 8.3 Hz, H-3b), 1.87(3H, s, H-12). ^{13}C-NMR(125 MHz, CD_3OD) δ: 173.0(C-11), 139.8(C-4), 130.2(C-6, 8), 129.3(C-5, 9), 127.2(C-7), 64.1(C-1), 54.2(C-2), 37.9(C-3) 22.6(C-12). Compound **5** was identified by comparing its NMR data with those of *N*-acetyl-L-phenylalaninol (Deena et al. 2013, Pan et al. 2014, Chen et al. 2015).

Compound **6.** Colorless amorphous powder, $C_{22}H_{30}O_6$, ESI-MS (positive) *m/z*: 413.34 $[M+Na]^+$. ^{1}H-NMR(500MHz, CD_3OD) δ:7.13 (4H, d, *J* = 8.8 Hz, H-3, 3', 5, 5'), 6.84 (2H, d, *J* = 8.8 Hz, H-2', 6'), 6.82 (2H, d, *J* = 8.8 Hz, H-2, 6), 4.05 (2H, m, H-8, 8'), 3.95(4H, m, H-7, 7') 3.54 (2H, dd, *J* = 9.5, 5.0 Hz, H-9'), 3.53 (2H, dd, *J* = 9.5, 5.0 Hz, H-9) 3.38 (3H, s, H-10), 1.61 (6H, s, H-12, 13) ^{13}C-NMR(125 MHz,CD_3OD) δ: 158.2(C-1), 158.1(C-1'), 144.7(C-4'), 144.6(C-4), 128.7(C-3, 3', 5, 5'), 115.0(C-2, 2', 6, 6'), 74.9(C-9), 71.8(C-8'), 70.5(C-7), 70.4(C-7'), 70.1(C-8), 64.2(C-9'), 59.4(C-10), 42.6(C-11), 31.5(C-12, 13). It was identified by comparing its NMR data with those of 1,2-propanediol,3-(4-(1-(4-(2-hydroxy-3-methoxypropoxy)phenyl) -1-methylethyl)phenoxy) (Zhang et al. 2011).

Compound **7.** Colorless amorphous powder, $C_{21}H_{28}O_8$, ESI-MS (positive) *m/z*: 399.31 $[M+Na]^+$. ^{1}H-NMR(500MHz, CD_3OD) δ:7.12 (4H, d, *J* = 8.9 Hz, H-3, 5), 6.84 (4H, d, *J* = 8.9 Hz, H-2, 6), 4.01 (6H, m, H-7, 8), 3.66 (4H, m, H-9), 1.60 (6H, s, H-11) ^{13}C-NMR(125 MHz,CD_3OD) δ: 158.1(C-1), 144.6(C-4), 128.7(C-3, 5), 115.0(C-2, 6), 71.8(C-8), 70.3(C-7), 64.2(C-9), 42.6(C-10), 31.5(C-11). It was identified by comparing its NMR data with those of 2,2-[bis-4-(2,3-dihydroxypropoxy)phenyl]propane (Ackerman et al. 2011).

Compound **8.** White needle, $C_5H_5NO_2$, ESI-MS *m/z*: 146.86 $[M+Cl]^-$. ^{1}H-NMR(500MHz, CD_3OD) δ: 6.94 (1H, dd, *J* = 2.6, 1.4 Hz, H-2) 6.87 (1H,

dd, *J* = 3.6, 1.4 Hz, H-4) 6.18 (1H, dd, *J* = 3.6, 2.6 Hz, H-3). ^{13}C-NMR(125 MHz,CD_3OD) *δ*: 164.5(C-6), 124.5(C-2), 123.7(C-5), 116.7(C-4), 110.6(C-3). It was identified by comparing its NMR data with those of pyrrole-2-carboxylic acid (Zhang et al. 2011).

Compound **9.** Colorless oil, $C_4H_{10}O_2$, ESI-MS (negative) *m/z*: 179.03 $[2M-H]^-$. ^{1}H-NMR(500MHz, CD_3OD) *δ*: 3.55 (2H, m, H-2, 3), 1.17 (6H, d, *J* = 6.3 Hz,H-1, 4). ^{13}C-NMR(125 MHz, CD_3OD) *δ*: 72.5(C-2, 3), 18.6(C-1, 4). It was identified by comparing its NMR data with those of 2,3-butanediol (Li et al. 2007).

Compound **10.** Colorless oil, $C_7H_{10}ClN_3O_3$, ESI-MS (positive) m/z： 219.99 $[M+H]^+$. ^{1}H-NMR (500MHz, CD_3OD) *δ*: 7.94 (1H, s, H-4), 4.71 (1H, dd, *J* = 14.3, 2.7 Hz,H-1'a), 4.29 (1H, dd, *J* = 14.3, 9.6 Hz,H-1'b), 4.12 (1H, m, H-2'), 3.69 (1H, dd, *J* = 5.1, 3.4 Hz,H-3'), 2.5 (3H, s, H-6). ^{13}C-NMR (125 MHz, CD_3OD) *δ*: 153.4(C-2), 140.1(C-5), 132.5(C-4), 71.1(C-2'), 50.6(C-1'), 47.4(C-3'), 14.4(C-6). It was identified by comparing its NMR data with those of *R*-(-)-l-(3-chloro-2-hydroxypropyl)-2-methyl-5-nitroimidazole (Skupin et al. 1997).

Compound **11.** White crystals, $C_{10}H_{14}N_2O_3$, ESI-MS (positive) *m/z*: 249.24 $[M+K]^+$. ^{1}H-NMR (500MHz, CD_3OD) *δ*: 4.60 (1H, dd, *J* = 10.6, 6.9 Hz, H-8), 4.49 (1H, t, *J* = 3.9 Hz,H-6), 4.39 (1H, t, *J* = 7.9 Hz, H-3), 3.63 (1H, m,H-9a), 3.51 (3H, m, H-9b, 12a, 12b), 2.29 (2H, m, H-7), 2.14 (4H, m, H-10a, 10b, 11). ^{13}C-NMR(125 MHz, CD_3OD) *δ*: 168.8(C-5), 168.7(C-2), 69.5(C-8), 61.6(C-6), 60.1(C-3), 54.7(C-9), 46.2(C-12), 37.6(C-7), 28.6(C-10), 24.1(C-11). It was identified by comparing its NMR data with those of cyclo(L-Pro-L-4Hyp) (Garbay-Jaureguiberry et al. 1980. Kazuharu et al. 1987).

Compound **12.** Yellow oil, $C_{14}H_{16}N_2O_3$, ESI-MS (positive) *m/z*: 261.12 $[M+H]^+$. ^{1}H-NMR(500MHz, CD_3OD) *δ*: 7.26 (5H, m, H-2', 3', 4', 5', 6'), 4.48 (1H, t, *J* = 5.0 Hz, H-3), 4.37 (1H, m, H-8), 4.28(1H, t, *J* = 4.8 Hz, H-6), 3.72(1H, dd, *J* = 13.0, 5.1 Hz, H-9a), 3.21(1H, m, H-9b), 3.17(2H, m, H-10), 2.08(1H, dd, *J* = 13.0, 5.9 Hz, H-7a) 1.41(1H, m, H-7b). ^{13}C-NMR(125 MHz, CD_3OD) *δ*: 171.2(C-5), 167.0(C-2), 137.3(C-11), 130.9(C-13, 15), 129.4(C-12, 16), 128.0(C-14), 68.5(C-8), 58.2(C-6), 57.5(C-3), 55.1(C-9), 38.7(C-10), 37.9(C-7). It was identified by

comparing its NMR data with those of cyclo-(*trans*-4-hydroxy-L-prolyl-L-phenylalanine) (Salbatore et al. 2003).

Compound **13.** Yellow Solid, $C_{11}H_{18}N_2O_3$, ESI-MS (positive) *m/z*: 227.13 $[M+H]^+$. ^{1}H-NMR(500MHz, CD_3OD) δ: 4.52 (1H, m, H-8), 4.46 (1H, t, *J* = 4.1 Hz, H-6), 4.17 (1H, m, H-3), 3.68(1H, dd, *J* = 12.8, 4.4 Hz, H-9a), 3.45(1H, d, *J* = 12.8 Hz, H-9b), 2.29(1H, m, H-7a), 2.09(1H, m, H-7b), 1.90(2H, m, H-10), 1.52(1H, m, H-11), 0.97(3H, d, *J* = 6.2 Hz, H-12) 0.95(3H, d, *J* = 6.2 Hz, H-13). ^{13}C-NMR(125 MHz, CD_3OD) δ: 173.0(C-5), 169.0(C-2), 69.0(C-8), 58.6(C-6), 55.1(C-9), 54.5(C-3), 39.3(C-10), 38.1(C-7), 25.7(C-11), 23.2(C-13), 22.1(C-12). It was identified by comparing its NMR data with those of cyclo-(*cis*-4-hydroxy-D-prolyl-L-leucine) (Salbatore et al. 2003).

Compound **14.** White powder, $C_7H_{10}N_2O_2$, ESI-MS (positive) *m/z*: 193.17 $[M+K]^+$. ^{1}H-NMR(500MHz, CD_3OD)δ: 4.25 (1H, t, *J* = 7.9 Hz,H-6), 4.12 (1H, dd, *J* = 16.8, 2.0 Hz,H-3a), 3.76 (1H, d, *J* = 16.8 Hz,H-3b), 3.55 (2H, m, H-9), 2.33 (1H, m, H-7a), 2.01 (1H, m, H-7b) 1.95 (2H, m, H-8). ^{13}C-NMR(125 MHz, CD_3OD) δ: 171.9(C-5), 166.4(C-2), 59.8(C-6), 46.9(C-3), 46.2(C-9), 29.3(C-7), 23.2(C-8). It was identified by comparing its NMR data with those of cyclo(L-Pro-Gly) (Chen et al. 2012).

Compound **15.** Yellow powder, $C_{16}H_{17}N_3O_2$, ESI-MS (positive) *m/z*: 588.74 $[2M+Na]^+$. ^{1}H-NMR(500MHz, CD_3OD) δ:7.58 (1H, d, *J* = 7.9 Hz, H-5), 7.34 (1H, d, *J* = 7.9 Hz, H-8), 7.10 (1H, m, H-2), 7.08 (1H, m, H-7), 7.03 (1H, m, H-6), 4.41 (1H, t, *J*= 4.5 Hz, H-11), 3.99 (1H, ddd, *J* = 10.2, 4.6, 0.9 Hz, H-14), 3.44 (1H, m, H-17a), 3.32 (1H, m, H-10a), 3.28 (1H, m, H-10b), 3.26 (1H, m, H-17b), 1.96 (1H, m, H-19a), 1.66 (1H, m, H-18a), 1.47 (1H, m, H-18b), 0.92 (1H, m, H-19b). ^{13}C-NMR(125 MHz,CD_3OD) δ: 170.7(C-13), 167.4(C-16), 137.9(C-9), 128.6(C-4), 125.5(C-2), 122.5(C-7), 119.8(C-6), 119.7(C-5), 112.2(C-8) 109.4(C-3), 60.0(C-14), 57.2(C-11), 45.9(C-17), 29.2(C-10), 29.1(C-19), 22.4(C-18). It was identified by comparing its NMR data with those of brevianamide F (Asiri et al. 2015).

Compound **16.** Colorless solid, $C_{12}H_{14}N_2O$, ESI-MS (positive) *m/z*: 225.23 $[M+Na]^+$. ^{1}H-NMR(500MHz, CD_3OD) δ:7.55 (1H, d, *J* = 8.3 Hz, H-4), 7.33(1H, d, *J*

= 8.3 Hz, H-7), 7.07(1H, m, H-5), 7.06(1H, s, H-2), 7.01(1H, dd, *J* = 8.2, 8.1 Hz, H-6), 3.45(2H, t, *J* = 7.3 Hz, H-11),2.93(2H, t, *J* = 7.3 Hz, H-10), 1.91(3H, s, H-14). ^{13}C-NMR(125 MHz,CD_3OD) *δ*: 173.3(C-13), 138.1(C-8), 128.7(C-9), 123.3(C-2), 122.2(C-6), 119.5(C-5), 119.2(C-4), 113,2(C-3), 112.2(C-7), 41.5(C-11), 26.1(C-10), 22.5 (C-14). It was identified by comparing its NMR data with those of N_b-acetyltryptamine (Li et al. 2003, Lin et al. 2008).

Compound **17.** White crystals, $C_8H_{14}N_2O_2$, ESI-MS (negative) *m/z*: 339.00 $[2M-H]^-$. ^{1}H-NMR(500MHz, CD_3OD) *δ*: 3.99 (1H, d, *J* = 17.8 Hz,H-6a), 3.88 (1H, t, *J* = 6.6 Hz,H-3),3.81 (1H, d, *J* = 17.8 Hz,H-6b), 1.81 (1H, m,H-8), 1.67 (2H, m, H-7), 0.97(3H, d, *J* = 6.8 Hz,H-9), 0.96(3H, d, *J* = 6.7 Hz,H-10). ^{13}C-NMR(125 MHz, CD_3OD) *δ*: 171.5(C-2), 168.9(C-5), 54.8(C-3), 45.2(C-6), 43.8(C-7), 25.2(C-8), 23.3(C-9) 22.1(C-10). It was identified by comparing its NMR data with those of cyclo(Gly-Leu) (Coursindel et al. 2010).

Compound **18.** White crystals, $C_8H_{14}N_2O_2$, ESI-MS (positive) *m/z*: 193.18 $[M+Na]^+$. ^{1}H-NMR(500MHz, CD_3OD) *δ*: 3.97 (1H, d, *J* = 18.0 Hz,H-6a), 3.81 (1H, d, *J* = 18.0 Hz,H-6b), 3.80 (1H, m,H-3), 1.95 (1H, m, H-7), 1.55 (1H, m, H-8a), 1.26 (1H, m, H-8b) 1.01 (3H, d, *J* = 7.0 Hz,H-10) 0.95 (3H, t, *J* = 7.4 Hz,H-9). ^{13}C-NMR(125 MHz, CD_3OD) *δ*: 170.1(C-2), 168.7(C-5), 61.0(C-3), 45.2(C-6), 41.2(C-7), 25.5(C-8), 15.4(C-10) 12.0(C-9). It was identified by comparing its NMR data with those of cyclo(Gly-Ile) (Coursindel et al. 2010).

Compound **19.** White solid, $C_{11}H_{12}N_2O_2$, ESI-MS (positive) *m/z*: 226.57 $[M+Na]^+$. ^{1}H-NMR(500MHz, CD_3OD) *δ*: 7.30 (3H, m, H-2', 4', 6'), 7.22 (2H, m, H-3', 5'), 4.23 (1H, m, H-6), 3.41(1H, d, *J* = 17.8 Hz,H-3a), 3.23(1H, dd, *J* = 13.7, 4.6 Hz,H-7a), 3.02(1H, dd, *J* = 13.7, 4.6 Hz,H-7b), 2.66(1H, d, *J* = 17.8 Hz, H-3b). ^{13}C-NMR(125 MHz, CD_3OD) *δ*: 170.0(C-1), 168.6(C-4), 136.3(C-1'), 131.4(C-3', 5'), 129.5(C-2', 6'), 128.4(C-4'), 57.5(C-6), 44.6(C-3), 40.8(C-7). It was identified by comparing its NMR data with those of cyclo(Phe-Gly) (Deslauriers et al. 1975, Coursindel et al. 2010).

Compound **20.** White powder, $C_4H_4N_2O_2$, ESI-MS (negative) *m/z*: 110.89 $[M-H]^-$. ^{1}H-NMR(500MHz, CD_3OD) *δ*:7.39 (1H, d, *J* = 7.7 Hz, H-6), 5.61 (1H, d, *J* =

7.7 Hz, H-5). ^{13}C-NMR(125 MHz,CD_3OD) δ: 167.3(C-4), 153.5(C-2), 143.5(C-6), 101.7(C-5). It was identified by comparing its NMR data with those of uracil (Staubmann et al. 1999).

Compound **21.** White powder, C_5H_9NO, ESI-MS(positive)m/z: 117.67$[M+NH_4]^+$ ^{1}H-NMR(500MHz, DMSO-d_6) δ: 3.10 (2H, m, H-3), 2.10 (2H, t, J = 6.5 Hz,H-6), 1.64 (4H, m, H-4, 5). ^{13}C-NMR(125 MHz, DMSO-d_6) δ: 170.3(C-1), 41.3(C-3), 31.4(C-6), 22.0(C-4), 20.7(C-5). It was identified by comparing its NMR data with those of 2-Piperidone (Morales-Serna et al. 2013).

Compound **22.** White powder, $C_8H_{14}N_2O_2$, ESI-MS (positive) *m/z*: 193.14 $[M+Na]^+$. ^{1}H-NMR(500MHz, CD_3OD) δ: 4.55 (1H, dd, J = 11.3, 1.3 Hz, H-7) 3.26 (2H, m, H-3) 1.97 (3H, s, H-10) 1.95-1.71 (4H, m, H-6, 4) 1.55 (1H, m, H-5a) 1.35 (1H, m, H-5b). ^{13}C-NMR(125 MHz,CD_3OD) δ: 177.2(C-1), 172.3(C-9), 53.3(C-7), 42.4(C-3), 32.1(C-6) 29.8(C-4), 29.0(C-5), 22.5(C-10). It was identified by comparing its NMR data with those of *N*-acetyl-cyclolysine (Adamczeski et al. 1989).

Compound **23.** White powder, $C_8H_9NO_2$, ESI-MS (negative) *m/z*: 149.97 $[M-H]^-$. ^{1}H-NMR(500MHz, CD_3OD) δ:7.58 (1H, dd, J = 8.0, 1.2 Hz, H-6), 7.02 (1H, td, J = 7.7, 1.4 Hz, H-4), 6.87 (1H, dd, J = 8.0, 1.0 Hz, H-3), 6.82 (1H, td, J = 7.7, 1.1 Hz, H-5), 2.16 (3H, s, H-2'). ^{13}C-NMR(125 MHz,CD_3OD) δ: 172.2(C-1'), 149.7(C-2), 127.1(C-1), 126.8(C-4), 123.9(C-6), 120.6(C-5), 117.3(C-3), 23.4(C-2'). It was identified by comparing its NMR data with those of *N*-(2-Hydroxyphenyl)-acetamide (Zhuo et al. 2012, Dashti et al. 2014).

Compound **24.** Colorless gum, $C_7H_6O_3$, ESI-MS (positive) *m/z*: 177.52 $[M+K]^+$. ^{1}H-NMR(500MHz, CD_3OD) δ: 7.88 (2H, d, J = 8.8 Hz, H-2, 6), 6.82 (2H, d, J = 8.8 Hz,H-3, 5). ^{13}C-NMR(125 MHz, CD_3OD) δ: 170.1(C-7), 163.3(C-4), 132.9(C-2, 6), 122.7(C-1), 116.0 (C-3, 5). It was identified by comparing its NMR data with those of *P*-hydroxybenzoic acid (Li et al. 2014).

Alpha-glucosidase inhibitory activity assay

The assay mixture (750 μL) contained 596.25 μL of 0.05 M phosphate buffer (pH 7.0), 112.5 μL of substrate solution (2 mM PNPG in 0.05 M phosphate buffer), 3.75 μL of enzyme solution(10 U/mL) and 37.5 μL indicated concentration of acarbose and other

inhibitors (dissolved well in DMSO and then further diluted with 0.05 M phosphate buffer). PBS, solution of inhibitors and enzyme solution were incubated at 37℃ for accurate 10 min, then substrate solution was added, after that anther 40 min, incubation at 37℃ was needed. The amount of PNP released was quantified (compare with standard curve) on a microplate spectrophotometer (Epoch，BioTek, USA) at 400 nm.

One set of reaction mixture prepared by an equivalent volume of DMSO and phosphate buffer. Acarbose was used as positive control. The inhibitory rates (%) were calculated according to the formular: (ODblank-ODtest)/ODblank×100%.

Table S1. Inhibitory effects of the isolates against α-glucosidase

compound	IC_{50} (μM)
1	174.2± 0.5
2	36.1± 0.4
3	47.4± 0.5
Other compounds	NONE
acarbose[a]	663.2± 0.4

[a]Positive control.

Molecular docking simulation of compound 2 with α-glucosidase

Although the X-ray crystal structures of some bacterial *α*-glucosidase have been reported, structural information is still unavailable for the eukaryotic *α*-glucosidase enzyme from Baker's yeast (the enzyme used in our biological assays). However, only a few homology models have been previously developed for this enzyme (Guerreiro et al. 2013, Barakat et al. 2015). We attempted to build the 3D structure for human α-glucosidase by comparative homology modeling technique using the samilar propriety as described by Assem Barakat (Barakat et al. 2015). The α-glucosidase sequence was retrieved from UniProt (access code P53341). The 1.30 Å resolving crystallographic structure of *Saccharomyces cerevisiae* isomaltase (PDB code: 3AJ7) (Yamamoto et al. 2010) with 72% similarity was selected as the template for modeling. The 3D structure was built by means of Modeller 9.15. The predicted 3D

model was subjected to energy minimization up to 0.05 gradients. Before docking simulation, ligands were searched for their conformers by MMFF94S in CONFLEX 6.7 package. Finally, all conformers were docked with α-glucosidase in Autodock Vina (Trott & Olson 2010). The binding site bounding box was set as 20 $Å^3$. The results are shown in Figures S1-S3.

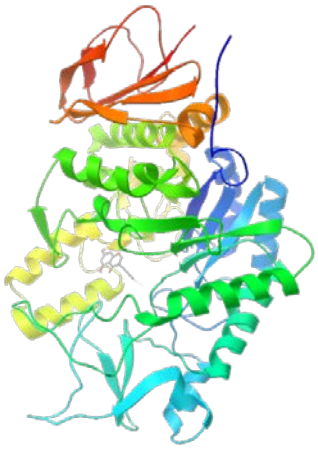


Figure S1. Binding modes of **2** in the active site of α-glucosidase

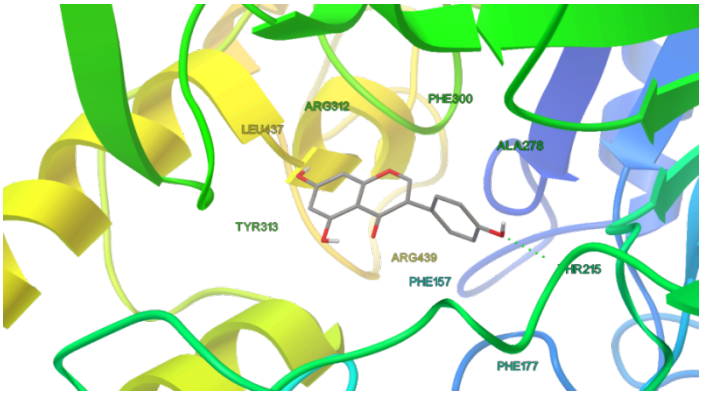


Figure S2. The binding modes and molecular interactions of **2** in the active sites of α-glucosidase

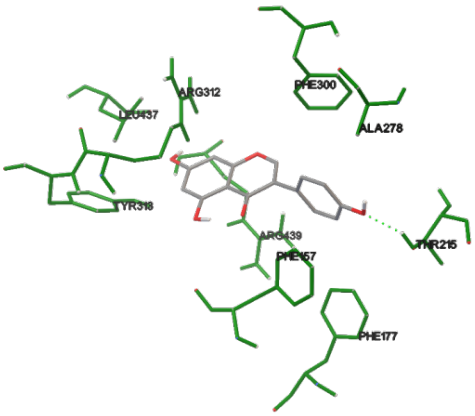


Figure S3. The binding sites of the compound **2** – α-glucosidase complex after molecular dynamics simulation

Phytotoxicity Bioassay

Phytotoxicity was assayed by the method reported previously (Ichihara et al. 1996, Zhang et al. 2013). Briefly, radish seedlings were washed by running water for 120 min and then soaked in 0.3% $KMnO_4$ for 15 min and flushed to colorless. An acetone solution containing a sample at a defined concentration (100 ppm) was poured on two sheets of filter paper in a 12-well microplate. After removal of the solvent *in vacuo*, 200 μL of aseptic water was added on it. Radish seedlings of uniform shape and size were placed on the filter paper. In this experiment the seedlings were grown for 4 days at 25 °C under completely dark conditions. The inhibitory effects were observed after 96 h. Glyphosate was used as the positive control. Acetone served as a blank control. Each experiment was conducted three times and presented as the mean ± standard deviation of three replicates. The results are shown in Tables S2 and S3 and Figure S4.

The inhibitory ratio(%) were calculated according to the formular:

Ratio(%) (root)= [root(control)-root(experiment)]/root(control) ×100%

Ratio(%)(hypocotyl)= [hypocotyl (control)-hypocotyl (experiment)]/ hypocotyl (control) ×100%

Table S2. Inhibitory effects of compounds **3, 8, 13, 15, 16** and **18** on the growth of radish seedlings at 100 ppm.

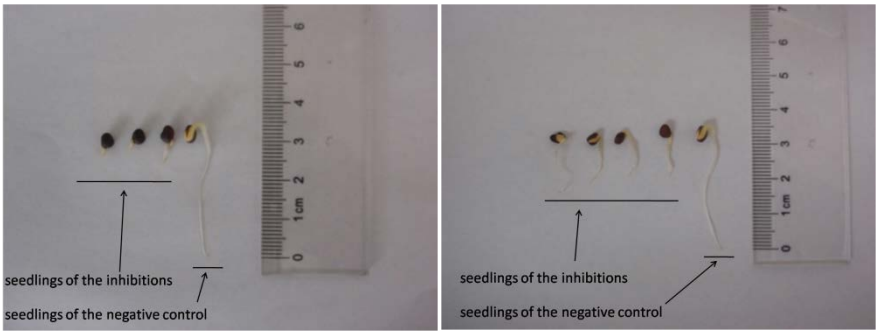
	inhibition ratio[a] (%)			inhibition ratio[a] (%)	
Comd	Root	Hypocotyls	Comd	Root	Hypocotyls
3	66.6± 2.3	33.0± 2.1	**16**	54.5± 1.5	-
8	66.6± 3.0	33.0± 2.9	**18**	60.6± 2.6	30.0± 2.0
13	87.7± 2.0	31.0± 2.8	glyphosate	54.5± 2.3	61.9± 2.6
15	60.6± 2.5	-			

[a]Mean ± SD.

Table S3. Length of root and hypocotyl

No.		length of root and hypocotyl (mm)															average	%
3	hypocotyl	2.05	1.90	2.60	2.45	2.40	2.30	2.30	2.40	2.30	—	—	—	—	—	—	2.30	33.0± 2.1
	root	12.25	7.20	10.50	14.10	13.20	9.70	12.00	9.25	8.10	—	—	—	—	—	—	10.70	66.6± 2.3
8	hypocotyl	2.50	2.80	2.10	2.30	2.00	2.15	2.30	1.80	2.40	2.65	2.30	—	—	—	—	2.30	33.0± 2.9
	root	14.40	15.75	8.20	9.50	9.30	10.00	8.10	7.35	8.30	14.30	12.50	—	—	—	—	10.70	66.6± 3.0
13	hypocotyl	2.20	2.50	2.30	2.70	2.70	1.90	2.05	2.50	2.30	—	—	—	—	—	—	2.35	31.0± 2.8
	root	1.70	3.35	4.50	6.80	5.70	1.55	1.50	5.40	5.05	—	—	—	—	—	—	3.95	87.7± 2.0
15	hypocotyl	4.00	4.90	3.75	4.45	3.90	4.50	5.10	4.60	4.30	4.80	4.10	—	—	—	—	4.40	—
	root	11.60	13.50	9.80	16.00	11.50	10.70	18.40	10.50	11.80	12.30	12.50	—	—	—	—	12.60	60.6± 2.5
16	hypocotyl	4.00	4.45	4.30	4.25	4.55	5.50	4.50	4.70	4.70	—	—	—	—	—	—	4.55	—
	root	14.50	14.50	13.25	12.75	14.55	18.20	14.50	14.50	14.2	—	—	—	—	—	—	14.55	54.5± 1.5
18	hypocotyl	2.30	2.70	2.50	2.05	2.60	2.40	2.35	2.30	—	—	—	—	—	—	—	2.40	30.0± 2.0
	root	12.50	15.50	9.20	8.90	15.30	11.10	13.60	14.70	—	—	—	—	—	—	—	12.60	60.6± 2.6
glyphosate	hypocotyl	1.10	1.20	1.50	0.90	1.60	1.70	1.00	1.60	1.20	1.30	1.20	—	—	—	—	1.30	61.9± 2.6
	root	14.00	15.55	12.60	10.50	16.50	17.65	12.2	18.00	14.50	14.00	14.55	—	—	—	—	14.55	54.5± 2.3
CK	hypocotyl	3.50	3.80	3.30	3.50	3.40	3.50	3.25	3.30	3.50	3.45	—	—	—	—	—	3.45	
	root	34.00	35.00	30.20	32.00	31.00	34.00	30.00	30.5	31.10	32.20	—	—	—	—	—	32.00	

—: indicated no seeds germination.



13 **3**

Figure S4. Phytotoxic effects of compounds **13** and **3** on the roots of radish seedlings.

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