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Synthesis of Cinnamamide Derivatives and Their α-Glucosidase Inhibitory Activities

(Sintesis Terbitan Sinamamida dan Aktiviti Rencatan α-Glucosidase)

TENI ERNAWATI*, ABDUL MUN'IM, MUHAMAD HANAFI & ARRY YANUAR

ABSTRACT

Chemically, methyl trans-cinnamate offers treatment at the three main reactive sites such as substitution at the phenyl ring, addition at the α,β-unsaturation, and substitution at the carboxylic methyl ester functionality. We focus our research to the amide and related derivatives of cinnamates because of their lesser known attributes towards antidiabetic activities. In this research, we modify methyl trans-cinnamate by amidating the carboxylic methyl ester functionality using several amines introduced in functional groups of methyl trans-cinnamate. A series of cinnamamide derivatives was synthesized and evaluated for α-glucosidase inhibitory effects. The structure of synthesized compounds was characterized by IR, melting point, UV, ^{1}H NMR, ^{13}C NMR, and mass spectral analysis. All 13 cinnamamide showed higher α-glucosidase activity than the starting compound. The substitution of cinnamic acid with an amide group altered the α-glucosidase inhibitory activity. Increased bulkiness and the chain length of the amine substituents decreased the inhibitory activity. Propylcinnamamide (3c) showed the most potent inhibitory activity among all the cinnamamide derivatives, all of which act through a competitive inhibitory mechanism. These compounds may be worth exploring further.

Keywords: α-glucosidase inhibitor; cinnamamide derivative; synthesis

ABSTRAK

Secara kimia, metil trans-sinamat menawarkan rawatan pada tiga tapak reaktif utama seperti penggantian pada gelang fenil, penambahan pada ketaktepuan α,β dan penggantian pada kefungsian karbosilik metil ester. Kami memfokuskan kajian ini kepada amida dan terbitan berkaitan sinamat kerana atribut yang kurang dikenali terhadap aktiviti antidiabetis. Dalam kajian ini kami mengubah suai metil trans-sinamat dengan keamidaan kefungsian karbosilik metil ester menggunakan beberapa amina yang diperkenalkan dalam kumpulan fungsian metil trans-sinamat. Beberapa terbitan sinamamida telah disintesis dan dinilai untuk kesan rencatan α-glucosidase. Struktur sintesis sebatian telah dicirikan oleh IR, titik lebur, UV, ^{1}H NMR, ^{13}C NMR dan analisis jisim spektrum. Kesemua 13 sinamamida menunjukkan aktiviti α-glucosidase yang tinggi berbanding sebatian pemula. Penggantian asid sinamik dengan kumpulan amida mengubah aktiviti perencatan α-glucosidase. Peningkatan berpukal dan panjang rantai bahan ganti amina mengurangkan aktiviti rencatan. Propilsinamamida (3c) menunjukkan aktiviti rencatan poten dalam kalangan terbitan sinamamida, dengan kesemuanya bertindak melalui mekanisme perencatan berpersaingan. Sebatian ini mungkin berbaloi untuk kajian lanjut.

Kata kunci: Perencat α-glucosidase; sintesis; terbitan sinamamida

INTRODUCTION

α-Glucosidase is a type of hydrolase enzyme that catalyzes the hydrolysis of terminal non-reducing carbohydrates into α-glucose (Park et al. 2013). Inhibition of α-glucosidase enzyme activity allows blood glucose levels to return within normal range (Bösenberg & van Zyl 2008). Glucosidase is responsible for the catalytic disruption of glycosidic bonds, which specifically depends on the amount of monosaccharides, the position of the cleavage site, and the configuration of the hydroxyl group in the substrate (Park et al. 2008a). Enzymatic hydrolysis of glycosidic bonds occurs through acid catalysis that requires two important residues such as proton donors and bases/ nucleophiles (Park et al. 2008b). α-Glucosidase inhibitors (IAGs), such as acarbose, nojirimycin, 1-deoxynojirimycin, miglitol, and voglibose, are widely used for the treatment of type 2 diabetes and obesity (Bösenberg & van Zyl 2008; Davies & Henrissat 1995; Liu et al. 2006; Mohammed et al. 2014; Van de Laar et al. 2008). They can also be used as a therapeutic target to treat viral infections, cancers, and hepatitis (Fischer et al. 1995; Mehta et al. 1998; Melo & Carvalho 2006).

Cinnamic acid derivatives have been reported to inhibit α-glucosidase (Babu et al. 2007; Huang et al. 2009; Narender et al. 2007; Phuwapraisirisan et al. 2008; Pili et al. 1995). The pharmacophore of cinnamic acid compound are phenyl ring, α,β-unsaturation and carboxylic acid. It is known that cinnamic acid derivatives act as IAGs. Their

structure includes a substitution at *para* position within the aromatic functional group functionalized with an alkylester group (Matsuura et al. 2004). However, no cinnamamide derivatives have been tested for their α-glucosidase inhibitory ability. Therefore, we replaced the carboxylic acid moiety of cinnamic acid by an alkyl amine and predicted that it can have better inhibitory activity against α-glucosidase. In this study, we synthesized a series of cinnamamide derivatives and elucidated their structures with H-NMR, C-NMR, FTIR, ESI QToF MS, GCMS. We report their inhibitory effects against *Saccharomyces cerevisiae* and rat intestinal α-glucosidase.

Materials and Methods

Chemical and Instruments

All solvents were dried and distilled according to standard procedure. ^{1}H were recorded on JEOL NMR ECZ500R using 500 MHz instrument and ^{13}C NMR spectra were recorded on JEOL NMR ECZ500R using 125 MHz instrument in deuterochloroform. Chemical shifts (δ) are reported in parts per million (ppm) downfield from tetramethylsilane (δ 0.00) or $CHCl_3$ (δ 7.26) for ^{1}H NMR and δ 77.0 for ^{13}C NMR as an internal standard, and coupling constants are reported in Hertz. ESI QToF MS Mass Spectra were recorded on Waters Xevo® G2-XS QToF. IR spectra were obtained on Bruker Tensor II. Analytical thin layer chromatography (TLC) was performed on Merck silica gel plates (Art5715 Kiesel gel $60F_{254}$ 0.25 mm) and preparative TLC was performed using Merck silica gel plates (Art5744 Kiesel gel $60F_{254}$ 0.5 mm). Silica gel column chromatography was carried out on Daisogel IR-60. Methyl trans-cinnamates obtained from isolation of *Alpinia malaccensis* using hydrodistillation methode was chosen as the starting material, which was characterized by GCMS, LCMS, ESI-QToF MS, ^{1}H NMR, ^{13}C NMR, and FTIR.

Experiments

Hydrolysis of methyl trans-*cinnamate*

In a 250 mL round-bottomed flask, methyl *trans*-cinnamate (0.012 mol), 2M NaOH, and 95% ethanol were added. The reaction mixture was heated at 50-60°C for 3 h. After completion (monitored by TLC), the reaction was neutralized with HCl 1M; the reaction mixture was extracted with butanol. The organic phase was washed with water, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to afford the product. Cinnamic acid was obtained by recrystallization. Identification of cinnamic acid was performed by using FT-IR, LC-MS, ^{1}H-NMR, and ^{13}C-NMR and GC-MS.

Cinnamic acid (2). Yield: (1.51 g, 85%). Mp: 133-134°C. ESI-QToF MS *m/z* 149.09745 $[M+H]^+$. FTIR: ν_{max} (cm^{-1}), 3680 (C-OH), 1685 (C=O). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 6.49 (*d*, 1H, *J* = 16 Hz), 7.83 (*d*, 1H, *J* = 16 Hz), 7.57 (*dd*, 2H, *J* = 2.0, Hz; *J* = 7.8 Hz), 7.42 (*dt*, 3H, *J* = 2.0, *J* =7.2 Hz), ^{13}C-NMR (125 MHz, $CDCl_3$) δ 172.8, 117.4, 147.2, 134.1, 128.5, 129.1, 130.8, 129.1, 128.5.

Amidation of cinnamic acid compound (series 3)

In a 250 mL flask, $SOCl_2$ (4 mL, 0.055 mol) was added dropwise to cinnamic acid (0.2961 g, 2 mmol) at 0°C and then heated for 5 h. The reaction mixture was cooled and anh. CH_2Cl_2 (10 mL), pyridine 0.2 mL, and (amine, ethylamine, propylamine, isopropylamine, octylamine, phenylamine, *p*-methoxyphenylamine) (6 mmol) were added and then stirred at room temperature for 3 h. The reaction mixture was washed with saturated NaCl and extracted with CH_2Cl_2. The organic layer was dried to give a crude product, which was purified by column chromatography (hex:AcOEt: 4:1) (Adisakwattana et al. 2004; Carvalho et al. 2008).

Cinnamamide (3a)

Yield: (0.235 g, 80%). Mp: 145-148°C. ESI-QToF MS *m/z* 148.07485 $[M+H]^+$. FTIR: ν_{max} (cm^{-1}), 3369 (NH), 1662 (C=O). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 6.43 (*d*, 1H, *J* = 16 Hz), 7.64 (*d*, 1H, *J* = 16 Hz), 7.47 (*dd*, 2H, *J* = 9.75 Hz), 7.35 (*dt*, 3H, *J* = 3.9 Hz, *J* = 9.75 Hz), ^{13}C-NMR (125 MHz, $CDCl_3$) δ 168.3, 119.2, 143.1, 134.5, 128.1, 128.9, 130.2, 128.9, 128.1.

Ethylcinnamamide (3b)

Yield: (0.262 g, 75%). Mp: 85-87°C. LCMS *m/z* 176.39 $[M+H]^+$, FTIR: ν_{max} (cm^{-1}), 3284 (NH), 1656 (C=O). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 6.43 (*d*, 1H, *J* = 16 Hz), 7.64 (*d*, 1H, *J* = 16 Hz), 7.47 (*dd*, 2H, *J* = 7.8 Hz), 7.35 (*t*, 3H, *J* = 1.95 Hz, *J* = 7.8 Hz), 3.42 (q, 2H, *J* = 2.6 Hz), 1.20 (*t*, 1H, *J* = 7.15 Hz), ^{13}C-NMR (125 MHz, $CDCl_3$) δ 166.4, 121.4, 140.5, 135.0, 127.8, 128.8, 129.6, 128.8, 127.8, 34.7, 14.8.

Propylcinnamamide (3c)

Yield: (0.31 g, 82%). Mp: 74-75°C. ESI-QToF MS *m/z* 190.15952 $[M+H]^+$. FTIR: ν_{max} (cm^{-1}), 3269 (NH), 1654 (C=O). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 6.48 (*d*, 1H, *J* = 16 Hz), 7.60 (*d*, 1H, *J* = 16 Hz), 7.45 (*dd*, 2H, *J* = 7.8 Hz), 7.30 (*dt*, 3H, *J* = 3.25 Hz, *J* = 7.8 Hz), 3.34 (*q*, 2H, *J* = 6.5 Hz), 1.58 (*m*, 2H, *J* = 7.15 Hz), 0.93 (*t*, 3H, *J* = 7.75 Hz), ^{13}C-NMR (125 MHz, $CDCl_3$) δ 166.4, 121.2, 140.7, 135.0, 127.8, 128.8, 129.6, 128.8, 127.8, 41.6, 22.9, 11.6.

Isopropylcinnamamide (3d)

Yield: (0.295 g, 78%). Mp: 95-97°C. ESI-QToF MS *m/z* 190.1568 $[M+H]^+$. FTIR: ν_{max} (cm^{-1}), 3265 (NH), 1656 (C=O). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 6.43 (*d*, 1H, *J* = 16 Hz), 7.63 (*d*, 1H, *J* = 16 Hz), 7.47 (*dd*, 2H, *J* = 7.8 Hz), 7.32 (*t*, 3H, *J* = 4.5 Hz, *J* = 7.8 Hz), 4.22 (*m*, 1H, *J* = 7.15 Hz), 1.22 (*d*, 3H, *J* = 7.1 Hz), 1.22 (*d*, 3H, *J* = 7.1 Hz),^{13}C-NMR (125 MHz, $CDCl_3$) δ 165.3, 121.2, 140.8, 134.9, 128.2, 128.8, 129.6, 128.8, 128.2, 41.7, 22.9, 22.9.

Octylcinnamamide (3e)

Yield: (0.477 g, 82.4%). Mp: 74-75°C. ESI-QToF MS *m/z* 259.23412 $[M+H]^+$. FTIR: ν_{max} (cm^{-1}), 3282 (NH), 1631 (C=O) 3059 and 2926 (CH aliphatic). ^{1}H-NMR (500 MHz, $CDCl_3$)

δ 0.85 (t, *J* = 6.5 Hz, 3H), 1.56 (*m*, 2H, *J* = 7.8 Hz), 1.24 (*m*, 2H, *J* = 3.9 Hz), 1.24 (*m*, 2H, *J* = 3.9 Hz), 1.24 (*m*, 2H, *J* = 3.9 Hz), 1.24 (*m*, 2H, *J* = 3.9 Hz), 3.37 (*q*, 2H, *J* = 6.5 Hz), 7.48 (*dd*, 2H, *J* =7.8 Hz), 7.32 (*dt*, 3H, *J* =1.95 Hz, *J* =9.05 Hz), 7.64 (*d*, 1H, *J* = 16 Hz), 6.46 (*d*, 1H, *J* = 16 Hz), ^{13}C-NMR (125 MHz, $CDCl_3$) δ 14.2, 22.7, 31.8, 29.3, 29.4, 27.1, 29.7, 40.0, 127.9, 128.9, 129.7, 128.9, 127.9, 134.9, 140.9, 120.8, 166.2.

Phenylcinnamamide (3f)
Yield: (0.348 g, 78%). Mp: 77-79°C. ESI-QToF MS *m/z* 224.10618 $[M+H]^+$. FTIR: ν_{max} (cm^{-1}), 3271 (NH), 1604 (C=O). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 6.69 (*d*, 1H, *J* = 16 Hz), 7.16 (*d*, 1H, *J* = 16 Hz), 7.18 (*dd*, 2H, *J* = 7.15 Hz), 6.78 (*dt*, 2H, *J* = 2.6 Hz, *J* = 8.4 Hz), 6.76 (*dt*, 1H, *J* = 2.6 Hz, *J* = 8.4 Hz), 7.18 (*dd*, 2H, *J* = 8.4 Hz), 6.71 (*dt*, 2H, *J* = 2.6 Hz, *J* = 8.4 Hz), 6.69 (*dt*, 1H, *J* = 2.6 Hz, *J* = 8.4 Hz), ^{13}C-NMR (125 MHz, $CDCl_3$) δ 164.7, 120.4, 142.4, 134.9, 129.1, 128.9, 124.6, 128.9, 129.1, 138.2, 121.2, 128.1, 130.0, 128.1, 121.2.

p-Methoxyphenylcinnamamide (3g)
Yield: (0.394 g, 78%). Mp: 151-153°C. ESI-QToF MS *m/z* 254.11655 $[M]^+$. FTIR: ν_{max} (cm^{-1}), 3250 (NH), 1610 (C=O). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 6.55 (*d*, 1H, *J* = 16 Hz), 7.75 (*d*, 1H, *J* = 16 Hz), 7.54 (*dd*, 2H, *J* = 8.4 Hz), 7.37 (*dt*, 3H, *J* = 2.6 Hz, *J* = 8.4 Hz), 7.52 (*dd*, 2H, *J* = 8.4 Hz), 6.87 (*dd*, 2H, *J* = 8.4 Hz), 3.85 (s, 3H), ^{13}C-NMR (125 MHz, $CDCl_3$) δ 165.1, 120.9, 141.0, 139.1, 127.6, 128.7, 129.6, 128.7, 127.6, 138.3, 121.5, 113.7, 156.7, 113.7, 121.5, 55.01.

Nitration of cinnamic acid (2c) and nitration of cinnamamide compound (series 4)
Cinnamamide/cinnamic acid compound (4.05 mmol) was added to a mixture of H_2SO_4 (8.10 mmol) and HNO_3 (8.10 mmol). The mixture was stirred in an oil bath at 50°C, and the progress of the reaction was monitored by TLC. At the end of the reaction, the resulting mixture was diluted with ethyl acetate/water. The filtrate was washed with water (2 × 10 mL) and dried with Na_2SO_4. The product was purified by column chromatography with the use of ethyl acetate-hexane (2:1) as the eluent (Hurth et al. 2015; Lu et al. 2013; Montalbetti & Falque 2005; Oxley et al. 2008; Rodrigues et al. 1999).

(E)3-(4-nitrophenyl)cinnamic acid (2c)
Yield: (1.51 g, 45%). Mp: 147-148°C. ESI-QToF MS *m/z* 193.04678 $[M+H]^+$. FTIR: ν_{max} (cm^{-1}), 3039 (NH), 1687 (C=O), 1396 (NO). ^{1}H-NMR (500 MHz, $CDCl_3$ δ 6.55 (*d*, 1H, *J* = 16 Hz), 7.75 (*d*, 1H, *J* = 16 Hz), 7.54 (*d*, 2H, *J* = 8.4 Hz), 7.37 (*d*, 2H, *J* = 8.4 Hz), ^{13}C-NMR (125 MHz, $CDCl_3$) δ 172.8, 117.4, 147.2, 134.1, 128.5, 129.1, 130.8, 129.1, 128.5.

(E)3-(4-nitrophenyl)cinnamamide (4a)
Yield: (0.392 g, 50.4%). Mp: 147-148°C. ESI-QToF MS *m/z* 193.05678 $[M+H]^+$. FTIR: ν_{max} (cm^{-1}), 3039 (NH), 1687 (C=O), 1396 (NO). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 6.55 (*d*, 1H, *J* = 16 Hz), 7.75 (*d*, 1H, *J* = 16 Hz), 7.54 (*d*, 2H, *J* = 8.4 Hz), 7.37 (*d*, 2H, *J* = 8.4 Hz), ^{13}C-NMR (125 MHz, $CDCl_3$) δ 168.3, 119.2, 143.1, 134.5, 128.1, 128.9, 130.2, 128.9, 128.1.

(E)N-ethyl-(4-nitrophenyl)cinnamamide (4b)
Yield: (0.490 g, 55%). Mp: 138-139°C. ESI-QToF MS *m/z* 221.10199 $[M+H]^+$, FTIR: ν_{max} (cm^{-1}), 3300 (NH), 1625 (C=O), 1418 (NO), ^{1}H-NMR (500 MHz, $CDCl_3$) δ 6.55 (*d*, 1H, *J* = 16 Hz), 7.75 (*d*, 1H, *J* = 16 Hz), 7.54 (*d*, 2H, *J* = 8.4 Hz), 7.37 (*d*, 2H, *J* = 8.4 Hz), 3.42 (*m,* 2H, *J* = 2.6 Hz), 1.20 (*t*, 1H, *J* = 7.15 Hz), ^{13}C-NMR (125 MHz, $CDCl_3$) δ 166.4, 121.3, 140.5, 135.0, 129.6, 128.8, 148.3, 129.6, 128.8, 34.7, 14.9.

(E)3-(4-nitrophenyl) N-propylcinnamamide (4c)
Yield: (0.62 g, 65.4%). Mp: 95-96°C. ESI-QToF MS *m/z* 235.10663 $[M+H]^+$. FTIR: ν_{max} (cm^{-1}), 3275 (NH), 1631 (C=O), 1336 (NO). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 6.48 (*d*, 1H, *J* = 16 Hz), 7.60 (*d*, 1H, *J* = 16 Hz), 7.45 (*d*, 2H, *J* = 7.8 Hz), 7.30 (*d*, 2H, *J* = 7.8 Hz), 3.34 (*q*, 2H, *J* = 6.5 Hz), 1.58 (*m*, 2H, *J* = 7.15 Hz), 0.93 (*t*, 3H, *J* = 7.75 Hz), ^{13}C-NMR (125 MHz, $CDCl_3$) δ 165.2, 124.9, 135.9, 133.5, 129.8, 129.2, 148.3, 129.2, 129.8, 41.7, 22.9, 11.5.

(E)3-(4-nitrophenyl) N-isopropylcinnamamide (4d)
Yield: (0.544 g, 57.4%). Mp: 154-155°C. ESI-QTOF MS *m/z* 235.10686 $[M+H]^+$, FTIR: ν_{max} (cm^{-1}), 3288 (NH), 1604 (C=O), 1344 (NO). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 6.46 (*d*, 1H, *J* = 15.5 Hz), 7.67 (*d*, 1H, *J* = 15.5 Hz), 7.62 (*d*, 2H, *J* = 7.75 Hz), 8.23 (*d*, 2H, *J* = 7.75 Hz), 4.22(*m*, 1H, *J* =6.25 Hz), 1.23(*d*, 6H, J = 6.75 Hz), ^{13}C-NMR (125 MHz, $CDCl_3$) δ 165.3, 121.2, 140.8, 134.9, 128.2, 128.8, 129.6, 128.8, 128.2, 41.7, 22.9, 22.9.

(E)3-(4-nitrophenyl) N-octylcinnamamide (4e)
Yield: (0.6 g, 48.8%). Mp: 84-85°C. ESI-QToF MS *m/z* 305.1897 $[M+H]^+$. FTIR: ν_{max} (cm^{-1}), 3300 (NH), 1625 (C=O), 1336 (NO). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 6.46 (*d*, 1H, *J* = 16 Hz), 7.64 (*d*, 1H, *J* = 16 Hz), 7.48 (*d*, 2H, *J* =7.8 Hz), 7.32 (*d*, 2H, *J* =7.8 Hz), 3.37 (*q*, 2H, *J* = 6.5 Hz), 1.24 (*m*, 10H, *J* = 7.8 Hz), 1.56 (*m*, 2H, *J* = 7.8 Hz), 0.85 (*t*, 3H, *J* = 6.5 Hz), ^{13}C-NMR (125 MHz, $CDCl_3$) δ 164.9, 124.9, 135.8, 133.4, 129.8, 129.2, 148.1, 129.2, 129.8, 40.04, 31.9, 27.1, 29.3, 29.3, 29.7, 22.7, 14.2.

Biological activity assays
Biological activity assays for the α-glucosidase enzyme inhibitory activity has been reported by Kim et al. (2004). *S. cerevisiae* α-glucosidase (0.5 mg) was dissolved in 5 mL phosphate buffer at pH7, and 100 mg bovine was dissolved in 5 mL phosphate buffer. Both enzyme and bovine solutions were mixed into 40 mL phosphate buffer at pH7. Into a test tube was added a solution of 250 µL of 20 mM *p*-NPG, 495 µL 100 mM phosphate buffer, and 5 µL sample solution of each compound synthesized and incubated for 5 min at 37°C. Next, 250 µL of the enzyme was added and incubated for 15 min at 37°C. The reaction

was stopped by adding 1000 µL of Na_2CO_3 solution. Absorbance was then measured using a spectrophotometer at a wavelength of 400 nm. Percentage enzyme inhibition was measured as ((absorbance control-absorbance test)/ absorbance control) × 100.

Kim et al. (2004) was reported biological activity assays for the α-glucosidase enzyme which have been modified. Rat intestinal acetone powder α-glucosidase (500 mg) was homogenized in 10 mL of 0.9% NaCl solution. After centrifugation (5000 rpm for 30 min), the solution was used in the experiment. Into a test tube was added a solution of 250 µL of 20 mM *p*-NPG, 595 µL 100 mM phosphate buffer, and 5 µL sample solution. After incubation for 5 min at 37°C, 150 µL of the enzyme was added and again incubated for 15 min at 37°C. The reaction was stopped by the addition of 1000 µL of Na_2CO_3 solution. Absorbance of the solution was then measured using a spectrophotometer at a wavelength of 400 nm. Percentage enzyme inhibition was measured as ((absorbance control-absorbance test)/absorbance control) × 100.

Measurement of kinetic constant

In order to investigate the type of inhibition, α-glucosidase enzyme kinetics analysis was performed according to a reported method (Adisakwattana et al. 2009). Kinetic analyses of the samples were performed using two reaction systems, i.e. the reaction system without the inhibitor and the reaction system with the sample inhibitor. The samples tested for their inhibitory kinetics were the best samples from previous inhibition test results (biological activity assay 2.2.4). The test was performed using different substrate concentrations (p-NPG), i.e. 0.5, 1, 3 5, 10 µM in phosphate buffer pH7. The reaction system mixture was similar to the mixture used for evaluating α-glucosidase inhibition. The absorbance was read at 400 nm, and the experiment was performed with 3 replicates. The type of inhibition was calculated on the basis of a Lineweaver-Burk plot using reciprocally plotted data (substrate concentration on the horizontal axis and velocity on the vertical axis).

Results and Discussion

Synthesis of cinnamamide derivatives

Synthetic cinnamamide derivatives were synthesized and assayed for their α-glucosidase inhibitory activities (Scheme 1 and Table 1). Basic hydrolysis of methyl *trans*-cinnamate 1 produced cinnamic acid 2. Treatment of cinnamic acid 2 with $SOCl_2$ and alkylamine afforded the corresponding cinnamamide derivatives (3a-3g) in good yields. Thus obtained cinnamamide derivatives (3a-3g) were then nitrated with sulfuric acid and nitric acid, and nitro cinnamamide derivatives (4a-4e) were obtained in moderate yields. The structures of cinnamamide derivatives (3a-3g) and (4a-4e) were characterized by UV, IR, melting point analysis, ^{1}H-NMR, ^{13}C-NMR, GCMS, LCMS, and ESI-QTOF mass spectral analysis. The UV absorption spectra

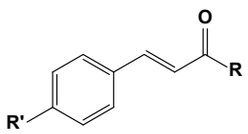
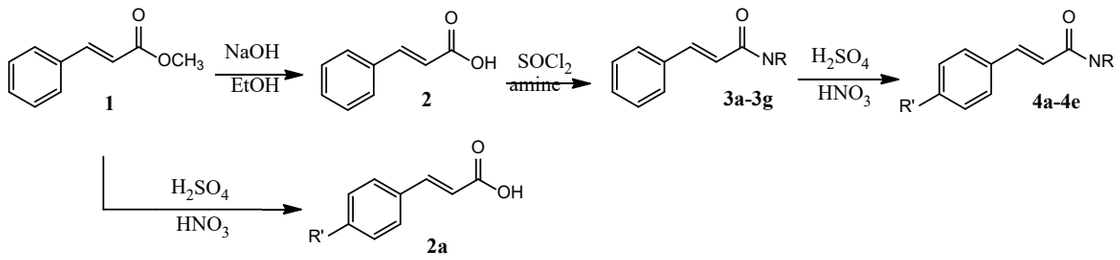


TABLE 1. Synthesis of cinnamamide derivatives

Entry	Sample	R	R'	Melting point (°C)	Yield (%)
1	Methyl cinnamate **1**	OCH_3	H	34-35	65
2	Cinnamic acid **2**	OH	H	133-134	85.5
3	Cinnamamide **3a**	NH_2	H	145-148	80
4	Ethylcinnamamide **3b**	$NHCH_2CH_3$	H	85-87	75
5	Propylcinnamamide **3c**	$NH\ CH_2CH_2CH_3$	H	74-75	82
6	Isopropylcinnamamide **3d**	$NHCH(CH_3)_2$	H	95-97	78
7	Octylcinnamamide **3e**	$NH(CH_2)_7CH_3$	H	74-75	82.4
8	Phenylcinnamamide **3f**	NHC_6H_5	H	77-79	78
9	*p*-Methoxyphenylcinnamamide **3g**	$NHC_6H_4OCH_3$	H	151-153	78
10	(*E*)3-(4-nitrophenyl)acrylic acid **2a**	OH	NO_2	165-168	56.4
11	(*E*)3-(4-nitrophenyl)acrylamide **4a**	NH_2	NO_2	147-148	50.4
12	(*E*)*N*-ethyl-(4-nitrophenyl) acrylamide **4b**	$NHCH_2CH_3$	NO_2	138-139	55
13	(*E*)3-(4-nitrophenyl)propylacrylamide **4c**	$NH\ CH_2CH_2CH_3$	NO_2	95-96	65.4
14	(*E*)3-(4-nitrophenyl)*N*-isopropylacrylamide**4d**	$NHCH(CH_3)_2$	NO_2	154-155	57.4
15	(*E*)3-(4-nitrophenyl) *N*-octylacrylamide **4e**	$NH(CH_2)_7CH_3$	NO_2	84-85	48.8



SCHEME 1. Synthesis of cinnamamide derivatives

of 3a-3g and 4a-4e exhibited their main peak at 280 nm, upon introduction of the carbonyl and amide group, respectively. The IR spectrum of 3a-3g showed an absorption peak at 3250-3369 cm^{-1} due to the amine (-NH) stretching, and at 1662-1656 cm^{-1} due to the carbonyl amide (-N-C=O) stretching. The IR spectrum of 4a-4e showed an absorption peak at 3039-3300 cm^{-1} due to the amine (-NH) stretching, at 1604-1687 cm^{-1} due to carbonyl amide (-N-C=O) stretching, and at 1336-1418 cm^{-1} due to the nitro (-NO_2) stretching. The ^{1}H NMR spectrum of 3a-3g and 4a-4e showed a cinnamic acid backbone through doublet (*d*) at δ 6.43 ppm, doublet at δ 7.64 ppm, doublet-doublet *(dd)* at δ 7.47 ppm, and doublet-triplet (*dt*) at δ 7.35 ppm, while an alkyl amine was evident by the following peak characteristics: ethylamide: triplets at δ 1.20 ppm, quartet at δ 3.42 ppm; propylamide: quartet at δ 3.34 ppm, multiplets at δ 1.58 ppm, triplets at δ 0.93 ppm; isopropylamide: multiplet at δ 4.22 ppm, doublets at δ 1.22 ppm; octylamide: quartet at δ 3.37 ppm, multiplets at δ 1.24 ppm, multiplets at δ 1.56 ppm, and triplets at δ 0.85 ppm. The ^{13}C NMR spectrum of 3a-3g and 4a-4e showed a cinnamic acid backbone through signals at δ 119.2, 128.1, 128.9, 130.2, 130.5, 134.5, 143.1, and 168.3 ppm, while an alkyl amine was evident by the following signals: ethylamide: 34.7 and 14.8 ppm; propylamide: 41.6, 22.9, and 11.6 ppm; isopropylamide: 41.7 and 22.9 ppm; octylamide: 40.0, 31.8, 29.7, 29.4, 29.3, 27.1, 22.7, and 14.2 ppm. Compounds 1, 2, 3a-3g, and 4a-4e were also identified by mass spectral analysis.

BIOLOGICAL ACTIVITY

α-Glucosidase inhibitory activity

Glucosidase is responsible for the catalytic disruption of glycosidic bonds, which depends explicitly on the amount of monosaccharides, the position of the cleavage site, and the configuration of the hydroxyl group in the substrate. Herein, we have synthesized 13 derivatives and report their inhibitory effects on α-glucosidase from *S. cerevisiae* and rat intestinal tissue. Ten compounds showed a more potent inhibition than standard acarbose, and five compounds showed a more potent inhibition than the standard 1-deoxynojirimycin. However, all cinnamamide derivatives showed better inhibition of α-glucosidase than the starting material.

Thirteen compounds showed α-glucosidase activity with IC_{50} values of 0.71 ± 0.492–4.02 ± 0.025 mM (1-deoxynojirimycin: 0.97 ± 0.019 mM) and acarbose (IC_{50} = 1.78 ± 0.727 mM). These compounds, based on the cinnamamide skeleton, only slightly differed from each other in their α-glucosidase inhibitory potential possible because of the different lengths of alkyl amine chain. Propylcinnamamide 3c (IC_{50} = 0.71 ± 0.492 mM), nitro propylcinnamamide 4c (IC_{50} = 0.75 ± 0.066 mM),

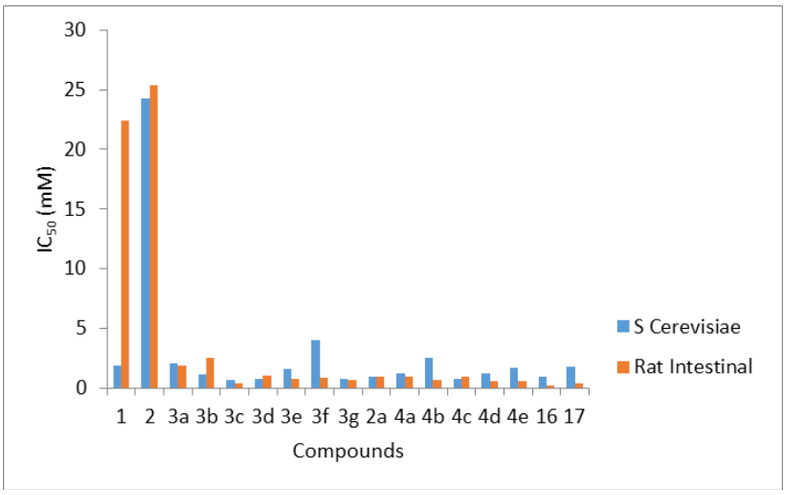


FIGURE 1. IC50 values of cinnamamide derivatives for inhibition of α-glucosidase

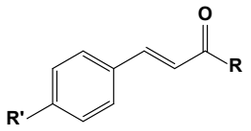


TABLE 2. IC50 values of cinnamamide derivatives for inhibition of α-glucosidase

Entry	Sample	R	R'	IC_{50} ± SEM* (mM)	
				S. cerevisiae	Rat intestinal
1	Methyl cinnamate **1**	OCH_3	H	1.88 ± 0.022	22.42 ± 0.011
2	Cinnamic acid **2**	OH	H	24.28 ± 0.019	25.37 ± 0.011
3	Cinnamamide **3a**	NH_2	H	2.05 ± 0.020	1.86 ± 0.047
4	Ethylcinnamamide **3b**	$NHCH_2CH_3$	H	1.15 ± 1.006	2.52 ± 0.253
5	Propylcinnamamide **3c**	NH $CH_2CH_2CH_3$	H	0.71 ± 0.492	0.37 ± 0.145
6	Isopropylcinnamamide **3d**	$NHCH(CH_3)_2$	H	0.81 ± 0.757	1.04 ± 0.054
7	Octylcinnamamide **3e**	$NH(CH_2)_7CH_3$	H	1.59 ± 0.008	0.80 ± 0.092
8	Phenylcinnamamide **3f**	NHC_6H_5	H	4.02 ± 0.025	0.86 ± 0.184
9	p-Methoxyphenylcinnamamide **3g**	$NHC_6H_4OCH_3$	H	0.80 ± 1.416	0.71 ± 0.160
10	(E)3-(4-nitrophenyl)acrylic acid **2a**	OH	NO_2	0.95 ± 0.008	0.99 ± 0.239
11	(E)3-(4-nitrophenyl)acrylamide **4a**	NH_2	NO_2	1.26 ± 0.047	0.96 ± 0.044
12	(E)N-ethyl-(4-nitrophenyl)acrylamide **4b**	$NHCH_2CH_3$	NO_2	2.59 ± 0.052	0.66 ± 0.055
13	(E)3-(4-nitrophenyl) N-propylacrylamide **4c**	NH $CH_2CH_2CH_3$	NO_2	0.75 ± 0.066	0.98 ± 0.217
14	(E)3-(4-nitrophenyl)N-isopropylacrylamide **4d**	$NHCH(CH_3)_2$	NO_2	1.21 ± 0.094	0.59 ± 0.418
15	(E)3-(4-nitrophenyl) N-octylacrylamide **4e**	$NH(CH_2)_7CH_3$	NO_2	1.73 ± 0.074	0.59 ± 0.017
16	1-Deoxynojirimycin			0.97 ± 0.019	0.20 ± 0.047
17	Acarbose			1.78 ± 0.727	0.46 ± 0.251

SEM, standard error of mean; 1-deoxynojirimycin and acarbose were used as standards

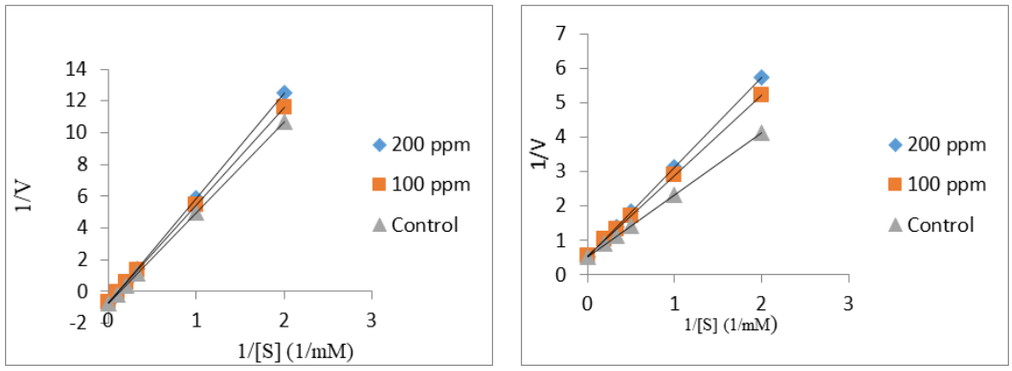
methoxyphenylcinnamamide 3g (IC_{50} = 0.80 ± 1.416), isopropylcinnamamide 3d (IC_{50} = 0.81 ± 0.757 mM), and nitro cinnamic acid 2a (IC_{50} = 0.95 ± 0.008 mM) showed the best activities, which were better than those of the standard 1-deoxynojirimycin and acarbose (Table 2).

In the assay for inhibitory potential against rat intestinal α-glucosidase, compounds 3c, 3g, 4b, 4d, and 4e (IC_{50} = 0.37± 0.145 mM, 0.71 ± 0.160 mM, 0.66 ± 0.055 mM, 0.59 ± 0.418 mM, and 0.59 ± 0.017 mM, respectively) had potent activities, which were equivalent to those of acarbose and 1-deoxynojirimycin (IC_{50} = 0.46 ± 0.251 mM and 0.20 ± 0.047 mM, respectively). Methyl trans-cinnamate (1) and trans-cinnamic acid (2) were found to be inactive (IC_{50} > 20 mM). Propylcinnamamide (3c) had very potent inhibitory activity (IC_{50} = 0.37 ± 0.145 mM) and was the most active among all cinnamamide derivatives. These results suggested that presence of the amide group at the carboxyl group of cinnamic acid moiety is necessary to increase α-glucosidase inhibitory activity. Increasing the chain length of alkylamine increased α-glucosidase inhibitory activity up to an optimum chain length, any increase beyond this length decreased the inhibitory activity; Propylcinnamamide had an optimum chain length of alkyl amine, and therefore, had the best activity on intestinal α-glucosidase. Isopropylcinamamide showed lesser activity than propylcinnamamide because its alkyl amine was bulkier.

Figure 1 shows that methyl *trans*-cinnamate and *trans*-cinnamic acid had a weak inhibitory activity against *S. cerevisiae* and rat intestinal α-glucosidase. Cinnamamide derivatives 3a-4e were more potent than methyl trans-cinnamate and trans-cinnamic acid. Propylcinnamamide showed the best activity on intestinal α-glucosidase inhibition among the cinnamamide derivatives and was more potent than acarbose.

Inhibition kinetics of propylcinnamamide on α-glucosidase

The Lineweaver-Burk plot of α-glucosidase inhibition kinetics is shown in Figure 2 and shows that the mechanism of α-glucosidase inhibition of propylcinnamamide (5) is competitive. The mechanism of inhibition of propylcinamide against the α-glucosidase enzyme in this study was studied through the Lineweaver-Burk curve based on the reaction of enzymes with substrates and enzymes with substrates and inhibitors. Based on the results of the Lineweaver-burk plot, the



(a) (b)

FIGURE 2. Lineweaver-Burk plot for inhibitory activity of propylcinnamamide against (a) S. cerevisiae α-glucosidase and (b) rat intestinal α-glucosidase

propylsamamide compound works competitively (Figure 2), when 1/[S] approaches 0, the maximum reaction speed (V max) is not affected by the presence of an inhibitor. Therefore, when the substrate concentration is high, V max in systems with inhibitors is equal to or close to V max with systems without inhibitors. Inhibitors that work competitively do not affect the value of Vmax, but increase the value of Km (Murray et al. 2003).

CONCLUSION

Thirteen compounds showed α-glucosidase activity (*S. cerevisiae*) with IC_{50} values of 0.71-4.02 mM. Propylcinnamamide (3c) (IC_{50} = 0.71 ± 0.492 mM), nitro propyl cinnamamide (4c) (IC_{50} = 0.75 ± 0.066), methoxyphenylcinnamamide (3g) (IC_{50} = 0.80 ± 1.416), isopropylcinnamamide (3d)) (IC_{50} = 0.81 ± 0.757), and nitro cinnamic acid (2a) (IC_{50} = 0.95 ± 0.008) showed the best activities, which were greater than those of the standards 1-deoxynojirimycin and acarbose. Compounds 5, 9, 12, 14, and 15 (IC_{50} = 0.37 mM, 0.71 mM, 0.66 mM, 0.59 mM, and 0.59 mM, respectively) showed potent α-glucosidase inhibitory activity (rat intestinal). The presence of amine substituents on cinnamic acid was responsible for the increase in the α-glucosidase inhibitory activity of the parent compound. An increase in the bulkiness and chain length of the amine substituents decreased the inhibitory activity. Propylcinnamamide (5) showed the highest inhibitory activity among all cinnamamide derivatives, and it acted via a competitive inhibitory mechanism. This study highlights compounds with the potential ability to regulate blood glucose levels and could be further developed for application in a clinical setting.

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Teni Ernawati* & Muhamad Hanafi
Research Center for Chemistry
Indonesian Institute of Sciences (LIPI)
Kawasan Puspiptek, Serpong Tangerang Selatan
Banten 15314
Indonesia

Abdul Mun'im & Arry Yanuar
Faculty of Pharmacy
Universitas Indonesia
Depok 16242
Indonesia

*Corresponding author; email: teni.ernawati.lipi@gmail.com

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