SUPPLEMENTARY MATERIAL

Isolation of polyphenol compounds from olive waste and inhibition of their derivatives for α-glucosidase and α-amylase

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ABSTRACT: Olive waste was used as a sustainable resource because it contained a variety of valuable compounds. The polyphenols active fraction from enrichment by microporous resin and extraction with ethyl acetate were analysed by different chromatographic methods. A total of 14 polyphenolic compounds were isolated and identified by structure elucidation. Based on the above obtained compounds, tyrosol was selected as a characteristic polyphenol and participated in transesterification reaction to synthesise β-ketoester using Yb(OTf)\textsubscript{3}. Then the Biginelli reaction with benzaldehyde, urea and ketoester (1:1.2:1.2) was performed at 90°C for 3.0 h under the acidic condition. In addition, the β-ketoester prepared using tyrosol with benzyl had a greater inhibitory effect on α-glucosidase and α-amylase, and the inhibition of enzyme activity for 3,4-dihydropyrimidinone derivatives prepared using abovementioned β-ketoester was improved significantly. Meanwhile, fluorine-containing dihydropyrimidinone derivatives were considerable inhibitors for both enzymes.

KEYWORDS: olive waste; polyphenol; isolation; derivative; enzyme activity inhibition

Experimental section

\textit{Chemical reagents and solvents}

Petroleum ether, ethyl acetate, n-butanol, dichloromethane ethanol and methanol were analytical grade and purchased from Chemical Reagent of Nanjin (Nanjin, China). Chloroform, dimethyl sulfoxide, ethyl ether, toluene and acetone were obtained from Tianjin Kemiu Chemical Reagent Co., Ltd (Tianjin, China). Acetonitrile and methanol used for HPLC were of chromatographic grade (Sinopharm Chemical Reagent Co., Ltd, China). All aqueous solutions were prepared with ultrapure water produced by EPED-10TF Laboratory water purifier. Methyl
acetoacetate, urea, aromatic aldehyde, \( \alpha \)-glucosidase and \( \alpha \)-amylase were purchased from Sigma-Aldrich (Shanghai, China). Other reagents were of analytical grade and were obtained from either Sigma-Aldrich or Sinopharm Chemical Reagent Co., Ltd.

XDA-1 macroporous resin was purchased from qinshi Co., Ltd (Zhengzhou, China). Column chromatography silica gel (200-300 mesh) and thin layer chromatography plates were obtained from Qingdao Haiyang Chemical Co., Ltd (Shandong, China). And Sephadex LH-20 were purchased from Beijing Rui Da Heng Hui Science & Technology Development Co., Ltd (Beijing, China).

Thin layer chromatography (TLC) was performed to identify main compounds in each fraction. Pre-coated silica TLC plates (2 × 10 cm, 5 × 20 cm) and preparation of TLC plates (20 × 20 cm) were used in the experiment. The plates were developed using different solvent system and visualised under UV light (\( \lambda \)=210, 254 and 365 nm) or iodine vapor. Semipreparative high performance liquid chromatography (HPLC) was carried out using Shimadzu LC-10AT pumps coupled with a Shimadzu SPD-M10A diode array detector. Methanol and water were used as the mobile phase in a proportion of 50:50 (v:v) for target compounds preparation. High performance liquid chromatography tandem mass spectrometry (HPLC-MS) analysis of target compounds were performed using an Agilent 1100 LC system, and the column outler was coupled to an Agilent 6310 Ion Trap XCT mass spectrometer equipped with an electrospray ionization (ESI) ion source. The mass spectra were recorded in the range of 50-800 m/z. Nuclear magnetic resonance (NMR) spectroscopy was performed on a Bruker Avance 400 spectrometer (Bruker, Germany). \(^1\)H NMR measurements were carried out at 400 MHz, \(^{13}\)C NMR spectra were at 100 MHz. Proton and carbon chemical shifts were recorded on \( \delta \) scale in ppm, relative to tetramethyl silane (TMS) (\( \delta \)=0.00 ppm) as internal reference, and coupling constant (J) was reported in Hz. CDCl\(_3\), CD\(_3\)OD or DMSO were chosen as solvents according to different sample characteristics.

**Preparation and isolation of sample**

Approximately 7.0 kg of olive waste was collected from the two-phase centrifugal system without removal the stone at an olive oil production company, namely Xiangyu (Gansu, China) in October 2016. The sample was stored at 4 °C until further analysis. According to the method of ultrasound-assisted enzymatic hydrolysis, after adding disodium hydrogen phosphate-citric acid buffer solution, olive stone was separated from the mixture system. The enzyme mixed (cellulose,
hemicellulose and pectinase at the ratio 1:1:1) was added to the solid-solution system and stirred evenly. The valuable components were extracted for 30 min through ultrasound assisted enzyme hydrolysis at 50 °C. The extract was filtered and then concentrated in a rotary evaporator until the solvent was completely removed. In accordance with polyphenol enrichment technology, 40% and 60% ethanol elution fractions were obtained. The samples were collected by vacuum concentration and dried in a vacuum freeze-drier. A suitable amount of distilled water was added so that the sample obtained could be evenly dispersed. Petroleum ether, ethyl acetate and n-butanol were sequentially used to extract the different polarity fractions, and the solvents were removed to obtain the target extracts. Ethyl acetate extract was further analysed by different chromatographic techniques. Some traditional methods, such as silica gel column chromatography, semipreparative HPLC and preparation TLC, were used to separate and purify polyphenol compounds from olive waste.

**Synthesis of β-keto ester and dihydropyrimidinone derivatives**

The reaction substrates (tyrosol or the compound from tyrosol protected for phenolic hydroxyl group)(1.2 equiv.) and methyl acetoacetate (1.0 equiv.) were charged into a round bottom flask, then an appropriate amount of catalyst (0.1, 0.2, 0.3 or 0.4 equiv.) and toluene (25 mL) were added sequentially. The reaction mixture was heated at 110 °C (oil bath) with constant stirring for 3.5 h. The methanol produced in the reaction need to be removed through reflux and distillation condenser. The reaction process was detected by TLC. After completion, the reaction solvent was removed by concentration under reduced pressure. The target product was directly separated and purified by silica gel column chromatography with petroleum ether and ethyl acetate (4:1).

Aromatic aldehyde, urea and β-ketoester selected (molar equivalent ratio, 1:1.2:1~1.5) were placed in a 25 mL Schlenk tube. A solution of alcohol (5.0 mL) was subsequently added to above mixture. And drop of HCl as a catalyst to promote the reaction was also added to the reaction tube. A homogeneous mixture was stirred with constant speed under suitable temperature condition (oil bath) (80, 90 or 100 °C) for 3 h. The process of reaction was monitored by TLC (hexane : ethyl acetate, 3:2) until the end of reaction. Then the samples were cooled to room temperature and crude reaction product was washed with chilled water (10 mL×3), and dried with vacuum. In order to obtain pure product, the solid product was washed again with ethyl ether (15 mL×2) to remove the residual solvents and impurities.
Enzyme inhibition activity assay

The α-glucosidase inhibition activity assay was conducted based on the method described by Mangala Gowri et al. and Tan et al. with some modifications. The potency of α-glucosidase inhibitor was evaluated by detecting the formation of 4-nitrophenol by α-glucosidase after reaction with 4-nitrophenyl α-D-glucopyranoside (PNPG), which was considered as reaction substance. And α-glucosidase solution (0.5 mg/mL) was prepared in phosphate buffer and stored at low temperature before use. Briefly, 200 μL of 0.5 mg/mL α-Glucosidase solution and 150 μL of target compound solutions at various concentrations were added to each tube containing 2.0 mL phosphate buffer. Before the 150 μL of 5.0 mmol/L PNPG solution was added to initiate reaction, the mixture systems need to be incubated at 37 °C for 10 min, then reaction samples were again subjected to incubation at 37 °C for another 10 min. Followed by adding 2.5 mL of Na₂CO₃ (0.1 mol/L) to stop the reaction. The absorbance of each tube was measured at 405 nm by a UV-visible spectrophotometer. For all tests, the inhibition assay was performed in triplicate. Acarbose was used as a positive control. A reaction mixture using equal amount of DMSO reagent to replace the sample solution was used as the control.

The α-amylase inhibition activity assay was performed following the method of previous reported with a slight modification. The solution of starch in phosphate buffer was heated at 100 °C until transparent and α-amylase solution was prepared using phosphate buffer before the experiment. Briefly, at the beginning of test, the mixture system was composed of α-amylase solution and sample solution at different concentrations (in DMSO). Following pre-incubated at 37 °C for 10 min. After that, 300 μL of soluble starch as a substrate was pipetted into each tube to start the reaction. Subsequently, the reaction system was incubated at 37 °C for 10 min. Then 1.0 mL of 96 mM 3, 5-dinitrosalicylic acid (DNS) color reagent was added for reaction termination. And the test tubes were placed in a boiling water to incubate for 5.0 min and cooled to room temperature. The final volume of reaction solution was made up to 10 mL using distilled water, and the absorbance was measured at 540 nm through the UV-visible spectrophotometer. Acarbose was used as a positive control. The value of IC₅₀ was calculated to evaluate the effect of enzyme inhibitors. All assays were carried out in triplicate and values were presented as IC₅₀.

Structural data

OPE-1: 3-(4-hydroxy-3-methoxyphenyl)acrylic acid, C₁₀H₁₀O₄, pale yellow crystal, relative
molecular weight: 194. $^1$H-NMR (400 MHz) $\delta$: 3.82 (3H, s, -CH$_3$), 7.29 (1H, s, H-2), 7.09 (1H, d, J=8.0 Hz, H-5), 6.80 (1H, d, J=8.0 Hz, H-6), 7.50 (1H, d, J=16 Hz, H-7), 6.37 (1H, d, J=16 Hz, H-8). $^{13}$C-NMR (100 MHz) $\delta$: 55.65 (-OCH$_3$), 125.74 (C-1), 111.13 (C-2), 149.04 (C-3), 147.88 (C-4), 115.60 (C-5), 122.78 (C-6), 144.46 (C-7), 115.48 (C-8), 167.95 (C-9, -CO-).

OPE-2: 2-(3, 4-dihydroxyphenyl)chroman-3, 5, 7-trirol, C$_{13}$H$_{14}$O$_6$, white crystal, relative molecular weight: 290. $^1$H-NMR (400 MHz) $\delta$: 5.85 (1H, d, J=2.4 Hz, H-8), 5.92 (1H, d, J=2.4 Hz, H-6), 2.50 (1H, dd, J=16, 8.0 Hz, H-4), 2.84 (1H, dd, J=16.2, 5.6 Hz, H-4), 3.97 (1H, m, H-3), 4.56 (1H, d, J=7.2 Hz, H-2), 6.75 (3H, m, H-2', 5', 6'). $^{13}$C-NMR (100 MHz) $\delta$: 82.88 (C-2), 68.84 (C-3), 28.54 (C-4), 156.94 (C-5), 96.33 (C-6), 157.86 (C-7), 85.54 (C-8), 157.60 (C-9), 100.85 (C-10), 132.26 (C-1'), 115.29 (C-2'), 146.28 (C-3'), 146.25 (C-4'), 116.11 (C-5'), 120.06 (C-6').

OPE-3: 4-hydroxy-3-methoxybenzaldehyde, C$_5$H$_9$O$_3$, white powder, relative molecular weight: 152. $^1$H-NMR (400 MHz) $\delta$: 3.94 (3H, s, -CH$_3$), 6.74 (1H, s, H-2), 7.42 (1H, d, J=6.4 Hz, H-6), 7.04 (1H, d, J=8.4 Hz, H-5). $^{13}$C-NMR (100 MHz) $\delta$: 123.08 (C-1), 108.97 (C-2), 147.30 (C-3), 151.93 (C-4), 114.54 (C-5), 127.57 (C-6), 191.13 (C-7).

OPE-4: 3, 4-dihydroxybenzoic acid, C$_7$H$_6$O$_4$, white crystal, relative molecular weight: 154. $^1$H-NMR (400 MHz) $\delta$: 7.50 (1H, d, J=2.0 Hz, H-2), 6.87 (1H, d, J=8.4 Hz, H-5), 7.51 (1H, m, H-6). $^{13}$C-NMR (100 MHz) $\delta$: 123.08 (C-1), 117.82 (C-2), 146.03 (C-3), 151.58 (C-4), 115.91 (C-5), 124.13 (C-6), 170.52 (C-7).

OPE-5: Ethyl 3, 4, 5-trihydroxybenzoate, C$_9$H$_{10}$O$_5$, white crystal, relative molecular weight: 198. $^1$H-NMR (400 MHz) $\delta$: 6.95 (2H, s, H-2, 6), 4.21 (2H, m, H-8), 1.27 (3H, t, J=7.2 Hz, H-9).

$^{13}$C-NMR (100 MHz) $\delta$: 119.56 (C-1), 108.43 (C-2), 145.52 (C-3), 138.29 (C-4), 145.52 (C-5), 108.43 (C-6), 165.78 (C-7, -CO-), 59.95 (C-8, -CH$_2$-), 14.22 (C-9, -CH$_3$).

OPE-6: 3-(4-hydroxyphenyl)acrylic acid, C$_5$H$_9$O$_3$, white crystal, relative molecular weight: 164. $^1$H-NMR (400 MHz) $\delta$: 6.80 (2H, d, J=8.8 Hz, H-3, 5), 7.51 (3H, m, H-2, 6, 7), 6.30 (1H, d, J=16 Hz, H-8). $^{13}$C-NMR (100 MHz) $\delta$: 125.24 (C-1), 130.04 (C-2), 115.70 (C-3), 159.55 (C-4), 115.30 (C-5), 130.04 (C-6), 144.13 (C-7), 115.30 (C-8), 167.90 (C-9, -CO-).

OPE-7: 4-(2-hydroxyethyl)phenol, C$_8$H$_{10}$O$_2$, white crystal, relative molecular weight: 138. $^1$H-NMR (400 MHz) $\delta$: 7.10 (2H, d, J=8.8 Hz H-2, 6), 6.79 (2H, d, J=8.4 Hz, H-3, 5), 2.81 (2H, t, J=6.8 Hz, H-7), 3.83 (2H, t, J=6.8 Hz, H-8). $^{13}$C-NMR (100 MHz) $\delta$: 130.66 (C-1), 130.38 (C-2, CO).
C-6), 115.68 (C-3, C-5), 154.51 (C-4), 38.47 (C-7, -CH$_2$), 64.05 (C-8, -CH$_2$).

OPE-8: 4-(2-hydroxyethyl)benzene-1, 2-diol, C$_8$H$_{10}$O$_4$, light yellow oil, relative molecular weight: 154. $^1$H-NMR (400 MHz) δ: 2.66 (2H, t, J=7.2 Hz, H-7), 3.68 (2H, t, J=7.2, H-8), 6.65 (1H, d, J=2.0 Hz, H-2), 6.68 (1H, d, J=8.0 Hz, H-5), 6.52 (1H, dd, J=8.0, 2.0 Hz H-6). $^{13}$C-NMR (100 MHz) δ: 131.84 (C-1), 117.13 (C-2), 146.15 (C-3), 144.62 (C-4), 116.37 (C-5), 121.29 (C-6), 39.66 (C-7, -CH$_2$), 64.36 (C-8, -CH$_2$).

OPE-9: 4-allyl-2-methoxyphenol, C$_{10}$H$_{12}$O$_2$, light yellow liquid, relative molecular weight: 164. $^1$H-NMR (400 MHz) δ: 6.66 (1H, s, H-2), 6.84 (1H, d, J=8.4 Hz, H-5'), 6.67 (1H, d, J=4.8 Hz, H-6), 3.30 (2H, d, J=6.8 Hz, H-7), 5.94 (1H, m, H-8), 5.06 (2H, m, H-9), 3.83 (3H, s, -CH$_3$). $^{13}$C-NMR (100 MHz) δ: 55.88 (-OCH$_3$), 131.96 (C-1), 111.19 (C-2), 146.51 (C-3), 143.95 (C-4), 114.35 (C-5), 121.23 (C-6), 39.94 (C-7), 137.90 (C-8), 115.56 (C-9).

OPE-10: 4-hydroxybenzoic acid, C$_7$H$_6$O$_3$, white crystal, relative molecular weight: 138. $^1$H-NMR (400 MHz) δ: 6.70 (2H, d, J=8.4 Hz, H-3, 5), 7.04 (2H, d, J=8.4 Hz, H-2, 6). $^{13}$C-NMR (100 MHz) δ: 125.10 (C-1), 130.24 (C-2), 115.00 (C-3), 156.01 (C-4), 115.0 (C-5), 130.24 (C-6), 173.15 (C-7, -CO-).

OPE-11: 2-(3, 4-dihydroxyphenyl)-3, 5, 7-trihydroxy-4H-chromen-4-one, C$_{15}$H$_{10}$O$_7$, light yellow powder, relative molecular weight: 302. $^1$H-NMR (400 MHz) δ: 6.88 (1H, d, J=8.0 Hz, H-2'), 7.62 (1H, dd, J=8.4, 2.0 Hz, H-5'), 7.73 (1H, d, J=2.0 Hz, H-6'), 6.17 (1H, d, J=2.0 Hz, H-6), 6.36 (1H, d, J=2.0 Hz, H-8). $^{13}$C-NMR (100 MHz) δ: 148.74 (C-2), 137.21 (C-3), 177.28 (C-4), 162.46 (C-5), 99.25 (C-6), 165.52 (C-7), 94.45 (C-8), 158.21 (C-9), 104.53 (C-10), 124.17 (C-1'), 116.02 (C-2'), 146.19 (C-3'), 147.96 (C-4'), 116.23 (C-5'), 121.72 (C-6').

OPE-12: 2-(3, 4-dihydroxyphenyl)-5, 7-dihydroxy-4H-chromen-4-one, C$_{15}$H$_{10}$O$_6$, pale yellow crystal, relative molecular weight: 286. $^1$H-NMR (400 MHz) δ: 6.67 (1H, s, H-3), 6.44 (1H, d, J=2.0 Hz, H-8), 6.19 (1H, d, J=2.0 Hz, H-6), 6.89 (1H, d, J=8.0 Hz, H-2'), 7.42 (2H, m, H-5', 6'). $^{13}$C-NMR (100 MHz) δ: 163.86 (C-2), 103.66 (C-3), 181.62 (C-4), 161.45 (C-5), 98.79 (C-6), 164.10 (C-7), 93.80 (C-8), 157.25 (C-9), 102.84 (C-10), 121.47 (C-1'), 113.34 (C-2'), 145.70 (C-3'), 149.67 (C-4'), 115.98 (C-5'), 118.95 (C-6').

OPE-13: 5, 7-dihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one, C$_{15}$H$_{10}$O$_5$, pale yellow crystal shape, relative molecular weight: 270. $^1$H-NMR (400 MHz) δ: 6.77 (1H, s, H-3), 6.20 (1H, s, H-6), 6.49 (1H, s, H-8), 7.92 (2H, d, J=8.4 Hz, H-2', 6'), 6.93 (2H, d, J=8.4 Hz, H-3', 5').
\(^{13}\)C-NMR (100 MHz) \(\delta\): 163.71 (C-2), 103.68 (C-3), 181.71 (C-4), 161.43 (C-5), 98.79 (C-6), 164.09 (C-7), 93.92 (C-8), 161.13 (C-9), 102.82 (C-10), 121.16 (C-1'), 128.44 (C-2'), 115.92 (C-3'), 157.28 (C-4'), 115.92 (C-5'), 128.44 (C-6').

OPE-14: 3-((3-(4-dihydroxyphenyl)acryloyl)oxy)-1, 4, 5-trihydroxycyclo hexanecarbonylic acid, C\(_{16}\)H\(_{18}\)O\(_9\), white crystal, relative molecular weight: 354.

\(^1\)H-NMR (400 MHz) \(\delta\): 2.13 (4H, m, H-2, 6), 4.16 (1H, m, H-3), 3.72 (1H, dd, J=8.6, 3.2 Hz, H-4), 3.30 (1H, m, H-5), 7.04 (1H, d, J=2.0 Hz, H-2'), 6.96 (1H, dd, J=8.2, 2.0 Hz, H-5'), 6.78 (1H, d, J=8.0 Hz, H-6'), 7.56 (1H, d, J=16 Hz, H-7').

\(^{13}\)C-NMR (100 MHz) \(\delta\): 76.16 (C-1), 38.81 (C-2), 71.99 (C-3), 73.50 (C-4), 38.24 (C-5), 71.32 (C-6), 177.03 (C-7), 127.83 (C-1'), 115.28 (C-2'), 147.10 (C-3'), 149.50 (C-4'), 116.50 (C-5'), 123.00 (C-6'), 127.83 (C-7'), 115.30 (C-8'), 168.69 (C-9').

Compound 1: 4-hydroxyphenethyl 3-oxobutanoate, relative molecular weight: 222. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\): 2.21 (s, 3H), 2.88 (t, J=7.2 Hz, 2H), 3.44 (s, 2H), 4.31 (t, J=6.8 Hz, 2H), 6.77 (m, 2H), 7.05 (m, 2H).

\(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\): 30.11, 24.05, 50.08, 66.13, 115.46, 129.28, 130.00, 154.56, 167.18, 200.97.

Compound 2: 4-(benzyloxy)phenethyl 3-oxobutanoate, relative molecular weight: 312. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\): 2.20 (s, 3H, -CH\(_3\)), 2.90 (t, J=7.2 Hz, 2H), 3.42 (s, 2H), 4.32 (t, J=7.2 Hz, 2H), 5.04 (s, 2H), 6.92 (m, 2H), 7.12 (m, 2H), 7.38 (m, 5H).

\(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\): 30.05, 34.09, 50.07, 65.97, 70.07, 114.99, 127.43, 127.93, 128.57, 129.73, 129.86, 137.07, 157.65, 167.00, 200.40.

Compound 2a: 5-Methoxycarbonyl-4-phenyl-6-methyl-3, 4-dihydropyrimidin-2(1H) –one, relative molecular weight: 246. \(^1\)H NMR (400 MHz, DMSO) \(\delta\): 2.26 (s, 3H, -CH\(_3\)), 3.56 (s, 3H, -CH\(_3\)), 5.15 (s, 1H), 7.29 (m, 5H), 7.76 (s, 1H), 9.22 (s, 1H). \(^{13}\)C NMR (100 MHz, DMSO) \(\delta\): 17.79, 50.74, 53.77, 99.00, 126.13, 127.25, 128.41, 144.65, 148.61, 152.12, 165.81.

Compound 2b: 5-Methoxycarbonyl-4-(4-fluorophenyl)-6-methyl-3, 4-dihydropyrimidin-2(1H) –one, relative molecular weight: 264. \(^1\)H NMR (400 MHz, DMSO) \(\delta\): 2.26 (s, 3H, -CH\(_3\)), 3.53 (s, 3H, -CH\(_3\)), 5.15 (s, 1H), 7.21 (m, 4H), 7.78 (s, 1H), 9.26 (s, 1H). \(^{13}\)C NMR (100 MHz, DMSO) \(\delta\): 17.80, 50.75, 53.16, 98.90, 115.03, 115.24, 128.10, 128.18, 140.89, 148.76, 152.00, 161.40, 165.73.

Compound 2c: 5-Methoxycarbonyl-4-(4-chlorophenyl)-6-methyl-3, 4-dihydropyrimidin-
2(1H)-one, relative molecular weight: 280. $^1$H NMR (400 MHz, DMSO) δ: 2.26 (s, 3H, -CH$_3$), 3.53 (s, 3H, -CH$_3$), 5.15 (d, J=3.2 Hz, 1H), 7.25 (m, 2H), 7.40 (m, 2H), 7.80 (s, 1H), 9.28 (s, 1H). $^{13}$C NMR (100 MHz, DMSO) δ: 17.82, 50.78, 53.24, 98.60, 128.08, 128.41, 131.80, 143.58, 148.95, 151.94, 165.68.

Compound 2d: 5-Methoxycarbonyl-4-(4-nitrophenyl)-6-methyl-3, 4-dihydropyrimidin-2(1H)-one, relative molecular weight: 291. $^1$H NMR (400 MHz, DMSO) δ: 2.28 (s, 3H, -CH$_3$), 3.54 (s, 3H, -CH$_3$), 5.29 (s, 1H), 7.52 (m, 2H), 7.92 (s, 1H), 8.21 (m, 2H), 9.39 (s, 1H). $^{13}$C NMR (100 MHz, DMSO) δ: 17.87, 50.84, 53.53, 97.99, 123.81, 127.55, 146.71, 149.58, 151.78, 165.54.

Compound 2a': 5-Phenylethoxycarbonyl - (4-benzyloxy)-4-phenyl-6-methyl-3, 4-dihydropyrimidin-2(1H)-one, relative molecular weight: 442. $^1$H NMR (400 MHz, DMSO) δ: 2.19 (s, 3H), 2.75 (t, J=6.4 Hz, 2H), 4.13 (m, 2H), 5.07 (s, 2H), 5.09 (d, J=3.2 Hz, 1H), 6.89 (m, 2H), 7.07 (m, 2H), 7.30 (m, 10H), 7.74 (s, 1H), 9.20 (s, 1H). $^{13}$C NMR (100 MHz, DMSO) δ: 17.79, 33.43, 53.71, 64.19, 69.14, 99.06, 114.63, 126.15, 127.19, 127.73, 128.36, 128.38, 129.68, 130.33, 137.19, 144.59, 148.64, 152.12, 156.85, 165.30.

Compound 2b': 5-Phenylethoxycarbonyl-(4-benzyloxy)-4-(4-fluorophenyl)-6-methyl-3, 4-dihydropyrimidin-2(1H)-one, relative molecular weight: 460. $^1$H NMR (400 MHz, DMSO) δ: 2.19 (s, 3H, -CH$_3$), 2.75 (t, J=6.4 Hz, 2H), 4.14 (m, 2H), 5.07 (s, 2H), 5.08 (d, J=3.6 Hz, 1H), 6.89 (m, 2H), 7.06 (m, 2H), 7.15 (m, 4H), 7.37 (m, 5H), 7.75 (s, 1H), 9.24 (s, 1H). $^{13}$C NMR (100 MHz, DMSO) δ: 17.79, 33.40, 53.07, 64.18, 69.14, 98.92, 114.60, 115.07, 127.58, 127.74, 128.38, 129.66, 130.31, 137.17, 140.84, 148.83, 151.97, 156.85, 160.05, 161.26, 165.21.

Compound 2c': 5-Phenylethoxycarbonyl-(4-benzyloxy)-4-(4-chlorophenyl)-6-methyl-3, 4-dihydropyrimidin-2(1H)-one, relative molecular weight: 476. $^1$H NMR (400 MHz, DMSO) δ: 2.19 (s, 3H, -CH$_3$), 2.75 (t, J=6.4 Hz, 2H), 4.14 (m, 2H), 5.06 (s, 2H), 5.08 (d, J=3.2 Hz, 1H), 6.89 (m, 2H), 7.05 (m, 2H), 7.14 (m, 2H), 7.37 (m, 7H), 7.77 (s, 1H), 9.25 (s, 1H). $^{13}$C NMR (100 MHz, DMSO) δ: 17.19, 33.39, 53.15, 64.21, 69.15, 98.61, 114.59, 127.57, 127.73, 128.07, 128.35, 128.37, 129.65, 130.33, 131.73, 137.16, 143.49, 149.05, 151.94, 156.86, 165.16.

Compound 2d': 5-Phenylethoxycarbonyl-(4-benzyloxy)-4-(4-nitrophenyl)-6-methyl-3, 4-dihydropyrimidin-2(1H)-one, relative molecular weight: 487. $^1$H NMR (400 MHz, DMSO) δ: 2.21 (s, 3H, -CH$_3$), 2.76 (t, J=6.4 Hz, 2H), 4.16 (m, 2H), 5.04 (s, 2H), 5.20 (d, J=3.2 Hz, 1H), 6.87 (m, 2H), 7.05 (m, 2H), 7.40 (m, 8H), 8.15 (m, 2H), 9.35 (s, 1H). $^{13}$C NMR (100 MHz, DMSO) δ:
17.84, 33.29, 53.40, 64.22, 69.14, 97.95, 114.56, 123.75, 127.48, 127.59, 127.73, 128.36, 129.61, 130.26, 137.14, 146.63, 149.72, 151.68, 151.73, 156.85, 165.01.
### Table S1. Effect of catalyst amount on transesterification reaction

| Compounds                        | Catalyst amount (equiv.) | Yield (%) |
|----------------------------------|--------------------------|-----------|
| Compound 1 (Direct transesterification) | 0.1                      | 21.8      |
|                                  | 0.2                      | 25.5      |
|                                  | 0.3                      | 34.6      |
|                                  | 0.4                      | 33.2      |
| Compound 2                       | 0.1                      | 51.7      |
| (1. Protection of phenolic hydroxyl) | 0.2                      | 63.2      |
|                                  | 0.3                      | 75.7      |
| (2. Transesterification)         | 0.4                      | 73.6      |
| n (Benzaldehyde) : n (Urea) : n (β-Keto ester) | Reaction temperature (°C) | Yield (%) |
|-----------------------------------------------|--------------------------|-----------|
| 1:1:1                                         | 90                       | 80.7      |
| 1:1.2:1                                       | 90                       | 81.3      |
| 1:1.2:1.2                                     | 90                       | 82.1      |
| 1:1.2:1.5                                     | 90                       | 82.6      |
| 1:1.2:1.2                                     | 80                       | 78.3      |
| 1:1.2:1.2                                     | 100                      | 79.6      |
| Number | Compounds | R   | R’       | Reaction time (h) | Yield (%) |
|--------|-----------|-----|----------|-------------------|-----------|
| 1      | 2a        | H   |          | 3.0               | 85.2      |
| 2      | 2b        | F   |          | 3.0               | 83.1      |
| 3      | 2c        | Cl  |          | 3.0               | 83.6      |
| 4      | 2d        | NO₂ |          | 3.0               | 76.8      |
| 5      | 2a’       | H   |          | 3.0               | 80.2      |
| 6      | 2b’       | F   |          | 3.0               | 75.4      |
| 7      | 2c’       | Cl  |          | 3.0               | 63.8      |
| 8      | 2d’       | NO₂ |          | 3.0               | 61.7      |
| Target compound | IC₅₀ (μmol/L) | α-Glucosidase inhibition | α-Amylase inhibition |
|-----------------|--------------|--------------------------|---------------------|
| 1               | 862.4±35.2ᵃ  | 900.6±5.90ᵃ              |                     |
| 2               | 833.0±29.7ᵃ  | 871.4±12.8ᵇ              |                     |
| 2a              | 668.3±25.6ᵇ  | 783.3±21.7ᵈ              |                     |
| 2b              | 617.5±12.3ᵈ  | 749.7±9.3⁵              |                     |
| 2c              | 632.6±11.6ᵇcd| 759.5±4.5¹ᵈᵉ           |                     |
| 2d              | 651.6±9.8⁰ᵇᶜ| 798.2±7.5⁸              |                     |
| 2a’             | 612.8±5.7⁰ᵈ | 674.3±13.6⁹h            |                     |
| 2b’             | 563.9±26.0ᵉ  | 659.6±11.²ᵇ            |                     |
| 2c’             | 596.4±15.⁴ᵇᵉ | 688.3±6.⁷⁸            |                     |
| 2d’             | 601.8±10.²ᵈᵉ | 718.9±18.⁷⁵f           |                     |
| Acarbose        | 591.3±9.7⁵ᶜᵉ | 667.5±12.⁹ᵈʰ           |                     |

Data were expressed as mean ± standard deviation (n=3), value with different letters with a column were significantly different (P<0.05).
Figure caption

Figure S1. Chemical composition separation flow chart.

Figure S2. NMR spectrum of compounds from active fraction.

Figure S3. Structure of compounds.

Figure S4. NMR spectrum of transesterification products.

Figure S5. NMR spectrum of dihydropyrimidine derivatives.
**Figure S1.** Chemical composition separation flow chart.
a1: $^1$H NMR spectra of compound OPE-1

a2: $^{13}$C NMR spectra of compound OPE-1

b1: $^1$H NMR spectra of compound OPE-2
b2: $^{13}$C NMR spectra of compound OPE-2

c1: $^1$H NMR spectra of compound OPE-3

c2: $^{13}$C NMR spectra of compound OPE-3
d1: $^1$H NMR spectra of compound OPE-4

d2: $^{13}$C NMR spectra of compound OPE-4

e1: $^1$H NMR spectra of compound OPE-5
e2: $^{13}$C NMR spectra of compound OPE-5

f1: $^1$H NMR spectra of compound OPE-6

f2: $^{13}$C NMR spectra of compound OPE-6
g1: $^1$H NMR spectra of compound OPE-7

h1: $^1$H NMR spectra of compound OPE-8

g2: $^{13}$C NMR spectra of compound OPE-7
$h_2$: $^{13}$C NMR spectra of compound OPE-8

$p_1$: $^1$H NMR spectra of compound OPE-9

$p_2$: $^{13}$C NMR spectra of compound OPE-9
j1: $^1$H NMR spectra of compound OPE-10

j2: $^{13}$C NMR spectra of compound OPE-10

k1: $^1$H NMR spectra of compound OPE-11
k2: $^{13}$C NMR spectra of compound OPE-11

l1: $^1$H NMR spectra of compound OPE-12

l2: $^{13}$C NMR spectra of compound OPE-12
m1: $^1$H NMR spectra of compound OPE-13

m2: $^{13}$C NMR spectra of compound OPE-13

n1: $^1$H NMR spectra of compound OPE-14
n2: $^{13}$C NMR spectra of compound OPE-14

**Figure S2.** NMR spectrum of compounds from active fraction
Figure S3. Structure of compounds.
a1: $^1$H NMR spectra of compound 1

a2: $^{13}$C NMR spectra of compound 1

b1: $^1$H NMR spectra of compound 2
b2. $^{13}$C NMR spectra of compound 2

Figure S4. NMR spectrum of transesterification products
a1: $^1$H NMR spectra of compound 2a

a2: $^{13}$C NMR spectra of compound 2a

b1: $^1$H NMR spectra of compound 2b
b2: $^{13}$C NMR spectra of compound 2b

c1: $^1$H NMR spectra of compound 2c

c2: $^{13}$C NMR spectra of compound 2c
d1: $^1$H NMR spectra of compound 2d

d2: $^{13}$C NMR spectra of compound 2d
e1: $^1$H NMR spectra of compound 2a'

e2: $^{13}$C NMR spectra of compound 2a'
f1: $^1$H NMR spectra of compound 2b'

f2: $^{13}$C NMR spectra of compound 2b'

g1: $^1$H NMR spectra of compound 2c'
g2: $^{13}$C NMR spectra of compound 2c'

h1: $^1$H NMR spectra of compound 2d'

h2: $^{13}$C NMR spectra of compound 2d'

Figure S5. NMR spectrum of dihydropyrimidine derivatives