Journal of Molecular Catalysis B: Enzymatic 97 (2013) 297–302

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

journal homepage: www.elsevier.com/locate/molcatb

Enzymatic synthesis of novel N-heterocycle-containing troxerutin derivatives

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ARTICLE INFO

Article history:
Received 16 April 2013
Received in revised form 31 August 2013
Accepted 2 September 2013
Available online 9 September 2013

Keywords:
Enzymatic
N-heterocycle
Troxerutin
Flavonoids
Amidation

ABSTRACT

Using vinyl-troxerutin esters (troxerutin—O—CO—$(CH_2)_n$—COO—CH=CH_2, n = 3, 4, 7, 8, 11) and N-heterocyclic amines (piperazine, methylpiperazine, piperidine, and morpholine) as substrates, twenty novel N-heterocycle-containing troxerutin derivatives were synthesized through enzymatic reaction. The products were characterized by ^{1}H NMR, ^{13}C NMR, ESI-MS and FT-IR. Among the ten kinds of commercially available enzymes tested, lipase LS-10 produced the highest yield (58%). Investigation of water content and different medium showed that pyridine containing water lower than 2% was the efficient medium. Increasing of the molar ratio of morpholine to vinylglutaryl-troxerutin to 6:1 produced the highest yield. The carbon chain length of vinyl-troxerutin esters did not show significant effect and the yields reached plateau after reaction at 50 °C for 24 h.

1. Introduction

Compounds containing N-heterocyclic unit are very important because of their important biological properties. Many modified natural products containing N-heterocycle were found to possess anticancer and antibiotic activities [1–3]. Flavonoids are a class of secondary phenolic metabolites, which are widely used in food, cosmetics, medicines and various commodity preparations [4,5] due to their antioxidant, anti-inflammatory [6], antibacterial [7], anticancer [8], and antiallergic activities [3]. To increase the bioactivities of flavonoids, introduction of N-containing building block has become a research hot spot. Series of N-containing flavonoids have been synthesized through chemical methods [9–12], such as Mannich reaction, amination of halogen derivatives, and the modification increased their physiological activities [13]. Through Mannich reaction, Zhang et al. [14] synthesized N-containing baicalein, quercetin, and chalcone analogs, and found that the N-containing derivatives showed increased CDKs inhibitory activity than the parent compounds. Babu et al. [15] prepared 8-aminomethylated derivatives of oroxylin A with appropriate primary or secondary amines, and some of the compounds demonstrated significantly better α-glucosidase inhibitory activity than oroxylin A. By the amination of halogen derivatives, N-containing flavonoids, such as luteolin, chrysin, and oroxylin A have been synthesized and studied for their structure activity relationships [16–20]. Compared to chemical modification of flavonoids, enzymatic synthesis offers higher selectivity under milder conditions and assures a relatively efficient synthetic approach to a large range of flavonoids derivatives [21]. Through enzymatic O-methylation [22,23], esterification [21], hydroxylation [24–26] or transglycosylation [27], flavonoids having several hydroxyl groups have been selectively modified. To the best of our knowledge, enzymatic synthesis of N-heterocycle-containing flavonoids has not been reported. In this paper, the enzymatic synthesis of series of N-heterocycle-containing troxerutin derivatives through the amidation between vinyl-troxerutin derivatives and N-heterocyclic amines (piperazine, methylpiperazine, piperidine, and morpholine) are reported. The influences of enzyme source, reaction medium, water content of the solvent, the molar ratio of the substrates, and the chain length of the vinyl-troxerutin derivatives on the yields were investigated.

2. Materials and methods

2.1. Enzymes and chemicals

Alkaline protease from *Bacillus subtilis* was obtained from Wuxi Xuemei Enzyme Co. Ltd. (Wuxi, P.R. China). Lipase IVK F-100 (I) was obtained from Shenzhen Lvweikang Biotechnology Co. Ltd. (Shenzhen, P.R. China). Lipase AY "Amano" 30G (II) and lipase AY "Amano" 50G (III) were obtained from Amano Enzyme Co. Ltd. (Japan). Lipase immobilized on acrylic resin from *Candida antarctica* (Novozym 435) (IV) and Lipozyme LA 35025 (V) were purchased

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http://dx.doi.org/10.1016/j.molcatb.2013.09.005

from Sigma. Lipase HZ-2 (VI) was purchased from Zhejiang Lilai Biotechnology Co. Ltd. (Zhejiang, P.R. China). Lipase LS-10 (VII) was purchased from Beijing Kaitai New Nentury Biotechnology Co. Ltd. (Beijing, P.R. China). Lipase type VIII from *candida rugosa* (VIII) was purchased from Shanghai Xibao Biotechnology Co. Ltd (Shanghai, P.R. China). Lipase from *hog pancreas* (IX) and Lipase from *candida cylindracea* (X) were purchased from Fluka. Troxerutin was provided by Sichuan Yabao Guangtai Pharmaceutical Co. Ltd. (Sichuan, P.R. China). All other chemicals were obtained from commercial sources and used without further purification. Divinyl carboxylates were prepared and purified as described in a patent [28]. Vinyl-troxerutin derivatives were synthesized according to the literature [29].

2.2. Analytical methods

The ^{1}H and ^{13}C NMR spectra were recorded with a Bruker Avance 400 MHz instrument using TMS as internal standard. FT-IR spectra were recorded using an IR-200 Fourier transform spectrometer in KBr pellets. Mass spectra were analyzed on a Bruker Esquire 3000 mass spectrometer fitted with an ion spray source (methanol, positive mode).

The progress of the amidation reaction was monitored by TLC (silica gel 60F254 glass sheets, ethyl acetate:methanol:water (15:3.6:1, by vol.)) and HPLC (waters e2695-2998, Inertsil® ODS-3 column, UV detector (λ_{max} = 350 nm)). The N-heterocycle-containing troxerutin derivatives for HPLC external standard (purity > 98%) were prepared and purified by column chromatography (silica, ethyl acetate:methanol:water (15:3.6:1, by vol.)). Aqueous methanol (50%) was employed as mobile phase with a flow rate of 1 mL/min (4.01 min (**3ai**), 4.20 min (**3bi**), 4.81 min (**3ci**), 5.24 min (**3di**), 6.64 min (**3ei**)). All experiments were carried out in duplicate.

2.3. General procedure for the synthesis of N-heterocycle-containing troxerutin derivatives

The enzymatic synthesis of N-heterocycle-containing troxerutin derivatives was carried out in the 250 mL flask on a shaker at 150 rpm. Enzyme was added to a mixture of vinyl-troxerutin derivative (0.11 mmol) and N-heterocyclic compound (piperazine, methylpiperazine, piperidine, or morpholine) (1.10 mmol) in different solvents, and the mixture was kept at 50 °C for specified time. The enzyme was filtered off and the filtrate was concentrated under reduced pressure. Column chromatography purification of the residue afforded the target product. The products were then characterized by NMR, IR and ESI-MS. Aliquots (0.2 mL) were transferred at various time intervals and stored at −20 °C for HPLC analysis later.

2.4. The compounds ***3ai*–*3ei***

3ai: yellow powder, yield 59%, R_f 0.19; ^{1}H NMR (DMSO-d_6), δ (ppm): 7.77 (s, 1H, $H_{2'}$), 7.73 (m, 1H, J = 8.4 Hz, $H_{6'}$), 7.15 (d, 1H, J = 8.0 Hz, $H_{5'}$), 6.74 (s, 1H, H_8), 6.38 (s, 1H, H_6), 5.41 (m, 1H, J = 7.2 Hz, $H_{1''}$), 4.41 (m, 3H, 2H of A acylated, 1H of B acylated), 4.32 (m, 1H, $H_{1'''}$), 4.26 (m, 1H, H of B acylated), 4.12–4.06 (m, 4H, H of A), 3.75 (m, 4H, H of B), 3.25–3.06 (10H, H of rhamnoglucosyl), 3.52, 3.23 (m, 8H, 8H of morpholine), 3.25–3.06 (m, 10H, H of rhamnoglucosyl), 2.40, 2.34 (m, 4H, 2H of —CH_2—CO—morpholine and 2H of —CH_2—COO—troxerutin), 1.76 (m, 2H, other CH_2 of glutaridioyl part), 0.97 (d, 3H, J = 4.4 Hz, CH_3 of rhamnosyl); ^{13}C NMR (DMSO-d_6): 177.7 (C-4), 173.1 (C=O), 171.5 (C=O), 165.0 (C-7), 161.2 (C-9), 156.7 (C-5), 156.4 (C-2), 151.6 (C-4′), 147.7 (C-3′), 134.2 (C-3), 123.6 (C-1′), 122.7 (C-6′), 114.6 (C-5′), 113.5 (C-2′), 105.5(C-10), 101.6 (C-1″), 101.2 (C-1‴), 98.8 (C-6), 93.3 (C-8), 76.6 (C-3″), 76.2 (C-5″), 74.6 (C-2″), 72.1 (C-4‴), 71.0 (C-3‴), 71.1 (C-2‴), 70.9 (C-4″), 70.7 (C-A), 70.5 (C-A), 68.4 (C-5‴), 67.1 (C-6″), 67.4 (C-A), 67.4 (morpholine), 67.1 (morpholine), 62.8 (C-B), 59.9 (C-B), 59.7 (C-B), 45.8 (morpholine), 41.8 (morpholine), 34.5 ($(CH_2)_n$), 33.2 ($(CH_2)_n$), 32.3 ($(CH_2)_n$), 18.1 (C-6‴); IR (KBr): 3356 cm^{-1} (OH), 1733 cm^{-1} (O=C—O), 1653 cm^{-1} (O=C—N), 1449 cm^{-1} (C—N); ESI-MS (m/z): 948.4 $[M+Na]^+$.

3bi: yellow powder, yield 60%, R_f 0.23; ^{1}H NMR (DMSO-d_6 + D_2O), δ (ppm): 7.81 (s, 1H, $H_{2'}$), 7.71 (m, 1H, J = 8.8 Hz, $H_{6'}$), 7.11 (d, 1H, J = 8.4 Hz, $H_{5'}$), 6.66 (s, 1H, H_8), 6.35 (s, 1H, H_6), 5.35 (m, 1H, J = 7.0 Hz, $H_{1''}$), 4.37 (m, 3H, 2H of A acylated, 1H of B acylated), 4.27 (m, 1H, $H_{1'''}$), 4.26 (m, 1H, H of B acylated), 4.07 (m, 4H, H of A), 3.74 (m, 4H, H of B), 3.32–3.03 (10H, H of rhamnoglucosyl), 3.37, 3.25 (m, 8H, 8H of morpholine), 2.33, 2.26 (m, 4H, 2H of —CH_2—CO—morpholine and 2H of —CH_2—COO—troxerutin), 1.48 (m, 4H, other CH_2 of hexanedioyl part), 0.90 (d, 3H, J = 4.4 Hz, CH_3 of rhamnosyl); ^{13}C NMR (DMSO-d_6): 177.8 (C-4), 173.3 (C=O), 170.8 (C=O), 165.1 (C-7), 161.2 (C-9), 156.8 (C-5), 157.0 (C-2), 151.4 (C-4′), 148.2 (C-3′), 134.4 (C-3), 123.6 (C-1′), 123.0 (C-6′), 115.6 (C-5′), 113.7 (C-2′), 105.7 (C-10), 101.8 (C-1″), 101.3 (C-1‴), 99.0 (C-6), 93.2 (C-8), 76.8 (C-3″), 76.5 (C-5″), 74.6 (C-2″), 72.3 (C-4‴), 71.2 (C-3‴), 71.1 (C-2‴), 71.0 (C-4″), 70.9 (C-A), 70.7 (C-A), 68.6 (C-5‴), 67.4 (C-6″), 67.4 (C-A), 67.3 (morpholine), 67.2 (morpholine), 62.9 (C-B), 60.1 (C-B), 59.9 (C-B), 45.7 (morpholine), 41.9 (morpholine), 33.7 ($(CH_2)_n$), 33.2 ($(CH_2)_n$), 29.0 ($(CH_2)_n$), 24.3 ($(CH_2)_n$), 18.1 (C-6‴); IR (KBr): 3357 cm^{-1} (OH), 1738 cm^{-1} (O=C—O), 1658 cm^{-1} (O=C—N), 1455 cm^{-1} (C—N); ESI-MS (m/z): 962.3 $[M+Na]^+$.

3ci: yellow powder, yield 61%, R_f 0.45; ^{1}H NMR (DMSO-d_6), δ (ppm): 7.84 (s, 1H, $H_{2'}$), 7.76 (m, 1H, J = 7.6 Hz, $H_{6'}$), 7.16 (d, 1H, J = 8.4 Hz, $H_{5'}$), 6.74 (s, 1H, H_8), 6.38 (s, 1H, H_6), 5.43 (m, 1H, J = 8.0 Hz, $H_{1''}$), 4.40 (m, 3H, 2H of A acylated, 1H of B acylated), 4.31 (m, 1H, $H_{1'''}$), 4.26 (m, 1H, H of B acylated), 4.12–4.06 (m, 4H, H of A), 3.75 (m, 4H, H of B), 3.40–3.05 (10H, H of rhamnoglucosyl), 3.52, 3.23 (m, 8H, 8H of morpholine), 2.35, 2.24 (m, 4H, 2H of —CH_2—CO—morpholine and 2H of —CH_2—COO—troxerutin), 1.52, 1.24 (m, 10H, other CH_2 of nonoanedioyl part), 0.97 (d, 3H, J = 5.2 Hz, CH_3 of rhamnoglucosyl); ^{13}C NMR (DMSO-d_6): 177.8 (C-4), 173.4 (C=O), 170.7 (C=O), 165.2 (C-7), 161.2 (C-9), 156.8 (C-5), 156.5 (C-2), 150.9 (C-4′), 147.4 (C-3′), 134.2 (C-3), 123.4 (C-1′), 122.8 (C-6′), 114.7 (C-5′), 113.1 (C-2′), 105.4 (C-10), 101.9 (C-1″), 101.2 (C-1‴), 98.8 (C-6), 93.1 (C-8), 76.9 (C-3″), 76.3 (C-5″), 74.6 (C-2″), 72.1 (C-4‴), 71.0 (C-3‴), 71.1 (C-2‴), 70.7 (C-4″), 70.7 (C-A), 70.5 (C-A), 68.5 (C-5‴), 67.4 (C-A), 67.2 (C-6″), 67.3 (morpholine), 67.0 (morpholine), 62.5 (C-B), 59.9 (C-B), 59.7 (C-B), 45.9 (morpholine), 42.0 (morpholine), 33.8 ($(CH_2)_n$), 33.7 ($(CH_2)_n$), 32.8 ($(CH_2)_n$), 28.9 ($(CH_2)_n$), 28.8 ($(CH_2)_n$), 28.8 ($(CH_2)_n$), 24.9 ($(CH_2)_n$), 18.1 (C-6‴); IR (KBr): 3350 cm^{-1} (OH), 1732 cm^{-1} (O=C—O), 1656 cm^{-1} (O=C—N), 1452 cm^{-1} (C—N); ESI-MS (m/z): 1004.4 $[M+Na]^+$.

3di: yellow powder, yield 58%, R_f 0.5; ^{1}H NMR (DMSO-d_6), δ (ppm): 7.84 (s, 1H, $H_{2'}$), 7.76 (m, 1H, J = 9.0 Hz, $H_{6'}$), 7.16 (s, 1H, $H_{5'}$), 6.74 (s, 1H, H_8), 6.38 (s, 1H, H_6), 5.41 (m, 1H, J = 10.0 Hz, $H_{1''}$), 4.40 (m, 3H, 2H of A acylated, 1H of B acylated), 4.31 (m, 1H, $H_{1'''}$), 4.26 (m, 1H, H of B acylated), 4.12–4.06 (m, 4H, H of A), 3.75 (m, 4H, H of B), 3.39–3.06 (10H, H of rhamnoglucosyl), 3.53, 3.24 (m, 8H, 8H of morpholine), 2.33, 2.25 (m, 4H, 2H of —CH_2—CO—morpholine and 2H of —CH_2—COO—troxerutin), 1.52, 1.45, 1.23 (m, 12H, other CH_2 of decanedioyl part), 0.96 (s, 3H, CH_3 of rhamnosyl); ^{13}C NMR (DMSO-d_6): 177.9 (C-4), 173.4 (C=O), 171.0 (C=O), 165.0 (C-7), 161.2 (C-9), 156.8 (C-5), 156.8 (C-2), 151.4 (C-4′), 147.9 (C-3′), 134.2 (C-3), 123.7 (C-1′), 123.3 (C-6′), 115.4 (C-5′), 113.7 (C-2′), 105.5 (C-10), 101.9 (C-1″), 101.3 (C-1‴), 98.8 (C-6), 93.3 (C-8), 76.8 (C-3″), 76.3 (C-5″), 74.6 (C-2″), 72.4 (C-4‴), 71.1 (C-3‴), 71.0 (C-2‴), 70.9 (C-4″), 70.8 (C-A), 70.5 (C-A), 68.5 (C-5‴), 67.4 (morpholine), 67.1 (morpholine), 67.2 (C-6″), 67.2 (C-A), 62.7 (C-B), 59.9 (C-B), 59.7 (C-B), 45.8 (morpholine), 41.8 (morpholine), 33.8 ($(CH_2)_n$), 32.8 ($(CH_2)_n$), 32.6 ($(CH_2)_n$), 29.2 ($(CH_2)_n$), 28.9 ($(CH_2)_n$), 28.8 ($(CH_2)_n$), 25.2 ($(CH_2)_n$),