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Application of Enzymatic Promiscuity in Pharmaceutical Synthesis: Papain-catalyzed One-pot Synthesis of 1,4-Dihydropyridine Calcium Channel Antagonists and Derivatives

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Abstract A new method for the synthesis of 1,4-dihydropyridine(1,4-DHP) calcium channel antagonists felodipine, nitrendipine and their derivatives *via* papain-catalyzed three-component reactions of aldehyde, methyl acetoacetate and ethyl 3-aminocrotonate was developed. Operational simplicity, mild reaction conditions and eco-friendliness are the key features of this protocol.

Keywords Papain; Catalytic promiscuity; Multicomponent reaction; 1,4-Dihydropyridine calcium channel antagonist

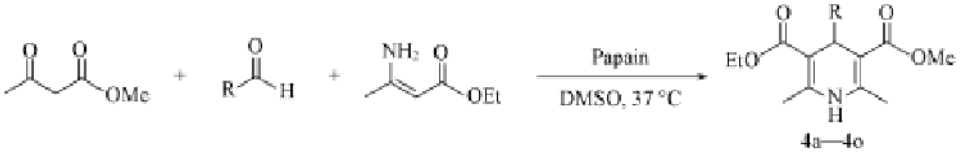
1 Introduction

Nowadays, hypertension(high blood pressure) affects approximately 14% of global population and is responsible for 9 million deaths annually[1,2]. In order to fight against this disease, several classes of antihypertensive drugs have been developed. Among them, 1,4-dihydropyridine(1,4-DHP) calcium channel antagonists, which lower blood pressure by affecting arterial vascular smooth muscle cells to cause vasodilation, are recommended for the first line treatment of hypertension[3].

1,4-DHP calcium channel antagonists can be readily prepared using the three-component reaction with microwave irradiation[4], catalyst micellar Keggin heteropolyacids[5], visible light[6], or in a continuous-flow microreactor[7]. However, many of these chemical methods reported so far suffer from disadvantages, such as harsh reaction conditions, high temperature, and tedious procedure of catalyst preparation. Therefore, a simple, convenient and environmentally friendly protocol is still desirable.

In the past twenty years, biocatalysis has emerged as an efficient and powerful tool in organic synthesis due to its valued features, such as mild reaction conditions, high efficiency and relatively low energy requirements[8]. One current frontier for biocatalysis is enzymatic promiscuity, which means the ability of enzymes to catalyze reactions other than their natural ones[9]. Some elegant works in this field have been reported in the last decade and enzymes, especially hydrolases, were verified as good catalysts for Aldol reaction[10], Michael reaction[11], Knoevenagel condensation[12], Morita-Baylis-Hillman reaction[13] and even multicomponent reactions[14—16]. However, to the best of our knowledge, the application of enzymatic promiscuity in pharmaceutical synthesis is rarely reported. The only two cases were reported by He *et al.*[17] and Guan *et al.*[18], respectively. He *et al.* reported a promiscuous enzyme-catalyzed synthesis of warfarin and derivatives. Five years later, Guan group found that the crude earthwarm extract also had the ability to catalyze the synthesis of warfarin and its derivatives. Although warfarin and derivatives can be obtained in high yields, the long reaction time(6 d and 5 d, respectively) in both cases restricts their application.

As the result of our continuous research in the field of enzymatic promiscuity[19,20], herein we reported a novel method for the synthesis of 1,4-DHP calcium channel antagonists felodipine, nitrendipine and their derivatives *via* papain-catalyzed three-component reactions(Scheme 1).



Scheme 1 Papain-catalyzed synthesis of 1,4-DHP calcium channel antagonists and derivatives

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2 Experimental

2.1 Materials and Characterization

Albumin bovine from bovine serum(BSA) was purchased from Aladdin Chemicals(Shanghai, China). Amano lipase AK(activity≥20000 U/g), Amano protease P-1(activity 10000 U/g) and Amano lipase L-3(activity 30000 U/g) were purchased from Amano Enzyme Inc.(Shanghai, China). Papain (activity 2000 U/mg) and pepsin(activity 3000—3500 U/g) were purchased from Sangon Biotech(Shanghai, China). Esterase from *Rhodobacter sphaeroides*(RspE, activity 200000 U/g) was expressed in *Escherichia coli* and applied without further purification[21]. Unless otherwise noted, all reagents were obtained from commercial suppliers and were used without further purification.

NMR spectra were recorded on a 400 MHz(101 MHz for ^{13}C NMR) spectrometer using $CDCl_3$ as the solvent and tetramethylsilane(TMS) as the internal reference. The mass spectra (MS) were measured by a Bruker microTOF-Q II spectrometer with electrospray ionization(ESI). High performance liquid chromography(HPLC) was carried out on a Shimadzu LC-2030 HPLC system equipped with an InertSustain C_{18} column (4.6 mm×250 mm×5 μm), using methanol/50 mmol/L KH_2PO_4 (pH=3.0)/acetonitrile(1:2:2, volume ratio) as the mobile phase at a flow rate of 1.0 mL/min and UV detection at 254 nm. Corresponding products were purified *via* column chromatography on silica gel[eluent: *V*(petroleum ether)/*V*(ethyl acetate)=6:1]. All melting points were determined on an Optimelt MPA100 melting point apparatus and were uncorrected.

2.2 Typical Enzymatic Procedure for the Synthesis of 1,4-DHP Calcium Channel Antagonists and Derivatives

A mixture of aldehyde **1**(1 mmol), methyl acetoacetate **2** (1 mmol), ethyl 3-aminocrotonate **3**(1 mmol) and papain (100 mg) in dimethyl sulfoxide(DMSO, 15 mL) was shaken at 240 r/min and 37 °C for specified period of time. Upon the complete consumption of aldehyde **1**(monitored by thin layer chromography, TLC), the reaction mixture was diluted with ethyl acetate(30 mL) and the enzyme was removed by simple filtration. The filtrate was washed with water(15 mL×3) and dried over anhydrous Na_2SO_4. The solvent was concentrated *in vacuo* and the residue was purified by column chromatography on silica gel(eluent: petroleum ether/ethyl acetate, volume ratio 6:1) to afford the title compounds.

2.3 Characterization

^1H NMR, ^{13}C NMR and mass spectra of compounds **4**a—**4**o are shown in the Electronic Supplementary Material of this paper.

3-Ethyl-5-methyl-4-(2,3-dichlorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate(**4**a, felodipine): a pale yellow solid; isolated yield 50.6%; m. p. 141.1—143.0 °C; ^1H NMR(400 MHz, $CDCl_3$), δ: 7.30(dd, J=16.0, 6.2 Hz, 2H, Ar-H), 7.08(t, J=7.8 Hz, 1H, Ar-H), 5.73(s, 1H, NH), 5.48(s, 1H, CH), 4.09(q, J=7.1 Hz, 2H, CH_2), 3.63(s, 3H, OCH_3), 2.33(d, J=4.2 Hz, 6H, CH_3), 1.20(t, J=7.2 Hz, 3H, CH_3); ^{13}C NMR(101 MHz, $CDCl_3$), δ: 167.90, 167.41, 148.13, 144.26, 144.16, 132.74, 130.95, 129.68, 128.23, 127.02, 103.87, 103.45, 59.87, 50.90, 38.55, 19.57, 19.51, 14.30; MS(ESI), *m/z*: 406.17$[M+Na]^+$.

3-Ethyl-5-methyl-2,6-dimethyl-4-phenyl-1,4-dihydropyridine-3,5-dicarboxylate(**4**b): a yellow solid; isolated yield 73.5%; m. p. 156.9—158.7 °C; ^1H NMR(400 MHz, $CDCl_3$), δ: 7.29(d, J=7.3 Hz, 2H, Ar-H), 7.23(t, J=7.5 Hz, 2H, Ar-H), 7.15(t, J=7.1 Hz, 1H, Ar-H), 5.70(s, 1H, NH), 5.02(s, 1H, CH), 4.21—4.03(m, 2H, CH_2), 3.66(s, 3H, OCH_3), 2.36(s, 6H, CH_3), 1.24(t, J=7.1 Hz, 3H, CH_3); ^{13}C NMR(101 MHz, $CDCl_3$), δ: 168.14, 167.67, 147.63, 144.32, 144.03, 127.93, 127.82, 126.16, 104.12, 103.78, 59.79, 51.00, 39.46, 19.57, 19.54, 14.28; MS(ESI), *m/z*: 338.23$[M+Na]^+$.

3-Ethyl-5-methyl-2,6-dimethyl-4-(*p*-tolyl)-1,4-dihydropyridine-3,5-dicarboxylate(**4**c): a yellow solid; isolated yield 63.0%; m. p. 128.3—130.9 °C; ^1H NMR(400 MHz, $CDCl_3$), δ: 7.19(d, J=8.0 Hz, 2H, Ar-H), 7.04(d, J=8.0 Hz, 2H, Ar-H), 5.81(d, J= 19.7 Hz, 1H, NH), 4.98(d, J=4.5 Hz, 1H, CH), 4.16—4.07(m, 2H, CH_2), 3.66(d, J=3.1 Hz, 3H, OCH_3), 2.34(d, J=2.7 Hz, 6H, CH_3), 2.30(s, 3H, Ar-CH_3), 1.26(td, J=7.1, 2.8 Hz, 3H, CH_3); ^{13}C NMR(101 MHz, $CDCl_3$), δ: 168.23, 167.76, 144.78, 144.37, 144.04, 135.59, 128.68, 127.65, 104.16, 103.79, 59.79, 51.00, 38.95, 21.08, 19.52, 19.51, 14.31; MS(ESI), *m/z*: 352.26$[M+Na]^+$.

3-Ethyl-5-methyl-4-(3-chlorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate(**4**d): a yellow solid; isolated yield 86.8%; m. p. 117.5—120.1 °C; ^1H NMR(400 MHz, $CDCl_3$), δ: 7.27—7.09(m, 4H, Ar-H), 5.72(s, 1H, NH), 4.99(s, 1H, CH), 4.23—4.03(m, 2H, CH_2), 3.67(d, J=2.8 Hz, 3H, OCH_3), 2.36(s, 6H, CH_3), 1.25(dd, J=12.3, 5.2 Hz, 3H, CH_3); ^{13}C NMR(101 MHz, $CDCl_3$), δ: 168.23, 167.76, 144.78, 144.37, 144.04, 135.59, 128.68, 127.65, 104.16, 103.79, 59.79, 51.00, 38.95, 21.08, 19.52, 19.51, 14.31; MS(ESI), *m/z*: 372.31 $[M+Na]^+$.

3-Ethyl-5-methyl-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate(**4**e, nitrendipine): a pale yellow solid; isolated yield 53.7%; m. p. 155.6—157.5 °C; ^1H NMR(400 MHz, $CDCl_3$), δ: 8.13(t, J=1.9 Hz, 1H, Ar-H), 8.02(dd, J=8.1, 2.0 Hz, 1H, Ar-H), 7.65(d, J=7.7 Hz, 1H, Ar-H), 7.39(t, J=7.9 Hz, 1H, Ar-H), 5.90(s, 1H, NH), 5.11(s, 1H, CH), 4.21—4.02(m, 2H, CH_2), 3.66(d, J=2.9 Hz, 3H, OCH_3), 2.38(d, J=2.2 Hz, 6H, CH_3), 1.24(td, J=7.1, 4.5 Hz, 3H, CH_3); ^{13}C NMR(101 MHz, $CDCl_3$), δ: 167.64, 167.13, 149.79, 148.23, 145.16, 144.90, 134.39, 128.67, 122.92, 121.37, 103.28, 102.98, 60.05, 51.17, 39.79, 19.60, 19.54, 14.26; MS(ESI), *m/z*: 360.92$[M+H]^+$.

3-Ethyl-5-methyl-4-(3-fluorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate(**4**f): a yellow solid; isolated yield 66.9%; m. p. 117.8—121.0 °C; ^1H NMR(400 MHz, $CDCl_3$), δ: 7.17(dd, J=14.3, 7.4 Hz, 1H, Ar-H), 7.08(d, J=7.6 Hz, 1H, Ar-H), 6.97(d, J=10.2 Hz, 1H, Ar-H), 6.83(t, J=8.2 Hz, 1H, Ar-H), 5.98(s, 1H, NH), 5.02(s, 1H, CH), 4.20—4.01(m, 2H, CH_2), 3.67(s, 3H, OCH_3), 2.34(s, 6H, CH_3), 1.25(t, J=

7.1 Hz, 3H, CH_3); ^{13}C NMR(101 MHz, $CDCl_3$), δ: 168.05, 167.57, 164.00, 161.57, 150.23, 150.17, 144.83, 144.57, 129.25, 129.17, 123.43, 123.41, 114.67, 114.46, 113.09, 112.88, 103.53, 103.21, 59.91, 51.07, 39.38, 39.37, 19.42, 19.40, 14.27; MS(ESI), *m/z*: 356.22$[M+Na]^+$.

3-Ethyl-5-methyl-4-(4-chlorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate(**4**g): a yellow solid; isolated yield 62.2%; m. p. 135.5—137.1 °C; ^1H NMR(400 MHz, $CDCl_3$), δ: 7.21(q, *J*=8.5 Hz, 4H, Ar-H), 5.76(s, 1H, NH), 4.98(s, 1H, CH), 4.21—4.02(m, 2H, CH_2), 3.66(s, 3H, OCH_3), 2.34(s, 6H, CH_3), 1.24(t, *J*=7.1 Hz, 3H, CH_3); ^{13}C NMR(101 MHz, $CDCl_3$), δ: 168.03, 167.55, 146.25, 144.63, 144.37, 131.72, 129.24, 128.03, 103.69, 103.39, 59.90, 51.06, 39.10, 19.47, 19.45, 14.29; MS(ESI), *m/z*: 372.16$[M+Na]^+$.

3′-Ethyl-5′-methyl-2′,6′-dimethyl-1′,4′-dihydro-[2,4′-bipyridine]-3′,5′-dicarboxylate(**4**h): a pale yellow solid; isolated yield 55.1%; m. p. 197.2—198.8 °C; ^1H NMR(400 MHz, $CDCl_3$), δ: 8.61(s, 1H, NH), 8.52(d, *J*=4.7 Hz, 1H, Py-H), 7.63(t, *J*=7.6 Hz, 1H, Py-H), 7.42(d, *J*=7.7 Hz, 1H, Py-H), 7.21—7.13(m, 1H, Py-H), 5.21(s, 1H, CH), 4.08(q, *J*=7.0 Hz, 2H, CH_2), 3.64(s, 3H, OCH_3), 2.27(s, 6H, CH_3), 1.22(t, *J*=7.1 Hz, 3H, CH_3); ^{13}C NMR(101 MHz, $CDCl_3$), δ: 168.03, 167.55, 146.25, 144.63, 144.37, 131.72, 129.24, 128.03, 103.69, 103.39, 59.90, 51.06, 39.10, 19.47, 19.45, 14.29; MS(ESI), *m/z*: 339.29$[M+Na]^+$.

3-Eethyl-5-methyl-2,6-dimethyl-4-(thiophen-2-yl)-1,4-dihydropyridine-3,5-dicarboxylate(**4**i): a pale yellow solid; isolated yield 38.5%; m. p. 141.1—143.0 °C; ^1H NMR(400 MHz, $CDCl_3$), δ: 7.08(d, *J*=3.8 Hz, 1H, Th-H), 6.87(s, 1H, Th-H), 6.81(s, 1H, Th-H), 5.97—5.82(m, 1H, NH), 5.36(s, 1H, CH), 4.29—4.12(m, 2H, CH_2), 3.73(s, 3H, OCH_3), 2.37(s, 6H, CH_3), 1.42—1.23(m, 3H, CH_3); ^{13}C NMR(101 MHz, $CDCl_3$), δ: 167.91, 167.45, 151.52, 145.20, 144.83, 126.43, 123.22, 123.02, 103.46, 103.03, 59.99, 51.16, 34.32, 19.39, 19.38, 14.38; MS(ESI), *m/z*: 344.20$[M+Na]^+$.

3-Ethyl-5-methyl-2,6-dimethyl-4-phenethyl-1,4-dihydropyridine-3,5-dicarboxylate(**4**j): a pale yellow solid; isolated yield 88.1%; m. p. 101.7—103.8 °C; ^1H NMR(400 MHz, $CDCl_3$), δ: 7.24(d, *J*=7.1 Hz, 2H, Ar-H), 7.16(d, *J*=6.8 Hz, 3H, Ar-H), 5.76(s, 1H, NH), 4.32—4.13(m, 2H, CH_2), 4.07(s, 1H, CH), 3.74(s, 3H, OCH_3), 2.63—2.50(m, 2H, CH_2), 2.32(s, 6H, CH_3), 1.77—1.62(m, 2H, CH_2), 1.32(t, *J*=6.9 Hz, 3H, CH_3); ^{13}C NMR(101 MHz, $CDCl_3$), δ: 168.53, 168.07, 145.69, 145.34, 143.04, 128.24, 128.15, 125.43, 102.77, 102.42, 59.75, 51.03, 38.39, 33.17, 31.24, 19.44, 19.41, 14.48; MS(ESI), *m/z*: 366.23$[M+Na]^+$.

3-Ethyl-5-methyl-4-(2-methoxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate(**4**k): a yellow solid; isolated yield 44.8%; m. p. 132.9—134.6 °C; ^1H NMR(400 MHz, $CDCl_3$), δ: 7.22(s, 1H, Ar-H), 7.13(t, *J*=7.5 Hz, 1H, Ar-H), 6.83(t, *J*=7.6 Hz, 2H, Ar-H), 5.72(s, 1H, NH), 5.29(s, 1H, CH), 4.15—4.00(m, 2H, CH_2), 3.81(s, 3H, OCH_3), 3.62(s, 3H, OCH_3), 2.30(s, 6H, CH_3), 1.21(t, *J*=6.9 Hz, 3H, CH_3); ^{13}C NMR(101 MHz, $CDCl_3$), δ: 168.65, 168.20, 156.99, 144.20, 144.02, 135.43, 130.19, 127.29, 120.14, 110.76, 103.03, 102.77, 59.58, 55.37, 50.88, 50.81, 35.05, 19.21, 14.22; MS(ESI), *m/z*: 368.26$[M+Na]^+$.

3-Ethyl-5-methyl-4-(4-bromophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate(**4**l): a pale yellow solid; isolated yield 69.3%; m. p. 152.4—155.0 °C; ^1H NMR (400 MHz, $CDCl_3$), δ: 7.34(d, *J*=7.4 Hz, 2H, Ar-H), 7.17(d, *J*=6.7 Hz, 2H, Ar-H), 5.73(s, 1H, NH), 4.97(s, 1H, CH), 4.19—4.02(m, 2H, CH_2), 3.66(s, 3H, OCH_3), 2.35(s, 6H, CH_3), 1.24(t, *J*=6.7 Hz, 3H, CH_3); ^{13}C NMR(101 MHz, $CDCl_3$), δ: 168.65, 168.20, 156.99, 144.20, 144.02, 135.43, 130.19, 127.29, 120.14, 110.76, 103.03, 102.77, 59.58, 55.37, 50.88, 50.81, 35.05, 19.21, 14.22; MS(ESI), *m/z*: 416.27$[M+Na]^+$.

3-Ethyl-5-methyl-4-(2-fluorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate(**4**m): a pale yellow solid; isolated yield 83.5%; m. p. 150.4—152.7 °C; ^1H NMR(400 MHz, $CDCl_3$), δ: 7.36—7.26(m, 1H, Ar-H), 7.11(d, *J*=4.9 Hz, 1H, Ar-H), 7.01(t, *J*=7.3 Hz, 1H, Ar-H), 6.92(t, *J*=9.1 Hz, 1H, Ar-H), 5.91—5.77(m, 1H, NH), 5.26(s, 1H, CH), 4.17—3.99(m, 2H, CH_2), 3.63(s, 3H, OCH_3), 2.33(s, 6H, CH_3), 1.21(t, *J*=6.9 Hz, 3H, CH_3); ^{13}C NMR(101 MHz, $CDCl_3$), δ: 168.15, 167.66, 161.04, 158.57, 144.80, 144.65, 134.94, 134.80, 131.01, 130.96, 127.77, 127.69, 123.71, 123.68, 115.13, 114.90, 102.82, 102.58, 59.78, 50.95, 34.22, 19.31, 19.25, 14.05; MS(ESI), *m/z*: 356.19$[M+Na]^+$.

3-Ethyl-5-methyl-2,6-dimethyl-4-(*m*-tolyl)-1,4-dihydropyridine-3,5-dicarboxylate(**4**n): a yellow solid; isolated yield 72.6%; m. p. 116.1—118.7 °C; ^1H NMR(400 MHz, $CDCl_3$), δ: 7.15—7.05(m, 3H, Ar-H), 6.96(d, *J*=6.8 Hz, 1H, Ar-H), 5.83(dd, *J*=34.3, 17.1 Hz, 1H, NH), 4.99(d, *J*=6.9 Hz, 1H, CH), 4.12(qq, *J*=10.9, 7.1 Hz, 2H, CH_2), 3.67(d, *J*=3.9 Hz, 3H, OCH_3), 2.35(d, *J*=4.2 Hz, 6H, CH_3), 2.31(s, 3H, Ar-CH_3), 1.25(td, *J*=7.1, 4.1 Hz, 3H, CH_3); ^{13}C NMR(101 MHz, $CDCl_3$), δ: 168.27, 167.80, 147.56, 144.48, 144.16, 137.29, 128.55, 127.80, 126.98, 124.87, 104.02, 103.64, 59.77, 50.99, 39.36, 21.61, 19.50, 19.47, 14.30; MS(ESI), *m/z*: 352.14$[M+Na]^+$.

3-Ethyl-5-methyl-4-(3-methoxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate(**4**o): a yellow solid; isolated yield 78.7%; m. p. 111.8—115.3 °C; ^1H NMR(400 MHz, $CDCl_3$), δ: 7.15(t, *J*=7.9 Hz, 1H, Ar-H), 6.88(dd, *J*=16.7, 4.8 Hz, 2H, Ar-H), 6.70(dd, *J*=8.1, 2.3 Hz, 1H, Ar-H), 5.80—5.67 (m, 1H, NH), 5.01(d, *J*=4.6 Hz, 1H, CH), 4.18—4.07(m, 2H, CH_2), 3.79(s, 3H, OCH_3), 3.67(d, *J*=2.8 Hz, 3H, OCH_3), 2.34 (d, *J*=2.0 Hz, 6H, CH_3), 1.26(t, *J*=7.1 Hz, 3H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$), δ: 168.16, 167.70, 159.28, 149.24, 144.57, 144.26, 128.81, 120.35, 114.09, 110.84, 103.83, 103.48, 59.81, 55.07, 51.01, 39.37, 19.47, 19.46, 14.33; MS(ESI), *m/z*: 368.40$[M+Na]^+$.

3 Results and Discussion

Initially, 2,3-dichlorobenzaldehyde, methyl acetoacetate and ethyl 3-aminocrotonate were used as the model substrates to optimize the reaction conditions including catalysts, solvents and catalyst loading. As shown in Table 1, several biocatalysts including lipases and proteases were investigated. When the reaction was performed in the absence of any catalysts, the expected product **4**a(felodipine) was obtained in only 17.9% yield, even after 48 h(Entries 1, 2, Table 1). Amano protease P-1, Amano lipase AK and Amano lipase L-3 exhibited low catalytic activity for the model reaction(Entries 3—5, Table 1).

Esterase from *Rhodobacter sphaeroides*(RspE) and pepsin showed acceptable catalytic activity, and the yields of compound **4**a were 44.7% and 45.3%, respectively(Entries 6, 7, Table 1). The best results were obtained by using papain as the catalyst(Entry 8, Table 1). In order to exclude protein catalysis, control experiments were conducted by using non-enzyme protein bovine serum albumin(BSA) and denatured papain. As expected, the reaction catalyzed by BSA or denatured papain gave product in yields of only 17.7% and 20.6%, respectively (Entries 2, 9, Table 1). All these results indicate that the special spatial conformation of papain is responsible for its ability to catalyze the model reaction.

Table 1 Catalytic activities of different catalysts in the model reaction[a]

Entry	Catalyst	Yield[b](%)
1	None	17.9
2	BSA	17.7
3	Amano protease P-1	22.8
4	Amano lipase AK	31.3
5	Amano lipase L-3	33.7
6	RspE	44.7
7	Pepsin	45.3
8	Papain	52.3
9	Denatured papain[c]	20.6

a. Reaction conditions: 2,3-dichlorobenzaldehyde(0.2 mmol), methyl acetoacetate(0.2 mmol), ethyl 3-aminocrotonate(0.2 mmol), catalyst(30 mg), DMSO(3 mL) at 240 r/min, 37 °C for 2 d; *b.* determined by HPLC using the external standard method; *c.* pre-treated with urea at 100 °C for 8 h.

Next, the reaction medium was optimized and the results are shown in Table 2. It could be seen that the catalytic activity of papain was obviously influenced by different solvents. A yield of no more than 5% was obtained when reaction was conducted in THF and *n*-hexane(Entries 1, 2, Table 2). With DMF and ethanol as solvents, the yields were improved to 12.2% and 18.4%, respectively. Among the solvents tested, DMSO exhibited evident advantage, which afforded a yield of 52.3%(Entry 5, Table 2). Based on the above experimental results, DMSO was chosen as the optimum solvent for the synthesis of 1,4-DHP calcium channel antagonists and derivatives.

Table 2 Effect of different solvents on the model reaction[a]

Entry	Solvent	Yield[b](%)
1	THF	1.9
2	*n*-Hexane	2.0
3	DMF	12.2
4	Ethanol	18.4
5	DMSO	52.3

a. Reaction conditions: 2,3-dichlorobenzaldehyde(0.2 mmol), methyl acetoacetate(0.2 mmol), ethyl 3-aminocrotonate(0.2 mmol), papain(30 mg), solvent(3 mL) at 240 r/min, 37 °C for 2 d; *b.* determined by HPLC using the external standard method.

Enzyme loading plays an important role in enzymatic reactions. When the enzyme loading was increased from 10 mg to 20 mg, the corresponding yield increased from 44.8% to 53.2%(Entries 1, 2, Table 3). When the enzyme loading was varied from 20 mg to 40 mg, the yield of product did not further increase and reached a plateau(Entries 2—4, Table 3). However, there was a significant decline in yield once the enzyme loading was beyond 40 mg(Entries 5—7, Table 3). A possible explanation for this phenomenon is that excessive enzyme loading may lead to protein agglomeration, which is unfavorable to the diffusion of substrates. From the economic point of view, 20 mg of papain was chosen for further investigation.

Table 3 Effect of enzyme loading on the yield of the model reaction[a]

Entry	Enzyme loading/mg	Yield[b](%)
1	10	44.8
2	20	53.2
3	30	52.3
4	40	54.5
5	50	50.9
6	70	45.4
7	90	40.5

a. Reaction conditions: 2,3-dichlorobenzaldehyde(0.2 mmol), methyl acetoacetate(0.2 mmol), ethyl 3-aminocrotonate(0.2 mmol), specified amount of papain, DMSO(3 mL) at 240 r/min, 37 °C for 2 d; *b.* determined by HPLC using the external standard method.

With the optimal conditions in hand, we then explored the scope of the reaction, and the results are summarized in Table 4. It was found that 1,4-DHP calcium channel antagonists felodipine and nitrendipine can be synthesized in moderate yields (Entries 1, 5, Table 4). Besides, other aldehydes bearing either electron-donating or electron-withdrawing groups on the benzene ring were converted into the corresponding analogues with moderate to high yields. For aromatic aldehydes bearing electron-donating groups(CH_3, OCH_3), the substituent on the *meta*-position gave higher yields than their *ortho*- and *para*-substituted analogues(Entry 14 *vs.* Entry 3, Entry 15 *vs.* Entry 11, Table 4). Moreover, it was interesting to find that 2-fluorobenzaldehyde was more active than 3-fluorobenzaldehyde(Entry 13 *vs.* Entry 6, Table 4), which means steric factors did not affect the reactivity greatly.

Table 4 Papain-catalyzed synthesis of 1,4-DHP calcium channel antagonist and derivatives[a]

Entry	R	Product	Time/d	Yield[b](%)
1	2,3-$Cl_2C_6H_3$	**4**a(Felodipine)	1	50.6
2	C_6H_5	**4**b	1	73.5
3	4-$CH_3C_6H_4$	**4**c	1	63.0
4	3-ClC_6H_4	**4**d	1	86.8
5	3-$NO_2C_6H_4$	**4**e(Nitrendipine)	1	53.7
6	3-FC_6H_4	**4**f	1	66.9
7	4-ClC_6H_4	**4**g	1	62.2
8	2-C_5H_4N	**4**h	1	55.1
9	2-C_4H_3S	**4**i	2	38.5
10	$C_6H_5CH_2CH_2$	**4**j	1	88.1
11	2-$CH_3OC_6H_4$	**4**k	1	44.8
12	4-BrC_6H_4	**4**l	1	69.3
13	2-FC_6H_4	**4**m	1	83.5
14	3-$CH_3C_6H_4$	**4**n	1	72.6
15	3-$CH_3OC_6H_4$	**4**o	1	78.7

a. All reactions were carried out by using aldehyde(1 mmol), methyl acetoacetate(1 mmol), ethyl 3-aminocrotonate(1 mmol), papain(100 mg), DMSO(15 mL) at 240 r/min, 37 °C; *b.* isolated yields.

4 Conclusions

1,4-DHP calcium channel antagonist and their derivatives were synthesized through papain-catalyzed one-pot three-

component reactions from readily available materials. This protocol is featured by operational simplicity, mild reaction conditions and eco-friendliness. The procedure demonstrates the potential application value of enzymatic promiscuity for pharmaceutical synthesis. Studies aiming at improving the activity and enantioselectivity of papain by enzyme engineering are underway in our laboratory.

Electronic Supplementary Material

Supplementary material is available in the online version of this article at http://dx.doi.org/10.1007/s40242-019-8273-8.

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