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A Novel One-Pot Multicomponent Enzymatic Synthesis of 2,4-Disubstituted Thiazoles

Huiping Zhang

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Abstract A novel one-pot multicomponent synthetic method of some new 2,4-disubstituted thiazoles catalyzed by enzyme was developed. Lipase from porcine pancreas (PPL) displayed good catalytic activity to promote this reaction starting from amines, aldehyde, thioacetic acid and methyl 3-(dimethyl amino)-2-isocyanoacrylate under mild conditions (35 °C) with good yields (up to 93 %). The experimental results revealed PPL showed broad catalytic activity to substrates. The blank and control experiments revealed that PPL played important catalytic role during the process. All the new compounds were confirmed by IR, ^{1}H NMR, ^{13}C NMR, MS and elemental analysis. This PPL-catalyzed one-pot multicomponent synthetic method provided a new strategy to synthesize 2,4-disubstituted thiazoles and expanded the application of enzymes in organic synthesis.

Keywords Enzymes · Multicomponent reactions · Heterocycles · Thiazoles · Synthesis

1 Introduction

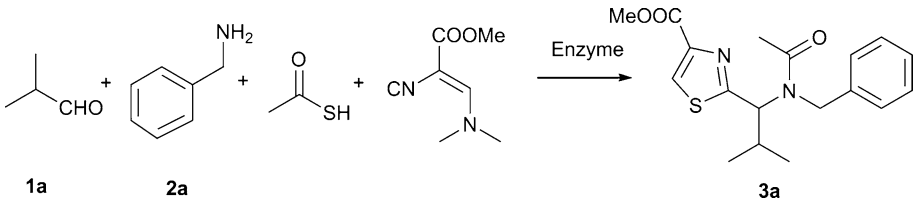
Thiazole derivatives have attracted much attention of medicinal and organic chemists for their varied biological activities, such as antitumor, antifungal, antibiotic and antiviral activities [1–4]. In addition, they are also important synthetic intermediates and common substructures in numerous biological compounds [5–7]. As one of the important thiazole derivatives, 2,4-disubstituted thiazoles exhibit multifarious bioactivities and the synthetic methods were developed continually [8–10]. Marder et al. reported the synthesis and applications of some 2,4-disubstituted thiazole derivatives as small molecule modulators of cellular development [11]. Srinivasan et al. developed an efficient synthesis of 2,4-disubstituted thiazoles using ionic liquid [12].

Multicomponent reactions (MCRs) have received much attention in modern organic chemistry for their high efficiency and greater atom-economy. It can provide new organic synthetic methods and easier access to get diverse compounds [13–15]. For the synthesis of thiazole derivatives, Sayyed-Alangi et al. reported the synthesis of functionalized thiazoles using MCRs from isothiocyanates with high yields and an easy work-up procedure [16]. Domling et al. found a novel MCRs to synthesize thiazole moiety from ss-aminothiocarboxylic acids, aldehydes, and 3-dimethylamino-2-isocyanoacylate [17] and they also reported the new MCRs of 2,4-disubstituted thiazoles under inert and water-free conditions at 0–10 °C stirred for 1–4 days [18]. This publication prompted us to research a mild synthetic method via MCRs for the synthesis of 2,4-disubstituted thiazoles.

Enzymatic synthesis, as a novel, green and mild catalytic method, flourished recently [19–21]. Especially for organic synthesis, enzymes showed enzymatic promiscuity to form C–C, C–N, C–S bonds through Henry reaction, Michael addition, Markovnikov addition and Aldol reactions [22–24]. There are some reports about the enzymatic synthesis of thiazole derivatives [25, 26]. For the importance of developing new methods, in this paper, the first chemoenzymatic multicomponent example of synthesizing 2,4-disubstituted thiazoles under mild conditions was developed. This method was another successful example of enzyme promiscuity in organic chemistry.

H. Zhang (✉)
Department of Chemistry and Chemical Engineering, Jining University, Qufu 273100, Shandong, China
e-mail: hpzhangjnu@163.com

Scheme 1 Model reaction of preparing 2,4-disubstituted thiazoles catalyzed by enzyme



This one-pot enzymatic reaction was performed by employing benzylamine **1a** (1 mmol), isobutyraldehyde **2a** (1 mmol), thioacetic acid and methyl 3-(dimethylamino)-2-isocyanoacrylate (1 mmol) as a model reaction (Scheme 1). Reaction progress was monitored by TLC and GC. The reaction conditions including enzyme, solvent, temperature, reaction time and catalysts amount were optimized respectively.

2 Experimental

All reagents and solvents were purchased without further purification, unless otherwise indicated. Reactions were performed in an end-over-end rotator. NMR was recorded on a Bruker Avance 400 spectrometer at 400 MHz in $CDCl_3$ using TMS as internal standard. IR spectra were recorded on a Bruker Equinox-55 spectrophotometer using KBr discs in the 4,000–400 cm^{-1} region. A Hewlett-Packard model 6890 gas chromatograph with a capillary column (HP-5) and flame-ionization detector was used to analyze the yields of products using tridecane as an internal standard. Melting points were recorded on an X4-Data microscopic melting point apparatus and were uncorrected. Elemental analyses were performed on an EA-1110 instrument. All the enzymes were purchased from Aldrich and used directly.

General procedure for synthesis of 2,4-disubstituted thiazoles: 1.0 mmol amines, 1.0 mmol aldehyde, 1.0 mmol thioacetic acid and 1.0 mmol methyl 3-(dimethylamino)-2-isocyanoacrylate were introduced accordingly into a test tube (10 ml), then 20 mg lipase from porcine pancreas (PPL) and 5 ml methanol were added and shaken at 160 rpm end-over-end rotation at 35 °C for 2 h. The reaction mixture was monitored by TLC to end (hexane/AcOEt = 3:2). The residue was purified on silica gel to afford the target compounds. All the new compounds were informed by IR, ^1H NMR, ^{13}C NMR, MS and elemental analysis.

2.1 Methyl 2-(1-(*N*-benzylacetamido)-2-methylpropyl)thiazole-4-carboxylate (**3a**) [13]

Yellow liquid. IR: ν_{max} 2957, 1727, 1648, 1472, 1443, 1410, 1321, 1244, 1210, 1109, 1058 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz, δ, ppm): 0.83 (d, $J = 6.8$ Hz, 3H, CH_3), 0.95 (d, $J = 6.8$ Hz, 3H, CH_3), 2.06 (s, 3H, CH_3-C=O), 2.84–2.93 (m, 1H, CH), 3.84 (s, 3H, CH_3O), 4.59 (d, $J = 17.2$ Hz, 1H, CH_2N), 4.78 (d, $J = 17.2$ Hz, 1H, CH_2N), 5.36 (d, $J = 7.2$ Hz, 1H, CHN), 6.84–7.14 (m, 5H, Ph-H), 8.01 (s, 1H, CHS). MS(EI): m/z (%) = 346 (M^+).

2.2 Methyl 2-(2-methyl-1-(*N*-(prop-2-ynyl) acetamido)propyl)thiazole-4-carboxylate (**3b**)

Yellow solid. M.p. 114–116 °C. IR (KBr): ν_{max} 3306, 3132, 2958, 1728, 1652, 1474, 1440, 1411, 1326, 1255, 1213, 1110, 1075 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz, δ, ppm): 0.89 (d, $J = 6.4$ Hz, 3H, CH_3), 1.05 (d, $J = 6.4$ Hz, 3H, CH_3), 2.18 (s, 1H, CH), 2.24 (s, 3H, CH_3C=O), 2.72–2.79 (m, 1H, CH), 3.94 (s, 3H, CH_3O), 4.01 (d, $J = 19.2$ Hz, 1H, CH_2N), 4.36 (d, $J = 19.2$ Hz, 1H, CH_2N), 5.51 (d, $J = 10.8$ Hz, 1H, CHN), 8.14 (s, 1H, CHS). ^{13}C NMR ($CDCl_3$, 100 MHz, δ, ppm): 19.2 (CH_3), 19.8 (CH_3), 22.4 (CH_3), 30.5 (CH), 36.8 (CH_2), 52.2 (CH_3), 67.8 (CH), 72.6 (CH), 79.3 (Cq), 129.6 (CH), 145.3 (Cq), 161.6 (Cq), 169.9 (Cq), 171.3 (Cq). MS(EI): m/z (%) = 294 (M^+). Anal. Calcd. for $C_{14}H_{18}N_2O_3S$: C, 57.12; H, 6.16; N, 9.52; Found: C, 56.99; H, 6.14; N, 9.45 %.

2.3 Methyl 2-(1-(*N*-benzylacetamido)butyl)thiazole-4-carboxylate (**3c**)

Yellow liquid. IR: ν_{max} 2958, 2360, 1735, 1648, 1408, 1323, 1212, 1081 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz, δ, ppm): 0.85 (t, $J = 7.2$ Hz, 3H, CH_3), 1.26–1.32 (m, 2H, CH_2), 1.95–2.01 (m, 1H, CH_2), 2.09 (s, 3H, CH_3C=O), 2.25–2.30 (m, 1H, CH_2), 3.90 (s, 3H, CH_3O), 4.63 (s, 2H, CH_2N), 5.81 (t, $J = 7.2$ Hz, 1H, CHN), 7.07–7.27 (m, 5H, Ph-H), 8.08 (s, 1H, CHS). ^{13}C NMR ($CDCl_3$, 100 MHz, δ, ppm): 13.8 (CH_3), 19.6 (CH_2), 22.1 (CH_3), 33.6 (CH_2), 50.6 (CH_3), 53.5 (CH_2), 61.8 (CH), 126.6 (CH), 127.2 (CH), 128.8 (CH), 129.7 (CH), 136.6 (Cq), 145.9 (Cq), 162.1 (Cq), 168.9 (Cq), 171.9 (Cq). MS(EI): m/z (%) = 346 (M^+). Anal. Calcd. for $C_{18}H_{22}N_2O_3S$: C, 62.40; H, 6.40; N, 8.09; Found: C, 62.39; H, 6.43; N, 8.05 %.

2.4 Methyl 2-(1-(*N*-(prop-2-ynyl)acetamido) butyl)thiazole-4-carboxylate (**3d**)

Yellow liquid. IR: ν_{max} 3254, 2957, 2872, 1696, 1622, 1431, 1296, 1215, 1094 cm^{-1}. ^1H NMR ($CDCl_3$,

400 MHz, δ, ppm): 0.79 (t, $J = 7.2$ Hz, 3H, CH_3), 1.24–1.28 (m, 2H, CH_2), 1.94–1.98 (m, 2H, CH_2), 2.22 (s, 3H, CH_3C=O), 2.13 (s, 1H, CH), 3.88 (s, 3H, CH_3O), 4.04 (d, $J = 16.2$ Hz, 1H, CH_2N), 4.38 (d, $J = 16.2$ Hz, 1H, CH_2N), 5.51–5.56 (m, 1H, CHN), 8.06 (s, 1H, CHS). ^{13}C NMR ($CDCl_3$, 100 MHz, δ, ppm): 13.1 (CH_3), 19.2 (CH_2), 22.4 (CH_3), 33.2 (CH_2), 37.1 (CH_2), 52.4 (CH_3), 65.8 (CH), 72.8 (CH), 79.9 (Cq), 128.3 (CH), 145.8 (Cq), 161.2 (Cq), 168.8 (Cq), 170.9 (Cq). MS(EI): m/z (%) = 294 (M^+). Anal. Calcd. for $C_{14}H_{18}N_2O_3S$: C, 57.12; H, 6.16; N, 9.52; Found: C, 57.16; H, 6.21; N, 9.55 %.

2.5 Methyl 2-(1-(*N*-benzylacetamido)pentyl)thiazole-4-carboxylate (**3e**)

Yellow liquid. IR: ν_{max} 2955, 2869, 1737, 1649, 1408, 1323, 1243, 1213, 1085, 1029 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz, δ, ppm): 0.74–0.77 (m, 2H, CH_2), 0.81 (t, $J = 6.4$ Hz, 3H, CH_3), 1.22–1.25 (m, 2H, CH_2), 2.01 (q, $J = 7.2$ Hz, $J = 16.2$ Hz, 2H, CH_2), 2.10 (s, 3H, CH_3), 3.90 (s, 3H, CH_3C=O), 4.63 (s, 2H, CH_2N), 5.78 (t, $J = 7.6$ Hz, 1H, CHN), 7.07–7.24 (m, 5H, Ph-H), 8.08 (s, 1H, CHS). ^{13}C NMR ($CDCl_3$, 100 MHz, δ, ppm): 13.9 (CH_3), 20.6 (CH_2), 22.6 (CH_3), 24.8 (CH_2), 33.6 (CH_2), 52.8 (CH_3), 53.7 (CH_2), 62.6 (CH), 126.3 (CH), 127.4 (CH), 128.7 (CH), 129.8 (CH), 136.8 (Cq), 145.4 (Cq), 161.8 (Cq), 169.5 (Cq), 171.8 (Cq). MS(EI): m/z (%) = 360 (M^+). Anal. Calcd. for $C_{19}H_{24}N_2O_3S$: C, 63.31; H, 6.71; N, 7.77; Found: C, 63.28; H, 6.69; N, 7.79 %.

2.6 Methyl 2-(1-(*N*-(prop-2-ynyl)acetamido) pentyl)thiazole-4-carboxylate (**3f**)

Yellow liquid. IR: ν_{max} 3242, 2956, 1733, 1655, 1408, 1320, 1244, 1216, 1086 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz, δ, ppm): 0.79 (t, $J = 6.4$ Hz, 3H, CH_3), 0.81–0.85 (m, 2H, CH_2), 1.20–1.23 (m, 2H, CH_2), 2.05 (q, $J = 7.2$ Hz, $J = 16.2$ Hz, 1H, CH_2), 2.20 (s, 3H, CH_3C=O), 2.5 (s, 1H, CH), 3.94 (s, 3H, CH_3O), 4.05 (d, $J = 16.2$ Hz, 1H, CH_2N), 4.39 (d, $J = 16.2$ Hz, 1H, CH_2N), 5.97 (t, $J = 7.6$ Hz, 1H, CHN), 8.14 (s, 1H, CHS). ^{13}C NMR ($CDCl_3$, 100 MHz, δ, ppm): 13.8 (CH_3), 21.1 (CH_2), 22.8 (CH_3), 24.7 (CH_2), 33.7 (CH_2), 36.6 (CH_2), 52.4 (CH_3), 64.7 (CH), 72.9 (CH), 79.8 (Cq), 128.9 (CH), 145.3 (Cq), 161.6 (Cq), 169.4 (Cq), 171.2 (Cq). MS(EI): m/z (%) = 308 (M^+). Anal. Calcd. for $C_{15}H_{20}N_2O_3S$: C, 58.42; H, 6.54; N, 9.08; Found: C, 58.39; H, 6.59; N, 9.11 %.

2.7 Methyl 2-(1-(*N*-cyclopentylacetamido)pentyl) thiazole-4-carboxylate (**3g**)

Yellow liquid. IR: ν_{max} 2958, 2925, 1730, 1649, 1418, 1315, 1265, 1220, 1077 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz, δ, ppm): 0.96 (t, $J = 6.8$ Hz, 3H, CH_3), 1.26–1.29 (m, 2H, CH_2), 1.31–1.34 (m, 2H, CH_2), 1.50–1.55 (m, 4H, C_5H_9), 1.72–1.75 (m, 4H, C_5H_9), 2.02 (s, 3H, CH_3C=O), 2.11–2.18 (m, 2H, CH_2), 3.61–3.65 (m, 1H, CHN), 3.88 (s, 3H, CH_3O), 5.48 (t, $J = 7.2$ Hz, 1H, CHN), 7.98 (s, 1H, CHS). ^{13}C NMR ($CDCl_3$, 100 MHz, δ, ppm): 13.7 (CH_3), 21.4 (CH_2), 22.5 (CH_3), 23.9 (CH_2), 24.6 (CH_2), 33.9 (CH_2), 34.8 (CH_2), 52.4 (CH_3), 57.6 (CH), 63.6 (CH), 128.4 (CH), 145.4 (Cq), 161.3 (Cq), 169.6 (Cq), 170.8 (Cq). MS(EI): m/z (%) = 338 (M^+). Anal. Calcd. for $C_{17}H_{26}N_2O_3S$: C, 60.33; H, 7.74; N, 8.28; Found: C, 60.38; H, 7.79; N, 8.31 %.

2.8 Methyl 2-(1-(*N*-butylacetamido)pentyl)thiazole-4-carboxylate (**3h**)

Yellow liquid. IR: ν_{max} 2968, 2921, 1740, 1638, 1413, 1321, 1243, 1209, 1076 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz, δ, ppm): 0.88 (t, $J = 6.8$ Hz, 3H, CH_3), 1.22–1.24 (m, 4H, $2CH_2$), 1.31 (t, $J = 5.6$ Hz, 3H, CH_3), 1.33–1.35 (m, 4H, $2CH_2$), 2.15 (s, 3H, CH_3C=O), 2.31 (q, $J = 7.2$ Hz, $J = 16.2$ Hz, 2H, CH_2), 3.24 (t, $J = 4.8$ Hz, 2H, CH_2), 3.94 (s, 3H, CH_3O), 5.55 (t, $J = 7.2$ Hz, 1H, CHN), 8.15 (s, 1H, CHS). ^{13}C NMR ($CDCl_3$, 100 MHz, δ, ppm): 13.6 (CH_3), 14.0 (CH_3), 20.1 (CH_2), 21.6 (CH_2), 22.4 (CH_3), 28.6 (CH_2), 32.1 (CH_2), 32.1 (CH_2), 34.8 (CH_2), 52.4 (CH_3), 59.3 (CH), 128.8 (CH), 145.5 (Cq), 161.9 (Cq), 169.9 (Cq), 171.3 (Cq). MS(EI): m/z (%) = 326 (M^+). Anal. Calcd. for $C_{16}H_{26}N_2O_3S$: C, 58.87; H, 8.03; N, 8.58; Found: C, 58.91; H, 7.99; N, 8.61 %.

2.9 Methyl 2-(cyclohexyl(*N*-cyclopentylacetamido) methyl)thiazole-4-carboxylate (**3i**)

Yellow liquid. IR: ν_{max} 2965, 1731, 1657, 1411, 1325, 1235, 1221, 1088 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz, δ, ppm): 1.40–1.44 (m, 10H, C_6H_{11}), 1.51–1.53 (m, 4H, C_5H_9), 1.73–1.75 (m, 4H, C_5H_9), 2.03 (s, 3H, CH_3C=O), 2.41–2.43 (m, 1H, CH), 3.86 (s, 3H, CH_3O), 4.95 (d, $J = 7.2$ Hz, 1H, CHN), 7.82 (s, 1H, CHS). ^{13}C NMR ($CDCl_3$, 100 MHz, δ, ppm): 22.4 (CH_3), 23.9 (CH_2), 24.6 (CH_2), 25.9 (CH_2), 29.8 (CH_2), 30.1 (CH_2), 32.3 (CH_2), 32.7 (CH_2), 39.6 (CH), 52.4 (CH_3), 55.4 (CH), 61.3 (CH), 128.1 (CH), 145.2 (Cq), 161.2 (Cq), 169.3 (Cq), 170.1 (Cq). MS(EI): m/z (%) = 364 (M^+). Anal. Calcd. for $C_{19}H_{28}N_2O_3S$: C, 62.61; H, 7.74; N, 7.69; Found: C, 62.58; H, 7.79; N, 7.72 %.

2.10 Methyl 2-((*N*-butylacetamido)(cyclohexyl) methyl)thiazole-4-carboxylate (**3j**)

Yellow liquid. IR: ν_{max} 2958, 1712, 1645, 1413, 1322, 1243, 1220, 1084 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz, δ, ppm): 0.33 (t, $J = 5.6$ Hz, 3H, CH_3), 1.35–1.37 (m, 4H, $2CH_2$), 1.40–1.44 (m, 10H, C_6H_{11}), 2.11 (s, 3H, CH_3C=O), 2.20 (t, $J = 4.8$ Hz, 2H, CH_2), 2.44–2.46 (m, 1H, CH), 3.94 (s, 3H, CH_3O), 5.52 (d, $J = 7.2$ Hz, 1H, CH), 8.17 (s,

1H, CHS). ^{13}C NMR ($CDCl_3$, 100 MHz, δ, ppm): 13.8 (CH_3), 20.4 (CH_2), 22.4 (CH_3), 24.6 (CH_2), 26.9 (CH_2), 28.6 (CH_2), 28.9 (CH_2), 32.1 (CH_2), 39.6 (CH), 47.9 (CH_2), 52.4 (CH_3), 61.3 (CH), 128.4 (CH), 145.6 (Cq), 161.6 (Cq), 169.8 (Cq), 171.1 (Cq). MS(EI): *m/z* (%) = 352 (M^+). Anal. Calcd. for $C_{18}H_{28}N_2O_3S$: C, 61.33; H, 8.01; N, 7.95; Found: C, 61.38; H, 7.98; N, 8.00 %.

2.11 Methyl 2-((*N*-cyclopentylacetamido)(cyclopropyl) methyl)thiazole-4-carboxylate (**3k**)

Yellow liquid. IR: ν_{max} 2944, 1738, 1650, 1415, 1323, 1248, 1219, 1083 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz, δ, ppm): 0.43 (q, J = 4.4 Hz, J = 8.8 Hz, 2H, CH_2), 0.64 (q, J = 4.4 Hz, J = 8.8 Hz, 2H, CH_2), 1.25–1.27 (m, 1H, CH), 1.67–1.69 (m, 4H, C_5H_9), 1.75–1.77 (m, 4H, C_5H_9), 2.20 (s, 3H, CH_3C=O), 3.92 (s, 3H, CH_3O), 4.04 (d, J = 7.6 Hz, 1H, CHN), 8.14 (s, 1H, CHS). ^{13}C NMR ($CDCl_3$, 100 MHz, δ, ppm): 6.2 (CH_2), 6.3 (CH_2), 15.8 (CH), 22.5 (CH_3), 23.3 (CH_2), 34.1 (CH_2), 34.3 (CH_2), 52.3 (CH_3), 56.4 (CH), 65.3 (CH), 128.2 (CH), 145.3 (Cq), 161.7 (Cq), 169.5 (Cq), 170.6 (Cq). MS(EI): *m/z* (%) = 322 (M^+). Anal. Calcd. for $C_{16}H_{22}N_2O_3S$: C, 59.60; H, 6.88; N, 8.69; Found: C, 59.58; H, 6.90; N, 8.71 %.

2.12 Methyl 2-((*N*-butylacetamido)(cyclopropyl) methyl)thiazole-4-carboxylate (**3l**)

Yellow liquid. IR: ν_{max} 2945, 1734, 1657, 1410, 1328, 1245, 1214, 1089 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz, δ, ppm): 0.46 (q, J = 4.8 Hz, J = 9.6 Hz, 2H, CH_2), 0.65 (q, J = 4.8 Hz, J = 9.6 Hz, 2H, CH_2), 0.90 (t, J = 7.2 Hz, 3H, CH_3), 1.27–1.29 (m, 1H, CH), 1.30–1.33 (m, 4H, $2CH_2$), 2.15 (s, 3H, CH_3C=O), 3.71 (t, J = 7.2 Hz, 2H, CH_2), 3.93 (s, 3H, CH_3O), 4.71 (d, J = 10.4 Hz, 1H, CH), 8.14 (s, 1H, CHS). ^{13}C NMR ($CDCl_3$, 100 MHz, δ, ppm): 6.7 (CH_2), 6.8 (CH_2), 13.6 (CH_3), 15.8 (CH), 20.1 (CH_2), 22.5 (CH_3), 34.1 (CH_2), 47.1 (CH_2), 52.4 (CH_3), 67.3 (CH), 128.1 (CH), 145.1 (Cq), 161.3 (Cq), 169.2 (Cq), 170.1 (Cq). MS(EI): *m/z* (%) = 310 (M^+). Anal. Calcd. for $C_{15}H_{22}N_2O_3S$: C, 58.04; H, 7.14; N, 9.02; Found: C, 58.11; H, 7.11; N, 8.99 %.

3 Results and Discussion

We initially screened the enzymes and the results were summarized in Table 1. Several enzymes displayed observable catalytic activities for this reaction. Especially, PPL showed better catalytic activity (Entry 1; Table 1) compared to other enzymes. Interestingly, α-amylase from hog pancreas had higher catalytic ability (Entry 2; Table 1) than that of α-amylase from *Aspergillus oryzae* and α-Amylase from *Bacillus subtilis* (Entries 7, 8; Table 1),

Table 1 Optimization of catalyst

Entry	Enzymes	Yield (%)[b]
1	Lipase from porcine pancreas	88
2	α-Amylase from hog pancreas	71
3	Typsin from porcine pancreas	52
4	Bovine serum albumin (BSA)	32
5	Diastase from *Aspergillus oryzae*	51
6	Amano lipase M from *Mucor javanicus*	32
7	α-Amylase from *Aspergillus oryzae*	33
8	α-Amylase from *Bacillus subtilis*	22
9	Blank	Trace
10	Denatured lipase from porcine pancreas[a]	Trace

Reaction conditions benzylamine (1 mmol), thioacetic acid (1 mmol), isobutyraldehyde (1 mmol), and methyl 3-(dimethylamino)-2-isocyanoacrylate (1 mmol), enzyme (20 mg), methanol (5 ml), shaken at 160 rpm at 35 °C for 2 h

[a] Lipase from porcine pancreas was refluxed in urea for 24 h at 100 °C [27]

[b] GC yields are based on tridecane as an internal standard

which maybe attribute to the different catalytic sites in their protein structures. To determine the catalytic effects coming from enzyme, the control experiments were performed (Entries 9, 10; Table 1) according to the literature [27]. The results revealed that only trace product was detected in the blank and denatured enzyme experiments. So we conclude that PPL played an important catalytic role in this reaction.

The reaction medium was one of the important influencing factors. Especially for the enzyme promiscuity, the solvents will affect the configuration of the enzyme. Several solvents were screened in this reaction. The experimental results demonstrated this reaction went smoothly in the presence of methanol with the highest yield (Entry 1; Table 2) compared to other organic solvents. Interestingly, as the similar polar solvents, ethanol and water seemed to be inefficient in this reaction (Entries 3, 9; Table 2). It maybe revealed that methanol was the most suitable candidate in term of polarity and structure. So we concluded that PPL had higher "promiscuous" activity than its "natural" activity in organic solvents. Based on the experimental results, methanol was selected as the optimum solvent in this reaction.

According to above conditions, we applied PPL to optimize other conditions. A GC yield was selected as the criterion to optimize the reaction temperature, time and concentration of PPL in this reaction. The experimental results were listed in Table 3. For the temperature, five experiments were performed. The results showed that the yields went up from 54–84 % at the region 25–35 °C. When the temperature continues to rise to 45 °C the yield decreased to 78 %. This maybe attributed to the partly

Table 2 Optimization of solvents

Entry	Solvent	T (°C)	Yield[a] (%)
1	Methanol	35	88
2	Acetonitrile	35	52
3	Ethanol	35	50
4	*n*-Hexane	35	41
5	Dichloromethane	35	39
6	1,4-Dioxane	35	37
7	Acetone	35	22
8	Tetrahydrofuran	35	20
9	Water	35	Trace

Reaction conditions benzylamine (1 mmol), thioacetic acid (1 mmol), isobutyraldehyde (1 mmol), and methyl 3-(dimethylamino)-2-isocyanoacrylate (1 mmol), lipase from porcine pancreas (20 mg), solution (5 ml), shaken at 160 rpm at 35 °C

[a] GC yields are based on tridecane as an internal standard

Table 3 Optimization of the reaction conditions

Entry	Amount (mg)	T (°C)	Time (h)	Yield (%)[a]
1	20	25	2	54
2	20	30	2	73
3	20	35	2	88
4	20	40	2	80
5	20	45	2	78
6	10	35	2	62
7	20	35	2	88
8	30	35	2	81
9	40	35	2	75
10	50	35	2	74
11	20	35	1	56
12	20	35	2	88
13	20	35	3	83

Reaction conditions benzylamine (1 mmol), thioacetic acid (1 mmol), isobutyraldehyde (1 mmol), and methyl 3-(dimethylamino)-2-isocyanoacrylate (1 mmol), PPL, methanol (5 ml), shaken at 160 rpm

[a] GC yields are based on tridecane as an internal standard

inactivated enzyme at higher temperature. So the optimum temperature was 35 °C. The optimal enzyme amount was screened from 10–50 mg. The results showed that the highest yield was achieved with 20 mg of enzyme, which maybe attributed to the reunion of enzyme when its concentration was high in solution. As to reaction time, the highest yield was obtained at 2 h. Synthetically, the optimum reaction condition is 20 mg PPL in 5 ml solvent and 2 h.

Based on these optimized conditions, we employed this reaction with various different amines and aldehydes in order to extend the substrate (Scheme 2; Table 4). We chose different amines and aldehydes bearing alkanes and

Table 4 Synthesis of different 2,4-disubstituted thiazoles catalyzed by PPL

Entry	Compound	R_1	R_2	Yield (%)[a]
1	**3a**	Isopropyl	Benzyl	88 (79)[b]
2	**3b**	Isopropyl	2-Propynyl	93 (86)
3	**3c**	*n*-Propyl	Benzyl	90 (84)
4	**3d**	*n*-Propyl	2-Propynyl	89 (82)
5	**3e**	*n*-Butyl	Benzyl	81 (72)
6	**3f**	*n*-Butyl	2-Propynyl	78 (67)
7	**3g**	*n*-Butyl	Cyclopentyl	67 (56)
8	**3h**	*n*-Butyl	*n*-Butyl	78 (65)
9	**3i**	Cyclohexyl	Cyclopentyl	75 (66)
10	**3j**	Cyclohexyl	*n*-Butyl	68 (57)
11	**3k**	Cyclopropyl	Cyclopentyl	71 (60)
12	**3l**	Cyclopropyl	*n*-Butyl	72 (61)

Reaction conditions amines (1 mmol), aldehyde (1 mmol), thioacetic acid (1 mmol) and methyl 3-(dimethylamino)-2-isocyanoacrylate (1 mmol), PPL (20 mg), methanol (5 ml), shaken at 160 rpm at 35 °C

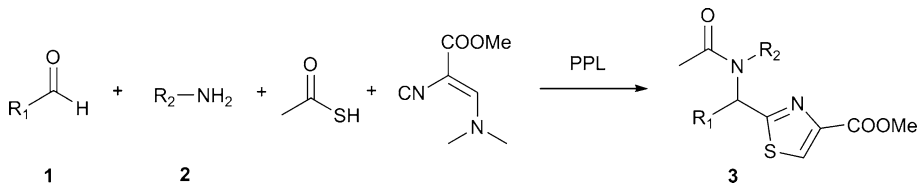
[a] GC yields are based on tridecane as an internal standard

[b] Isolated yields

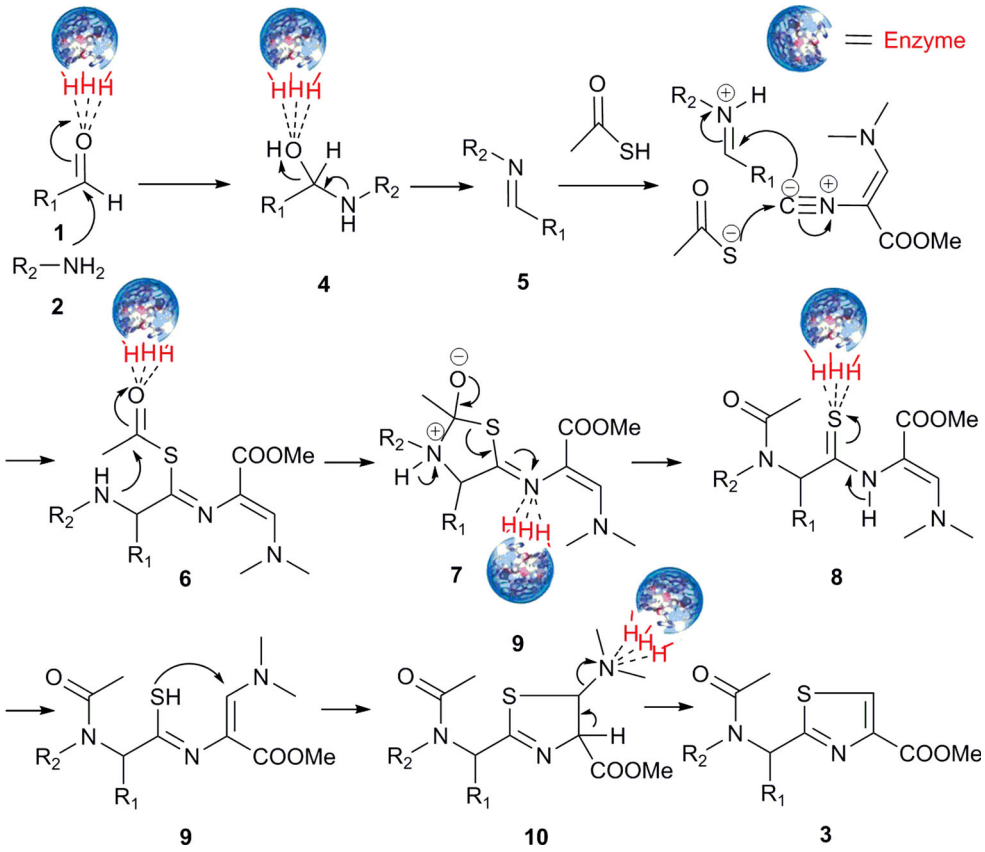
cycloalkanes to check the application scope of this reaction. The yields were relatively higher when the substituent groups R_1 were isopropyl and *n*-propyl (Entries 1–4; Table 4). When R_1 was *n*-butyl in entries 5–8 (Table 4), the yields kept moderate to good (Entries 5–8; Table 4). When the substituent groups R_1 were cycloalkanes, the yields were a little lower (Entries 9–12; Table 4). It seemed that the higher yields could be obtained when the shorter chain aldehydes were employed. Therefore, two conclusions can be drawn from the above experimental results. One, the steric effect in aldehydes have some influence on yield, namely bulkier groups provide lower yields. The other one, the amines and aldehydes with different groups can react smoothly to afford 2,4-disubstituted thiazoles with moderate to high yields, so the enzyme PPL have a wide tolerance range towards amines and aldehydes in this reaction.

A plausible reaction mechanism for this process is proposed as depicted in Scheme 3. It is likely that the presence of the enzyme activates the relevant substrates via the hydrogen bond effects in the whole process [28]. It seems that the carbonyl group in substrates **1** accelerates dehydration via hydrogen bond to produce imine **5**, which generates ion pair and reacts with methyl 3-(dimethylamino)-2-isocyano-acrylate to obtain the intermediate **6**. Then, the carbonyl group in **6** is activated via hydrogen bond of enzyme and attacked by secondary amine group to give intermediate **7**, which transform to **8** via hydrogen bond of enzyme. After that, the C=S bond is activated by enzyme to form **9**. Subsequently, the intermediate **10** was obtained by intramolecular cyclization from **9** and the dimethylamine leaves from **10** to give product **3**. This

Scheme 2 One-pot multicomponent enzymatic synthesis of 2,4-disubstituted thiazoles



Scheme 3 A plausible mechanism for the enzymatic synthesis of 2,4-disubstituted thiazoles



plausible reaction mechanism is similar with literature [18], and the further enzymatic mechanism is still under research.

4 Conclusion

In summary, we herein firstly reported an efficient PPL-catalyzed one-pot multicomponent synthesis of 2,4-disubstituted thiazoles reaction. PPL displayed great catalytic activity in this reaction and showed a wide tolerance range towards aldehydes and amines. This one-pot enzymatic multicomponent conversion method provides a novel strategy and useful tool for the synthesis of 2,4-disubstituted thiazoles and expands the toolbox of enzyme promiscuous in organic synthesis.

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