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Research paper

Unexpected three-component domino synthesis of pyridin-2-ones catalyzed by promiscuous acylase in non-aqueous solvent

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ABSTRACT

The Acylase "Amano" (AA)-catalyzed synthesis of valuable pyridin-2-ones via domino Knoevenagel condensation–Michael addition–intramolecular cyclization–oxidization reaction between aldehyde, cyanoacetamide and ethyl acetoacetate or cyclohexyl acetoacetate was developed in the sense of a one-pot strategy. Various aliphatic, aromatic and hetero-aromatic pyridin-2-ones could also be produced in the reaction. The mechanism was illustrated according to the controlled reaction, pyridin-2-one was formed via the oxidization by oxygen at the final step. This simple and efficient enzymatic domino reaction not only widens its application of AA to organic synthesis, but is also an attractive way for the synthesis of heterocyclic compounds.

1. Introduction

Functionalized pyridin-2-ones have been considered as an important class of organic heterocycles, not only because of its successful application as a building block in the natural synthesis [1], but numerous compounds with this scaffold have also exhibited significant pharmacological activities [2–5]. Much research has focused on the development of efficient and green synthetic methods for such nitrogen-containing heterocycles and continuing works are in progress. So far, a variety of methods have been established, including Vilsmeier–Haack reaction of readily available 1-acetyl and 1-carbamoyl cyclopropanes [6], regioselective synthesis from aminoenones and malononitrile [7], intramolecular Dieckmann-type condensation [8], metal-mediated cycloaddition [9,10]. However, they usually suffered from multistep procedures, harsh conditions or low yields. So developing an efficient and green approach for the preparation of pyridin-2-ones derivatives using commercially available and simple materials is of great importance.

Klibanov pioneered non-aqueous enzymology in the early 1980s [11]. Recently many enzymes have demonstrated the capability of catalyzing one or more alternative reactions besides their natural ones [12–18]. This catalytic promiscuity has led to the evolution of biocatalysts and largely enriches the application in organic and bioorganic synthesis. Due to its efficiency and environmental friendliness, biocatalysis as a new tool has attracted much attention of chemists. A variety of reactions catalyzed by enzyme such as aldol reaction [19], Michael [20,21] and Markovnikov addition [22], Knoevenagel condensation [23,24] have been reported. However, to the best of our knowledge, single-enzyme mediated domino multistep processes in the absence of any other chemical catalyst are extremely scarce [25,26], especially the construction of more complex and bioactive heterocycles.

We have systematically studied enzymatic promiscuity for years [22,27–31]. Following this work, our initial endeavor focused on evaluating the synthetic potential between aldehyde, cyanoacetamide and 1,3-dicarbonyl compound catalyzed by promiscuous enzyme in organic solvent. To our surprise, pyridin-2-ones were obtained when ethyl acetoacetate and cyclohexyl acetoacetate acted as 1,3-dicarbonyl compound. Herein we describe the unexpectedly enzymatic three-component reaction in detail.

2. Material and methods

2.1. General information for reagents and analytical methods

D-Aminoacylase from *Escherichia coli* (10,000 U/mg, 1 U is defined as enzyme quantity, which produces 1 mmol of D-amino acid per 30 min), Acylase 'Amano' (AA) from *Aspergillus oryzae* (≥30,000 U/g, 1 U is defined as enzyme quantity, which produces 1 mmol of L-amino acid per 30 min) and Amano Lipase M from *Mucor javanicus* (≥10,000 U/g enzyme activity, pH 7.0, 40 °C) were purchased from Amano Enzyme Inc. (Japan). Lipase immobilized on

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acrylic resin from *Candida antarctica* (≥10,000 U/g, recombinant, expressed in *A. oryzae*) was purchased from Sigma (Steinheim, Germany). Lipase from *porcin pancreas* (PPL) (30–90 U/mg protein, one unit will hydrolyze 1.0 equiv of triacetin in 1 h at pH 7.7 at 37 °C) was purchased from Sigma (Steinheim, Germany). Lipase Type VII from *Candida rugosa* (CRL) was purchased from Sigma (Steinheim, Germany). All solvents were analytical grade and were dried by storing over activated 3 Å molecular sieves for 24 h prior to use. All reagents were obtained from commercial suppliers and were used without further purification.

The ^{1}H and ^{13}C NMR spectra were recorded with TMS as internal standard using a Bruker AMX-400 MHz spectrometer. Chemical shifts were expressed in parts per million and coupling constants (*J*) in hertz. Analytical HPLC was performed using an Agilent 1100 series with a reversed-phase Shim-Pack VP-ODS column (150 × 4.6 mm) and a UV detector (290 nm). IR spectra were measured with a Nicolet Nexus FTIR 670 spectrophotometer. Melting points were determined using XT-4 apparatus and were not corrected. All the known products were characterized by comparing the ^{1}H NMR data with those reported in the literature. The structures of new compounds were confirmed by ^{1}H, ^{13}C NMR, and HRMS.

2.2. Typical procedure for the enzymatic multicomponent reaction

Aldehyde (1.5 mmol), cyanoacetamide (2.25 mmol), 1,3-dicarbonyl compound (0.75 mmol) and AA (30 mg) were added to a 10 mL conical flask containing 3 mL ethylene glycol (EG), the mixture was shaken at 60 °C for 24 h, and monitored by thin layer chromatography (TLC). After completion of the reaction, the crude product was purified by silica gel column chromatography with an eluent consisting of petroleum ether/ethyl acetate (1/1 v/v).

3. Results and discussion

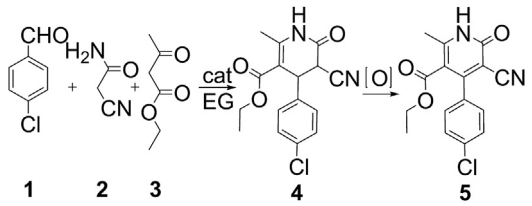
3.1. The catalytic activity of different enzymes for the domino reaction

In our initial research, 4-chlorobenzaldehyde (**1**), cyanoacetamide (**2**) and ethyl acetoacetate (**3**) were mixed in EG at 50 °C, compound **4** which was named as 3,4-dihydropyridin-2-one should be obtained [32]. However, we got compound **5** instead of **4**. It seemed that something acted as an oxidant in this process. In order to get good efficiency, we decided to examine the catalytic activities of several lipases for the model reaction firstly. As shown in Table 1, poor yield was obtained in the absence of lipase (Table 1, entry 10). Meanwhile the yield was not improved when the reaction was incubated with denatured AA or bovine serum albumin (BSA) (Table 1, entries 8 and 9). To our delight, AA exhibited the highest promiscuous catalytic activity in the experiment (Table 1, entry 1). However, other lipases, such as DA, MJL, CAL-B, LAY-30, PPL and CRL showed no or low activity toward the reaction (Table 1, entries 2–7). So AA was identified as the biocatalyst for further optimization of the reaction conditions.

3.2. The effect of solvents on the reaction

Solvents play an important role in the biocatalytic transformation. Therefore, the effect of several solvents on the reaction was investigated. We found that the catalytic activity of AA was considerably influenced by the reaction media (Fig. 1). From the data listed in Fig. 1, commonly used solvents including dioxane, *N*,*N*-dimethylformamide (DMF), toluene and *tert*-pentanol showed no promotion for the reaction with yields less than 5%. Surprisingly, we found that polar protic solvents, EG, Di-EG and 1,4-butylene glycol which were not usually used in multicomponent reactions, were favorable for the reaction, especially for EG which gave the best yield of 31%. While the reactions in Di-EG and 1,4-butylene gave yields of 25% and 18% respectively. The yield in dimethyl sulfoxide (DMSO) was 8%. So EG was chosen as the optimum solvent for the enzymatic reaction.

Table 1
The catalytic activities of different lipases.[a]



Entry	Enzyme	**5** Yield (%)[b]
1	Acylase "Amano"(AA)	31
2	D-Aminoacylase (DA)	24
3	Amano Lipase M from *Mucor javanicus* (MJL)	23
4	*Candida antarctica* lipase B (CAL-B)	3
5	Lipase AY-30 (LAY-30)	16
6	Lipase from *porcine pancreas* (PPL)	8
7	Lipase from *Candida rugosa* (CRL)	12
8	Acylase "Amano"[c]	10
9	Acylase "Amano"[d]	<5
9	Bovine serum albumin (BSA)	9
10	–	9

[a] Reaction conditions: **1** (0.5 mmol), **2** (0.5 mmol), **3** (0.5 mmol), catalyst (30 mg), EG (1 mL), 50 °C, 24 h.
[b] Determined by HPLC.
[c] Denatured by EDTA at 100 °C for 24 h.
[d] Protected under nitrogen atmosphere.

3.3. The effect of molar ratio of substrates, catalyst loading and reaction temperature

Further screenings of the molar ratio of substrates, catalyst loading and reaction temperature were performed under the optimal catalyst and solvent. We firstly probed into the effect of molar ratio (Table 2, entries 1, 2 and 3). The best result was obtained

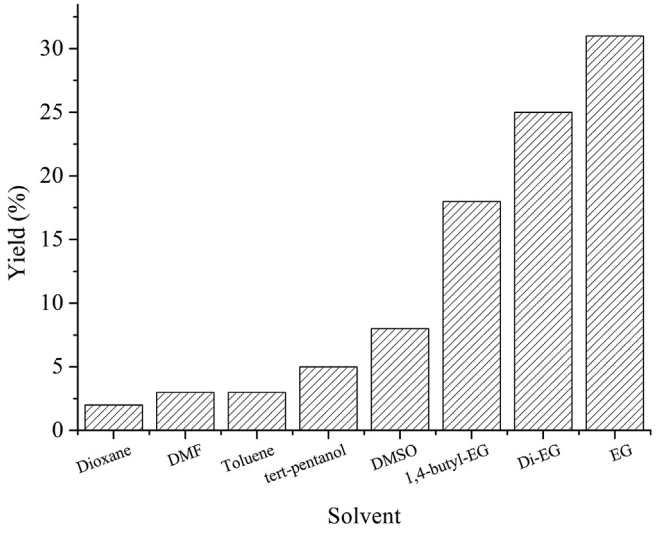


Fig. 1. The effect of solvent on the AA catalyzed domino reaction. Conditions: *p*-chlorobenzaldehyde (0.5 mmol), cyanoacetamide (0.5 mmol), ethyl acetoacetate (0.5 mmol), AA (30 mg), solvent (1 mL) at 50 °C for 24 h.