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Lipase-Mediated Amidation of Anilines with 1,3-Diketones via C–C Bond Cleavage

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Abstract: In this work, an efficient and green lipase-mediated technique has been mined for the amidation of anilines with 1,3-diketones via C–C bond cleavage. Under the optimal conditions, high yields (64.3%–96.2%) could be obtained when Novozym 435 was used as the catalyst. Furthermore, Novozym 435 exhibited a satisfying reusability and more than 80% of yield can be obtained after seven cycles. This work provides a more rapid and mild strategy for amide synthesis with high yield and expands the application of enzyme in organic synthesis.

Keywords: lipase; promiscuity; amidation; 1,3-diketones; C–C bond cleavage

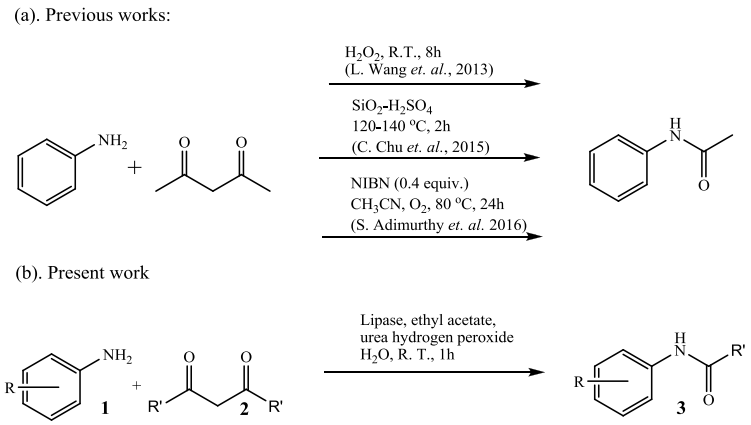
1. Introduction

As a fundamental reaction in organic synthesis, the formation of amides is used in the production of a broad range of bulk commodities, high-value fine chemicals, agrochemicals, and pharmaceuticals, etc. [1–3]. Up to now, various methods have been used for this purpose, including amidation using carboxylic acids or their derivatives as acyl donors, the transition-metal-catalyzed amidation of aryl halides or nitriles with nitrogen-containing reagents, and the amidation between alcohols or aldehydes with amines by oxidative coupling [4–7]. However, most of these methods suffer from unstable substrates, the use of transition metals, expensive reagents, or extreme reaction conditions. To overcome these problems, more efficient and mild methods for the synthesis of amides are urgently needed. Biocatalysis is a green and sustainable technique that can answer this need well. Some methods for the acylation of amines catalyzed by enzyme (lipase, protease, amidase, penicillin G acylase, etc.) have been developed by utilizing carboxylic acids, anhydrides, or esters as acyl donors [8–13]. Nevertheless, one of the main drawbacks of enzymatic synthesis of amides is the low reaction rate, which seriously limits its industrial applications.

1,3-Diketones, the inexpensive and widely available starting chemicals in organic synthesis, have been employed as novel acylation agents for synthesizing esters or amides and have received much attention in the last decade [14–18]. Several research groups have realized the transformations of 1,3-diketones to amides via C–C bond cleavage (Scheme 1a) [19–21]. Due to the importance of the novel oxidative amidation, it is still a fascinating theme for researchers in organic synthesis.

Recently, research has shown that lipase can catalyze the in situ generation of peracids by the perhydrolysis of carboxylic acids or esters [22–24]. This process is a representative case of enzyme catalytic promiscuity, which indicates the capability of an enzyme to catalyze chemical reactions different from its physiological reactions [25–27]. The in situ generated peracids from

the lipase-catalyzed perhydrolysis have been successfully utilized in Baeyer–Villiger reactions, the epoxidation of alkenes, and the oxidation of amines [28–33]. In this study, we designed a new strategy for the lipase-mediated amidation of anilines with 1,3-diketones via C–C bond cleavage (Scheme 1b). This mild method can afford excellent yields of amides in a shorter reaction time (1 h) under room temperature (R.T.) than the results from the reported literature (Scheme 1a). Moreover, compared with traditional amide synthesis catalyzed by lipase using carboxylic acids, anhydrides, or esters as acyl donors reported so far [8–10], lipase-mediated oxidative amidation presents the highest catalytic efficiency—even at room temperature. To the best of our knowledge, no other reports have presented the lipase-mediated oxidative formation of amides.



Scheme 1. Amidation of anilines with 1,3-diketones as acyl donors.

2. Results and Discussion

Initially, aniline (**1a**) and acetylacetone (**2a**) were selected as model substrates for the oxidative formation of acetanilide (**3aa**). Generally, hydrogen peroxide (H_2O_2) is a simple and mild oxidant in enzymatic oxidations. However, the enzyme is unstable for its sensitivity to high concentrations of H_2O_2 [34,35]. Many papers have reported the stabilization of lipases versus hydrogen peroxide inactivation (via genetic tools, immobilization, chemical modification, etc.) [36–38]. According to previous lipase-mediated oxidations, urea hydrogen peroxide (UHP) was selected for its ability to generate the oxidant in a controlled manner and avoid the inactivition of lipase [39,40]. Thus, we adapted UHP as the oxidizing agent for the perhydrolysis of ethyl acetate (EA) to generate peracid in this study. Several lipases of different origin were used to catalyze the reaction, and the results are summarized in Table 1. It could be observed that ANL, CalB, APE1547, and Novozym 435 (a commercial immobilized CalB) can catalyze this reaction (Entries 1–4). Among these used lipases, CalB and Novozym 435, which are supplied by Novozymes, are the widly used lipase in biocatalysis [41]. Considering the relatively inexpensive price, CalB and Novozym 435 are more attractive in this lipase-mediated amidation. However, BSL2 and CSL exhibited no activity for the synthesis of acetanilide (Entries 5 and 6). When the denatured Novozym 435 was used as the catalyst (Entry 7), we found a similar result to that obtained from the control (Entry 9), which indicated that a special spatial conformation of enzyme plays an important role in this reaction. As for the oxidant, the oxidative amidation could not occur when UHP was absent in this reaction (Entry 8). It was noteworthy that the 92.5% yield of the oxidative amidation mediated by Novozym 435 could be obtained in 1 h, which indicated that this green and efficient means has great potential in industrial production.

An appropriate reaction medium is one of the influencing factors on the catalytic performance and stability of enzyme. Thus, six solvents were investigated for this lipase-mediated amidation, and the results are shown in Figure 1. Compared with other solvents, the high yield (>90%) could be observed while acetonitrile or water was used in this reaction. Generally, the substitution of hazardous solvents with more environmentally friendly alternatives is a major purpose for green organic synthesis [42,43].

It is also interesting that almost no hydrolysis of amide was induced in the short reaction time on the basis of high yield of acetanilide when water was used as the solvent. Thus, considering acetonitrile is more toxic, we chose water as the solvent, which fulfilled our main goal of exploiting a green protocol for the synthesis of amides.

Table 1. Lipase-mediated synthesis of acetanilide (**3aa**) from aniline (**1a**) and pentane-2,4-dione (**2a**) [a].

Entry	Enzyme	Yield [b] (%)
1	ANL (Lipase from *Aspergillus niger*)	64.2 ± 1.9
2	CalB (*Candida antarctica* lipase B)	87.6 ± 2.2
3	APE1547 (*Aeropyrum pernix* esterase)	79.3 ± 1.4
4	Novozym 435	92.5 ± 1.5
5	BSL2 (*Bacillus subtilis* lipase)	ND [d]
6	CSL (Lipase from *Candida* sp. 99–125)	ND [d]
7	denatured Novozym 435 [c]	ND [d]
8	No UHP	ND [d]
9	Control	ND [d]

[a] Reaction conditions: **1a** (1 mmol), **2a** (1 mmol), H_2O (2 mL), UHP (1.2 mmol), EA (0.2 mL), enzyme (150 U), room temperature, 1 h; [b] isolated yield; [c] Pretreated Novozym 435 by heating it for 1 h in boiling water; [d] ND: not detected.

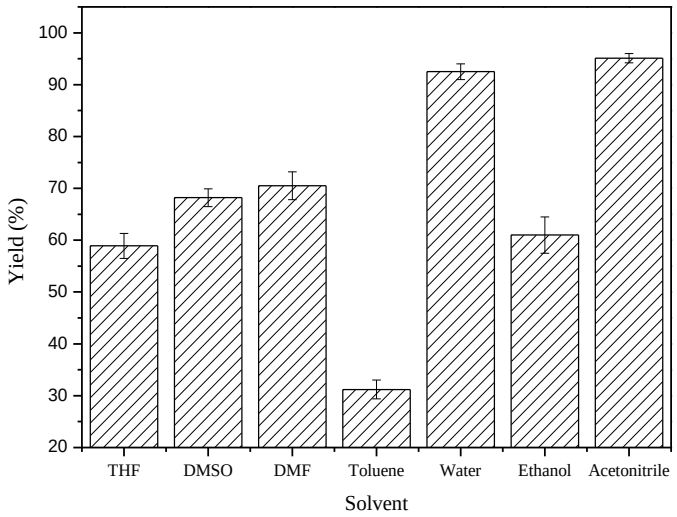


Figure 1. Effect of reaction medium on the oxidative formation of acetanilide (**3aa**). Reaction conditions: **1a** (1 mmol), **2a** (1 mmol), solvent (2 mL), UHP (1.2 mmol), EA (0.2 mL), Novozym 435 (150 U), room temperature, 1 h. THF: tetrahydrofuran; DMSO: dimethyl sulfoxide; DMF: dimethylformamide.

The effects of enzyme dosage and oxidant loading were studied (Table 2). It was found that a high dosage of Novozym 435 could enhance the yield of acetanilide (**3aa**). A more than linear increment from 50 to 100 U was observed, which indicated that the peracid must reach a certain concentration to afford the product. However, the yield could not be improved by further increasing the dosage of Novozym 435 (>150 U). Therefore, 150 U of Novozym 435 turned out to be sufficient in this oxidative amidation. With respect to the oxidant loading, the yield of amide increased as the oxidant loading was elevated from 1 to 1.2 equiv, and the yield changed slightly at higher oxidant loadings. In this work, EA was used as the substrate of enzyme to generate peroxyacetic acid in situ. Therefore, the amount of EA has also been investigated (data not shown here). It was found that a lower amount of EA resulted in a longer reaction time to obtain a high yield. More peracid could be generated by increasing the amount of EA and that may help to increase the yield of amide. EA with a concentration of 10%$_{v/v}$ was sufficient for this reaction.

Table 2. Optimization of enzyme dosage and oxidant loading for the synthesis of acetanilide (**3aa**).

Entry	Enzyme Dosage (U)	Oxidant Loading (Equiv.)	Yield (%)
1	50	1	11.8 ± 1.3
2	100	1	64.5 ± 2.1
3	150	1	81.1 ± 1.8
4	180	1	82.6 ± 2.2
5	200	1	84.7 ± 1.1
6	150	1.2	92.5 ± 1.5
7	150	1.4	93.7 ± 0.7

Reaction conditions: **1a** (1 mmol), **2a** (1 mmol), H_2O (2 mL), EA (0.2 mL), room temperature, 1 h.

It is known that immobilization can improve the stability of enzyme and increase its reusability, which makes the enzymatic reaction economically viable [44–46]. In this work, the reusability of Novozym 435 was studied. It can be seen in Figure 2 that the yield of this reaction slightly decreased as the number of reaction cycles increased and that a yield of more than 80% can be obtained even after seven cycles. The lost of enzyme activity is probably due to the leakage of enzyme from the support or the deactivation of enzyme by the peracid during the amidation [47–50].

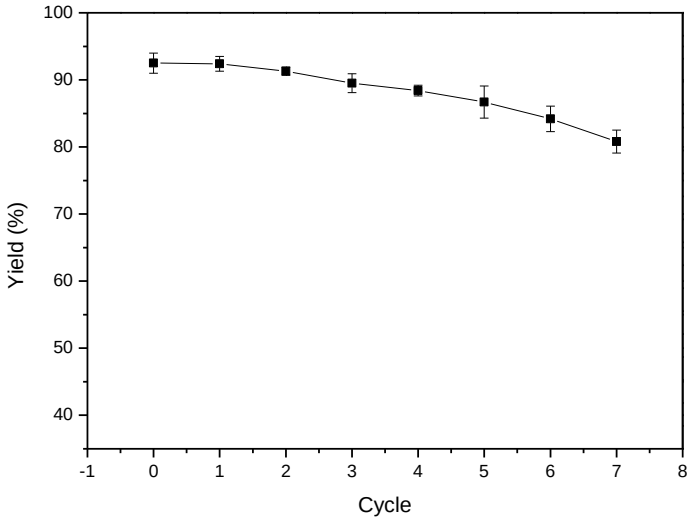
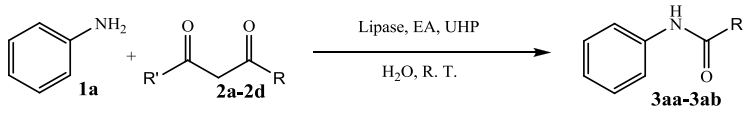
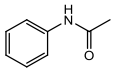
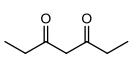
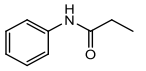
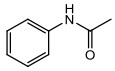
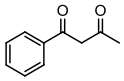
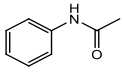


Figure 2. The reusability of Novozym 435 Reaction conditions: **1a** (1 mmol), **2a** (1 mmol), H_2O (2 mL), UHP (1.2 mmol), EA (0.2 mL), Novozym 435 (150 U), room temperature, 1 h.

Various anilines were selected to be acylated by different 1,3-diketones via C–C bond cleavage to evaluate the scope of this protocol. Table 3 shows that all the selected 1,3-diketones (**2a**–**2d**) can react with aniline and afford the desired amides in good yields (76.6%–92.5%). The less sterically hindered 1,3-diketones (**2a** and **2b**) can react more easily with aniline than Compound **2c**. When Compound **2d** was employed, the acetylation product could be obtained with only an 87.4% yield. For the substrate of aryl amines (Table 4), anilines containing electronic donating groups (**1b**–**1g**) can afford higher yields than anilines bearing electron withdrawing groups (**1h**–**1p**). Furthermore, no reaction could be observed with strong electron withdrawing groups (**1r** and **1s**) or hindered group (**1t**) present at the ortho position of anilines.

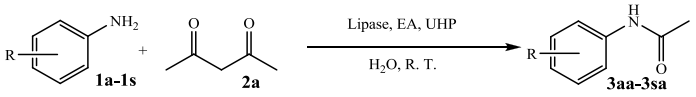
Table 3. Amidation of aniline (**1a**) with various 1,3-diketones as the acyl donor.

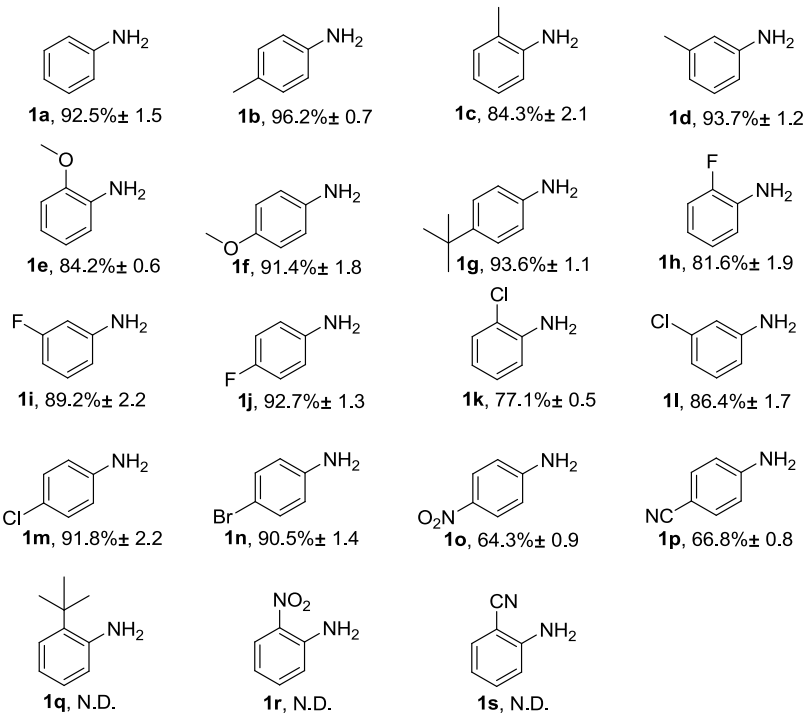


Entry	1,3-Diketone	Product	Yield (%)
1	 2a	 3aa	92.5 ± 1.5
2	 2b	 3ab	89.2 ± 1.4
3	 2c	 3aa	76.6 ± 0.8
4	 2d	 3aa	87.4 ± 1.7

Reaction conditions: **1a** (1 mmol), **2** (1 mmol), H_2O (2 mL), UHP (1.2 mmol), EA (0.2 mL), Novozym 435 (150 U), room temperature, 1 h.

Table 4. Amidation of substituted anilines with pentane-2,4-dione (**2a**) as the acyl donor.

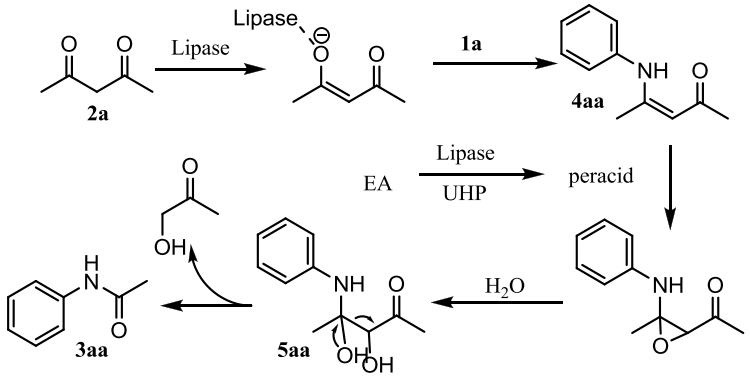




Reaction conditions: **1** (1 mmol), **2a** (1 mmol), H_2O (2 mL), UHP (1.2 mmol), EA (0.2 mL), Novozym 435 (150 U), room temperature, 1 h.

A possible reaction pathway for this lipase-mediated amidation was proposed (Scheme 2). At first, the substrate **2a** was deprotonated by lipase to form an enolate ion. Next, another substrate aniline **1a** connected the enolate ion to obtain an intermediate **4aa** (enaminone). Then, the enaminone could be

epoxidize by peroxyacetic acid which was in situ generated by lipase, followed by its reaction with water, leading to **5aa**. Finally, a rearrangement of the intermediate **5aa** was made to produce acetanilide **3aa**. To confirm the proposed reaction mechanism, control experiments were designed (data not shown here). We carried out the enamination of acetylacetone (**2a**) with aniline (**1a**) at room temperature for 0.5 h and obtained the corresponding enaminone (**4aa**) with a 93% yield. Then, the purified enaminone (**4aa**) could be oxidized by the commercial peroxyacetic acid to produce acetanilide (**3aa**) in 15 min with a high yield (97%). These experimental results verified our hypothesized mechanism to some extent.



Scheme 2. The possible mechanism of lipase-mediated oxidative amidation.

3. Materials and Methods

3.1. Materials

Lipase from *Aspergillus niger* (53,000 U/g), *Candida antarctica* lipase B (CalB, 10,000 U/mL), and Novozym 435 (15,000 U/g) were purchased from Sigma (Beijing, China). Lipase from *Candida* sp. 99–125 (CSL, 7500 U/g) was purchased from Beijing CTA New Century Biotechnology Co., Ltd. (Beijing, China). *Bacillus subtilis* lipase (BSL2, 3600 U/g) was expressed from a homely constructed *Bacillus subtilis* strain BSL2 [51]. *Aeropyrum pernix* esterase (APE1547, 5500 U/g) was expressed from a hyperthermophilic archaeon strain [52]. One unit (U) of enzyme activity was defined as the amount of enzyme that hydrolyzes 1 μmol of 4-nitrophenyl acetate per min at 30 °C. All the enzymes were used after lyophilization for the lipase-mediated amidation directly. The chemical reagents were of analytical reagent grade and purchased from Shanghai Chemical Reagent Co., Ltd. (Shanghai, China). NMR spectra were taken with an Inova 500 (500 MHz) spectrometer.

3.2. General Procedure of the Lipase-Mediated Oxidative Formation of Amides

A mixture of substituted aniline (1 mmol), 1,3-diketone (1 mmol), Novozym 435 (150 U), EA (0.2 mL), and UHP (1.2 mmol) in water (2 mL) was shaken (200 rpm) at room temperature in a round-bottom flask for 1 h. The reaction mixture was extracted twice by ethyl acetate. The organic layers were combined, dried over sodium sulfate, and concentrated to obtain the crude amide, which was further purified by column chromatography (ethyl acetate/hexane) on silica gel to yield the pure product. Each experiment was performed triplicate, and all the obtained data were based on the average values.

4. Conclusions

In this work, we have communicated a green and efficient method for the amidation of anilines with 1,3-diketones via C–C bond cleavage. This lipase-mediated amidation can proceed more rapidly (1 h) in water with high yields (64.3%–96.2%) under room temperature than the traditional amidations catalyzed by Novozym 435 and the reported amidations via C–C bond cleavage. Furthermore, Novozym 435 can be reutilized for seven cycles and retain its high catalytic performance. The strategy

described herein provides a new case for enzyme catalytic promiscuity which can contribute to the progress of novel synthetic methodology and green technology.

Supplementary Materials: The following are available online at www.mdpi.com/2073-4344/7/4/115/s1.

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Author Contributions: Liu Zhang, Fengxi Li, and Lu Zhang performed the experiments; Chunyu Wang, Zhi Wang, and Rui Zhao analyzed the data; Rui Zhao and Lei Wang wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest.

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