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Lipase-Catalyzed Synthesis of Indolyl 4H-Chromenes via a Multicomponent Reaction in Ionic Liquid

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Abstract: Synthesis of indolyl 4H-chromenes via a three-component reaction catalyzed by lipase in ionic liquidsis reported here for the first time. High yields (77–98%) were obtained when *Mucor miehei* lipase was used as the catalyst in [EMIM][BF_4]. Furthermore, [EMIM][BF_4] exhibited good reusability in this enzymatic reaction. This study affords a new example of lipase catalytic promiscuity and broadens the application range of ionic liquid in biocatalysis.

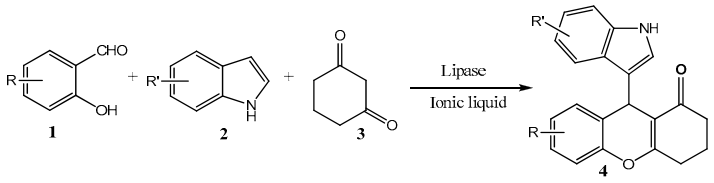
Keywords: lipase; promiscuity; multicomponent; ionic liquid; indolyl-4H-chromene

1. Introduction

Chromene and its derivatives can be found in many natural products [1]. With their vital role in pharmacology, they have been utilized in many fields, such as medicinal, bioorganic, and pharmaceutical chemistry [2–7]. The substructure of indole is a key structural motif in a variety of bioactive compounds [8,9]. Therefore, indoyl 4H-chromene has the combined properties of both indole and chromene moieties that provide new promising biological activities [10,11]. Many methods have been presented for the synthesis of these derivatives and various catalysts have been used, such as ZnO nanoparticle, polystyrene-supported *p*-toluenesulfonic acid, 4-dimethylaminopyridine (DMAP), β-cyclodextrin, oleic acid, 1,4-diazabicyclo[2.2.2]octane (DABCO), microbial cyclosophoraose, etc. [12–17]. However, most of the reported methods have encountered drawbacks, such as the utilization of environmentally hazardous and expensive catalysts, long reaction times, high reaction temperatures, and complicated reaction processes. Thus, a new alternative synthetic method for the indolyl 4H-chromene is still highly desirable.

Enzyme catalytic promiscuity is the "hidden skill" of the enzyme to catalyze different type of organic reactions [18–25]. This useful enzyme property makes it possible to catalyze multistep reactions in a multicomponent reaction (MCR). Recently, a series of enzymatic MCRs have been described to produce complex skeletons, and these findings have significantly broadened the use of enzymes in organic synthesis [26–29]. However, most of these enzymatic promiscuous reactions used organic solvent as the reaction media, which are volatile and toxic to the environment. Moreover, the deactivation of enzymes could be observed in these organic solvents, particularly at high temperatures.

It is known that room temperature ionic liquids (ILs) have been broadly used as attractive media in enzymatic reactions for their distinct features, such as the negligible vapor pressure, high thermal stability, and excellent biocompatibility [30,31]. Another advantage of ILs is that they can be easily recovered as the reaction media in biocatalysis [32,33]. However, only a few studies have used ionic liquid as the reaction media for enzyme catalytic promiscuous reactions. Sharma et al. reported a novel combination of enzyme and ionic liquid [HMIM] Br for the oxidation of aryl alcohols/acetates [34]. Yu and co-workers exhibited the asymmetric cross aldol reactions of aromatic aldehydes with ketones catalyzed by lipase in [BMIM][PF_6] [35]. As part of our investigation on the enzymatic synthesis of heterocyclic compounds, a mild and efficient method for the synthesis of indolyl 4H-chromenes via a MCR catalyzed by lipase in ionic liquid (Scheme 1) is herein reported for the first time.



Scheme 1. Synthesis of indolyl 4H-chromenes catalyzed by lipase in ionic liquid.

2. Results and Discussion

Initially, we carried out the model MCR in [EMIM][BF_4] with salicylaldehyde (**1a**), indole (**2a**), and cyclohexane-1,3-dione (**3**) as the substrates catalyzed by different enzymes. The results are presented in Table 1. It could be observed that the catalytic activity was affected dramatically by the organism from which the enzyme was obtained. *Mucor miehei* lipase (MML) was identified to be the most efficient catalyst for this MCR in [EMIM][BF_4]. The denatured MML and BSA did not exhibit activity for this MCR (Entries 6 and 7), and no reaction was observed in the absence of enzyme (Entry 8), which suggests that a specific conformation of lipase is necessary for the catalytic activity.

Table 1. The effect of enzyme origin on the synthesis of indolyl 4H-chromene [a].

Entry	Enzyme	Isolated Yield (%) [b]
1	MML (*Mucor miehei* lipase)	93
2	PPL (Porcine pancreatic lipase)	81
3	PSL (Lipase from *Pseudomonas* sp.)	62
4	CRL (*C. rugosa* lipase)	48
5	CalB (*C. antarctica* lipase B)	68
6	denatured MML [c]	N.D. [d]
7	Albumin from bovine serum (BSA)	N.D. [d]
8	Control	N.D. [d]

[a] Reaction condition: **1a** (1 mmol), **2a** (1 mmol), **3** (1 mmol), [EMIM][BF_4] (5 mL), enzyme (60 mg, protein content), 60 °C, 3 h. [b] Isolation procedure was described in Section 3.2. [c] The denatured MML was obtained by heating MML to 100 °C for 12 h in water before lyophilization. [d] N.D.: Not detected.

In this study, eight ILs of imidazolium type were selected for this enzymatic synthesis of indolyl 4H-chromene. As shown in Table 2, the yields were dramatically affected by the anion of ILs. MML was active in [BMIM][OTf], [BMIM]N($Tf)_2$, [BMIM][BF_4], and [BMIM][PF_6], but exhibited low activities in [BMIM][Ac] and [BMIM][NO_3]. The low nucleophilicity of anion (PF_6, BF_4, N($Tf)_2$, or OTf) may have helped the ILs maintain the enzyme performance [36,37]. Higher yields were obtained in hydrophilic ionic liquid ([BMIM][BF_4] and [BMIM][OTf]), which could be attributed to the preferable solubility of substrates in ILs. The effect of the cation of ionic liquid has also been studied (Entries 6–8). An apparent decrease of yield could be found in the results when the alkyl chain of the cation was elongated. One plausible explanation is that the high viscosity of ILs is harmful to the mass transfer [38–40].

Compared to the solvents (ethanol and water), MML exhibited a higher enzyme performance in [EMIM][BF_4]. Therefore, [EMIM][BF_4] was chosen as the suitable IL for further study.

Table 2. The effect of ionic liquid on the synthesis of indolyl 4H-chromene.

Entry	Ionic Liquid	Isolated Yield (%)
1	[BMIM][Otf]	79
2	[BMIM]$N(Tf)_2$	57
3	[BMIM][PF_6]	68
4	[BMIM][Ac]	11
5	[BMIM][NO_3]	15
6	[BMIM][BF_4]	85
7	[EMIM][BF_4]	93
8	[HMIM][BF_4]	72
9	Ethanol	80
10	Water	67

Reaction condition: **1a** (1 mmol), **2a** (1 mmol), **3** (1 mmol), solvent (5 mL), *Mucor miehei* lipase (MML) (60 mg, protein content), 60 °C, 3 h.

Lipase catalyzed synthesis of indolyl 4H-chromene was carried out at a temperature range of 30 to 80 °C in [EMIM][BF_4]. The effect of temperature on the reaction is demonstrated in Figure 1. The yield increased with the enhancement of temperature from 30 °C to 60 °C, and the maximum yield was achieved at 60 °C. Further increase in the reaction temperature resulted in an appreciable loss of the reaction yield. Generally, high temperature is known to increase the colliding probability between enzyme and substrate, which is conducive to form the enzyme-substrate complexes and improve the reaction rate. However, excess temperature could destroy the conformation of enzyme and decrease enzyme catalytic performance.

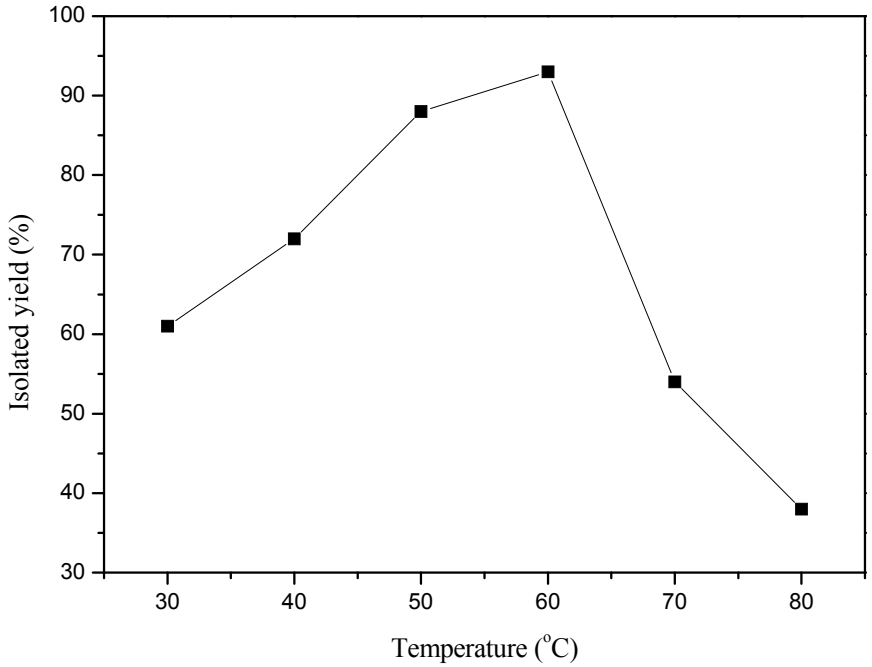


Figure 1. The effect of temperature on the synthesis of indolyl 4H-chromene. Reaction condition: **1a** (1 mmol), **2a** (1 mmol), **3** (1 mmol), [EMIM][BF_4] (5 mL), MML (60 mg, protein content), 3 h.

The effect of enzyme dosage was also studied (Figure 2). It could be found in Figure 2 that the yield increased along with the increasing amount of MML from 20 to 60 mg. But the yield could not

be enhanced by further increasing the dosage of MML. Therefore, 60 mg of MML turned out to be sufficient to catalyze the reaction.

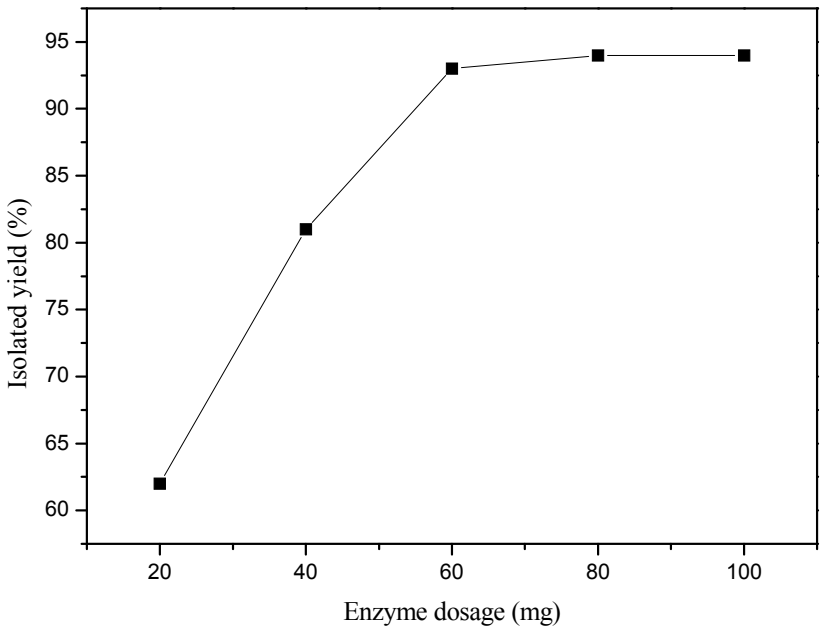


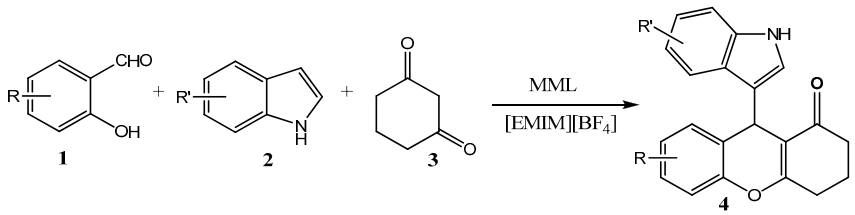
Figure 2. The effect of enzyme dosage on the synthesis of indolyl 4H-chromene. Reaction condition: **1a** (1 mmol), **2a** (1 mmol), **3** (1 mmol), [EMIM][BF_4] (5 mL), MML, 60 °C, 3 h.

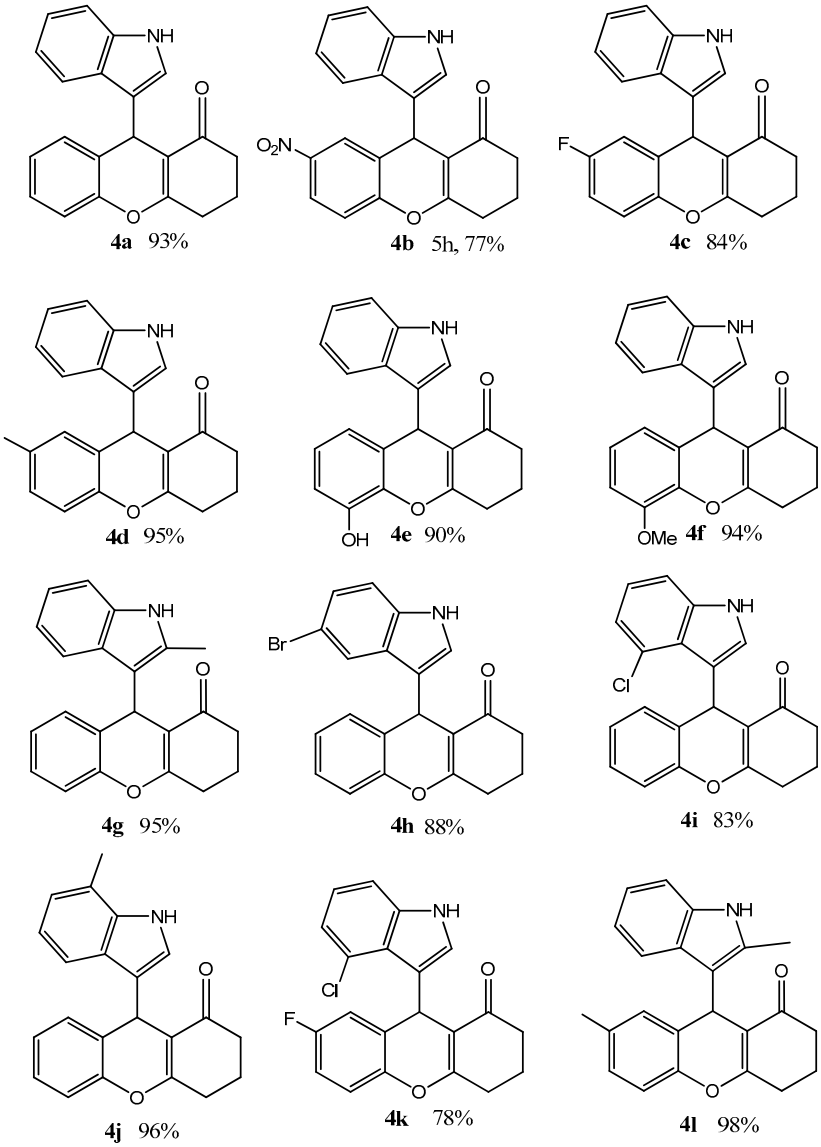
To test the robustness of the reaction, various substituted salicylaldehydes or indoles have been used for the synthesis of indolyl 4H-chromenes. As shown in Table 3, all reactions provided indolyl 4H-chromenes in good to excellent isolated yields (77–98%). It is noteworthy that the salicylaldehydes or indoles with electron donating groups (OH, methyl or methoxyl) provided higher yields than those substrates with electron withdrawing groups (F, Cl, Br, or NO_2).

Another significant property of ILs is their reusability, which is responsible for their environmental friendliness character and the industrial applications. In this study, the IL was recovered by filtration from the reaction system and washed with cold ethyl acetate to remove the residual substrates. It was then dried under vacuum and reused for the next cycle. The results shown in Figure 3 indicated that the recovered IL was recycled for ten runs without any negative effect on the yield of indolyl 4H-chromene.

Generally, the immobilization is a powerful tool to enhance the reusability and stability of enzymes in modern biotechnology [41–43]. In this study, MML was immobilized on SBA-15 mesoporous silica via physical adsorption according to the previous study in our Lab [44,45], and the immobilized MML was used in the synthesis of indolyl 4H-chromenes. Our results indicated that the immobilized MML showed a lower catalytic performance than free MML (Table 4), but exhibited a satisfactory reusability (Figure 4). When the immobilized enzyme was reused, a slight loss of the catalytic activity could be observed, which may be due to the leakage of protein from SBA-15. Considering the operational simplicity of the immobilized enzyme on magnetic nanoparticles [46–48], research on using immobilized MML attached onto the magnetic Fe_3O_4 nanoparticles by covalent attachment for recycling is currently undergoing and will be reported in the future.

Table 3. Synthesis of functionalized indolyl 4H-chromene derivatives.





Reaction condition: **1** (1 mmol), **2** (1 mmol), **3** (1 mmol), [EMIM][BF_4] (5 mL), MML (60 mg, protein content), 60 °C, 3 h.

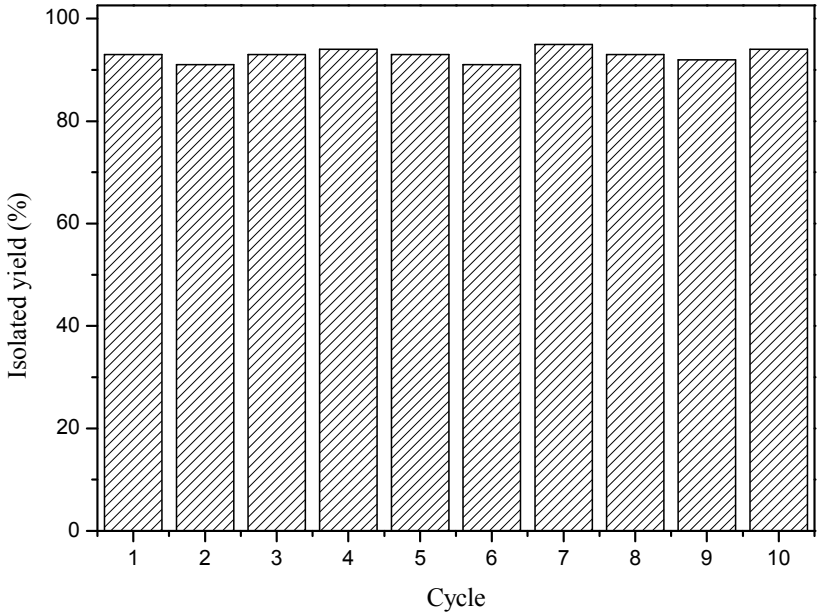


Figure 3. The reusability of [EMIM][BF_4]. Reaction condition: **1a** (1 mmol), **2a** (1 mmol), **3** (1 mmol), [EMIM][BF_4] (5 mL), MML (60 mg, protein content), 60 °C, 3 h.

Table 4. Comparison of free MML and the immobilized MML.

Catalyst	Bound Protein (mg/g)	Isolated Yield (%)
Free MML	-	93
Immobilized MML	180	86

Reaction condition: **1a** (1 mmol), **2a** (1 mmol), **3** (1 mmol), [EMIM][BF_4] (5 mL), enzyme (60 mg, protein content), 60 °C, 3 h.

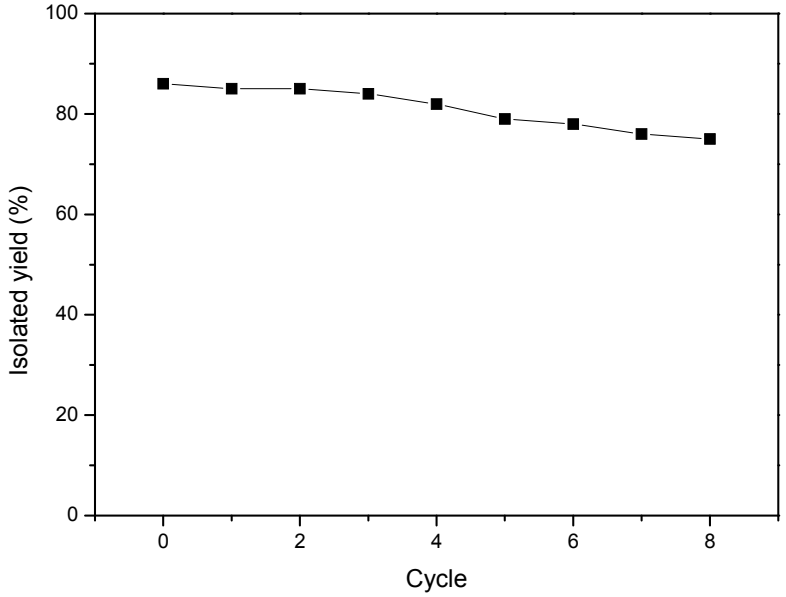


Figure 4. The reusability of immobilized MML in the synthesis of indolyl 4H-chromenes. Reaction condition: **1a** (1 mmol), **2a** (1 mmol), **3** (1 mmol), [EMIM][BF_4] (5 mL), enzyme (60 mg, protein content), 60 °C, 3 h.

3. Materials and Methods

3.1. Materials

C. rugosa lipase (CRL), Porcine pancreatic lipase (PPL), and *Candida antarctica* lipase B (CalB) were purchased from Sigma (Beijing, China). *Mucor miehei* lipase (MML) was purchased from Shanghai Dongfeng Biochemical Reagent Co., Ltd. (Shanghai, China). Lipase from *Pseudomonas* sp. (PSL) was purchased from Amano Pharmaceutical Co., Ltd. (Tokyo, Japan). All enzymes were lyophilized before being used in the reaction. Substituted salicylaldehydes, substituted indoles, and cyclohexane-1,3-dione were purchased from J&K Scientific (Beijing, China). Ionic liquids were purchased from Shanghai Chengjie Chemical Co. Ltd. (Shanghai, China). The ionic liquids used and their abbreviations are listed in the Supplementary Materials. SBA-15 was donated by Dr. Yazhuo Li from College of Chemistry, Jilin University. All the other chemical reagents were purchased from Shanghai Chemical Reagent Company (Shanghai, China). All the commercially available reagents and solvents were used without further purification. NMR spectra were recorded on an Inova 500 (500 MHz) spectrometer (Vernon Hills, IL, USA).

3.2. General Procedure of the Synthesis of Indolyl 4H-Chromenes Catalyzed by Lipase

A mixture of substituted salicylaldehyde (1 mmol), substituted indole (1 mmol), and cyclohexane-1, 3-dione (1 mmol), MML (60 mg, protein content) in [EMIM][BF_4] (5 mL) was stirred at 60 °C in a round-bottom flask for 3 h. The enzymatic reaction was monitored by TLC (0.5-mm silica gel plates, eluent: ethyl acetate/*n*-hexane: 1/4). Then, the mixture was filtered, and the filter cake was washed with cold ethanol and water. The resulting residue was dried under vacuum to provide the pure product. All the products were well characterized by their ^{1}H-NMR spectral analysis. Each experiment was performed triplicate, and all the data were obtained based on the average values.

3.3. Reusability of [EMIM][BF_4]s

To test the reusability of [EMIM][BF_4] in repeated use, batch reaction was conducted under the optimal reaction conditions for 3 h. After the reaction had been completed, enzyme and the product were filtered, and the IL was washed with cold ethyl acetate in order to extract the residual substrates. Then, the recycled [EMIM][BF_4] was dried under vacuum and reused for the next cycle under the same conditions.

3.4. Immobilization of MML

The commercial MML (1 g) was dissolved in phosphate buffer (0.1 L, pH 7.5, 0.1 M) at 4 °C for 120 min, and the insoluble residue was removed by centrifugation (8000 rpm, 5 min). Then, the entire supernatant was lyophilized. MML solution (10 mg/mL) was obtained by dispersing the lyophilized MML in phosphate buffer (pH 7.5, 0.1 M). MML solution (10 mL, 10 mg/mL) was put into the tube containing SBA-15 (0.5 g) at 4 °C for 120 min under stirring. After that, the immobilized MML was obtained from the supernatant by centrifugation and washed with the deionized water more than three times. The immobilized MML was dried for 24 h and the enzyme loading of the immobilized MML was determined according to Lowry method for protein concentration [49].

3.5. Systhesis of Indolyl 4H-Chromenes Catalyzed by the Immobilized MML

A mixture of **1a** (1 mmol), **2a** (1 mmol), and **3** (1 mmol), immobilized MML (60 mg, protein content) in [EMIM][BF_4] (5 mL) was stirred at 60 °C in a round-bottom flask for 3 h. The reaction was monitored by TLC (0.5-mm silica gel plates, eluent: ethyl acetate/*n*-hexane: 1/4). Then, the mixture was isolated by filtration, and the obtained precipitation was washed with cold ethanol and water. The resulting residue was dissolved in $CHCl_3$, and the insoluble immobilized MML was then recovered by centrifugation. The organic phase was dried under vacuum to provide the pure product.

4. Conclusions

In conclusion, an efficient synthesis of indolyl 4H-chromenes via a three-component reaction catalyzed by lipase in ionic liquid has been developed for the first time. This novel strategy offers several advantages including a simple workup procedure, environmental friendliness, shorter reaction time with excellent yields, as well as the ability to convert a variety of substrates. Excitingly, ionic liquid exhibited a good reusability in the lipase catalyzed reaction. This green method provides not only a new case of lipase promiscuous reaction in organic synthesis, but also expands the utility of ionic liquid in enzyme promiscuous reaction.

Supplementary Materials: The following are available online at www.mdpi.com/2073-4344/7/6/185/s1, Figure S1: Data of products, Figure S2: Spectra of products, Table S1: Ionic liquids and the abbreviations.

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Author Contributions: Weian Zhang, Ziyuan Zhao, Zhi Wang, and Chao Guo performed the experiments; Chunyu Wang and Weian Zhang 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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