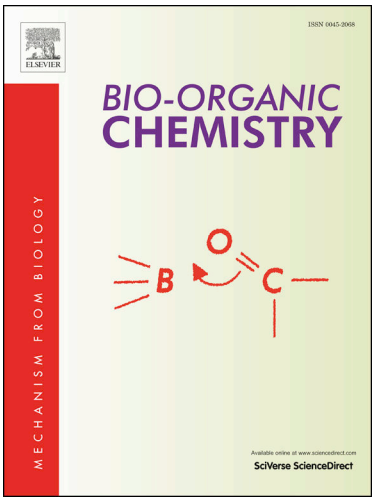
Journal Pre-proofs



One-Pot Cascade Synthesis of Benzopyrans and Dihydropyrano[c]chromenes Catalyzed by Lipase TLIM

Yajie Fu, Zeping Lu, Xiaolong Ma, Ke Fang, Xinyi He, Huajin Xu, Yi Hu

PII: S0045-2068(19)32105-4
DOI: https://doi.org/10.1016/j.bioorg.2020.103888
Reference: YBIOO 103888

To appear in: *Bioorganic Chemistry*

Received Date: 9 December 2019
Revised Date: 23 April 2020
Accepted Date: 24 April 2020

Please cite this article as: Y. Fu, Z. Lu, X. Ma, K. Fang, X. He, H. Xu, Y. Hu, One-Pot Cascade Synthesis of Benzopyrans and Dihydropyrano[c]chromenes Catalyzed by Lipase TLIM, *Bioorganic Chemistry* (2020), doi: https://doi.org/10.1016/j.bioorg.2020.103888

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2020 Published by Elsevier Inc.

Journal Pre-proofs

One-Pot Cascade Synthesis of Benzopyrans and Dihydropyrano[c]chromenes Catalyzed by Lipase TLIM

Yajie Fu, Zeping Lu, Xiaolong Ma, Ke Fang, Xinyi He, Huajin Xu, Yi Hu*

(State Key Laboratory of Materials-Oriented Chemical Engineering, School of Pharmaceutical Sciences, Nanjing Tech University, Nanjing 210009, China)

*Corresponding author, email: huyi@njtech.edu.cn

Abstract

Lipase TLIM was reported to be an efficient, commercially available and reusable catalyst for the Knoevenagel-Michael cascade reactions of aldehydes, malononitrile / ethyl cyanoacetate and 4-hydroxycoumarin/ 1, 3-cyclohexanedione/ dimedone in aqueous DMSO. This methodology presents many superiorities such as simple procedure, mild reaction conditions, commercially available and reusable catalyst, high substrate applicability, the ability to be scaled up, and good to excellent yields.

Keywords cascade reactions, enzymatic synthesis, benzopyrans, chromene, lipase

1 Introduction

Benzopyrans and dihydropyrano[c]chromenes have received more and more concerns due to their prominent pharmacological and biological activities. They are able to inhibit the activities of acetylcholinesterase and butyrylcholinesterase[1, 2], which means that they play a role in the treatment of neurodegenerative disorders such as Parkinson disease, Alzheimer's, Huntington's disease , etc. They can act as xanthine oxidase inhibitors[3], which means that they make contributions in reducing risks of many diseases like gout, hyperuricemia, chronic kidney disease, hypertension, atherosclerosis, etc. They also show notable cytotoxic and anti-tyrosinase activities[4], which means that they work in the remedy of Albinism. Benzopyrans and dihydropyrano[c]chromenes are usually synthesized by the three-component tandem reaction of aldehydes, active methylene compounds and diverse enolizable C-H activated acidic compounds employing various catalysts, such as IL@CNTs under ultrasonic radiation[5], [DMAP-PEG_{1000}-DIL][BF_4][6], Fe_3O_4@SiO_2-guanidine-PAA[7], Fe_3O_4@SiO_2-imid-PMA^{n}[8], {[HMIM]$C(CN)_3$}NMS[9], $H_5BW_{12}O_{40}$[10], CTMAB-bentonite[11], Ru@imine-Z[12]. Although the reported protocols have their own advantages, they also have limits such as the use of expensive or troublesomely-prepared catalysts, relative high temperature, and sometimes only moderate yields could be obtained. Also, most of the reported procedures have limited substrate spectrum. Thence, it is necessary to develop a green, convenient and efficient procedure to synthesize a wide range of benzopyrans and dihydropyrano[c]chromenes.

Biocatalytic promiscuity is attracting more and more attention due to its wide application in C-C bond forming reactions like Knoevenagel condensation, Aldol condensation, Henry reaction, Mannich reaction, Michael addition, Baylis-Hillman reaction and so on[13-16]. In our previous studies, lipase LPL was reported to catalyze the Knoevenagel condensation of aromatic aldehydes with active methylene

compounds in anhydrous DMSO and 76-81% yields of products were obtained [17], lipase TLIM demonstrated noticeable promiscuity for the Knoevenagel-Michael cascade reactions of 1, 3-dicarbonyl compounds with aldehydes to synthesize xanthone derivatives in anhydrous n-hexane and 80-97% yields of products were obtained[18], lipase RMIM was applied in the synthesis of dicoumarol derivatives in pure water and 81-98% yields of products were achieved [19]. Here we report a simple, efficient enzymatic method for the multicomponent Knoevenagel-Michael cascade reactions of aldehydes, malononitrile / ethyl cyanoacetate and 4-hydroxycoumarin/ 1, 3-cyclohexanedione/ dimedone catalyzed by lipase TLIM in aqueous DMSO to synthesize benzopyrans and dihydropyrano[c]chromenes.

2. Materials and Methods

Porcine pancreas lipase (PPL), Amano lipase PS from *burkholderia cepacia* (BCL) and *candida rugosa* lipase (CRL) were purchased from Sigma. Lipase from *thermomyces lanuginosus* immobilized on particle silica gel (TLIM), lipase from *rhizomucor miehei* immobilized on anion exchange resin (RMIM), lipase B from *candida antarctica* immobilized on macroporous acrylic resin (Novozym 435) and papain were purchased from Novo Nordisk. Bovine serum albumin (BSA) was purchased from Shanghai Huixing. Lipase XHlip-F was donated by XHSynbio. Lipase lipoprotein from *aspergillus niger* (LPL) was donated by Ningxia Sunson group corporation. Lipase DF was donated by Amano Enzyme China Ltd. Other reagents were commercially available and were used without further purification.

The melting points were determined on a WRS-1B digital melting point instrument and were not corrected. The ^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker Advance 2B 300 MHz instrument with DMSO-d_6 as solvent and TMS as internal standard. The HRMS were measured on Agilent LC/MS mass spectrometer. The progress of the reaction was monitored by TLC using pre-coated Haiyang GF254 silica gel plates. HPLC data was obtained using Dionex Liquid Chromatography (diamonsil C18 (2) (4.6×250mm, 5μm), water with 0.1% formic acid /methanol (v/v=7/13,1mL/min, 20min, radiant elution), 40℃, 254nm).

2.1 General procedure for the synthesis of dihydropyrano[c]chromenes

A mixture of 4-hydroxycoumarin (1mmol), aldehydes (1mmol), malononitrile (1mmol)/ ethyl cyanoacetate (1mmol), and TLIM (45mg) in 5mL solvent (DMSO/water, v/v=9/1) were stirred at 45℃. The progress of the reaction was monitored by TLC (ethyl acetate / n-hexane = 2/1). Upon completion of the reaction, lipase TLIM was recovered by centrifugation and could be reused in the next run directly. The reaction solution was steamed to get crude product, which was then purified by recrystallization in ethanol.

2.2 General procedure for the synthesis of benzopyrans

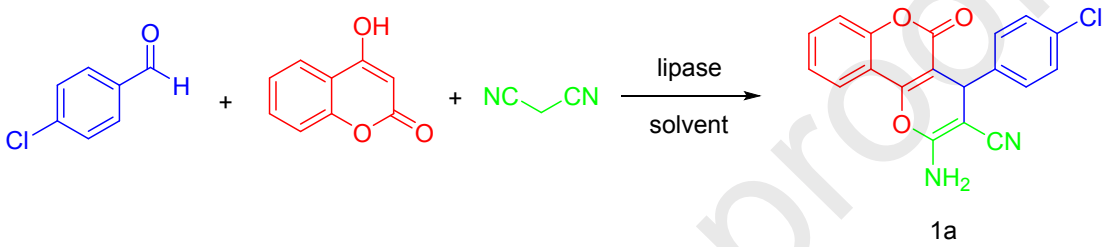
A mixture of 1, 3-cyclohexanedione (1mmol) / dimedone (1mmol), aldehydes (1mmol), malononitrile (1mmol) / ethyl cyanoacetate (1mmol), and TLIM (45mg) in 5mL solvent (DMSO/water, v/v=9/1) were stirred at 45℃. The progress of the reaction was monitored by TLC (ethyl acetate / n-hexane = 2/1).

Journal Pre-proofs

Upon completion of the reaction, lipase TLIM was recovered by centrifugation and could be reused in the next run directly. The reaction solution was steamed to get crude product, which was then purified by recrystallization in ethanol.

3. Results and discussion

3.1 Optimization of enzyme sources for the synthesis of **1a**



1a

Scheme 1 Enzymatic reaction of 4-chlorobenzaldehyde, 4-hydroxycoumarin and malononitrile

Table1. Effect of enzyme sources on the yield of **1a**

Entry	Enzyme[a]	Yield (%)[b]	Entry	Enzyme[a]	Yield (%)[b]
1	PPL[c]	11	9	PPL	28
2	RMIM[d]	35	10	DF	37
3	TLIM[e]	31	11	LPL	40
4	No enzyme	ND	12	CRL	41
5	Novozym435	17	13	TLIM	46（44[g]）
6	Hlip-F	19	14	Papain	35
7	BCL	19	15	BSA	42
8	RMIM	25	16	TLIM[f]	29

[a] Reaction conditions: lipase (50 mg), 4-chlorobenzaldehyde (1mmol), 4-hydroxycoumarin (1mmol), malononitrile (1mmol), n-hexane (5 mL), 45 °C, 24 h.

[b] HPLC yield.

[c] Reaction conditions: PPL (30 mg), 4-chlorobenzaldehyde (1mmol), 4-hydroxycoumarin (1mmol), malononitrile (1mmol), solvent (5mL, ethanol /water, v/v=4/1), 35 °C, 1 h.

[d] Reaction conditions: RMIM (50 mg), 4-chlorobenzaldehyde (1mmol), 4-hydroxycoumarin (1mmol), malononitrile (1mmol), water (5 mL), 45 °C, 20 h.

[e] Reaction conditions: TLIM (50 mg), 4-chlorobenzaldehyde (1mmol), 4-hydroxycoumarin (1mmol), malononitrile (1mmol), n-hexane (5 mL), 35 °C, 12 h.

[f] Denatured TLIM was obtained by treating with acetone for 24 h.

[g] Isolated yield.

Journal Pre-proofs

In the initial research, we chose the reaction between 4-chlorobenzaldehyde, 4-hydroxycoumarin and malononitrile as the model reaction (Scheme 1). Based on the reported reaction conditions[18-20], no satisfactory result was obtained (Table 1, Entries1-3). Obviously, the previous methods are not suitable for this three-component reactions. It is necessary to seek out better reaction conditions for the model reaction. As can be seen in Table 1, no product could be detected in the blank control reaction (Table 1, Entry 4). Lipases such as Novozym435, Hlip-F, BCL, RMIM, PPL, DF, LPL, CRL and TLIM could promote the reaction (Table 1, Entries5-13), and TLIM showed the best catalytic activity among these lipases to generate the target product with 46% yield (Table 1, Entry13). Other enzymes such as papain and BSA could also promote the model reaction with yields of 35% and 42% separately (Table 1, Entries14-15). Compared to the untreated TLIM, the denatured TLIM showed lower catalytic activity and generated the target product with 29% yield. It can be supposed that enzyme active center is excluded from reaction and only the enzyme functional groups catalyze the reaction.

3.2 Optimization of solvent and water content for the synthesis of **1a**

Table2. Effect of solvent source and water content on the yield of **1a**

Entry	Solvent[a]	Yield (%)[b]	Log P
1	n-Hexane	46	3
2	Toluene	50	2.5
3	THF	4	0.4
4	Isopropanol	28	0.4
5	Acetonitrile	5	0.2
6	DMF	38	-0.6
7	H_2O	26	-
8	DMSO	97	-1.5
9	DMSO[c]	99	-1.5
10	DMSO[d]	48	-1.5
11	DMSO[e]	39	-1.5

[a] Reaction conditions: TLIM (50 mg), 4-chlorobenzaldehyde (1mmol), 4-hydroxycoumarin (1mmol), malononitrile (1mmol), solvent (5 mL), 45 °C, 24 h.

[b] HPLC yield.

[c] Reaction conditions: TLIM (50 mg), 4-chlorobenzaldehyde (1mmol), 4-hydroxycoumarin (1mmol), malononitrile (1mmol), DMSO (4.5mL), water (0.5mL), 45 °C, 24 h.

[d] Reaction conditions: TLIM (50 mg), 4-chlorobenzaldehyde (1mmol), 4-hydroxycoumarin (1mmol), malononitrile (1mmol), DMSO (3.75mL), water (1.25mL), 45 °C, 24 h.

[e] Reaction conditions: TLIM (50 mg), 4-chlorobenzaldehyde (1mmol), 4-hydroxycoumarin (1mmol), malononitrile (1mmol), DMSO (2.5mL), water (2.5mL), 45 °C, 24 h.

Subsequently, we explored the effects of various solvents on the reactions to synthetize **1a** using TLIM as catalyst (Table 2, Entries1-8). As can be seen in Table 2, trace target product could be obtained in THF (Table 2, Entry 3) and acetonitrile (Table 2, Entry 5). Low to moderate yield of target product could be obtained in solvents like n-hexane (Table 2, Entry1), toluene (Table 2, Entry2), isopropanol (Table 2, Entry4), DMF (Table 2, Entry6) and water (Table 2, Entry7). DMSO exhibited extraordinarily excellent

Journal Pre-proofs

activity to promote the model reaction to generate the target product in 97% yield (Table 2, Entry8), which may result from its good solubility for substrates and its quality to keep the catalytic activity of enzymes. It was reported that the LogP value of the reaction medium influenced the catalytic activity of enzymes[21]. While no obvious correlation was observed in the TLIM catalyzed model reaction, which was consistent with literatures[17, 18, 22]. In our previous researches, lipase LPL could catalyze the Knoevenagel condensation reaction in anhydrous DMSO to generate target products efficiently[17] and lipase TLIM could catalyze the Knoevenagel-Michael cascade reaction in anhydrous n -hexane to give target products in excellent yields[18]. As for this research, a gentle rise in the yield from 97% to 99% was observed as the water content increased from 0 to 10% (Table 2, Entries8-9), and a sharp decrease in the yield from 99% to 39% was detected as the water content increased from 10% to 50% (Table 2, Entries9-11). This may be related to the fact that the right amount of water is useful in keeping the structural stability and catalytic activity of lipase TLIM. Consequently, 10% of water content was selected as an optimum condition for the synthesis of **1a**.

3.3 Optimization of enzyme quantity, temperature and reaction time for the synthesis of **1a**

Table3. Effects of enzyme quantity, temperature and reaction time on the yield of **1a**

Entry[a]	Temperature(℃)	Enzyme quantity (mg)	Time (h)	Yield (%)[b]
1	45	35	24	63
2	45	45	24	99
3	45	50	24	99
4	45	55	24	89
5	35	45	24	43
6	55	45	24	60
7	45	45	8	72
8	45	45	16	99

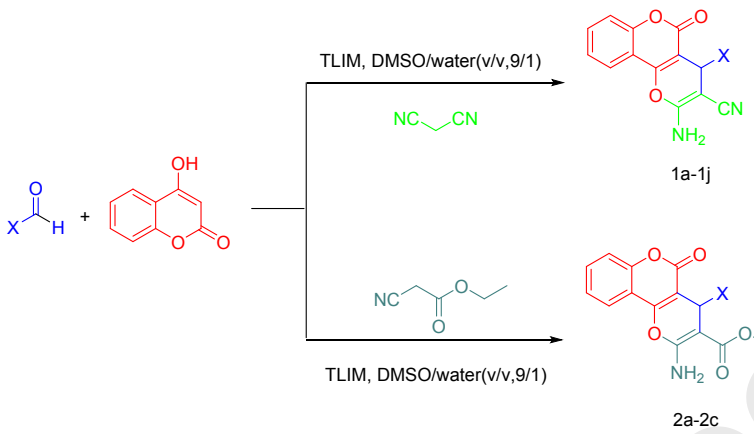
[a] Reaction conditions: TLIM, 4-chlorobenzaldehyde (1mmol), 4-hydroxycoumarin (1mmol), malononitrile (1mmol), DMSO (4.5mL), water (0.5mL).

[b] HPLC yield.

The effect of enzyme quantity on the yield of **1a** was investigated (Table 3, Entries1-4). The yield of **1a** kept increasing as the enzyme quantity increased from 35mg to 45 mg and tended to decline as the enzyme quantity is 55mg. It was reported that temperature could affect the catalytic activity and substrate specificity of enzymes[23]. We explored the influence of temperature on the yield of **1a** (Table 3, Entry2 and Entries5-6), and the suitable temperature for this enzymatic reaction was 45℃. The yield of **1a** kept increasing as the reaction time increased from 8h to 16h (Table 3, Entries7-8) and tended to be stable after 16h (Table 3, Entry2).

Journal Pre-proofs

3.4 TLIM-catalyzed cascade synthesis of dihydropyrano[c]chromenes



Scheme 2 TLIM-catalyzed synthesis of dihydropyrano[c]chromenes

Table 4. TLIM-catalyzed synthesis of dihydropyrano[c]chromenes

Entry[a]	X	Product	Time/h	Yield/%[b]
1	4-ClC_6H_5	1a	16	99
2	4-$NO_2C_6H_5$	1b	16	98
3	4-CNC_6H_5	1c	16	98
4	4-$CH_3C_6H_5$	1d	24	93
5	4-OH-3-$OCH_3C_6H_5$	1e	24	91
6	4-$OCH_3C_6H_5$	1f	24	91
7	4-OHC_6H_5	1g	24	80
8	4-t-C_4H_9	1h	48	75
9	2-thienyl	1i	16	91
10	butyl	1j	48	80
11	4-$CF_3C_6H_5$	2a	16	93
12	4-$CH_3C_6H_5$	2b	24	90
13	2-thienyl	2c	24	85
14	4-ClC_6H_5	1a	16	95[c]

[a] Reaction conditions: TLIM (45 mg), aldehyde (1mmol), 4-hydroxycoumarin (1mmol), malononitrile/ ethyl cyanoacetate (1mmol), DMSO (4.5mL), water (0.5mL), 45 °C.

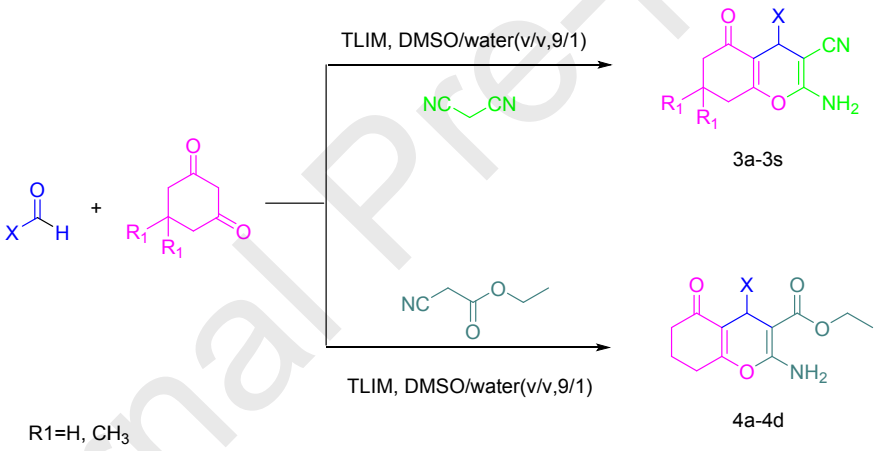
[b] Isolated yield.

[c] Reaction conditions: TLIM (450 mg), 4-chlorobenzaldehyde (10mmol), 4-hydroxycoumarin (10mmol), malononitrile (10mmol), DMSO (45mL), water (5mL), 45 °C.

Journal Pre-proofs

With the optimal reaction conditions in hand, substrate applicability of the multicomponent cascade reactions catalyzed by lipase TLIM was further explored (Scheme 2). As can be seen in Table 4, aromatic aldehydes with electron-withdrawing substituents (Table 4, Entries1-3) and aromatic aldehydes with electron-donating substituents (Table 4, Entries4-7) all reacted efficiently with 4-hydroxycoumarin and malononitrile to generate target products in excellent yields (80-99%). It took relatively longer time for aromatic aldehydes with electron-donating substituents to finish the multicomponent cascade reactions than aromatic aldehydes with electron-withdrawing substituents. It was noteworthy that heterocyclic aldehydes like thiophene-2-aldehyde could react readily with 4-hydroxycoumarin and malononitrile to get the target product in 91% yield after 16h (Table 4, Entry 9). Unexpectedly, aromatic aldehydes having large steric hindrance such as 4-tert-butylbenzaldehyde (Table 4, Entry 8) and aliphatic aldehydes like butyraldehyde (Table 4, Entry 10) could also react smoothly with 4-hydroxycoumarin and malononitrile to generate **1h** and **1j** respectively in good yields after 48h. Outstanding results could also be obtained when ethyl cyanoacetate was used as the substrate instead of malononitrile (Table 4, Entries11-13). In addition, the developed method was applied to a gram-scale synthesis and 95% yield of product was obtained (Table 4, entry 14).

3.5 TLIM-catalyzed cascade synthesis of benzopyrans



Scheme 3 TLIM-catalyzed synthesis of benzopyrans

Journal Pre-proofs

Table 5. TLIM-catalyzed cascade synthesis of benzopyrans

Entry[a]	X	R1	Product	Time/h	Yield/%[b]
1	4-ClC_6H_5	H	3a	12	99
2	4-$NO_2C_6H_5$	H	3b	12	98
3	4-CNC_6H_5	H	3c	12	99
4	4-$CH_3C_6H_5$	H	3d	12	91
5	4-OH-3-$OCH_3C_6H_5$	H	3e	12	99
6	4-$OCH_3C_6H_5$	H	3f	12	99
7	4-OHC_6H_5	H	3g	12	95
8	4-t-C_4H_9	H	3h	24	98
9	2-thienyl	H	3i	12	92
10	butyl	H	3j	36	96
11	4-$NO_2C_6H_5$	CH_3	3k	12	99
12	4-CNC_6H_5	CH_3	3l	12	98
13	4-$CH_3C_6H_5$	CH_3	3m	12	99
14	4-OH-3-$OCH_3C_6H_5$	CH_3	3n	12	98
15	4-$OCH_3C_6H_5$	CH_3	3o	12	96
16	4-OHC_6H_5	CH_3	3p	12	99
17	4-t-C_4H_9	CH_3	3q	12	98
18	2-thienyl	CH_3	3r	12	98
19	butyl	CH_3	3s	36	98
20	4-$NO_2C_6H_5$	H	4a	12	90
21	4-OH-3-$OCH_3C_6H_5$	H	4b	12	82
22	4-$CF_3C_6H_5$	H	4c	12	95
23	2-thienyl	H	4d	24	84

[a] Reaction conditions: TLIM (45 mg), aldehyde (1mmol), 1,3-diketones (1mmol), malononitrile/ ethyl cyanoacetate (1mmol), DMSO (4.5mL), water (0.5mL), 45 °C.

[b] Isolated yield.

As can be seen in Table 5, the optimum conditions are suitable not only for the synthesis of dihydropyrano[c]chromenes but also for the synthesis of benzopyrans. Aromatic aldehydes with electron-withdrawing substituents (Table 5, Entries1-3) and aromatic aldehydes with electron-donating substituents (Table 5, Entries4-7) all reacted smoothly with 1, 3-cyclohexanedione and malononitrile to generate target products in excellent yields (91-99%). Excellent results could also be acquired for the reactions of aromatic aldehydes having large steric hindrance (Table 5, Entry8)/heterocyclic aldehydes (Table 5, Entry9)/aliphatic aldehydes (Table 5, Entry10) , 1, 3-cyclohexanedione and malononitrile. Moreover, the reactions of dimedone, malononitrile and aromatic aldehydes (Table 5, Entries11-17)/ heterocyclic aldehydes (Table 5, Entry18)/ aliphatic aldehydes (Table 5, Entry19) could also go well to generate target products in 96-99% yields. Zhang et al. reported that PPL could efficiently catalyze the

one-pot multiple reaction to synthesize benzopyrans, but only moderate yields of target products could be obtained when it came to ethyl cyanoacetate[20]. While ethyl cyanoacetate could react easily with 1, 3-cyclohexanedione and aromatic aldehydes (Table 5, Entries20-22)/ heterocyclic aldehydes (Table 5, Entry23) to give target products in excellent yields using our procedure.

3.6 Reusability of lipase TLIM for the synthesis of **1a**

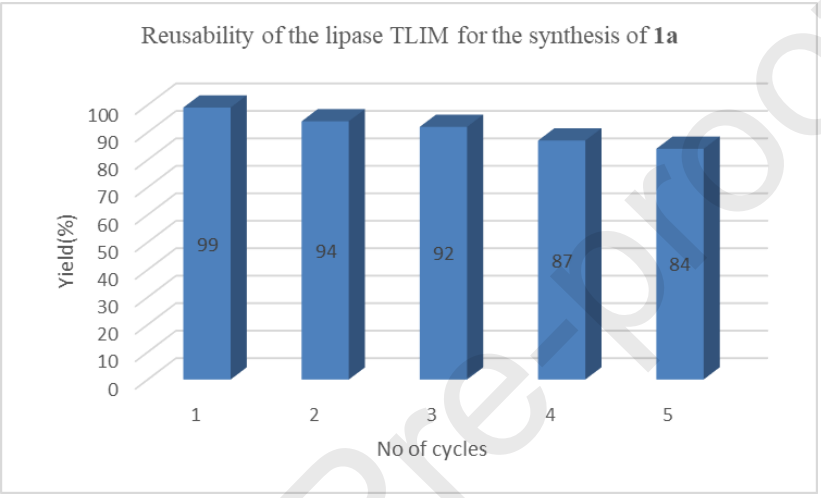


Fig.1. Reusability of lipase TLIM for the synthesis of **1a** Reaction conditions: TLIM (45 mg), 4-chlorobenzaldehyde (1mmol), 4-hydroxycoumarin (1mmol), malononitrile (1mmol), DMSO (4.5mL), water (0.5mL), 45 °C, 24h, HPLC yield.

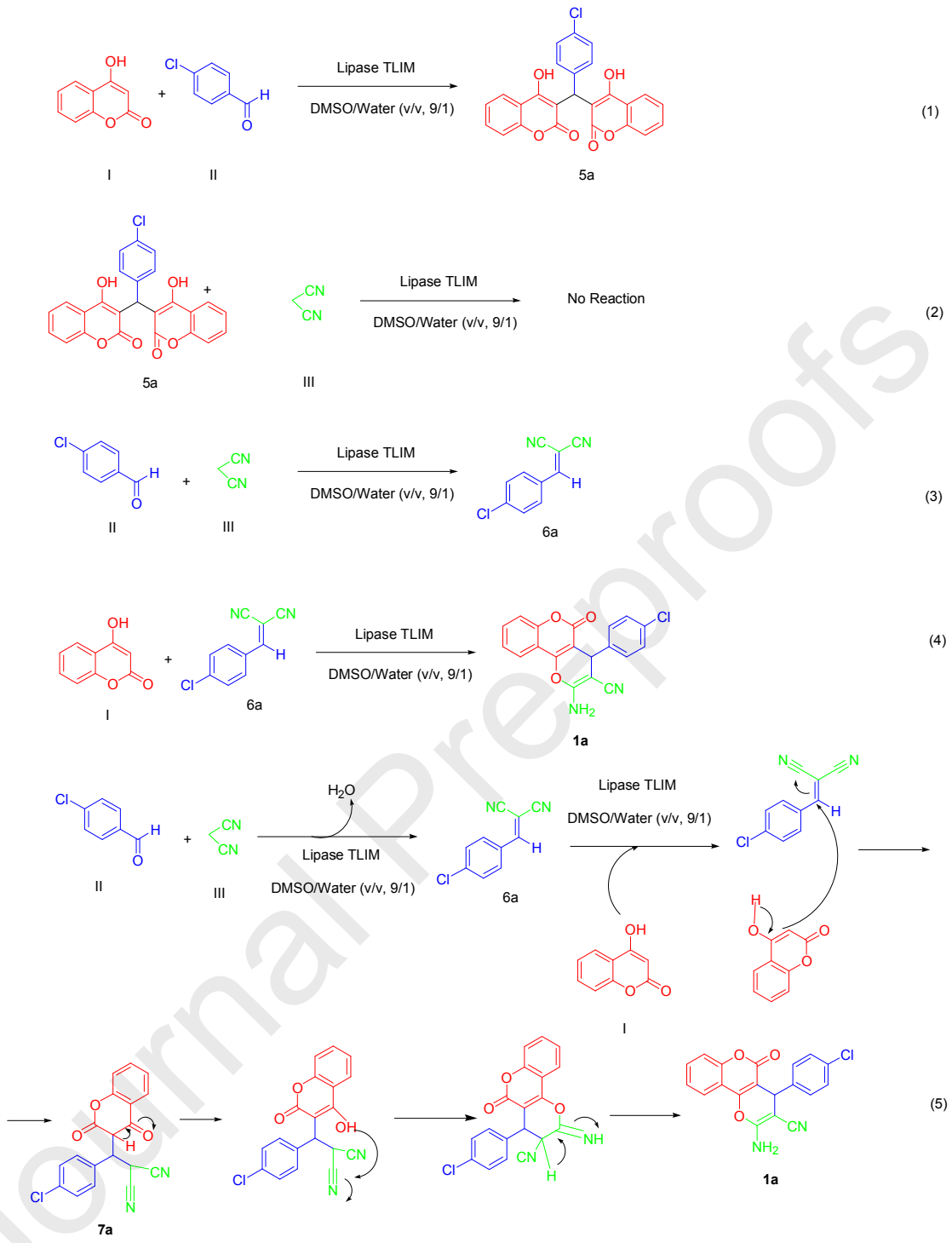
Reusability of catalyst is a vital factor to inspect the economical and environmental performance of reactions. We investigated the reusability of catalyst by using 4-chlorobenzaldehyde, 4-hydroxycoumarin and malononitrile as model substrates. The immobilized lipase TLIM could be collected by centrifugation after the reaction and used in the next run directly. As can be seen in Fig.1, the immobilized lipase TLIM showed satisfactory reusability and 84% yield of target product could be obtained after five cycles. Actually, after the fifth recycling, we measured that the hydrolytic activity of lipase TLIM decreased from original 1161U/g to 668U/g, which was consistent with the decrease of yield. It is noteworthy that excellent yields could be obtained for reactions in anhydrous DMSO (Table2, Entry8). After the fifth recycling, the activity of lipase TLIM in anhydrous DMSO decreased from original 1161U/g to 20U/g and only 11% yield of the target product could be obtained in anhydrous DMSO. As a result, the right amount of water is useful in keeping the structural stability and catalytic activity of lipase TLIM.

Journal Pre-proofs

3.7 Possible mechanism for the synthesis of **1a** catalyzed by TLIM

Taking the model reaction (Scheme 1) as an example, we proposed the possible mechanism based on literatures[9, 20, 24] and experiments. We chose the reaction between 4-hydroxycoumarin, 4-chlorobenzaldehyde and malononitrile as the model reaction (Scheme 1). Firstly, we obtained the product (**5a**) based on 4-hydroxycoumarin and 4-chlorobenzaldehyde (Scheme 4, (1)). No products were detected based on **5a** and malononitrile (Scheme 4, (2)). Secondly, we obtained the product (**6a**) based on 4-chlorobenzaldehyde and malononitrile (Scheme 4, (3)). 96% Yield of target product (**1a**) were obtained based on **6a** and 4-hydroxycoumarin (Scheme 4, (4)). What's more, we detected the intermediate product by TLC and obtained it by column chromatography. The intermediate product was turned out to be **6a**. As a result, we surmised that 4-chlorobenzaldehyde reacted firstly with malononitrile instead of 4-hydroxycoumarin. We assumed that the Knoevenagel condensation reaction occurred between malononitrile and 4-chlorobenzaldehyde to generate the intermediate compound (**6a**) and water firstly. Then the Michael addition reaction occurred between 4-hydroxycoumarin and **6a** to give **7a**, which finally turned to the target product (**1a**) by cyclization and tautomerization (Scheme 4, (5)).

Journal Pre-proofs



Scheme 4. Possible mechanism for the synthesis of **1a** catalyzed by TLIM

3.8 Comparison of the catalytic efficiency of TLIM with some reported catalysts

Table 6 Comparison of the catalytic efficiency of TLIM with some reported catalysts

Entry	Catalyst	Temperature/ ℃	Reusability of catalyst	Yield/ %	Reference

Journal Pre-proofs

1	TLIM	45	Reusable	99	This work
2	I_2	120	Non reusable	80	[25]
3	$(CTA)_3[SiW_{12}]$-Li^+-MMT	Reflux	Reusable	91	[26]
4	LiBr	Reflux	Non reusable	90	[27]
5	SiO_2-Cu(Ⅱ)	Reflux	Reusable	88	[28]
6	Borax	Reflux	Reusable	70	[29]
7	$NH_4Al(SO_4)_2 \cdot 12H_2O$	80	Reusable	85	[30]

Furthermore, we took the reaction of dimedone, 4-hydroxybenzaldehyde and malononitrile as an example and compared the catalytic efficiency of TLIM with other reported catalysts in terms of temperature, reusability of catalyst and yields (Table6). As can be seen in Table6, this work used commercially available and recyclable catalyst and much lower temperature was needed than other reported protocols. Moreover, 99% yield of corresponding product (**3p**) could be obtained, which was absolutely superior to other reported methods.

4 Spectroscopic data for representative products

Subsequently, TLIM-catalyzed condensation of malononitrile / ethyl cyanoacetate, 4-hydroxycoumarin/ 1, 3-cyclohexanedione/ dimedone and a variety of aldehydes in aqueous DMSO was investigated. All the products were characterized by ^{1}H NMR, ^{13}C NMR, HRMS and melting points，and the data of known products are consistent with the literature(see SI）. Here is the spectroscopic data for representative products.

2-amino-4-(4-chlorophenyl)-5-oxo-4H,5H-pyrano[3,2-c]chromene-3-carbonitrile（1a）:
Yield: 0.3472g (99%); white solid; m p 260℃-261℃.
1H NMR (300 MHz, DMSO-*d*6) δ 7.95 – 7.86 (m, 1H), 7.76 – 7.68 (m, 1H), 7.59 – 7.44 (m, 2H), 7.44 (d, *J* = 6.2 Hz, 2H), 7.37 (d, *J* = 8.5 Hz, 2H), 7.30 (d, *J* = 8.5 Hz, 2H), 4.49 (s, 1H).
HRMS (EI-TOF): m/z Calcd. for $C_{19}H_{11}ClN_2O_3[M+Na]^+$: 350.0401, found 350.0404.

Ethyl 2-amino-5-oxo-4-(p-tolyl)-4H,5H-pyrano[3,2-c]chromene-3-carboxylate (2b)：
Yield: 0.3396g (90%); white solid; m p 118℃-119℃.
^{1}H NMR (300 MHz, DMSO-d_6) δ 8.47 (s, 3H), 7.90 (d, *J* = 7.8 Hz, 2H), 7.59 (t, *J* = 7.7 Hz, 2H), 7.45 – 7.21 (m, 4H), 7.03 (s, 4H), 6.31 (s, 1H), 2.24 (s, 3H).
HRMS (EI-TOF): m/z Calcd. for $C_{22}H_{19}NO_5[M+Na]^+$: 377.1212, found 377.1207.

2-amino-4-(4-chlorophenyl)-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (3a)：
Yield: 0.2977g (99%); white solid; m p 241℃-243℃.
^{1}H NMR (400 MHz, DMSO-d6) δ 7.38 – 7.28 (m, 2H), 7.23 – 7.15 (m, 2H), 7.06 (s, 2H), 4.20 (s, 1H), 2.61 (q, J = 4.6, 4.0 Hz, 2H), 2.27 (dtq, J = 16.6, 11.1, 5.2 Hz, 2H), 1.92 (ddq, J = 23.8, 10.6, 5.8 Hz, 2H).
HRMS (EI-TOF): m/z Calcd. for $C_{16}H_{13}ClN_2O_2[M+Na]^+$: 300.0603, found 300.0601.

2-amino-7,7-dimethyl-4-(4-nitrophenyl)-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile

Journal Pre-proofs

(3k)：

Yield: 0.3360g (99%); white solid; m p 180℃-182℃.

^{1}H NMR (300 MHz, DMSO-d_6) δ 8.17 (d, J = 8.6 Hz, 2H), 7.45 (d, J = 8.6 Hz, 2H), 7.16 (s, 2H), 4.37 (s, 1H), 2.54 (s, 2H), 2.26 (d, J = 16.1 Hz, 1H), 2.11 (d, J = 16.1 Hz, 1H), 1.04 (s, 3H), 0.96 (s, 3H).

HRMS (EI-TOF): m/z Calcd. for $C_{18}H_{17}N_3O_4$[M+Na]$^+$: 339.1109, found 339.1105.

Ethyl 2-amino-4-(4-nitrophenyl)-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carboxylate (4a)：

Yield: 0.3225g (90%); yellow solid; m p 181℃-182℃.

^{1}H NMR (300 MHz, DMSO-d_6) δ 8.09 (d, J = 8.6 Hz, 2H), 7.67 (s, 2H), 7.42 (d, J = 8.7 Hz, 2H), 4.63 (s, 1H), 3.94 (q, J = 7.0 Hz, 2H), 2.63 (t, J = 5.9 Hz, 2H), 2.39 – 2.18 (m, 2H), 2.04 – 1.90 (m, 1H), 1.85 (dd, J = 11.1, 5.0 Hz, 1H), 1.07 (t, J = 7.1 Hz, 3H).

HRMS (EI-TOF): m/z Calcd. for $C_{18}H_{18}N_2O_6$[M+Na]$^+$: 358.1107, found 358.1110

5 Conclusion

In conclusion, TLIM is firstly reported to be a commercially available, reusable and efficient catalyst in the Knoevenagel-Michael cascade reactions of aldehydes, malononitrile / ethyl cyanoacetate and 4-hydroxycoumarin. Moreover, excellent yields could also be obtained when the substrate was 1, 3-cyclohexanedione/ dimedone instead of 4-hydroxycoumarin. This methodology offers many superiorities such as simple procedure, mild reaction conditions, high substrate applicability and the ability to be scaled up.

Acknowledgments

This research was financially supported by the National Natural Science Foundation of China (No.21676143), the self-owned research project from key laboratory of Materials-Oriented Chemical Engineering (No. ZK201603), the Jiangsu Synergetic Innovation Center for Advanced Bio-Manufacture, and the Qing Lan Project of Jiangsu Province.

References

1. B. Sameem; M. Saeedi; M. Mahdavi; H. Nadri; F. H. Moghadam; N. Edraki; M. I. Khan; M. Amini, *Bioorg Med Chem* **2017,** 25, (15), 3980-3988.

2. V. Bhaskar; R. Chowdary; S. R. Dixit; S. D. Joshi, *Bioorg Chem* **2019,** 84, 202-210.

3. M. Kaur; A. Kaur; S. Mankotia; H. Singh; A. Singh; J. V. Singh; M. K. Gupta; S. Sharma; K. Nepali; P. M. S. Bedi, *Eur J Med Chem* **2017,** 131, 14-28.

Journal Pre-proofs

4. M. Gardelly; B. Trimech; M. A. Belkacem; M. Harbach; S. Abdelwahed; A. Mosbah; J. Bouajila; H. Ben Jannet, *Bioorg Med Chem Lett* **2016,** 26, (10), 2450-2454.

5. Mayank; B. Kaur Billing; P. K. Agnihotri; N. Kaur; N. Singh; D. O. Jang, *ACS Sustainable Chemistry & Engineering* **2018,** 6, (3), 3714-3722.

6. Y. Wang; H. Ye; G. Zuo; J. Luo, *Journal of Molecular Liquids* **2015,** 212, 418-422.

7. P. Mohammadi; H. Sheibani, *Materials Chemistry and Physics* **2019,** 228, 140-146.

8. M. Esmaeilpour; J. Javidi; F. Dehghani; F. Nowroozi Dodeji, *RSC Advances* **2015,** 5, (34), 26625-26633.

9. M. A. Zolfigol; N. Bahrami-Nejad; F. Afsharnadery; S. Baghery, *Journal of Molecular Liquids* **2016,** 221, 851-859.

10. M. M. Heravi; M. Mirzaei; S. Y. S. Beheshtiha; V. Zadsirjan; F. Mashayekh Ameli; M. Bazargan, *Applied Organometallic Chemistry* **2018,** 32, (9), e4479.

11. M. E. Sedaghat; M. Rajabpour Booshehri; M. R. Nazarifar; F. Farhadi, *Applied Clay Science* **2014,** 95, 55-59.

12. K. Tabatabaeian; M. A. Zanjanchi; M. Mamaghani; A. Dadashi, *Canadian Journal of Chemistry* **2014,** 92, (11), 1086-1091.

13. M. Kapoor; M. N. Gupta, *Process Biochemistry* **2012,** 47, (4), 555-569.

14. Y. Miao; M. Rahimi; E. M. Geertsema; G. J. Poelarends, *Current Opinion In Chemical Biology* **2015,** 25, 115-123.

15. M. Lopez-Iglesias; V. Gotor-Fernandez, *Chemical Record* **2015,** 15, (4), 743-759.

16. Y. Ding; H. Huang; Y. Hu, *Chinese Journal Of Organic Chemistry* **2013,** 33, (5), 905-914.

17. Y. Ding; X. Ni; M. Gu; S. Li; H. Huang; Y. Hu, *Catalysis Communications* **2015,** 64, 101-104.

18. Y. Fu; B. Fan; H. Chen; H. Huang; Y. Hu, *Bioorg Chem* **2018,** 80, 555-559.

19. Y. Fu; Z. Lu; K. Fang; X. He; H. Huang; Y. Hu, *Bioorg Med Chem Lett* **2019,** 29, (10), 1236-1240.

20. J.-C. Xu; W.-M. Li; H. Zheng; Y.-F. Lai; P.-F. Zhang, *Tetrahedron* **2011,** 67, (49), 9582-9587.

21. E. Cernia; C. Palocci; S. Soro, *Chemistry And Physics Of Lipids* **1998,** 93, (1-2), 157-168.

22. D. Koszelewski; D. Paprocki; A. Madej; F. Borys; A. Brodzka; R. Ostaszewski, *European Journal of Organic Chemistry* **2017,** 2017, (31), 4572-4579.

23. A. Zaks; A. M. Klibanov, *Science* **1984,** 224, (4654), 1249-1251.

24. Z. B. Xie; D. Z. Sun; G. F. Jiang; Z. G. Le, *Molecules* **2014,** 19, (12), 19665-77.

25. R. S. Bhosale; C. V. Magar; K. S. Solanke; S. B. Mane; S. S. Choudhary; R. P. Pawar, *Synthetic Communications* **2007,** 37, (24), 4353-4357.

26. E. Abbaspour-Gilandeh; M. Aghaei-Hashjin; A. Yahyazadeh; H. Salemi, *RSC Advances* **2016,** 6, (60), 55444-55462.

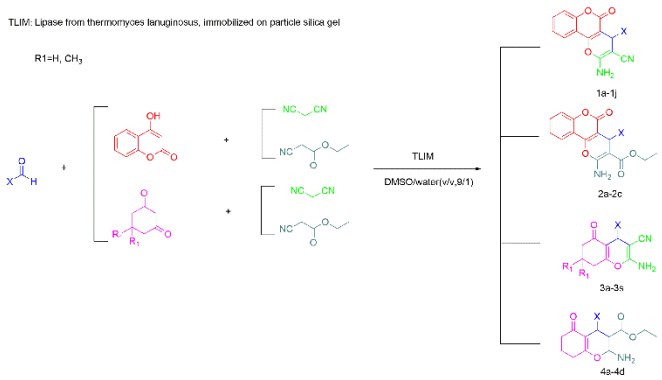
27. W.-B. Sun; P. Zhang; J. Fan; S.-H. Chen; Z.-H. Zhang, *Synthetic Communications* **2010,** 40, (4), 587-594.

28. M. Gupta; M. Gupta; R. Rajnikant; V. K. Gupta, *New Journal of Chemistry* **2015,** 39, (5), 3578-3587.

29. A. Molla; E. Hossain; S. Hussain, *RSC Advances* **2013,** 3, (44), 21517.

30. A. A. Mohammadi; M. R. Asghariganjeh; A. Hadadzahmatkesh, *Arabian Journal of Chemistry* **2017,** 10, S2213-S2216.

Journal Pre-proofs



Journal Pre-proofs

● An efficient enzymatic method for the synthesis of dihydropyrano[c]chromenes was developed using three-component tandem reactions of aldehydes, malononitrile / ethyl cyanoacetate and 4-hydroxycoumarin.

●An efficient method for the synthesis of benzopyrans was developed by one-pot enzymatic cascade reactions of aldehydes, malononitrile / ethyl cyanoacetate and 1, 3-cyclohexanedione/ dimedone.

● Simple procedure, mild reaction conditions, high substrate applicability, the ability to be scaled up, and good to excellent yields, are the important attributes of the present protocol.

Journal Pre-proofs

Journal Pre-proofs

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Journal Pre-proofs