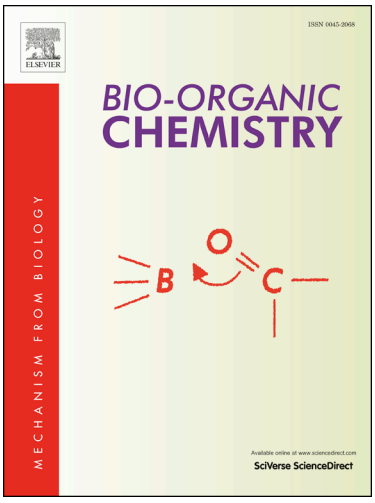

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Promiscuous Enzyme-catalyzed Cascade Reaction: Synthesis of Xanthone Derivatives

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Abstract

Based on the screening of biocatalysts and reaction conditions including organic solvent, water content, lipase loading, reaction temperature and time, lipase TLIM exhibited the prominent promiscuity for the Knoevenagel-Michael cascade reactions of 1, 3-diketones with aromatic aldehydes to synthesize xanthone derivatives. This procedure provides satisfactory advantages such as environmental begin, simple work-up, generality, obtaining in excellent yields (80-97%), and potential for recycling of biocatalyst.

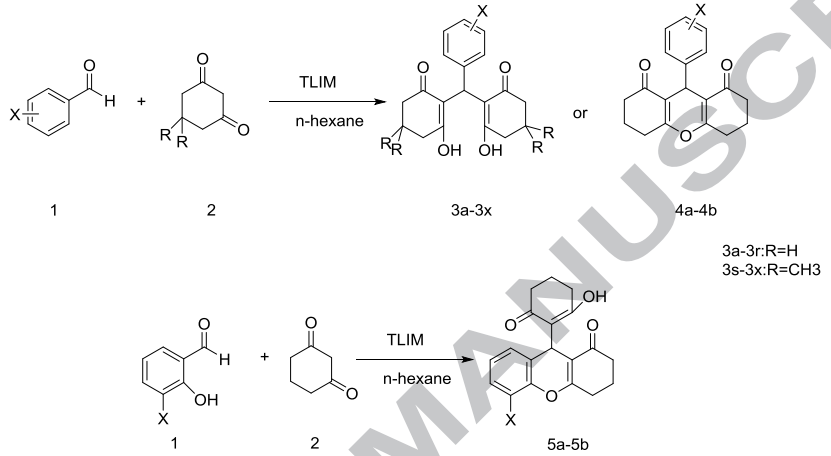
Keywords lipase, Knoevenagel condensation, aromatic aldehyde, xanthone, catalysis

1 Introduction

Xanthone derivatives comprise key structural unit in a variety of natural products and are found to possess significant pharmacological and biological activities[1-3]. Besides, these compounds can be applied in the field of pH sensitive fluorescent material[4], laser technology[5] and dyestuff[6]. Owing to its important contributions in different areas, these compounds are getting an upsurge of interest in recent years. Xanthones are generally obtained from Knoevenagel condensation and Michael addition of 1, 3-diketones with aromatic aldehydes. Various catalysts for synthesizing xanthones have been reported such as phase transfer catalysts TEBA[7], CsF[8], urea under ultrasound[9], nano zeolites Fe/NaY[10], supercritical diethyl ether[11], natural phosphates NP[12], taurine[13], acidic ionic liquids under microwave irradiation[14], magnetic nanoparticles $CuFe_2O_4$@SiO_2- OP_2O_5H[15], MCM-41@Schiff Base-$Mn(OAc)_2$[16], and Oleylamine[17]. However, these methods are usually restricted by volatile toxic organic solvents needed, expensive and non reusable catalysts reused, impractical for large scale production, and sometimes only moderate yields obtained.

In recent years, enzymes have gained a wide interest of researchers for their increasingly important role in organic synthesis due to their high efficiency and environmental friendliness[18, 19]. It is well reported that enzymes play an important catalytic role in various C-C bond forming reactions such as Aldol condensation, Mannich reaction, Henry reaction, Knoevenagel condensation, Michael addition, Baylis-Hillman reaction and so on[20, 21]. Ye *et al*[22] reported the use of "Amano"

lipase DF to catalyze the Knoevenagel-Michael addition cascade reaction in the synthesis of xanthone derivatives. However, the protocol suffers some limitations such as the use of strong polar solvent DMF which posed difficulty in the work-up procedure, requirement of excessive aromatic aldehyde and the consumption of relatively large amount of enzyme which couldn't be reused in this protocol. Continuing our interest in enzyme-catalyzed C-C bond forming reactions[23, 24], we, herein, reported the use of immobilized lipase TLIM as catalyst in the Knoevenagel-Michael cascade reactions of 1, 3-diketones with aromatic aldehydes to synthesize xanthone derivatives (Scheme 1).



Scheme 1. Enzymatic reaction of aromatic aldehydes with 1, 3-diketones

2. Materials and Methods

Porcine pancreas lipase (PPL), Amano Lipase PS from *Burkholderia cepacia* (BCL) and *Candida rugosa* lipase (CRL) were purchased from Sigma. Pancreatin from porcine pancreas (PPP) was purchased from Aladdin Industrial Corporation. TLIM （Lipase from *Thermomyces lanuginosus*, immobilized on particle silica gel）, Papain and, Novozym 435 (lipase B from *Candida antarctica*, immobilized on a macroporous acrylic resin) were purchased from Novo Nordisk. Lipase XHlip-F was given by XHSynbio. Lipase lipoprotein from *Aspergillus niger* (LPL) was purchased from Ningxia Sunson group corporation. Lipase DF was donated by Amano Enzyme China Ltd. Other reagents were commercially available and were used without further purification.

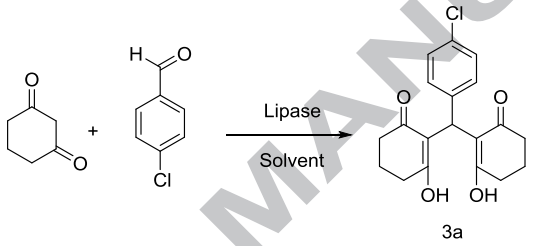
The melting points were determined on a WRS-1B digital melting point instrument and were not corrected. The ^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker Advance 2B 400 MHz instrument with $CDCl_3$ as solvent and TMS as internal standard. The HRMS were measured on Agilent LC/MS mass spectrometer. 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 (Amethyst C18-H (4.6×250mm, 5μm), formic acid with 0.1% water/methanol (v:v=2/3-3/7,15min, radiant elution), 45℃, 254nm).

2.1 *General procedure for the synthesis of xanthone derivatives*

A mixture of 1, 3-cyclohexanedione (2mmol), aromatic aldehyde (1mmol) and TLIM (50mg) in n-hexane (5 mL) was stirred at 35℃. The progress of the reaction was monitored by TLC (dichloromethane: ethyl acetate = 2:1). Upon completion of the reaction, the reaction mixture was filtered, and the residue obtained was subsequently dissolved in an appropriate amount of dichloromethane to separate the product and TLIM. By simple filtration, the immobilized biocatalyst TLIM was recovered and subjected to the next run directly, the filtrate was evaporated to obtain the crude product which was further purified by recrystallization using 95% ethanol.

3. Results and discussion

3.1 *Optimization of enzyme sources for the Knoevenagel -Michael cascade reactions*



Scheme 2. Enzymatic reaction of *p*-chlorobenzaldehyde with 1, 3-cyclohexanedione

Table1. Effect of enzyme sources on the yield of **3a**

Entry[a]	Enzyme	Yield(%)[b]	Entry[a]	Enzyme	Yield(%)[b]
1	No enzyme	trace	7	CRL	33
2	TLIM	53	8	Lipase DF	46
3	BCL	32	9	LPL	40
4	XHlip-F	41	10	PPP	30
5	Novozym 435	43	11	Papain	28
6	PPL	45	12	TLIM[c]	25

[a] Reaction conditions: lipase (50 mg), 4-chlorobenzaldehyde (1mmol), cyclohexane-1,3-dione (2mmol), CH_2Cl_2 (5mL),35℃,18 h.

[b] HPLC yield.

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

In an initial study, we chose *p*-chlorobenzaldehyde and 1, 3-cyclohexanedione as the model substrates to generate

2,2'-((4-chlorophenyl)methylene)bis(3-hydroxycyclohex-2-en-1-one) (**3a**), as shown in Scheme 2. Our previous study showed that LPL and PPL efficiently catalyzed the Knoevenagel condensation[23, 24]. Ye *et al* reported that Lipase DF posses the efficient biocatalytic promiscuity for the Knoevenagel -Michael addition cascade reaction in the synthesis of xanthone derivatives[25]. Different from the reported results, TLIM (Table 1, entry 2) showed a relatively better catalytic efficiency than PPL, Lipase DF and LPL (Table 1, entries 6-9). To explore the effect of specific structure of the lipase on its catalytic efficiency, we conducted the reaction using PPP (Table 1, entry 10), papain (Table 1, entry 11) and denatured TLIM (Table 1, entry 12) as catalyst. It turned out that all these three enzymes showed lower catalytic efficiency compared with lipases investigated before (Table1, entries 2-9). From these results, it could be speculated that the specific structure of lipase imparts significant effect on the catalytic performance during this reaction.

Table2. Effect of solvent source and water content on the yield of **3a**

Entry[a]	Solvent	Yield(%)[b]	Dielectric constant	LogP
1	H_2O	66	78.5	-
2	Isopropanol	49	19.9	0.4
3	Methylbenzene	48	2.4	2.5
4	CH_2Cl_2	53	8.9	1.0
5	THF	35	7.5	0.4
6	Acetonitrile	56	37.5	0.2
7	DMSO	56	47.2	-1.5
8	DMF	64	38.3	-0.6
9	n-Hexane	95	1.9	3
10	n-Hexane[c]	82	1.9	3
11	n-Hexane[d]	74	1.9	3

[a] Reaction conditions: TLIM(50 mg), 4-chlorobenzaldehyde (1mmol), cyclohexane-1,3-dione (2mmol), solvent (5mL),35℃,18 h

[b] HPLC yield.

[c] n-hexane(3.5mL),water(1.5mL).

[d] n-hexane(2.5mL),water(2.5mL).

3.2 *Optimization of solvent and water content for the Knoevenagel -Michael cascade reactions*

In the non-aqueous enzymatic reactions, it is reported that LogP value of the reaction medium influences the catalytic activity[26]. It is generally believed that the catalytic activity of an enzyme increases as the solvent LogP increases[27]. However, our results were not found consistent with this belief as shown in Table 2. We assume that the solubility of the substrates and products in the desired solvent also affects the reaction rate. Table 2 showed up the joint action of solubility and LogP. Subsequently, we chose the combination of TLIM and n-hexane to screen for the most suitable water content. It is known that water is important for conformational flexibility of enzyme and the biocatalytic efficiency of the enzyme increases with increase in water content consumed by the reaction system[28, 29]. We, however, discovered that the product yield decreased from 95% to 74% (Table 2, entries 9- 11) with the addition of water, which is consistent with the result of Knoevenagel condensation of aromatic aldehydes with acyclic active methylene compounds catalyzed by LPL[24].

3.3 *Optimization of enzyme quantity, reaction time and temperature for the Knoevenagel -Michael cascade reactions*

Table3. Effects of enzyme amount and reaction time on the yield of **3a**

Entry[a]	Enzyme amount(mg)	Time (h)	Temperature(℃)	Yield (%)[b]
1	30	18	35	78
2	70	18	35	90
3	50	18	35	95
4	50	14	35	79
5	50	16	35	93
6	50	24	35	93

[a] Reaction conditions: TLIM, 4-chlorobenzaldehyde (1mmol), cyclohexane-1,3-dione (2mmol), n-hexane (5mL).

[b] HPLC yield.

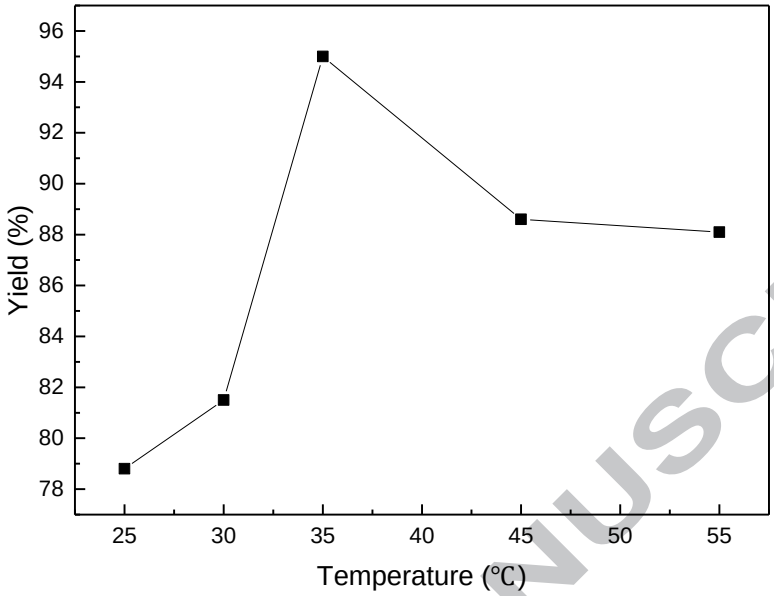


Fig.1. Effects of temperature on the yield of **3a**. Reaction conditions: TLIM (50mg), 4-chlorobenzaldehyde (1mmol), cyclohexane-1, 3-dione (2mmol), n-hexane (5mL), 18h. HPLC yield.

The concentration of enzyme exerts significant effect on the proximity of substrate and enzyme. As shown in Table 3 (Entries 1-3), the optimum concentration of enzyme used was 50mg, as compared to use of 200mg enzyme for 1mmol aromatic aldehydes in the Lipase DF-catalyzed reaction system[25]. It was observed that the yield kept increasing until the reaction time reached 18 hours (Table 3, entries 3-5). After 18 hours, the reaction yield was found nearly constant (Table 3, entry 6). The reaction temperature also exerted very important influence on the activity of enzyme (Fig.1.). Under these experimental conditions, the optimal reaction temperature of the enzyme was selected as 35°C.

3.4 *TLIM-catalyzed synthesis of oxindanedione derivatives*

After the optimal reaction conditions were determined, the substrate applicability of Knoevenagel-Michael cascade reactions catalyzed by lipase TLIM was further investigated (Table 4). TLC was used to monitor the progress of reactions. It was observed that aromatic aldehydes with electron-withdrawing substituents reacted more readily with 1, 3-diketones as compared to aromatic aldehydes with electron-donating substituents to generate product **3** in excellent yields (80%-97%). 1, 3 - cyclohexanedione showed higher reactivity than dimedone. Also, it was noteworthy that satisfactory results were obtained for heterocyclic aldehydes like 2-thiophenealdehyde (Table 4, entry 18) and pyridine-2-carbaldehyde (Table 4, entry 26), as well as with aromatic aldehydes having large steric hindrance such as 4-tert-butylbenzaldehyde (Table 4, entry 13) and 2, 6-dichlorobenzaldehyde (Table 4,

entry 25). Unexpectedly, the reaction of 2, 6-dichlorobenzaldehyde and pyridine-2-carbaldehyde with 1,3-cyclohexanedione produced **4a** and **4b** respectively, which were usually obtained in presence of acid and at relatively higher temperature[7, 12, 30]. Benzaldehydes with hydroxyl group in orbit position reacted with 1, 3-cyclohexanedione to generate **5** (Table 4, entries 27-28), which could be attributed to the dehydration reaction of phenolic hydroxyl and enol hydroxyl groups. This result was different from the reaction of dimedone with aromatic aldehydes in water without catalyst[31] while found similar to the reaction of dimedone with aromatic aldehydes catalyzed by TEBA and p-toluenesulfonic acid[7]. Moreover, the developed method was applied to a gram-scale synthesis and high yield was obtained (Table 4, entry 29). Finally, recyclability and reusability of TLIM were investigated for the synthesis of desired product **3a** in a good yield (Table 4, entries 30-31).

Table 4. Comparing the yield of xanthenediones catalyzed by lipase TLIM

Entry[a]	X	Product	Time/h	Yield/%[b]
1	4-Cl	3a	12	95
2	4-Br	3b	18	95
3	3-NO_2	3c	22	91
4	4-NO_2	3d	14	97
5	3-Cl	3e	18	87
6	4-F	3f	18	95
7	2,4-di-Cl	3g	32	82
8	4-CN	3h	14	94
9	H	3i	18	93
10	4-OCH_3	3j	16	88
11	4-CH_3	3k	20	84
12	4-OH	3l	16	90
13	4-C_4H_9	3m	24	82
14	4-CF_3	3n	14	93
15	4-OH-3-OCH_3	3o	20	92

Entry[a]	X	Product	Time/h	Yield/%[b]
16	2-F	3p	14	94
17	2-NO_2	3q	18	85
18	2-Thienyl	3r	13	85
19	4-NO2	3s	18	96
20	4-Cl	3t	18	93
21	2-F	3u	14	92
22	2-NO_2	3v	20	86
23	H	3w	24	90
24	4-CH3	3x	30	82
25	2,6-di-Cl	4a	32	80
26	2-pyridyl	4b	30	87
27	2-OH	5a	14	85
28	2-OH-3-OCH_3	5b	20	92
29	4-Cl	3a	18	94[c]
30	4-Cl	3a	18	85[d]
31	4-Cl	3a	18	82[e]

[a] Reaction conditions: TLIM (50mg), aldehyde (1mmol), 1,3-diketones(2mmol), n-hexane(5mL), 35℃.

[b] Isolated yield.

[c] Reaction conditions: TLIM (500mg), *p*-chlorobenzaldehyde (10mmol), 1,3-cyclohexanedione (20mmol), n-Hexane(50mL), 35℃, isolated yield.

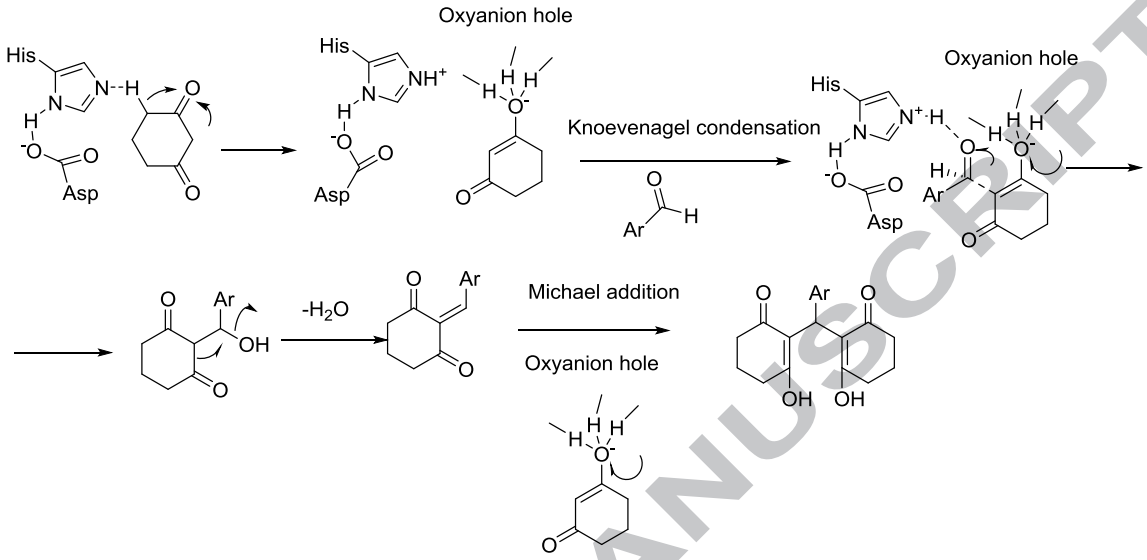
[d] HPLC yield of 3a (run 2).

[e] HPLC yield of 3a (run 3).

3.5 *Possible mechanism for the synthesis of oxindanedione derivatives catalyzed by TLIM*

Enzymatic synthesis of xanthone derivatives was carried out using Knoevenagel condensation-Michael addition as two-step tandem reaction. A possible mechanism was proposed in Scheme 3: Firstly, 1, 3-cyclohexanedione or dimedone interacted with Asp-His residue of lipase TLIM to form an enol anion, which was stable in the

oxygen ion pore of the lipase TLIM. Further, the enol anion attacked the aromatic aldehyde to undergo Knoevenagel condensation. As a result, an intermediate was obtained, which underwent Michael addition with the next enolate anion to produce the desired xanthone derivatives.



Scheme 3. Possible mechanism for the synthesis of oxindanedione derivatives catalyzed by TLIM

Spectroscopic data for representative products

2,2'-((4-chlorophenyl)methylene)bis(3-hydroxycyclohex-2-en-1-one) (3a): White solid; mp:203-205℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.33 (s, 1H), 12.03 (s, 1H), 7.21 (d, *J* = 8.5 Hz, 2H), 7.02 (d, *J* = 8.0 Hz, 2H), 5.40 (s, 1H), 2.68 – 2.52 (m, 4H), 2.50 – 2.33 (m, 4H), 2.02 (dt, *J* = 13.6, 7.6 Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.27, 190.90, 136.49, 131.57, 128.27, 127.95, 116.19, 33.49, 32.99, 32.57, 20.07; HRMS(ESI-TOF) m/z $[M+Na]^+$：369.0864，found: 369.0880.

9-(2,6-dichlorophenyl)-3,4,5,6,7,9-hexahydro-1H-xanthene-1,8(2H)-dione (4a): White solid; mp:260-262℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 (d, *J* = 7.8 Hz, 1H), 7.09 (d, *J* = 7.8 Hz, 1H), 6.99 (t, *J* = 8.0 Hz, 1H), 5.52 (s, 1H), 2.65 – 2.46 (m, 4H), 2.36 – 2.28 (m, 4H), 2.06 – 1.90 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.74, 165.36, 136.26, 129.53, 128.60, 127.77, 113.37, 37.02, 30.29, 27.15, 20.29; HRMS(ESI-TOF) m/z $[M+Na]^+$：385.0369, found: 385.0383.

9-(2-hydroxy-6-oxocyclohex-1-en-1-yl)-2,3,4,9-tetrahydro-1H-xanthen-1-one (5a):

White solid; mp:230-232℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.86 (s, 1H), 7.15 (dq, *J* = 7.9, 3.9 Hz, 1H), 7.04 – 6.99 (m, 3H), 4.64 (s, 1H), 2.76 (dt, *J* = 17.8, 4.7 Hz, 1H), 2.65 – 2.48 (m, 3H), 2.42 (ddd, *J* = 17.0, 11.4, 5.5 Hz, 2H), 2.35 – 2.15 (m, 2H), δ 2.11 – 1.87 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 196.62, 167.25, 150.03, 128.88, 127.31, 126.19, 124.71, 121.86, 121.84, 120.03, 115.69, 112.42, 37.13, 34.25, 27.72, 20.86, 20.75. HRMS(ESI-TOF) m/z $[M+Na]^+$：333.1097, found: 333.1110.

4. Conclusion

In summary, an efficient enzymatic method for the synthesis of xanthone derivatives was developed using Knoevenagel-Michael cascade reactions of 1, 3-diketones with aromatic aldehydes. The use of separable solvent, recyclable biocatalyst, high yields and the ability to be scaled up, are the important attributes of the present protocol. The present methodology has extended the potential use of TLIM in organic and pharmaceutical synthesis.

Acknowledgments

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Abstract

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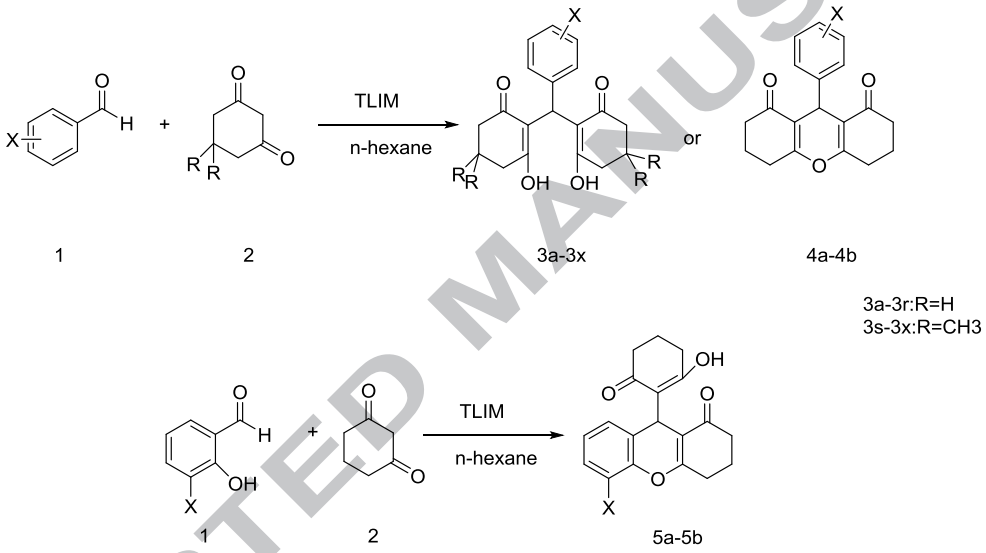
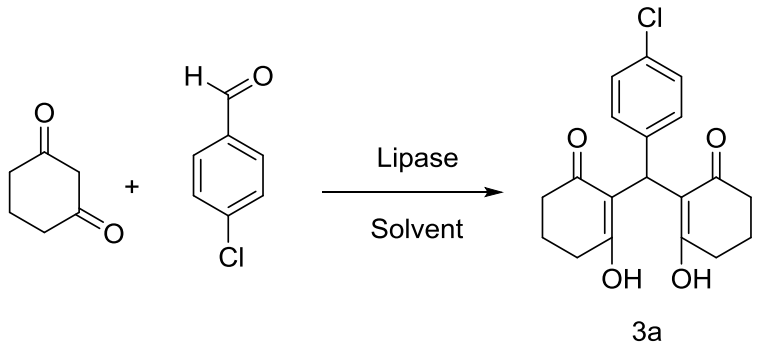


Scheme 1. Enzymatic reaction of aromatic aldehydes with 1, 3-diketones



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6	PPL	45	12	TLIM[c]	25

[a] Reaction conditions: lipase (50 mg), 4-chlorobenzaldehyde (1mmol), cyclohexane-1,3-dione (2mmol), CH_2Cl_2 (5mL),35℃,18 h.

[b] HPLC yield.

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

Table2. Effect of solvent source and water content on the yield of **3a**

Entry[a]	**Solvent**	**Yield(%)[b]**	**Dielectric constant**	**LogP**
1	H_2O	66	78.5	-
2	Isopropanol	49	19.9	0.4
3	Methylbenzene	48	2.4	2.5
4	CH_2Cl_2	53	8.9	1.0
5	THF	35	7.5	0.4
6	Acetonitrile	56	37.5	0.2
7	DMSO	56	47.2	-1.5
8	DMF	64	38.3	-0.6
9	n-Hexane	95	1.9	3
10	n-Hexane[c]	82	1.9	3
11	n-Hexane[d]	74	1.9	3

[a] Reaction conditions: TLIM(50 mg), 4-chlorobenzaldehyde (1mmol), cyclohexane-1,3-dione (2mmol), solvent (5mL),35℃,18 h

[b] HPLC yield.

[c] n-hexane(3.5mL),water(1.5mL).

[d] n-hexane(2.5mL),water(2.5mL).

Table3. Effects of enzyme amount and reaction time on the yield of **3a**

Entry[a]	Enzyme amount(mg)	Time (h)	Temperature(℃)	Yield (%)[b]
1	30	18	35	78
2	70	18	35	90
3	50	18	35	95
4	50	14	35	79
5	50	16	35	93
6	50	24	35	93

[a] Reaction conditions: TLIM, 4-chlorobenzaldehyde (1mmol), cyclohexane-1,3-dione (2mmol), n-hexane (5mL).

[b] HPLC yield.

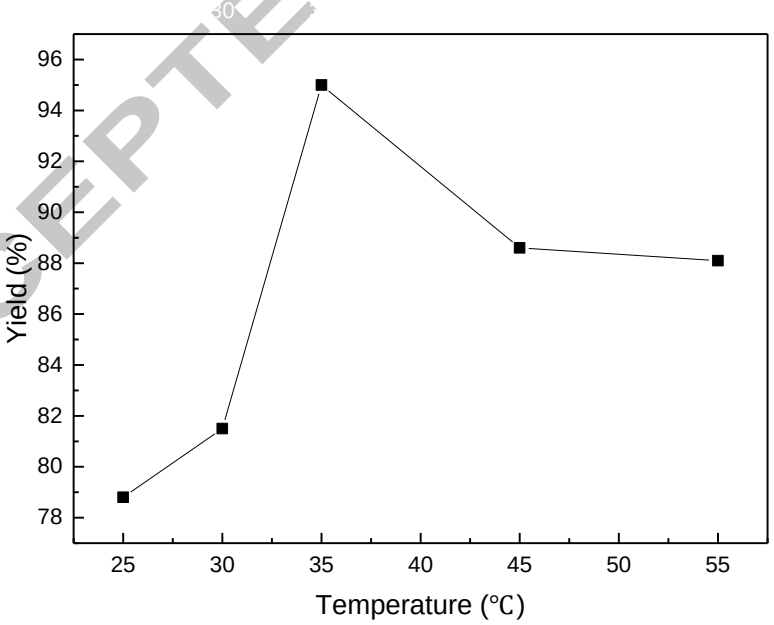


Fig.1. Effects of temperature on the yield of **3a**. Reaction conditions: TLIM (50mg), 4-chlorobenzaldehyde (1mmol), cyclohexane-1, 3-dione (2mmol), n-hexane (5mL), 18h. HPLC yield.

Table 4. Comparing the yield of xanthenediones catalyzed by lipase TLIM

Entry[a]	X	Product	Time/h	Yield/%[b]
1	4-Cl	3a	12	95
2	4-Br	3b	18	95
3	3-NO_2	3c	22	91
4	4-NO_2	3d	14	97
5	3-Cl	3e	18	87
6	4-F	3f	18	95
7	2,4-di-Cl	3g	32	82
8	4-CN	3h	14	94
9	H	3i	18	93
10	4-OCH_3	3j	16	88
11	4-CH_3	3k	20	84
12	4-OH	3l	16	90
13	4-C_4H_9	3m	24	82
14	4-CF_3	3n	14	93
15	4-OH-3-OCH_3	3o	20	92
16	2-F	3p	14	94
17	2-NO_2	3q	18	85
18	2-Thienyl	3r	13	85
19	4-NO2	3s	18	96
20	4-Cl	3t	18	93
21	2-F	3u	14	92
22	2-NO_2	3v	20	86

Entry[a]	X	Product	Time/h	Yield/%[b]
23	H	3w	24	90
24	4-CH3	3x	30	82
25	2,6-di-Cl	4a	32	80
26	2-pyridyl	4b	30	87
27	2-OH	5a	14	85
28	2-OH-3-OCH_3	5b	20	92
29	4-Cl	3a	18	94[c]
30	4-Cl	3a	18	85[d]
31	4-Cl	3a	18	82[e]

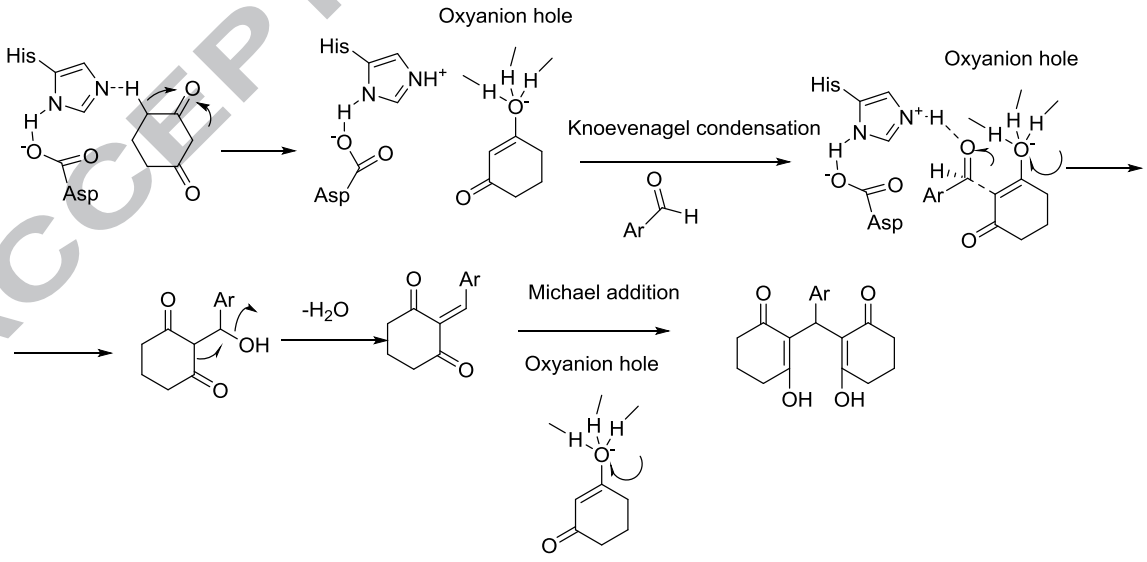
[a] Reaction conditions: TLIM (50mg), aldehyde (1mmol), 1,3-diketones(2mmol), n-hexane(5mL), 35℃.

[b] Isolated yield.

[c] Reaction conditions: TLIM (500mg), *p*-chlorobenzaldehyde (10mmol), 1,3-cyclohexanedione (20mmol), n-Hexane(50mL), 35℃, isolated yield.

[d] HPLC yield of 3a (run 2).

[e] HPLC yield of 3a (run 3).



Scheme 3. Possible mechanism for the synthesis of oxindanedione derivatives catalyzed by TLIM

An efficient enzymatic method for the synthesis of xanthone derivatives was developed using Knoevenagel-Michael cascade reactions of 1, 3-diketones with aromatic aldehydes. The use of separable solvent, recyclable biocatalyst, high yields and the ability to be scaled up, are the important attributes of the present protocol. The present methodology has extended the potential use of TLIM in organic and pharmaceutical synthesis.