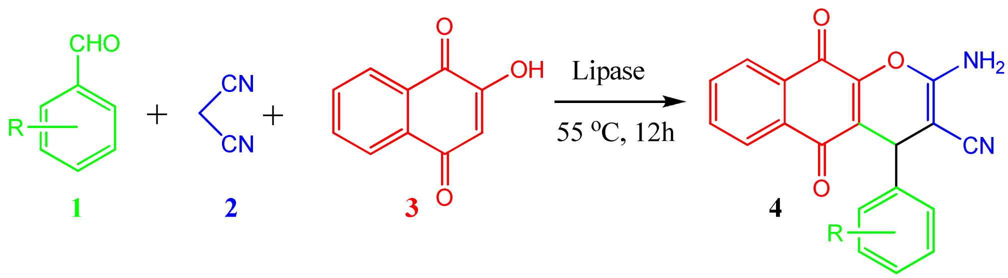


**Lipase-catalyzed synthesis of benzo[g]chromene derivatives**

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**ARTICLE TYPE**

# A green and one-pot synthesis of benzo[g]chromene derivatives through a multi-component reaction catalyzed by lipase

**Fengjuan Yang,[a,b] Haoran Wang,[a,b] Liyan Jiang,[a,b] Hong Yue,[a,b] Hong Zhang,[a,c] Zhi Wang*[a,b] and Lei Wang*[a,b]**

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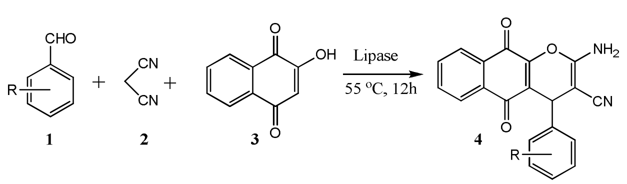
**The synthesis of benzo[g]chromene derivatives through a multi-component reaction catalyzed by lipase was reported in the first time. This novel efficient method has the advantages of environmental friendliness, high yield and simple work-up. Moreover, this protocol extends the phenomenon of enzyme promiscuity.**

A multi-component reaction is generally defined as a reaction in which three or more reactants combine in one pot to form a single product that contains essentially all of the atoms of the starting materials (with the exception of condensation products, such as $H_2O$, HCl, or MeOH) [1-2]. Compared with the conventional chemical reactions, the multi-component reaction strategies can offer the advantage of simplicity and synthetic efficiency [3-4].

Enzyme catalytic promiscuity is the ability of an enzyme to catalyze more than one type of chemical transformation. As a "hidden skill" of enzyme, it can provide novel synthesis pathway that are currently not available [5,6] and can widen the application of enzyme. In this area, lipase is the most used enzyme due to its broad specificity and excellent stability in various media. Many elegant works of lipase catalytic promiscuity in the carbon–carbon bond-forming reactions (Aldol condensation, Morita–Baylis–Hillman reaction, Michael addition, Markovnikov addition, and Knoevenagel reaction, *et al.*) have been reported in the past few years [7-14]. As a part of our interest to explore the applications of lipase in this new area, we are focusing on the multi-component reaction of synthesis of benzo[g]chromene derivatives catalyzed by lipase in this study. Benzo[g]chromene derivatives have extensive bioactivities, such as antibacterial, antiproliferation and antitumor activities [15-20]. To the best of our knowledge, it is reported for the first time that the synthesis of benzo[g]chromene derivatives can be catalyzed by lipase (Scheme 1).




**Scheme 1** Lipase-catalyzed synthesis of benzo[g]chromene derivatives

Initial studies were undertaken using benzaldehyde, malononitrile and 2-hydroxy-1,4-naphthoquinone as a model reaction. Several kinds of lipases were selected to catalyze this multi-component reaction and the results are listed in Table 1. It could be found that all the selected lipases can catalyze this multi-component reaction and the catalytic activities depend mainly on the lipase origin. When the denatured lipase or bovine serum albumin (BSA) was used as the catalyst, almost no product could be detected which suggested a special active conformation of enzyme play a crucial role in this multi-component reaction. Among of the selected lipases, *Candida sp.* lipase (CSL) exhibited the highest catalytic activity. Therefore, we chose CSL as the catalyst for the multi-component reaction. ‡

**Table 1** The catalytic activities of different lipases in the synthesis of benzo[g]chromene derivatives [a]

| Entry | Enzyme | Isolated yield (%) |
|---|---|---|
| 1 | *Candida antarctica* Lipase B (CALB) | 57 |
| 2 | Porcine pancreas Lipase (PPL) | 79 |
| 3 | *Candida sp.* Lipase (CSL) | 88 |
| 4 | *Pseudomonas fluorescens* Lipase (PFL) | 54 |
| 5 | *Pseudomonas sp.* Lipase (PSL) | 66 |
| 6 | *Bacillus subtillus* Lipase (BSL2) | 70 |
| 7 | *C. rugosa* Lipase (CRL) | 61 |
| 8 | Bovine serum albumin (BSA) | Trace |
| 9 | *Candida sp.* Lipase (denatured) [b] | Trace |
| 10 | No enzyme | Trace |

[a] Reaction condition: 2-hydroxy-1,4-naphthoquinone (1 mmol), malononitrile (1 mmol) and benzaldehyde (1 mmol), ethanol (2mL), enzyme (20 mg, protein content), 55 °C, 12h. [b] CSL was denatured by heating it to 100 °C for 6 h in water before lyophilization.

Choosing a suitable solvent is of crucial importance for the enzyme catalytic performance [21]. Thus, seven organic solvents were screened for this reaction and the results are presented in Figure 1. Compared with other solvents, the highest yield (88%) could be obtained while ethanol was used as the reaction media. It's believed that the solvent can lead to a conformational change of the enzyme and then affect the enzyme activity [22].

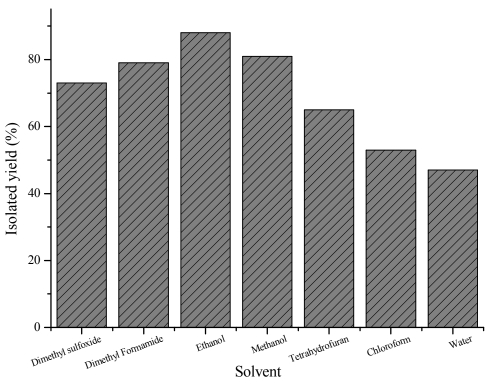



**Fig. 1** Solvent effect on the synthesis of benzo[g]chromene derivatives catalyzed by lipase

Reaction condition: 2-hydroxy-1,4-naphthoquinone (1 mmol), malononitrile (1 mmol) and benzaldehyde (1 mmol), solvent (2mL), CSL (20 mg, protein content), 55 ºC, 12h.

It's generally believed that reaction temperature is another vital influence factor for all enzymatic reactions [23]. In this study, the reaction temperature was varied from 25 to 75 ºC to investigate its effect. As shown in Figure 2, the yield increased as temperature was enhanced from 25 to 55 ºC and dropped dramatically at higher temperature. The increased collision chance between the enzyme and substrate at the elevated temperature may improve the reaction rate. Further increasing temperature may disrupt the enzyme conformation and decrease the enzyme activity. Since the yield was found to be the highest at 55 ºC, the optimum temperature for this reaction was 55 ºC.

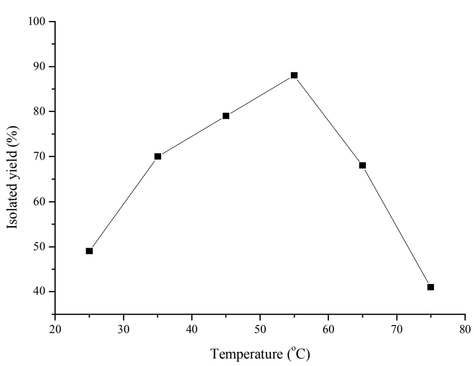
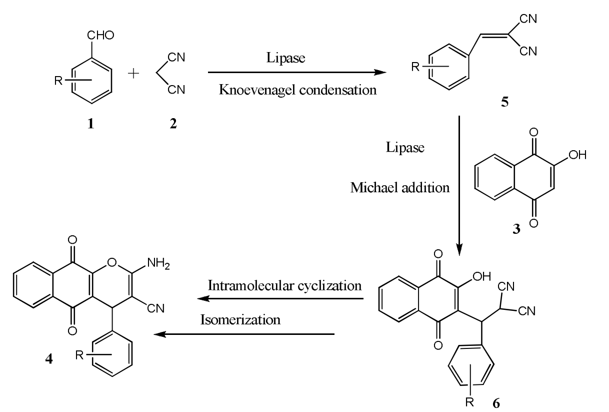



**Fig. 2** Temperature effect on the synthesis of benzo[g]chromene derivatives catalyzed by lipase

Reaction condition: 2-hydroxy-1,4-naphthoquinone (1 mmol), malononitrile (1 mmol) and benzaldehyde (1 mmol), ethanol (2mL), CSL (20 mg, protein content), 12h at different temperature.

To explore the scope and feasibility of this multi-component reaction, a series of benzo[g]chromene derivatives were synthesized under the optimum reaction conditions. The results in Table 2 demonstrated that the protocol could be applied to aromatic aldehydes either with electron-withdrawing groups (Entry 2-6) or electron-donating groups (Entry 7-9) with satisfied yields (from 81% to 93%). It is noteworthy that the electronic nature of substituents of the aromatic aldehyde has no distinct effect on the multi-component reaction.

The lipase-catalyzed Knoevenagel condensation and Michael addition have been reported previously [10, 24]. According to these reports and our results in this study, a plausible mechanism was proposed in Scheme 2. The synthesis of benzo[g]chromene derivatives involves a Knoevenagel condensation, Michael addition, cyclization and isomerization, respectively. As shown in Scheme 2, lipase could catalyze the steps of Knoevenagel condensation and Michael addition during the reaction process. Firstly, enzymatic Knoevenagel condensation of the aldehyde **1** to malononitrile **2** was occurred to produce an intermediate **5**. Secondly, Michael addition of the 2-Hydroxy-1,4-naphthoquinone **3** on the intermediate **5** could be catalyzed by lipase to produce an intermediate **6**. Finally, intramolecular cyclization and isomerization formed the product **4** automatically. It's important to note that the benzo[g]chromene derivatives obtained in this study were racemic when all the screened lipases were used as catalyst, which indicated that lipase didn't exhibit the stereoselectivity in the Michael addition of this reaction. This phenomenon was in accordance with the recent reported literatures [25, 26].




**Scheme 2** Mechanism of the lipase-catalyzed synthesis of benzo[g]chromene derivatives

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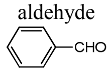
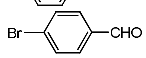
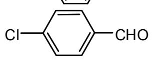
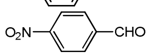
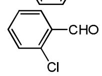
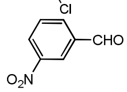
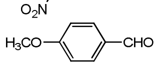
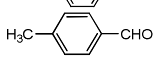
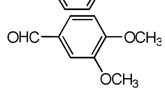
**Table 2** Lipase-catalyzed synthesis of benzo[g]chromene derivatives with different aromatic aldehydes [a]

| Entry | Aromatic aldehyde | Product | Isolated yield (%) |
|---|---|---|---|
| 1 |  | **4a** | 88 |
| 2 |  | **4b** | 90 |
| 3 |  | **4c** | 91 |
| 4 |  | **4d** | 93 |
| 5 |  | **4e** | 89 |
| 6 |  | **4f** | 85 |
| 7 |  | **4g** | 86 |
| 8 |  | **4h** | 83 |
| 9 |  | **4i** | 81 |

[a] Reaction condition: 2-Hydroxy-1,4-naphthoquinone (1 mmol), malononitrile (1 mmol) and aromatic aldehyde (1 mmol), ethanol (2mL), CSL (20 mg, protein content), 55 °C, 12h.

## Conclusions

In summary, an efficient and simple method for the synthesis of benzo[g]chromene derivatives catalyzed by lipase was reported for the first time. After thorough optimization of reaction conditions, all the products could be obtained in high yields (from 81% to 93%). Compared with the reported methods [27-33], the notable features of this new synthetic route are not only atom economy, environmental friendliness and simple operational process, but more importantly, this work significantly expands the utility of lipase in organic synthesis and encourages us to use the current tools of enzyme engineering and directed evolution to increase the catalytic performance of lipase. It's known that immobilization is a powerful tool to avoid the enzyme aggregation in organic solvent and recover and reuse of the enzyme with high remnant activity [34-37]. Further study of the immobilization enzyme on the lipase-catalyzed synthesis of benzo[g]chromene derivatives is now in progress in our laboratory.

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## Notes and references

[a] *Key Laboratory of Molecular Enzymology and Engineering of Ministry of Education, Jilin University, Changchun 130023, P R China;Email: wangzhi@jlu.edu.cn, w_lei@jlu.edu.cn*
[b] *School of life sciences, Jilin University, Changchun 130023, P R China;*
[c] *College of Chemistry, Jilin University, Changchun 130023, P R China;*

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‡ A typical enzymatic procedure of the reaction: CSL (20 mg, protein content) was added to a 25 mL round-bottom flask containing aromatic aldehyde (1 mmol), malononitrile (1 mmol) and ethanol (2 mL). The suspension was maintained at 55 °C for 10 min. Then, 2-hydroxy-1,4-naphthoquinone (1 mmol) was added to the reaction mixture. After completion of the reaction (after 12 h, monitored by TLC), the reaction mixture was concentrated under vacuum. The residue was washed with water and cold diethyl ether three times to remove unreacted starting materials and other organic contaminations, and then the filter cake was recrystallized from 95% ethanol to give products **4** with high purity. The experiments were performed triplicate, and all data were obtained based on the average values. The products were characterized by NMR and ESI-MS experiments.

1 E. Ruijter, R. Scheffelaar and R. V. A. Orru, *Angew. Chem. Int. Ed.*, 2011, **50**, 6234.
2 B. Ganem, *Accounts Chem. Res.*, 2009, **42**, 463.
3 H. Bienaymé, C. Hulme, G. Oddon and P. Schmitt, *Chem. - Eur. J.*, 2000, **6**, 3321.
4 R. M. Armstrong, A. P. Combs, P. A. Tempest, S. D. Brown and T. A. Keating, *Acc. Chem. Res.*, 1996, **29**, 123.
5 I. Nobeli, A. D. Favia and J. M. Thornton, *Nat. Biotechnol.*, 2009, **27**, 157.
6 L. L. Torres, A. Schließmann, M. Schmidt, N. Silva-Martin, J. A. Hermoso, J. Berenguer, U. T. Bornscheuer and A. Hidalgo, *Org. Biomol. Chem.*, 2012, **10**, 3388.
7 C. Branneby, P. Carlqvist, A. Magnusson, K. Hult, T. Brinck and P. Berglund, *J. Am. Chem. Soc.*, 2003, **125**, 874.
8 M. Svedendahl, K. Hult and P. Berglund, J. Am. Chem. Soc., 2005, **127**, 17988.
9 M. T. Reetz, R. Mondiere and J. D. Carballeira, Tetrahedron Lett., 2007, **48**, 1679.
10 W. B. Wu, N. Wang, J. M. Xu, Q. Wu and X. F. Lin, Chem. Commun., 2005, 2348.
11 Y. F. Lai, H. Zheng, S. J. Chai, P. F. Zhang and X. Z. Chen, *Green Chem.*, 2010, **12**, 1917.
12 H. R. Wang, Z. Wang, C. Y. Wang, F. J. Yang, H. Zhang, H. Yue and L. Wang, *RSC adv.*, 2014, **4**, 35686.
13 Z. G. Le, L. T. Guo, G. F. Jiang, X. B. Yang and H. Q. Liu, *Green Chem. Lett. Rev.*, 2013, **6**, 277.
14 F. J. Yang, Z. Wang, H. R. Wang, H. Zhang, H. Yue and L. Wang, *RSC Adv.*, 2014, **4**, 25633.
15 A. M. El-Agrody, M. H. El-Hakim, M. S. A. Abd El-Latif, A. H. Fakery, M. El-Sayed and K. A. El-Ghareab, *Acta Pharm.*, 2000, **50**, 111.
16 J. Zamocka, E. Misikova and J. Durinda, *Cesk Farm*, 1992, **41**, 170.
17 S. J. Mohr, M. A. Chirigos, F. S. Fuhrman and J. W. Pryor, *Cancer Res.*, 1975, **35**, 3750.

18 M. Brunavs, C. P. Dell and W. M. Owton, *J. Fluor. Chem.*, 1994, **68**, 201.
19 V. K. Tandon, M. Vaish, S. Jain, D. S. Bhakuni and R. C. Srimal, *Indian J. Pharm. Sci.*, 1991, **53**, 22.
20 P. Coudert, J. M. Coyquelet, J. Bastide, Y. Marion and J. Fialip, *Ann. Pharm. Fr.*, 1988, **46**, 91.
21Y. K. Huang, S. W. Tsai, *Appl. Microbiol. Biotechnol.*, 2014, **98**, 621.
22 Y. S. Lin, P. Y. Wang, A. C. Wu and S. W. Tsai, *J. Mol. Catal. B-Enzym.*, 2011, **68**, 245.
23 R. Tian, C. H. Yang, X. F. Wei, E. N. Xun, R. Wang, S. G. Cao, Z. Wang and L. Wang, *Biotechnol. Bioprocess Eng.*, 2011, **16**, 337.
24 Z. Wang, C. Y. Wang, H. R. Wang, H. Zhang, Y. L. Su, T. F. Ji and L. Wang, *Chin. Chem. Lett.*, 2014, **25**, 802.
25 P. Steunenberg, M. Sijm, H. Zuilhof, J. P. M. Sanders, E. L. Scott and M. C. R. Franssen, *J. Org. Chem.,* 2013, **78**, 3802.
26 J. L. Wang, J. M. Xu, Q. Wu, D. S. Lv and X. F. Lin, *Tetrahedron*, 2009, **65**, 2531.
27 X. H. Wang, X. H. Zhang, S. J. Tu, F. Shi, X. Zou, S. Yan, Z. G. Han, W. J. Hao, X. D. Cao and S. S. Wu, J. Heterocyclic Chem., 2009, **46**, 832.
28 K. Azizi and A. Heydari, *RSC Adv.*, 2014, **4**, 6508.
29 C. S. Yao, C. X. Yu, T. J. Li and S. J. Tu, *Chin. J. Chem.*, 2009, **27**, 1989.
30 Y. Yu, H. Y. Guo and X. J. Li, *J. Heterocyclic Chem.*, 2010, **48**, 1264.
31 M. G. Dekamin, M. Eslami and A. Maleki, *Tetrahedron*, 2013, **69**, 1074.
32 J. M. Khurana, B. Nand and P. Saluja, *Tetrahedron*, 2010, **66**, 5637.
33 J. M. Khurana, D. Magoo and A. Chaudhary, *Synth. Comm.*, 2012, **42**, 3211.
34 R. C. Rodrigues, C. Ortiz, A. Berenguer-Murcia, R. Torres and R. Fernández-Lafuente, *Chem. Soc. Rev.*, 2013, **42**, 6290.
35 E. N. Xun, X. L. Lv, W. Kang, J. X. Wang, H. Zhang, L. Wang and Z. Wang, *Appl. Biochem. Biotechnol.*, 2012, **168**, 697.
36 K. Hernandez and R. Fernández-Lafuente, *Enzyme Microb. Technol.*, 2011, **48**, 107.
37 C. Garcia-Galan, A. Berenguer-Murcia, R. Fernández-Lafuente and R. C. Rodrigues, *Adv. Synth. Catal.*, 2011, **353**, 2885.