Efficient synthesis of 3-hydroxy chromones via oxidative cyclization mediated by lipase

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ABSTRACT
In this work, an efficient synthesis of 3-hydroxy chromones was accomplished through oxidative cyclization mediated by lipase. This enzymatic process has salient features of environmental friendliness, mild reaction condition, satisfactory yield, green reusable biocatalyst, and short reaction time. The proposed method expands the application of lipase in organic synthesis.

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Introduction
Chromones are privileged structural skeleton in flavonoid natural products and functional molecules with a vast range of applications in clinical medicine and functional materials (1, 2). Among all chromones, 3-hydroxy-chromnone (3-HC) and its derivatives represent an important class, which exhibits fluorescent properties due to the excited state of the intramolecular proton transfer (ESIPT) process (3). The noticeable spectral properties make 3-HC derivatives a useful fluorescent sensor of normal and supercritical liquids, lipid membranes, DNA, and proteins (4–6). Therefore, the synthesis of 3-HC derivatives has drawn great attention among organic chemists over the last few decades.

Many synthesis methods for 3-HCs have been reported using different substrates. Syzuku’s group reported a frequently used method that involves the oxidation of the α-position of carbonyl group and subsequent cleavage of C–N bond by K₃Fe(CN)₆ to afford 3-HCs (Scheme 1, a) (7). Reddy and co-workers described the synthesis of 3-HC from 3-formylchromone via Bayer–Villiger oxidation (Scheme 1, b) (8). Moriarty et al. reported a cascade reaction to synthesize 3-HC involving the oxidation of chromone and hydroxylation (Scheme 1, c) (9). In 2020, Behera’s group developed an efficient method for synthesis of various 3-HCs via enamino ketone followed by oxidative cyclization using mCPBA (Scheme 1, d) (10). Most of these methods require expensive and environmentally harmful reagents, such as organic peracids or metal catalysts. Therefore, the development of new mild oxidative methodologies for the synthesis of 3-HCs is an urgent issue.

Lipase (EC 3.1.1.3) is a widely used enzyme in organic synthesis due to its broad enzyme catalytic promiscuity,
which refers to the ability of an enzyme to catalyze non-natural reactions in its active site (11–15). Lipase can cata-lyze the synthesis of peracids via the perhydrolysis of carboxylic acids or esters. In situ generated peracids are used in many controllable oxidation reactions, such as epoxidation, Baeyer–Villagers reaction, and oxidations of amines and alcohols (16–22). Compared with chemical methods, these lipase-mediated oxidation techniques have several advantages, such as mild reaction condition, environmental friendliness, and green reusable biocatalyst. In our continuous effort to develop new applications of lipase in organic synthesis, we report a new lipase-mediated oxidative synthesis of 3-HCs for the first time (Scheme 2).

Results and discussion

In this study, ethyl acetate was used as a reaction medium and a substrate to in situ generate peroxyacetic acid. Urea–hydrogen peroxide (UHP) was used as oxidant. Lipase-mediated oxidation initially used o-hydroxyphenyl enaminone (1a) as a model substrate for the synthesis of 3-HC (2a). As shown in Table 1, MML and CSL exhibited a relatively lower catalytic ability (entries 1–2), and CalB afforded a moderate yield (68 ± 2.6%) of 3-HC in this oxidation (entry 3), while an extremely high yield (97 ± 1.3%) could be obtained within a short reaction time (0.5 h) when Novozym 435 (an immobilized CalB) was used (entry 7). The reason for this might be that the hydrophobic immobilized carrier used in Novozym 435 helped to maintain the active conformation of the enzyme molecule in the reaction medium, stabilize the active center of enzyme catalysis and allow the reaction to proceed efficiently. Only trace products were observed when BSL2, PPL or PSL was used in the reaction (entries 4–6). Furthermore, the denatured Novozym 435 (denatured by PMSF or heating) or BSA (without catalytic function) failed to mediate oxidation (entries 8–10). Hence, active center and conformation of enzyme are required for the in situ generation of peracid. Compared with lipase-mediated method, chemical method afforded a poor yield of 3-HC (23 ± 3.9%) even with 3 equiv. mCPBA in 5 h (entry 12). We also optimized the

Table 1. Lipase-mediated oxidation for the synthesis of 3-hydroxy chromone.

| Entry | Catalyst     | Yield (%) |
|-------|--------------|-----------|
| 1     | MML          | 31 ± 2.4  |
| 2     | CSL          | 10 ± 4.5  |
| 3     | CALB         | 68 ± 2.6  |
| 4     | BSL2         | Trace     |
| 5     | PPL          | Trace     |
| 6     | PSL          | Trace     |
| 7     | Novozym 435 | 97 ± 1.3  |
| 8     | Novozym 435 (denatured by PMSF) | N.D. |
| 9     | Novozym 435 (denatured by heating) | N.D. |
| 10    | BSA          | N.D.      |
| 11    | m-CPBA       | 23 ± 3.9  |

*aReaction condition: 1a (1 mmol), UHP (1.2 mmol), enzyme (10 mg, protein content), ethyl acetate (1 mL), R.T., 0.5 h.

bCalB (C. antarctica lipase B), MML (Lipase from Aspergillus niger), CSL (Lipase from Candida sp. 99–125), BSL2(Lipase from Bacillus subtilis), PPL (Porcine pancreatic lipase), PSL (Lipase from Pseudomonas sp.), BSA (Albumin from bovine serum).

cIsolated yield.

dNot detected.

a1a (1 mmol), m-CPBA (3 mmol), DCM (5 mL), R.T., 5 h.
reaction conditions, such as enzyme dosage, reaction time and reaction medium (data shown in Figure S1–S3).

The effect of the amount of UHP was also investigated. As shown in Figure 1, the yield of 3-HC increased with increasing amount of UHP from 0.2 mmol to 1.2 mmol, and the yield increased slightly by further increasing the amount of UHP. Therefore, the optimal amount of UHP was 1.2 mmol in lipase-mediated oxidation. To evaluate the potential of the proposed method, we scaled up the lipase-mediated synthesis of 3-HC to 50-fold [1a (50 mmol), UHP (60 mmol), Novozym 435 (500 mg, protein content), ethyl acetate (50 mL), R.T., (0.5 h)]. A high yield (98%) of 3-HC was obtained. Therefore, this lipase-mediated method is efficient and attractive for the practical synthesis of 3-HC.

Enzyme instability and non-reusability limit their applications, which can be overcome by immobilization (23–25). As a widely used immobilized lipase, Novozym 435 exhibits excellent reusability in many practical applications. In this work, the reusability of Novozym 435 was evaluated under the optimized reaction conditions. After each batch, Novozym 435 obtained by filtering was washed with ethyl acetate and reused in the same reaction. As shown in Figure 2, the yield of 3-HC (2a) remained relatively high (81%) after five cycles. Thereafter, the catalytic performance of Novozym 435 decreased obviously. This result might be attributed to not only the slight leakage of lipase from support, but also the inactivation of enzyme by the oxidant (UHP) and peroxyacetic acid in the oxidation system. The inactivation of enzyme was also proven indirectly by the release of p-nitrophenol during the hydrolysis of p-nitrophenyl acetate.

Under the optimal conditions, a spectrum of substituted o-hydroxyphenyl enaminoles (1) were employed to investigate the scope and feasibility of this enzymatic method (Table 2). As predicted, the lipase-mediated reaction was conducted smoothly under mild condition within a short reaction time. Steric effects were found to affect the yield of 3-HCs, and para-substituted o-hydroxyphenyl enaminoles afforded the corresponding 3-HCs with higher yields (2b–2e). However, the electronic effect only slightly influenced the yield of 3-HCs. This enzymatic method exhibited good substrate tolerance and high efficiency for the synthesis of 3-HCs.

According to the experimental results and previous reports (26, 27), the possible mechanism of the reaction is proposed. As shown in Scheme 3, o-hydroxyphenyl enaminoles (1a) were epoxidized by peroxyacetic acid, which was in situ generated by Novozym 435. Spontaneous cyclization occurred to form intermediate I. Finally, the corresponding product (2a) was formed following the deamination of intermediate I.

**Experimental section**

**Materials**

CalB (C. antarctica lipase B), MML (Lipase from Aspergillus niger), CSL (Lipase from Candida sp. 99–125), PPL (Porcine pancreatic lipase), PSL (Lipase from Pseudomonas sp.) and BSA (Albumin from bovine serum) were purchased from Sigma-Aldrich China Co. (Beijing, China). BSL2 (Lipase from Bacillus subtilis) was expressed from a homely constructed Bacillus subtilis strain BSL2 (28). Novozym 435 (protein content: 10%) was purchased from Novo Nordisk Co., Ltd (Beijing, China). The quantity (per gram of carrier) of protein bound to the carrier in the commercial immobilized lipases were 100 mg for Novozym 435...
Table 2. Scope of lipase-mediated synthesis of 3-hydroxyl chromones.

| Reaction condition: | 1 (1 mmol), UHP (1.2 mmol), Novozym 435 (10 mg, protein content), ethyl acetate (1 mL), R.T., 0.5 h. |
|-------------------|-------------------------------------------------------------------------------------------------|

when determined using a conventional Kjeldahl method. o-Hydroxyacetophenone, DMF-DMA, urea hydrogen peroxide and all other chemical reagents were purchased from Bide Pharmatech. Ltd. and Energy-Chemical Ltd. (Shanghai, China). All commercially available reagents and solvents were used as received without further purification. Proton nuclear magnetic resonance (1H NMR) spectra were recorded on a 400 MHz spectrometer. Chemical shifts for protons are reported in parts per million downfield from tetramethylsilane (TMS) and are referenced to the residual protium in the NMR solvent. Analytical normal-phase high-performance liquid chromatography (HPLC) was carried out using a Shimadzu LC-20AT series instrument. The column purchased by Shanghai Chiralway Biotech. Co., Ltd. The chemical purity of products was determined with an HPLC equipped with a Superchiral S-OD (150 × 4.6 mm).

Scheme 3. Plausible mechanism for the synthesis of 3-HCs.

General procedure for the lipase-mediated synthesis of 3-HCs (2)

A mixture of o-hydroxyphenyl enaminone (1, 1 mmol), UHP (1.2 mmol), Novozym 435 (10 mg, protein content)
in ethyl acetate (1 mL) was stirred at room temperature in a round-bottom flask for 0.5 h. The reaction was monitored by TLC on 0.5 mm Silica gel sheet with ethyl acetate/hexane (1/2). Then, the mixture was filtered and the residue was washed with ethyl acetate. The combined organic phases were concentrated under vacuum, and the resulting residue was purified by column chromatography on silica gel with ethyl acetate/hexane (1/2) to afford the purified 3-HCs. The isolated yield of 3-HC (2) was calculated based on the purified 3-HC (2) and 1 mmol of starting substrate (1).

Isolated yield (2) = \frac{\text{Molarity of purified 3-HC (2)}}{\text{Molarity of adopted o-hydroxyphenyl enaminoine (1, 1 mmol)}}

Each experiment was performed triplicate, and all the data were obtained based on the average values. All the isolated products were well characterized by their \textsuperscript{1}H-NMR spectral analysis and HPLC analysis.

Conclusions

In this work, we reported a reliable route for the synthesis of 3-HCs via oxidative cyclization in high yields (85–97%). Compared with chemical methods, the proposed enzymatic process is characterized by environmental friendliness, mild reaction condition, satisfactory yield, green reusable biocatalyst, and short reaction time. Furthermore, Novozym 435 was reused for over five cycles and remained to have high catalytic performance. This work not only presents the widespread application of the proposed method for the synthesis of chromone derivatives but also provides a new case for enzyme catalytic promiscuity to contribute to the progress of novel synthesis methodology in organic synthesis.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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References

[1] Gaspar, A.; Matos, M.J.; Garrido, J.; Uriarte, E.; Borges, F. Chromone: A Valid Scaffold in Medicinal Chemistry. Chem. Rev. 2014, 114 (9), 4960–4992.
[2] Dziuba, D.; Karpenko, I.A.; Barthes, N.P.F.; Michel, B.Y.; Klymchenko, A.S.; Benhida, R.; Demchenko, A.P.; Mely, Y.; Burger, A. Rational Design of a Solvatochromic Fluorescent Uracil Analogue with a Dual-band Ratiometric Response Based on 3-Hydroxy Chromone. Chem.-Eur. J. 2014, 20 (7), 1998–2009.
[3] Perveaux, A.; Lorphelin, M.; Lasorne, B.; Laugernat, D. Fast and Slow Excited-State Intramolecular Proton Transfer in 3-Hydroxychromone: A Two-State Story? Phys. Chem. Chem. Phys. 2017, 19 (9), 6579–6593.
[4] Li, X.; Li, J.; Dong, X.W.; Gao, X.; Zhang, D.; Liu, C.L. A Novel 3-Hydroxychromone Fluorescence Sensor for Intracellular Zn\textsuperscript{2+} and its Application in the Recognition of Prostate Cancer Cells. Sens. Actuators B Chem. 2017, 245, 129–136.
[5] Klymchenko, A.S.; Ozturk, T.; Pivovarenko, V.G.; Demchenko, A.P. A 3-Hydroxychromone with Dramatically Improved Fluorescence Properties. Tetrahedron Lett. 2001, 42 (45), 7967–7970.
[6] Liu, J.J.; Chen, X.Z.; Zhang, Y.Y.; Gao, G.; Zhang, X.Y.; Hou, S.C.; Hou, Y.X. A Novel 3-Hydroxychromone Fluorescent Probe for Hydrogen Sulfide Based on an Excited-State Intramolecular Proton Transfer Mechanism. New J. Chem. 2018, 42 (15), 12918–12923.
[7] Murata, A.; Ito, T.; Fujiyasu, K.; Suzuki, T. Reaction of 3-Hydroxychromone with Metallic Ions. Bunseki Kagaku 1966, 15, 143–149.
[8] Reddy, K.C.; Mallaiiah, B.V.; Sriramannayana, G. Bayer-Villiger Oxidation of Chromone 3-Carboxaldehydes—a Facile Method for the Synthesis of 3-Hydroxychromones. Curr. Sci. 1980, 49 (1), 18–19.
[9] Constantino, M.G.; Lacerda, V.J.; Da Silva, G.V.J. An Efficient Synthesis of 3-Hydroxychromone Using Niobium Pentachloride. J. Heterocycl. Chem. 2003, 34 (3), 369–371.
[10] Gudipati, R.; Kandula, V.; Raghavulu, K.; Basavaiah, K.; Yennam, S.; Behera, M. Peroxy-benzoic Acid Mediated Domino C(sp2) Hydroxylation /Annulation of Enaminones for the Synthesis of 3-Hydroxy Chromones. Chemistry Select 2020, 5 (23), 7093–7097.
[11] Hult, K.; Berglund, P. Enzyme Promiscuity: Mechanism and Applications. Trends Biotechnol. 2007, 25 (5), 231–238.
[12] Leveson-Gower, R.B.; Mayer, C.; Roelfes, G. The Importance of Catalytic Promiscuity for Enzyme Design and Evolution. Nat. Rev. Chem. 2019, 3 (12), 687–705.
[13] Singla, P.; Bhardwaj, R.D. Enzyme Promiscuity – A Light on the “Darker” Side of Enzyme Specificity. Biocatal. Biotransform. 2020, 38 (2), 81–92.
[14] Li, F.; Tang, X.Y.; Xu, Y.N.; Wang, C.; Wang, Z.J.; Li, Z.Q.; Wang, L. A Dual-Protein Cascade Reaction for the Regioselective Synthesis of Quinoxalines. Org. Lett. 2020, 22 (10), 3900–3904.
[15] Xu, Y.N.; Li, F.X.; Zhao, N.; Su, J.L.; Wang, C.Y.; Wang, C.D.; Li, Z.Q.; Wang, L. Environment-friendly and Efficient Synthesis of 2-Aminobenzo-Xazoles and 2-Aminobenzothiazoles Catalyzed by Vitreoscilla Hemoglobin Incorporating a Cobalt Porphyrin Cofactor. Green Chem. 2021, 23 (20), 8047–8052.
[16] Zhang, J.X.; Qian, W.W.; Wang, C.Y.; Cao, Z.Y.; Chen, S.S.; Zhang, L.; Zhang, Y.; Wang, L. Lipase-mediated Epoxidation of Alkenes in Supercritical Carbon Dioxide. Green Chem. Lett. Rev. 2018, 11 (4), 508–512.

[17] Wang, Z.; Chen, X.; Wang, C.Y.; Zhang, L.; Li, F.X.; Zhang, W.A.; Chen, P.; Wang, L. A Mild and Efficient Dakin Reaction Mediated by Lipase. Green Chem. Lett. Rev. 2017, 10 (4), 269–273.

[18] Yang, F.J.; Zhang, X.W.; Li, F.X.; Wang, Z.; Wang, L. A Lipase-Glucose Oxidase System for the Efficient Oxidation of N-Heteroaromatic Compounds and Tertiary Amines. Green Chem. 2016, 18 (12), 3518–3521.

[19] Silva, W.S.D.; Lapis, A.A.M.; Suarez, P.A.Z.; Neto, B.A.D. Enzyme-mediated Epoxidation of Methyl Oleate Supported by Imidazolium-Based Ionic Liquids. J. Mol. Catal. B: Enzym. 2011, 68 (1), 98–103.

[20] Mendez-Sanchez, D.; Rios-Lombardia, N.; Gotor, V.; Gotor-Fernandez, V. Chemoenzymatic Epoxidation of Alkenes Based on Peracid Formation by a Rhizomucor Miehei Lipase-Catalyzed Perhydrolysis Reaction. Tetrahedron 2014, 70 (6), 1144–1148.

[21] Kotlewksa, A.J.; van Rantwijk, F.; Sheldon, R.A.; Arends, I. Epoxidation and Baeyer-Villiger Oxidation Using Hydrogen Peroxide and a Lipase Dissolved in Ionic Liquids. Green Chem. 2011, 13 (8), 2154–2160.

[22] Zhao, Z.Y.; Zhang, L.; Li, F.X.; Tang, X.Y.; Ma, Y.W.; Wang, C.Y.; Wang, Z.; Zhao, R.; Wang, L. A Novel Oxidation of Salicyl Alcohols Catalyzed by Lipase. Catalysts 2017, 7 (12), 354.

[23] Boudrant, J.; Woodley, J.M.; Fernandez-Lafuente, R. Parameters Necessary to Define an Immobilized Enzyme Preparation. Process Biochem. 2020, 90, 66–80.

[24] Arana-Pena, S.; Carballares, D.; Morellon-Sterlling, R.; Berenguer-Murcia, A.; Alcantara, A.R.; Rodrigues, R.C.; Fernandez-Lafuente, R. Enzyme Co-immobilization: Always the Biocatalyst Designers’ Choice … or Not? Biotechnol. Adv. 2021, 51, 107584.

[25] Rodrigues, R.C.; Virgen-Ortiz, J.J.; dos Santo, J.C.S.; Berenguer-Murcia, A.; Alcantara, A.R.; Barbosa, O.; Ortiz, C.; Fernandez-Lafuente, R. Immobilization of Lipases on Hydrophobic Supports: Immobilization Mechanism, Advantages, Problems, and Solutions. Biotechnol. Adv. 2019, 37 (5), 746–770.

[26] Hussain, H.; Al-Harrasi, A.; Green, I.R.; Ahmed, I.; Abbas, G.; Rehman, N.U. meta-Chloroperbenzoic Acid (mCPBA): A Versatile Reagent in Organic Synthesis. RSC Adv. 2014, 4 (25), 12882–12917.

[27] Guo, Y.H.; Xiang, Y.F.; Wei, L.; Wan, J.P. Thermoinduced Free-Radical C-H Acyloxylation of Tertiary Enaminones: Catalyst-Free Synthesis of Acyloxyl Chromones and Enaminones. Org. Lett. 2018, 20 (13), 3971–3974.

[28] Ma, J.S.; Zhang, Z.M.; Wang, B.J.; Kong, X.J.; Wang, Y.G.; Cao, S.G.; Feng, Y. Overexpression and Characterization of a Lipase from Bacillus subtilis. Protein Expression Purif. 2006, 45 (1), 22–29.