Synthesis of functionalized 4H-Chromenes catalyzed by lipase immobilized on magnetic nanoparticles

Weian Zhang\textsuperscript{a}, Peng Chen\textsuperscript{b}, Ziyuan Zhao\textsuperscript{a}, Lei Wang\textsuperscript{a}, Shujin Wang\textsuperscript{c}, Ying Tang\textsuperscript{c}, Baoqin Wang\textsuperscript{c}, Zhi Wang\textsuperscript{a} and Hong Zhuang\textsuperscript{c}

\textsuperscript{a}Key Laboratory of Molecular Enzymology and Engineering of Ministry of Education, School of Life Sciences, Jilin University, Changchun, People’s Republic of China; \textsuperscript{b}The Second Hospital of Jilin University, Changchun, People’s Republic of China; \textsuperscript{c}College of Food Science and Engineering, Jilin University, Changchun, People’s Republic of China

ABSTRACT

In the work, \textit{mucor miehei} lipase (MML) was covalently immobilized on the 2,4,6-trichloro-1,3,5-triazine (TCT)-modified magnetite nanoparticles. Then, the immobilized MML was utilized in the synthesis of functionalized 4H-Chromenes via a multicomponent reaction firstly. Under the optimized reaction conditions, immobilized MML displayed high catalytic performance (Yield: 81–96\%) and excellent reusability, indicating a high potential for practical operation.

ARTICLE HISTORY

Received 22 January 2018
Accepted 2 May 2018

KEYWORDS

Lipase; multicomponent; magnetic nanoparticle; immobilization; 4H-chromene

Introduction

Multicomponent reaction (MCR), a simple and atom-economic synthetic approach, offers a remarkable handy protocol to synthesize the structurally diversified products with relevant biological activity (1–3). Therefore, MCR-based synthetic methodology is currently gaining more importance in synthetic chemistry. Recently, the use of enzyme has been extended to catalytic promiscuity to promote new reactions (4,5). Enzyme catalytic promiscuity is the ability of an enzyme active site to catalyze several different chemical transformations, such as Michael addition, aldol addition, Morita-Baylis–Hillman reaction, Knoevenagel reaction and perhydrolysis (6–10). Based on these findings, many enzymatic MCRs have been reported to generate complex bioactive molecules, and these researches have dramatically widened the application of enzyme catalytic promiscuity in synthetic chemistry (11–15). However, most of these enzymatic MCRs have encountered the problems of poor stability and difficulties in recovery and recycling for enzymes when these reactions were taken under the conditions far from their physiological environment. It’s known that these problems could be avoided by immobilization which is a useful technique in modern biotechnology (16–20).

Enzyme immobilization on magnetite nanoparticles (MNP) has attracted considerable attention recently for its unique advantages for the high specific surface area, low mass transfer resistance and convenient separation from the reaction mixture under a magnetic field (21,22). Many works have been reported that lipase could be immobilized on MNPs to enhance its stability and catalytic activity in enzymatic esterifications or resolutions (23–26). However, improvement of lipase catalytic performance on MCRs by immobilization on MNPs has not been reported yet. Among these MNPs, 2,4,6-trichloro-1,3,5-triazine (TCT) modified MNPs are the excellent support materials for covalent immobilization of enzyme which can improve the thermal and operational stability of enzyme (27). TCT-modified MNPs could be prepared in a two-step modification process (Scheme 1). The first modification step is functionalization of the surface of silica-coated MNPs by 3-animopropyltriethoxy silane (APTMS) to introduce amino groups on silica-coated MNPs. Subsequently, amino silica is treated with TCT and the resulting TCT-modified MNPs can be used as support for
covalent immobilization of enzyme. For example, Abbasi et al. reported the immobilization of glucose oxidase on TCT-modified MNPs, and the immobilized glucose oxidase presents excellent stability and activity (28). Porcine pancreas lipase (PPL) has been covalently immobilized on TCT-modified MNPs to increase its stability and hydrolysis activity (29). These findings encouraged us to enhance the catalytic performance and stability of lipase in enzymatic promiscuous reactions, especially MCRs, by immobilization on TCT-modified MNPs.

Functionalized 4H-Chromenes are important compounds which are known to possess various pharmaceutical activities such as spasmolytic, diuretic, anticoagulant, antimicrobial, antitumor, and antianaphylactic activities (30). Synthesis of functionalized 4H-Chromenes catalyzed by lipase has been proven to be a greener way than other methods (31,32). However, there are some drawbacks in these reports, such as the using of expensive enzyme and the difficulty to recycle enzyme from solid product even if enzyme was immobilized on SBA-15. Therefore, a suitable immobilized enzyme with high catalytic performance and easy separation from the reaction system is needed. To cut down the cost and make this enzymatic process attractive for industrial applications, in the current work, the immobilized *mucor miehei* lipase (MML) was prepared by covalent chemical attachment on TCT-modified MNPs for the first time. Then, this immobilized MML was utilized for the synthesis of functionalized 4H-Chromenes via MCR (Scheme 2). The reaction was carried out efficiently and the immobilized lipase exhibited an excellent reusability and operational simplicity.

**Results and discussion**

As a starting point, TCT-modified MNPs were prepared according to the method reported by Ranjbakhsh (29). The size and morphology of silica-coated MNPs were characterized by TEM technique. As shown in Figure 1, the average size of MNPs was estimated to be 10–15 nm.

![Figure 1. The TEM of silica-coated MNPs (Fe₃O₄@SiO₂).](image-url)

**Scheme 1.** Covalent immobilization of enzyme on TCT-modified MNPs.

**Scheme 2.** Synthesis of functionalized 4H-Chromenes catalyzed by immobilized lipase.

![Figure 2. FT-IR spectra of Fe₃O₄@SiO₂ (a), TCT-APTES-Fe₃O₄@SiO₂ (b) and immobilized MML (c).](image-url)
Table 1. The catalytic performance of free MML and immobilized MML.

| Entry | Catalyst             | Yield (%) |
|-------|----------------------|-----------|
| 1     | Free MML             | 32        |
| 2     | Immobilized MML      | 51        |
| 3     | None                 | N.D.      |
| 4     | TCT-modified MNPs    | N.D.      |

Reaction condition: salicylaldehyde (1.0 mmol), dimedone (1.0 mmol), β-naphthol (1.0 mmol), immobilized MML (270 mg, protein content: 20 mg, 98 U), 40°C, 3 h, ethanol 5 mL.

Figure 2 showed the FT-IR spectra of silica-coated MNPs (Fe3O4@SiO2, a), TCT-modified MNPs (TCT-APTES-Fe3O4@SiO2, b) and immobilized MML (c). The peak at 1650 cm\(^{-1}\) (the characteristic band of MML) revealed that MML was successfully attached to the TCT-modified MNPs.

The catalytic performance of immobilized MML was evaluated in the synthesis of functionalized 4H-Chromenes. The model MCR was performed with salicylaldehyde (1.0 mmol), dimedone (1.0 mmol), and β-naphthol (1.0 mmol) as the substrates and results were demonstrated in Table 1. It could be found that MML could catalyze the model MCR, and the immobilized MML exhibited a better activity for the synthesis of desired 4H-Chromene (4a) than free MML. The results of control experiments (entry 3–4) suggested that lipase was indeed an efficient catalyst in this MCR. Because MNPs were small and nonporous, lipase molecules might be dispersed on the surface of MNPs with a good orientation resulting in a higher affinity to substrate and more available active sites.

The solvent is important in the enzymatic reactions due to its effect on the enzyme activity. In this study, six organic solvents were used and the results were illustrated in Table 2. The reaction was obviously faster in protic/polar solvents (entry 1–2) than other solvents. Due to the stabilization of charged intermediates by protic/polar solvent, they can promote proton-transfer reactions, such as this lipase-catalyzed MCR.

Furthermore, the solvent can change the conformation of enzyme and then affect the enzyme catalytic performance obviously. Based on the above results, we chose ethanol as the optimum solvent for the next study.

Table 2. Effect of organic solvents on the synthesis of functionalized 4H-Chromenes.

| Entry | Solvent          | Yield (%) |
|-------|------------------|-----------|
| 1     | Methanol         | 42        |
| 2     | Ethanol          | 51        |
| 3     | Tetrahydrofuran  | 26        |
| 4     | Acetonitrile     | 20        |
| 5     | Dimethyl sulfoxide | 18     |
| 6     | Water            | 35        |

Reaction condition: salicylaldehyde (1.0 mmol), dimedone (1.0 mmol), β-naphthol (1.0 mmol), immobilized MML (270 mg, protein content: 20 mg, 98 U), 40°C, 3 h, solvent 5 mL.

The effect of temperature on the immobilized lipase-catalyzed synthesis of compound 4a was investigated (Figure 3). The yield kept an upward tendency as the reaction temperature increased (35–65°C), and slightly decreased by further elevating the temperature (65–75°C). Generally, high temperature can increase the collision between lipase and substrate, and then accelerate the lipase-catalyzed reaction. The good stability of immobilized MML in higher temperature is probably due to covalent conjugation of the enzyme with MNPs, which increases the rigidity of enzyme conformation and protects immobilized MML against thermal denaturation in organic solvent.

The effect of enzyme dosage on this enzymatic MCR was evaluated. As shown in Figure 4, the yields were obviously increased along with the increasing amounts of enzyme from 5 to 20 mg (protein content). However, a slight increase in yield could be obtained when enzyme dosage exceeded 20 mg. Considering the cost of enzyme and obtained yield, 20 mg (protein content) of immobilized MML was proved to be sufficient for this reaction.

The reusability of immobilized enzymes is a key factor affecting the utilization of an immobilized enzyme in practical application (33). In this work, the immobilized MML was recovered by magnetic separation easily, washed with ethanol, and then used again for the next batch. The results in Figure 5 demonstrated that 80% yield of compound 4a could be obtained even after six cycles. We also illustrated the residual activity of MML after each batch in Figure 6. After six cycles, the relative activity of the immobilized MML was 85% of initial.
activity (using p-nitrophenyl acetate). The excellent operational stability and convenience indicated that immobilized MML on TCT-modified MNPs has a high potential for industrial production.

With the optimized reaction conditions in hand, we then explored the scope and limitation of this method. Substituted salicylaldehydes were reacted with an active methylene compound (dimedone, 1,3-cyclohexanedione or N,N-dimethylbarbituric acid) and a nucleophile (β-naphthol or indole) in the synthesis of functional 4H-chromenes. It was found that substituted salicylaldehydes gave the desired 4H-chromenes in moderate to excellent yields. The salicylaldehydes with electron donating groups (Entry 2–4) afforded higher yields than those with electron withdrawing groups (Entry 5–7). All the active methylene compounds could be successfully employed to react with salicylaldehyde and β-naphthol, affording 9-substituted 4H-chromenes in high yields (Entry 8–9). Furthermore, a high yield could also be obtained when indole was used as nucleophile (Entry 10). It’s important to note that all the functionalized 4H-Chromenes obtained in this study were racemic when lipase was used as catalyst, which suggested that lipase didn’t present the stereoselectivity in this MCR.

**Experimental**

**Materials**

Silica-coated MNP (10–15 nm) was donated by Changchun BC&HC Pharmaceutical Technology Co. (Changchun, China). MML (3500 U) was obtained from Shanghai Dongfeng Biochemical Reagent Co., Ltd. (Shanghai, China). One unit of the enzyme activity was defined as the amount of enzyme required to hydrolyze 1 μmol of p-nitrophenyl acetate per minute at 30°C. All the other chemical reagents were purchased from J&K Scientific Ltd. (Beijing, China) and used as received. NMR spectra were measured on an Inova 500 (500 MHz) spectrometer (Vernon Hills, IL, USA). The infrared transmittance spectra were measured by an FTIR spectrometer (Nicolet 5700, Thermo Fisher, USA). Transmission electron microscopy analysis was recorded using a JEM-2100F electron microscope (JEOL, Japan).
Table 3. Synthesis of functionalized 4H-Chromenes catalyzed by immobilized MML.

| Entry | Aldehyde | Active methylene compound | Nucleophile | Product 4 | Yield (%) |
|-------|----------|---------------------------|-------------|-----------|-----------|
| 1     | ![Image](image1.png) | ![Image](image2.png) | ![Image](image3.png) | ![Image](image4.png) | 89        |
| 2     | ![Image](image5.png) | ![Image](image6.png) | ![Image](image7.png) | ![Image](image8.png) | 96        |
| 3     | ![Image](image9.png) | ![Image](image10.png) | ![Image](image11.png) | ![Image](image12.png) | 93        |
| 4     | ![Image](image13.png) | ![Image](image14.png) | ![Image](image15.png) | ![Image](image16.png) | 92        |
| 5     | ![Image](image17.png) | ![Image](image18.png) | ![Image](image19.png) | ![Image](image20.png) | 83        |
| 6     | ![Image](image21.png) | ![Image](image22.png) | ![Image](image23.png) | ![Image](image24.png) | 84        |
| 7     | ![Image](image25.png) | ![Image](image26.png) | ![Image](image27.png) | ![Image](image28.png) | 81        |
| 8     | ![Image](image29.png) | ![Image](image30.png) | ![Image](image31.png) | ![Image](image32.png) | 91        |

(Continued)
Silica-coated MNPs surface modification by TCT

The silica-coated MNPs were modified by TCT according to the reported method (29). First, the silica-coated MNPs (2 g) were reacted with APTES (60 mL) in organic solvent (ethanol: 100 mL) at 25°C for 2 h, which was followed by 1.5 h at 50°C. The amine-modified MNPs could be obtained from the cooled reaction system and washed with ethanol and tetrahydrofuran. Subsequently, the amine-modified MNPs were mixed with TCT (8 g) in tetrahydrofuran (200 mL) at 25°C for 3 h. The desired MNPs were washed with tetrahydrofuran, ethanol and deionized water. Finally, the TCT-modified MNPs were dried under vacuum at 30°C.

Immobilization of MML on TCT-modified MNPs

The TCT-modified MNPs (1 g) were suspended in 80 mL of PBS buffer solution (pH = 8.0). Then, the MML solution (protein content: 200 mg) was dropped into the solution and the mixture was shaken for 2 h at 25°C. The immobilized MML was separated and washed with PBS buffer solution (3*10 mL). Enzyme loading on MNPs was calculated by taking away the amount of free MML from the total added enzyme. The immobilization yield was 40% under the above reaction conditions. The specific activity of the immobilized MML (4900 U, using p-nitrophenyl acetate) increased about 1.4-fold when compared with that of the free lipase.

Typical procedure of the synthesis of functionalized 4H-Chromenes catalyzed by immobilized MML

Substrate 1 (1 mmol), 2 (1 mmol), 3 (1 mmol) and immobilized MML (98 U, 20 mg, protein content) were added in ethanol (5 mL) and incubated at 65°C for 3 h. The reaction was monitored by thin layer chromatography. After completion of this MCR, immobilized MML was separated in the presence of a magnetic stirrer bar. Then the reaction mixture was filtered, and the precipitate was flushed with cold ethanol and deionized water. The obtained solid was dried in vacuum at 30°C to afford the pure product. Structures of all the desired 4H-Chromenes were confirmed by the 1H-NMR spectral analysis.

Reusability of immobilized MML

To investigate the reusability of immobilized MML, batch reaction was performed under the optimized reaction conditions for 3 h. Then, immobilized MML was separated in the presence of a magnetic stirrer bar, and washed by cold ethanol. The recycled immobilized enzyme was reused directly for the next run.

Conclusions

In conclusions, mucor miehei lipase (MML) was successfully immobilized on TCT-modified MNPs and exhibited a satisfactory catalytic performance in the synthesis of functionalized 4H-Chromenes. Under the optimal conditions, high yields of functionalized 4H-Chromenes (81–96%) could be obtained in 3 h. It was noteworthy that immobilized MML presented a satisfactory reusability in this MCR. The notable advantages of this methodology are environmental friendliness, operational simplicity, short reaction time and good reusability. Furthermore, this study expands the utilization of lipase in synthetic chemistry.
Acknowledgments
We gratefully acknowledge the Foundation of Changchun BC&HC Pharmaceutical Technology Co., Ltd (no. 3R117W391465).

Disclosure statement
No potential conflict of interest was reported by the authors.

Notes on contributors
Weian Zhang is studying as a master candidate in School of Life Sciences, Jilin University. His research has focused on the synthesis and development of new drugs.

Peng Chen is working at Department of Pediatrics, the Second Hospital of Jilin University. Now, she is mainly investigating the biological activity of chiral drugs.

Ziyuan Zhao is studying as a master candidate in School of Life Sciences, Jilin University. His research has focused on the new synthetic methodologies to synthesize bioactive compounds.

Lei Wang is working in Key Laboratory of Molecular Enzymology and Engineering of Ministry of Education (Jilin University, Changchun, China) as a professor. Recently, his research has focused on the new synthetic methodologies to synthesize bioactive compounds.

Shujin Wang is studying as a master candidate in College of Food Science and Engineering, Jilin University. Her research has focused on the development and detection of functional foods.

Ying Tang is studying as a master candidate in College of Food Science and Engineering, Jilin University. Her research has focused on food chemistry.

Baoqin Wang is studying as a master candidate in College of Food Science and Engineering, Jilin University. Her research has focused on food chemistry.

Zhi Wang was born in 1973. He obtained his Ph.D. degree in 2003. Recently, his research has focused on the enzymatic resolution of chiral compounds.

Prof. Hong Zhuang, born in 1974. She is working in College of Food Science and Engineering, Jilin University, Changchun. Her research interest is food chemistry, mainly engaged in the design, development and detection of functional foods.

References
[1] Estévez, V.; Villacampa, M.; Menéndez, J.C. Recent Advances in the Synthesis of Pyroles by Multicomponent Reactions. Chem. Soc. Rev. 2014, 43 (13), 4633–4657.
[2] Rotstein, B.H.; Zaretsky, S.; Rai, V.; Yudin, A.K. Small Heterocycles in Multicomponent Reactions. Chem. Rev. 2014, 114 (16), 8323–8359.
[3] Cioc, R.C.; Ruijter, E.; Orru, R.V. Multicomponent Reactions: Advanced Tools for Sustainable Organic Synthesis. Green Chem. 2014, 16 (6), 2958–2975.
[4] Kapoor, M.; Gupta, M.N. Lipase Promiscuity and its Biochemical Applications. Process Biochem. 2012, 47,555–569.
[5] Miao, Y.; Rahimi, M.; Geerntema, E.M.; Poelarends, G.J. Recent Developments in Enzyme Promiscuity for Carbon-Carbon Bond-forming Reactions. Curr. Opin. Chem. Biol. 2015, 25, 115–123.
[6] Zhang, M.J.; Li, R.; He, Y.H.; Guan, Z. Pepsin-catalyzed Vinylogous Michael Addition of Deconjugated Butenolides and Maleimides in Water. Catal. Commun. 2017, 98, 85–89.
[7] Li, C.; Feng, X.W.; Wang, N.; Zhou, Y.J.; Yu, X.Q. Biocatalytic Promiscuity: The First Lipase-catalysed Asymmetric Aldol Reaction. Green Chem. 2008, 10 (6), 616–618.
[8] Reetz, M.T.; Mondièrè, R.; Carballeira, J.D. Enzyme Promiscuity: First Protein-catalyzed Morita-Baylis-Hillman Reaction. Tetrahedron Lett. 2007, 48 (10), 1679–1681.
[9] Hu, W.; Guan, Z.; Deng, X.; He, Y.H. Enzyme Catalytic Promiscuity: The Papain-catalysed Knoevenagel Reaction. Biochimie 2012, 94 (3), 656–661.
[10] Wang, Z.; Chen, X.; Wang, C.; 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.
[11] Chen, X.; Zhang, W.A.; Yang, F.J.; Guo, C.; Zhao, Z.Y.; Ji, D.; Zhou, F.; Wang, Z.; Zhao, R.; Wang, L. Synthesis of Dihydropyran[4,3-b]pyranes via a Multicomponent Reaction Catalyzed by Lipase. Green Chem. Lett. Rev. 2017, 10,54–58.
[12] Yang, F.J.; Wang, Z.; Wang, H.R.; Zhang, H.; Yue, H.; Wang, L. Enzyme Catalytic Promiscuity: Lipase Catalyzed Synthesis of Substituted 2H-Chromenes by a Three-component Reaction. RSC Adv. 2014, 4,25633–25636.
[13] Yin, D. H.; Liu, W.; Wang, Z.X.; Huang, X.; Zhang, J.; Huang, D.C. Enzyme-catalyzed Direct Three-component Aza-Diels-Alder Reaction Using Lipase from Candida sp. 99–125. Chin. Chem. Lett. 2017, 28 (1), 153–158.
[14] Kłossowski, S.; Wiraszka, B.; Berlożewski, S.; Ostaszewski, R. Model Studies on the First Enzyme-catalyzed Ugi Reaction. Org. Lett. 2013, 15,566–569.
[15] Yang, F.J.; Wang, H.R.; Jiang, L.Y.; Yue, H.; Zhang, H.; Wang, Z.; Wang, L. A Green and One-pot Synthesis of Benzo[g] Chromene Derivatives through a Multi-component Reaction Catalyzed by Lipase. RSC Adv. 2015, 5 (7), 5213–5216.
[16] Rodrigues, R.C.; Ortiz, C.; Berenguer-Murcia, Á.; Torres, R.; Fernández-Lafuente, R. Modifying Enzyme Activity and Selectivity by Immobilization. Chem. Soc. Rev. 2013, 42,6290–6307.
[17] Manoel, E.A.; dos Santos, J.C.; Freire, D.M.G.; Rueda, N.; Fernandez-Lafuente, R. Immobilization of Lipases on Hydrophobic Supports Involves the Open form of the Enzyme. Enzyme Microb. Technol. 2015, 71,53–57.
[18] Sheldon, R. A. Enzyme Immobilization: The Quest for Optimum Performance. Adv. Synth. Catal. 2007, 349 (8–9), 1289–1307.
[19] Rueda, N.; dos Santos, J.C.S.; Ortiz, C.; Torres, R.; Barbosa, O.; Rodrigues, R.C.; Berenguer-Murcia, Á.; Fernandez-Lafuente, R. Chemical Modification in the Design of Immobilized Enzyme Biocatalysts: Drawbacks and Opportunities. Chem. Rec. 2016, 16,1436–1455.
[20] Mehta, J.; Bhardwaj, N.; Bhardwaj, S.K.; Kim, K.H.; Deep, A. Recent Advances in Enzyme Immobilization Techniques: Metal-organic Frameworks as Novel Substrates. *Coord. Chem. Rev.* 2016, 322, 30–40.

[21] Vaghari, H.; Jafarizadeh-Malmiri, H.; Mohammadlou, M.; Berenjian, A.; Anarjan, N.; Jafari, N.; Nasiri, S. Application of Magnetic Nanoparticles in Smart Enzyme Immobilization. *Biotechnol. Lett.* 2016, 38 (2), 223–233.

[22] Hola, K.; Markova, Z.; Zoppellaro, G.; Tucek, J.; Zboril, R. Tailored Functionalization of Iron Oxide Nanoparticles for MRI, Drug Delivery, Magnetic Separation and Immobilization of Biosubstances. *Biotechnol. Adv.* 2015, 33 (6), 1162–1176.

[23] Akil, E.; Carvalho, T.; Bárea, B.; Finotelli, P.; Lecomte, J.; Torres, A.G.; Amaral, P.; Villeneuve, P. Accessing Regio- and Typo-selectivity of Yarrowia lipolytica Lipase in its Free Form and Immobilized onto Magnetic Nanoparticles. *Biochem. Eng. J.* 2016, 109, 101–111.

[24] Dyal, A.; Loos, K.; Noto, M.; Chang, S.W.; Spagnoli, C.; Shafi, K.V.; Ulman, A.; Cowman, M.; Gross, R. A. Activity of Candida rugosa Lipase Immobilized on γ-Fe₂O₃ Magnetic Nanoparticles. *J. Am. Chem. Soc.* 2003, 125 (7), 1684–1685.

[25] Huang, S.H.; Liao, M.H.; Chen, D.H. Direct Binding and Characterization of Lipase onto Magnetic Nanoparticles. *Biotechnol. Progr.* 2003, 19 (3), 1095–1100.

[26] Osuna, Y.; Sandoval, J.; Saade, H.; López, R. G.; Martinez, J.L.; Colunga, E.M.; de la Cruz, G.; Segura, E.P.; Arévalo, F.J.; Zon, M.A.; Fernández, H.; Ilyina, A. Immobilization of Aspergillus niger Lipase on Chitosan-coated Magnetic Nanoparticles Using Two Covalent-binding Methods. *Bioproc. Biosyst. Eng.* 2015, 38 (8), 1437–1445.

[27] Shahrestani, H.; Taheri-Kafkani, A.; Soozanipour, A.; Tavakoli, O. Enzymatic Clarification of Fruit Juices Using Xylanase Immobilized on 1, 3, 5-Triazine-functionalized Silica-encapsulated Magnetic Nanoparticles. *Biochem. Eng. J.* 2016, 109, 51–58.

[28] Abbasi, M.; Amiri, R.; Bordbar, A.K.; Ranjbakhsh, E.; Khosropour, A.R. Improvement of the Stability and Activity of Immobilized Glucose Oxidase on Modified Iron Oxide Magnetic Nanoparticles. *Appl. Surf. Sci.* 2016, 364, 752–757.

[29] Ranjbakhsh, E.; Bordbar, A.K.; Abbasi, M.; Khosropour, A.R.; Shams, E. Enhancement of Stability and Catalytic Activity of Immobilized Lipase on Silica-coated Modified Magnetite Nanoparticles. *Chem. Eng. J.* 2012, 179, 272–276.

[30] Hossein nia, R.; Mamaghani, M.; Tabatabaeian, K.; Shirini, F.; Rassa, M. An Expedient Regioselective Synthesis of Novel Bioactive Indole-substituted Chromene Derivatives via One-pot Three-component Reaction. *Bioorg. Med. Chem. Lett.* 2012, 22 (18), 5956–5960.

[31] Zhang, W.A.; Zhao, Z.Y.; Wang, Z.; Guo, C.; Wang, C.Y.; Zhao, R.; Wang, L. Lipase-catalyzed Synthesis of Indolyl 4H-Chromenes via a Multicomponent Reaction in Ionic Liquid. *Catalysts* 2017, 7 (6), 185.

[32] Xu, J.C.; Li, W.M.; Zheng, H.; Lai, Y.F.; Zhang, P.F. One-pot Synthesis of Tetrahydrochromene Derivatives Catalyzed by Lipase. *Tetrahedron* 2011, 67 (49), 9582–9587.

[33] Barbosa, O.; Ortiz, C.; Berenguer-Murcia, Á.; Torres, R.; Rodrigues, R.C.; Fernandez-Lafuente, R. Strategies for the One-step Immobilization-Purification of Enzymes as Industrial Biocatalysts. *Biotechnol. Adv.* 2015, 33 (5), 435–456.