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PROTEIN-PROMOTED SYNTHESIS OF CHROMENE DERIVATIVES VIA BIOCATALYTIC DOMINO REACTION

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and Yu-Ling Zhu**

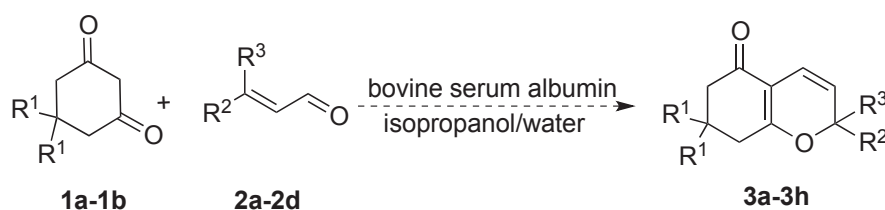
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Abstract – An efficient and green procedure has been developed for the synthesis of chromenes using 1,3-diketones and α,β -unsaturated aldehydes. The effect of reaction conditions including solvents, water content, molar ratio of substrates and catalyst loading and reaction temperature were investigated. A series of chromene derivatives were prepared with moderate to high yields under the catalysis of bovine serum albumin (BSA) in 2-propanol successfully. This novel activity of BSA to catalyze the domino reaction is not only of practical significance in expanding the application of biocatalysts, but also providing more convenient synthetic method for green chemistry.

INTRODUCTION

Chromenes are dominant heterocycle structural elements distributed in a wide range of natural products and as privileged precursors to a variety of bioactive molecules.¹ These structures are not only present in compounds which are important in biomedicine and pharmacy, but can also be used as pigments, photo-active materials and biodegradable agrochemicals.^{2,3} Owing to their frequent occurrence in nature, and the vast array of biological and photochromic properties, the development of new efficient synthesis protocols for the “privileged” structural motif is of great significance. Numerous synthetic approaches have been developed for the construction of structurally diverse and densely functionalized chromenes.⁴⁻⁷ However, biocatalysis methods for the synthesis of this type of compounds still remain scarce.^{8,9} Since the past decade, biocatalysis has emerged as an eco-friendly and sustainable synthetic methodology due to its simple processing requirements and high selectivity.¹⁰ Moreover, enzyme promiscuity, an emerging area in the field of biocatalysis, has largely extended the application of enzymes in organic synthesis and provided novel synthetic pathways that are not currently available.¹¹⁻¹⁵ Moreover, some proteins which have no natural catalytic function are able to catalyze certain synthetic reactions. Among

the proteins tested, bovine serum albumin (BSA) is considered to be a powerful one which has been reported with many kinds of catalytic activity. In the new study highlighted here, an efficient and green procedure has been attempted for the synthesis of chromenes using 1,3-diketones and α,β -unsaturated aldehydes (Scheme 1). The effect of reaction conditions including solvents, water content, molar ratio of substrates and catalyst loading and reaction temperature were investigated. A series of chromene derivatives were prepared with moderate to high yields under the catalysis of BSA in 2-propanol successfully.



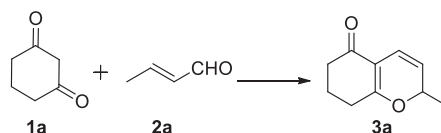
Scheme 1. BSA-catalyzed synthesis of chromenes

RESULTS AND DISCUSSION

Initial efforts were undertaken using 1,3-cyclohexanedione **1a** and crotonaldehyde **2a** as a model reaction. To select the appropriate biocatalyst, a series of commercially available lipases were screened (Table 1). For example, some lipases tested demonstrated the ability to catalyze the reaction with moderate yields (Table 1, entries 2-6). In order to exclude background activity, some additional control experiments were conducted. When the reaction was incubated in the absence of the catalyst (Table 1, entry 8), only 8% of the desired product was detected. To our surprise, denatured CRL (Table 1, entry 7) also showed moderate catalytic activity, not completely lost the activity. Instead, bovine serum albumin (BSA), which has no enzymatic activity, was preferred for the model reaction with 63% yield. All the results demonstrated that the catalysis was simply due to non-specific amino acid residues on the surface of the enzymes and that the specific catalytic site and spatial conformation of the natural lipases were not essential for their activities during the reaction. The BSA exhibited the highest activity may be due to the rich variety of surface amino acids of it. So far, several reactions catalyzed by BSA have been reported. For instance, in 2011, Gotor and colleagues¹⁶ discovered that BSA could promote the Henry reaction between aromatic aldehydes and 1-nitroalkanes in aqueous medium efficiently. Moreover, Sharma *et al.*¹⁷ have successfully developed a simple, inexpensive and waste-free methodology for the synthesis of bioactive 3,4-dihydropyrimidin-2(1*H*)-ones using BSA while exploring the promiscuous lipase catalyzed system for the asymmetric version of Biginelli reaction. In 2013, Lin and co-workers¹⁸ reported that the Gewald reaction could be catalyzed by BSA with satisfying yields. In 2014, Fadnavis group¹⁹ developed the Knoevenagel condensation of aldehydes with diethyl malonate in an environmentally friendly fashion

with immobilized BSA in DMSO at room temperature. In 2015, Guan *et al.*²⁰ described a green domino reaction for the synthesis of 2-amino-4*H*-chromene derivatives using BSA as a catalyst in ethanol. Recently, Li group²¹ discovered that BSA could serve as an efficient, retrievable catalyst in the one-pot four-component reaction of aryl aldehydes, malononitrile, hydrazine hydrate, and ethyl acetoacetate for the synthesis of pyrano[2,3-*c*]pyrazole derivatives under mild reaction conditions.

Table 1. Optimization of catalysts^a



Entry	Catalyst	Yield ^b [%]
1	bovine serum albumin (BSA)	63
2	lipase from <i>Candida antarctica</i> B (CAL-B)	54
3	lipase from <i>Mucor jaranicus</i> (MJL)	56
4	lipase from <i>Candida rugosa</i> (CRL)	54
5	lipase from <i>Candida cylindracea</i> (CCL)	40
6	lipase from <i>Candida rugosa</i> (CRL) ^c	30
7	—	8

^a Reaction conditions: **1a** (0.1 mmol), **2a** (0.5 mmol), catalyst (10 mg), DCM (950 μ L), 50 μ L H₂O, 270 rpm, 30 °C, 24 h. ^b Determined by HPLC. ^c Heated at 200 °C for 8 h.

To gain a much deeper insight of the reaction, several solvents with different polarities or other properties were investigated for the model reaction (**Figure 1**), and the results revealed that the solvent had a great

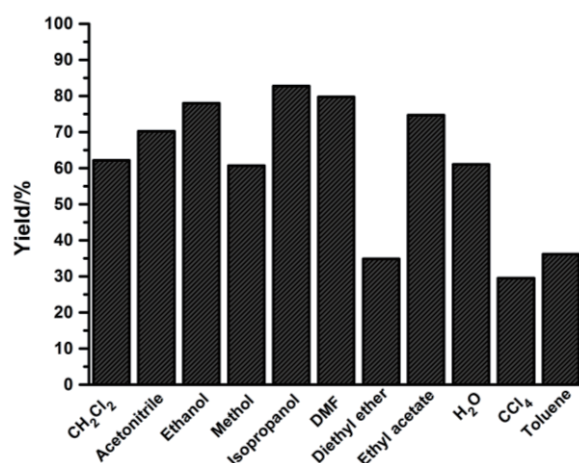


Figure 1. The effect of solvent on the BSA-catalyzed domino reaction^{a,b}

^a Reaction conditions: **1a** (0.1 mmol), **2a** (0.5 mmol), BSA (10 mg), organic solvent (950 μ L), H₂O (50 μ L) were added to a 10 mL test tube, and shaking at 270 rpm and 30 °C for 24 h. ^b All yields were determined by HPLC.

effect on the reaction process. The reaction in 2-propanol afforded the highest yield of 82% after 24 h. The reactions in other polar solvents also provided relatively good yields that ranged from 60 to 79%.

Non-polar solvents, however, produced relatively lower yields. Based on the above results, it seems that polar solvents favor the reaction may be potentially due to the good solubility of substrates and the surface amino acids of BSA were easier to expose to the substrates. From these findings, 2-propanol was chosen as the reaction medium in the following investigation.

Notably, the content of water was of great significance to the biocatalytic domino reaction. Therefore, experiments were performed with different amounts of water in the reaction, and the results were shown in **Figure 2**. It was found that the yield of the reaction could be enhanced by increasing the concentration of water slightly, this might be explained by the fact the change of microenvironment and cause the unfolding of the protein, which leads to the free basic amino group of BSA exposed to the substrates. The desired product could be obtained in the highest yield of 86% in 2-propanol at 10% (water/[water+2-propanol], v/v) water content. It is suggested that the yield of the reaction was decreased with increasing the concentration of water - the probable reason maybe that the increasing of water will decrease the solubility of the substrates.

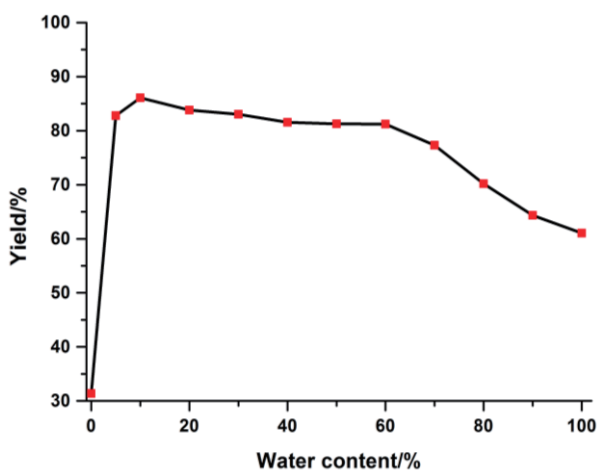


Figure 2. The effect of water content on the BSA-catalyzed domino reaction^{a,b}

^a Reaction conditions: **1a** (0.1 mmol), **2a** (0.5 mmol), solvent (1 mL), BSA (10 mg) were added to a 10 mL test tube, and shaking at 270 rpm and 30 °C for 24 h. ^b All yields were determined by HPLC.

Then the influence of molar ratio of substrates on the reaction between 1,3-cyclohexanedione (**1a**) to crotonaldehyde (**2a**) was investigated. As shown in **Figure 3**, it was found that the molar ratio had important influence on the yield of domino reaction. When the molar ratio of 1,3-cyclohexanedione to crotonaldehyde varied from 1/1 to 1/2, the yield was improved from 41 to 90%. Then the yield was slightly declined when the molar ratio continually increased from 1/2 to 1/40. This may be due to that excess crotonaldehyde was combined with the free basic amino group of BSA which may lead to some inactivation. However, the yield would decline sharply at 2/1 and the bell-shaped curves presented, no obvious difference was observed when continually increased from 5/1 to 20/1.

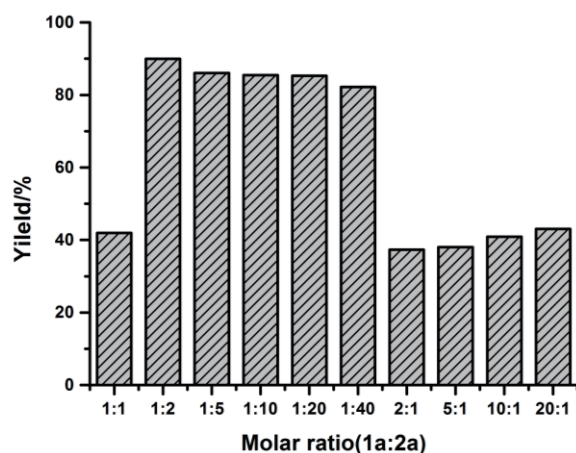


Figure 3. The effect of molar ratio on the BSA-catalyzed domino reaction^{a,b}

^a Reaction conditions: **1a**, **2a**, BSA (10 mg), 2-propanol (900 μ L), H₂O (100 μ L) were added to a 10 mL test tube, and shaking at 270 rpm and 30 °C for 24 h. ^b All yields were determined by HPLC.

The influence of protein loading on the reaction was examined to select the appropriate enzyme amount. The results are shown in **Figure 4**. When the catalyst loading was increased from 2 to 15 mg, the evident difference in the yields (16 to 95%) was observed. However, once the quantity of BSA surpasses 15 mg, there was a slightly decline in yield. The possible reason might be that more catalyst loading was unfavorable to the diffusion of the substrates. The results revealed that 15 mg BSA was the optimum quantity for the reaction. In addition, the influence of temperature (20-60 °C) on the reaction was investigated (Figure S1 in the Supporting Information). As shown in Figure S1, it was found that the reaction temperature had a very minimal impact on the reaction process. Consequently, 20 °C was chosen as the optimum temperature for further experiments.

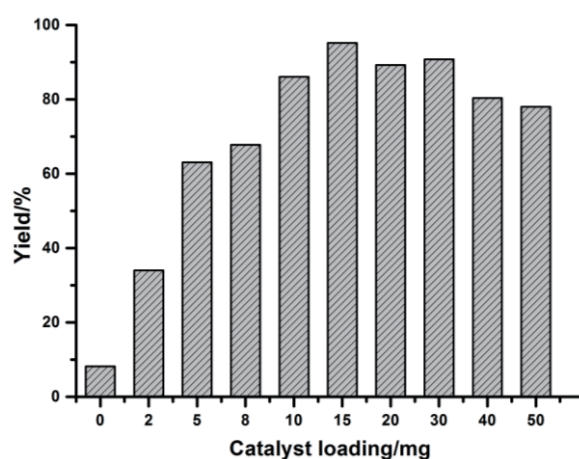


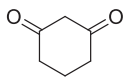
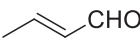
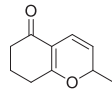
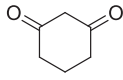
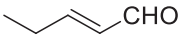
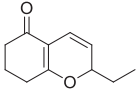
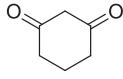
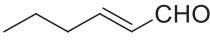
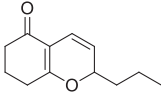
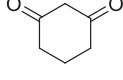
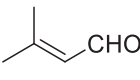
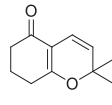
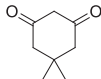
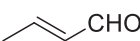
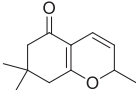
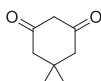
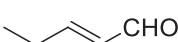
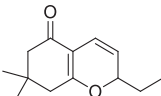
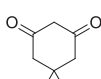
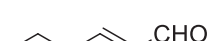
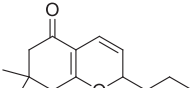
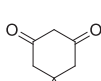
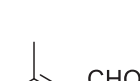
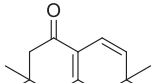
Figure 4. The effect of catalyst loading on the BSA-catalyzed domino reaction^{a,b}

^a Reaction conditions: **1a** (0.1 mmol), **2a** (0.2 mmol), BSA (0-50 mg), 2-propanol (900 μ L), H₂O (100 μ L) were added to a 10 mL test tube, and shaking at 270 rpm and 30 °C for 24 h. ^b All yields were determined by HPLC.

Next, in order to extend the scope of this reaction, several combinations of 1,3-diones and α,β -unsaturated

aldehydes were tested under the previously optimized reaction condition. According to Table 2, various substrates could smoothly react with each other and gave corresponding products with moderate to good yields. It was found that there was no distinctly difference between 5,5-dimethyl-1,3-cyclohexanedione

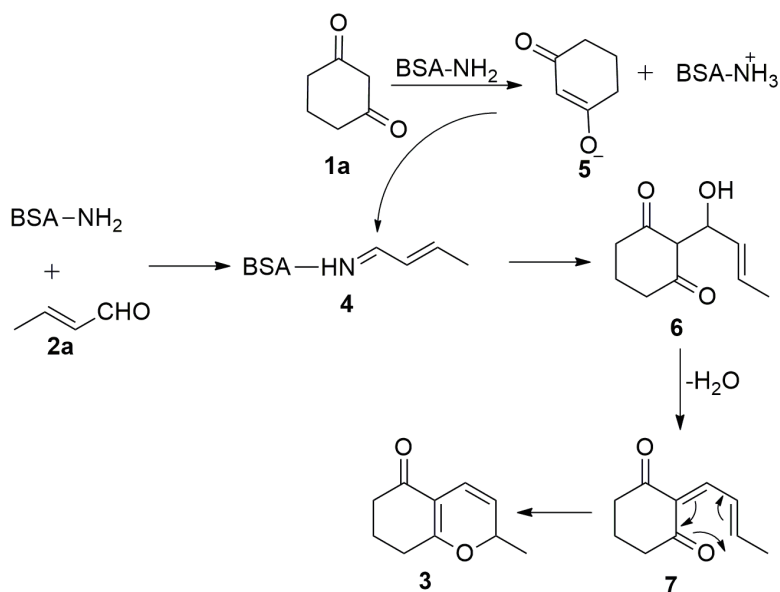
Table 2. Substrate scope of the BSA-catalyzed domino reaction^a

Entry	1	2	Product 3	Yield ^b [%]
1	 1a	 2a	 3a	95
2	 1a	 2b	 3b	83
3	 1a	 2c	 3c	71
4	 1a	 2d	 3d	73
5	 1b	 2a	 3e	93
6	 1b	 2b	 3f	79
7	 1b	 2c	 3g	69
8	 1b	 2d	 3h	65

^a Reaction conditions: **1** (1 mmol), **2** (2 mmol), BSA (150 mg), 2-propanol (4.5 mL), H₂O (500 μL) were added to a 25 mL round-bottom flask, and shaking at 20 °C and 270 rmp for 24 h. ^b Yield of the isolated product after chromatography on silica gel.

(Table 2, entry 5) and 1,3-cyclohexanedione (Table 2, entry 1) for that R^1 is far away from the active methylene group, hardly affects the domino process. Dimethyl substituted olefin aldehydes (Table 2, entry 4) at C3 olefin aldehydes exhibited lower reactivities than crotonaldehyde (Table 2, entry 1). Furthermore, the length of R^2 also influenced the reaction; ethyl substituted and *n*-propyl substituted olefin aldehydes (Table 2, entries 2 and 3) at C3 gave lower yields when compared to crotonaldehyde (Table 2, entry 1). This is probably owing to the relatively greater steric hinderance of R^2 and R^3 which would hinder the domino process and reduce the generation of chromenones.

According to the previous research and the above results, some amino acid side chain in BSA as the catalytic base for the domino reaction was responsible for the catalysis.^{4-7,18-23} Thus, a possible mechanism for the BSA-catalyzed domino reaction was deduced (Scheme 2). Firstly, free amine of the BSA attacks on the formyl group of crotonaldehyde **2a** leading to the formation of a covalent enamine intermediate **4**. Simultaneously, the removal of proton from active methylene group of 1,3-cyclohexanedione **1a** by the free basic amino group of BSA to form **5**. Next, an aldol reaction proceeded with the forming of **6**. Subsequently, the dehydration of **6** to give intermediate **7**, which finally underwent an intra-molecular oxy-Michael addition to afford the product **3**.



Scheme 2. The proposed mechanism

CONCLUSION

In summary, a highly efficient process for the preparation of chromenone derivatives mediated by BSA, with cyclic 1,3-diketone and α,β -unsaturated aldehydes as the starting materials were developed. This newly found reaction conditions allowed us a convenient synthetic method in green chemistry, and is supposed to expand the application of BSA-catalyzed organocatalytic reactions.

EXPERIMENTAL

Materials. MJL (Lipase M from *Mucor javanicus*, 10,000 U/g), CRL (Lipase from *Candida rugosa*, 700 U/mg), CCL (Lipase from *Candida cylindracea*, 42.8 U/mg), CAL-B (Lipase B from *Candida antarctica*, 2 U/mg) were purchased from Sigma-Aldrich. BSA (Bovine Serum Albumin) was purchased from Aladdin Co., Ltd. (Shanghai, China). Unless otherwise mentioned, all reagents were obtained from commercial suppliers and used without further purification. The NMR spectra were recorded on a Bruker 400 MHz instrument using CDCl₃ as solvents. Chemical shifts (δ) were expressed in ppm with TMS as internal standard, and coupling constants (J) were reported in Hz. HPLC was carried out on UltiMate3000 using Acclaim 120, C18 column (4.6 mm \times 250 mm). Elution was performed with a mixture of MeOH/H₂O (5/95-70/30, v/v) by gradient elution method at 0.8 mL/min at 30 °C and UV detection at 242 nm. HRMS were performed on Bruker Daltonics Bio TOF mass spectrometer. Column chromatography was carried on silica gel (200-300 mesh) using ethyl acetate-petroleum ether as mobile phase.

General procedure. A typical reaction mixture contained **1** (1 mmol), **2** (2 mmol), 150 mg BSA, 2-propanol (4.5 mL), H₂O (500 μ L) was shaken at 20 °C and 270 rpm. Then, after completion of the reaction, the residue was filtered off and the solvent was evaporated. The crude product was purified by flash column chromatography with EtOAc/petroleum ether (1:20 to 1:10) to obtain the pure product.

2-Methyl-7,8-dihydro-2H-chromen-5(6H)-one (3a). Pale yellow liquid; ¹H NMR (400 MHz, CDCl₃) δ 6.51-6.36 (m, 1H), 5.27 (dd, J = 10.0, 3.1 Hz, 1H), 5.00 (qdd, J = 6.5, 3.1, 1.7 Hz, 1H), 2.38 (ddd, J = 9.1, 7.4, 4.3 Hz, 4H), 1.98-1.93 (m, 2H), 1.40 (d, J = 6.6 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 194.87, 172.29, 118.86, 117.30, 111.33, 73.92, 36.42, 28.32, 21.67, 20.62; HRMS (ESI, m/z): [M+H]⁺ calcd. for C₁₀H₁₂O₂, 165.0916; found 165.0915.

2-Ethyl-7,8-dihydro-2H-chromen-5(6H)-one (3b). Pale yellow liquid; ¹H NMR (400 MHz, CDCl₃) δ 6.44 (dd, J = 10.1, 1.7 Hz, 1H), 5.35-5.19 (m, 1H), 4.83 (dtd, J = 7.6, 3.1, 1.6 Hz, 1H), 2.45-2.30 (m, 4H), 2.01-1.89 (m, 2H), 1.79-1.65 (m, 2H), 1.00-0.90 (m, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 194.99, 172.82, 117.71, 117.50, 111.33, 78.77, 36.34, 28.78, 28.24, 20.58, 8.55; HRMS (ESI, m/z): [M+H]⁺ calcd. for C₁₁H₁₄O₂, 177.0916; found 177.0911.

2-Propyl-7,8-dihydro-2H-chromen-5(6H)-one (3c). Pale yellow liquid; ¹H NMR (400 MHz, CDCl₃) δ 6.47-6.35 (m, 1H), 5.25 (dd, J = 10.0, 3.2 Hz, 1H), 4.92-4.81 (m, 1H), 2.42-2.29 (m, 4H), 1.97-1.88 (m, 2H), 1.76-1.65 (m, 1H), 1.64-1.54 (m, 1H), 1.42 (dddt, J = 17.3, 13.3, 10.0, 7.4 Hz, 2H), 0.91 (t, J = 7.4 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 194.71, 172.45, 117.81, 117.52, 111.37, 76.81, 37.83, 36.37, 28.24, 20.59, 17.53, 13.84; HRMS (ESI, m/z): [M+H]⁺ calcd. for C₁₂H₁₆O₂, 193.1229; found 193.1225.

2,2-Dimethyl-7,8-dihydro-2H-chromen-5(6H)-one (3d). Pale yellow liquid; ¹H NMR (400 MHz, CDCl₃) δ 6.35 (d, J = 10.0 Hz, 1H), 5.19 (d, J = 10.0 Hz, 1H), 2.34 (dt, J = 9.1, 6.7 Hz, 4H), 1.93 (dd, J = 13.0, 6.5 Hz, 2H), 1.35 (s, 6H); ¹³C NMR (100 MHz, CDCl₃) δ 194.78, 171.56, 122.82, 115.75, 110.47,

76.85, 36.36, 28.58, 28.35, 20.60. HRMS (ESI, m/z): $[M+H]^+$ calcd. for $C_{11}H_{14}O_2$, 179.1072; found 179.1072.

2,7,7-Trimethyl-7,8-dihydro-2H-chromen-5(6H)-one (3e). Pale yellow liquid; 1H NMR (400 MHz, $CDCl_3$) δ 6.40 (d, $J = 9.9$ Hz, 1H), 5.36-5.18 (m, 1H), 4.97 (ddd, $J = 6.5, 3.2, 1.6$ Hz, 1H), 2.30-2.18 (m, 4H), 1.43-1.34 (m, 3H), 1.06-1.01 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 194.55, 170.81, 118.61, 117.13, 110.22, 73.94, 50.34, 42.07, 32.20, 28.43, 28.34, 21.62; HRMS (ESI, m/z): $[M+H]^+$ calcd. for $C_{12}H_{16}O_2$ 193.1229; found 193.1229.

2-Ethyl-7,7-dimethyl-7,8-dihydro-2H-chromen-5(6H)-one (3f). Pale yellow liquid; 1H NMR (400 MHz, $CDCl_3$) δ 6.43 (dd, $J = 10.0, 1.4$ Hz, 1H), 5.25 (dd, $J = 10.0, 3.2$ Hz, 1H), 4.95-4.78 (m, 1H), 2.30-2.20 (m, 4H), 1.69 (ddd, $J = 13.0, 7.4, 4.6$ Hz, 2H), 1.03 (d, $J = 3.8$ Hz, 6H), 0.95 (t, $J = 7.4$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 194.51, 171.18, 117.55, 117.24, 110.18, 78.80, 50.31, 42.03, 32.14, 28.77, 28.40, 28.35, 8.52; HRMS (ESI, m/z): $[M+H]^+$ calcd. for $C_{13}H_{18}O_2$ 207.1385; found 207.1382.

7,7-Dimethyl-2-propyl-7,8-dihydro-2H-chromen-5(6H)-one (3g). Pale yellow liquid; 1H NMR (400 MHz, $CDCl_3$) δ 6.42 (dd, $J = 10.0, 1.2$ Hz, 1H), 5.26 (dd, $J = 10.0, 3.2$ Hz, 1H), 4.99-4.79 (m, 1H), 2.28-2.20 (m, 4H), 1.76-1.67 (m, 1H), 1.64-1.56 (m, 1H), 1.49-1.35 (m, 2H), 1.03 (d, $J = 2.2$ Hz, 6H), 0.92 (t, $J = 7.3$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 194.49, 171.03, 117.61, 117.34, 110.26, 77.50, 50.33, 42.05, 37.83, 32.16, 28.41, 28.35, 17.53, 13.87; HRMS (ESI, m/z): $[M+H]^+$ calcd. for $C_{14}H_{20}O_2$ 221.1542; found 221.1515.

2,2,7,7-Tetramethyl-7,8-dihydro-2H-chromen-5(6H)-one (3h). Pale yellow liquid; 1H NMR (400 MHz, $CDCl_3$) δ 6.39 (d, $J = 9.9$ Hz, 1H), 5.22 (d, $J = 9.9$ Hz, 1H), 2.24 (s, 4H), 1.38 (s, 6H), 1.06 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 194.50, 170.10, 122.68, 115.75, 109.54, 79.76, 50.41, 42.44, 32.24, 28.43, 28.34; HRMS (ESI, m/z): $[M+H]^+$ calcd. for $C_{13}H_{18}O_2$, 207.1385; found 207.1384.

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