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Synthesis, Structure Revision, and Anti-inflammatory Activity Investigation of Putative Blumeatin

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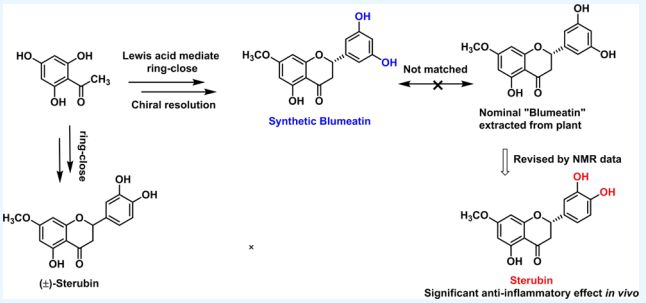
ABSTRACT: Blumeatin, reported herein, bearing two hydroxyl groups at C3′ and C5′ of ring B, is isolated from the traditional Chinese medicine *Blumea balsamifera*. But the isolation procedure of blumeatin from plants has limitations of prolonged duration and high cost. A procedure featuring Lewis acid-catalyzed ring closure and chiral resolution via Schiff base intermediates is provided here to prepare optically pure blumeatin and its *R*-isomer efficiently. Furthermore, the structure revision of putative blumeatin based on a logically synthetic procedure and NMR spectroscopic analysis was conducted. The 1D and 2D NMR data analysis unambiguously confirmed our proposal that the reported blumeatin structure has been misassigned as it corresponds to sterubin, which contains two hydroxyl groups at C3′ and C4′ of ring B. Finally, the results of the ear-swelling test exhibited that synthetic (±)-blumeatin and (±)-sterubin had moderate anti-inflammatory activity which was less than that of (−)-sterubin.



1. INTRODUCTION

Blumea balsamifera is a renowned medicinal plant in the Chinese Pharmacopoeia.[1−5] It is rich in chemical components such as flavonoids, sesquiterpenes, volatile oils, and sterols.[6,7] Blumeatin (Figure 1) is one of the flavanones with bioactivities

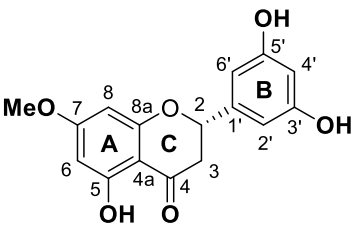


Figure 1. Structure of putative blumeatin.

extracted from *B. balsamifera*. The structure of blumeatin was reported to be (*S*)-2-(3,5-dihydroxyphenyl)-5-hydroxy-7-methoxychroman-4-one in the literature.[1,5,8−13] Particularly, blumeatin exhibited significant anti-inflammatory activity and promoted fat cell differentiation.[14,15] Our previous research indicated that the anti-inflammatory activity of blumeatin was slightly less than that of dexamethasone in an in vitro experiment.[16] However, isolation from *B. balsamifera* represents the main way to obtain blumeatin currently, which has low efficiency and high cost. Therefore, establishing a synthetic approach to obtain blumeatin could have an important significance in the use of blumeatin.

There are many ways to chemically synthesize flavonoids. Patel and Shah[17] reported a one-step procedure for flavone preparation via an iodine-mediated oxidative ring-closure reaction with chalcone. However, acquisition of chalcone precursors waas not very convenient, and the yields of the ring-closure reactions were not high enough. A *β*-diketone formed by the Baker−Venkataraman method[18,19] suffered from an acid-catalyzed ring-closure reaction to afford flavones. But this procedure was not environmentally friendly due to the large amount of waste acid produced. Applications of transition-metal catalysts could efficiently construct flavones,[20,21] but they were costly and tough operating conditions were required. Zhang's group[22] provided an approach for the preparation of flavones: a ring-closure reaction from substituted acetophenones and substituted benzaldehydes in the presence of boric acid and piperidine to generate flavones. This procedure was simple, efficient, and easy to operate. Therefore, we planned to prepare blumeatin based on Zhang's method.

We herein report a synthetic procedure to obtain blumeatin (Figure 2) involving the methylation of (2,4,6-trihydroxyphenyl)ethan-1-one, construction of the flavanone nucleus

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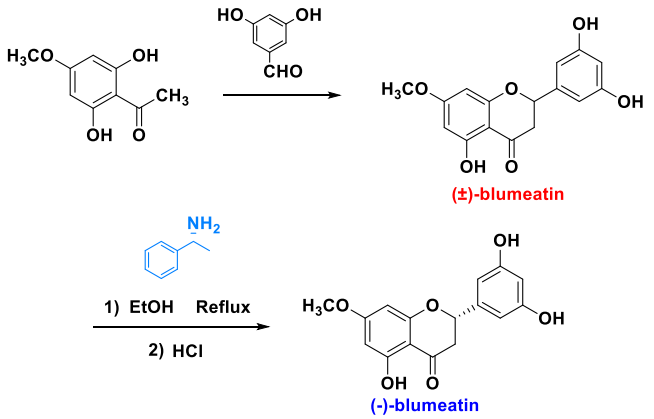

Figure 2. Synthetic route of (−)-blumeatin.

under Lewis acid catalysis, and chiral resolution via a Schiff base to produce optically active blumeatin.

2. RESULTS AND DISCUSSION

2.1. Synthesis of Optically Active (−)-Blumeatin and (+)-Blumeatin. The key step to construct blumeatin was the closure of the C-ring. It could be completed by the reaction of 3,5-dihydroxybenzaldehyde and 1-(2,6-dihydroxy-4-methoxyphenyl) ethan-1-one in the presence of boric acid, piperidine, and silica gel. Then, (±)-blumeatin (**1**) was obtained with a 75% total yield (Scheme 1). Fortunately, followed by imidization of 4-carbonyl of (±)-blumeatin with an optically pure (*R*)-1-phenylethan-1-amine, the diastereomeric imine intermediates could be separated favorably by silica column chromatography to give the same amount of optically active **2A** and **2B**. Finally, an acid-mediated hydrolysis produced optically pure (+)-blumeatin (**1A**) or (−)-blumeatin (**1B**), respectively.

2.2. Electronic Circular Dichroism Analysis of 1A and 1B. To clarify the absolute configurations of **1A** and **1B**, the electronic circular dichroism (ECD) spectra of **1A** and **1B** were tested in MeOH (0.2668 mg/mL and 0.2735 mg/mL, respectively) in the 200−400 nm range. In the ECD spectrum of **1A**, a negative cotton effect is visible at 280 nm (Figure 3).

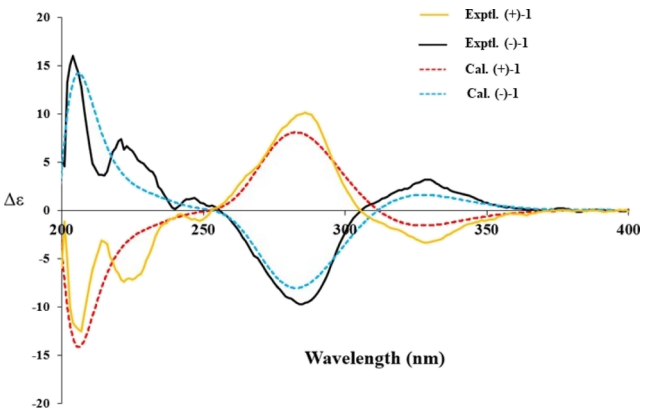

Figure 3. ECD spectra of **1A** and **1B**.

For **1B**, a positive cotton effect is observed at 290 nm. Simultaneously, ECD calculations were performed by the time-dependent density functional theory-predicted curve calculated at the quantum mechanical level. The calculated ECD spectra of **1A** and **1B** were consistent with their experimental counterparts. According to the ECD spectra rule of flavanones reported by Gaffield,[23] **1A** was assigned as an *R*-configuration and **1B** was an *S*-isomer.

2.3. Structure Revision of Putative Blumeatin. According to HRMS, ECD, ^{1}H NMR, ^{13}C NMR, and 2D-NMR data, combined with the watertight procedure and synthetic raw materials, the target molecules **1A** and **1B** were

Scheme 1. Syntheses of (+)-Blumeatin and (−)-Blumeatin

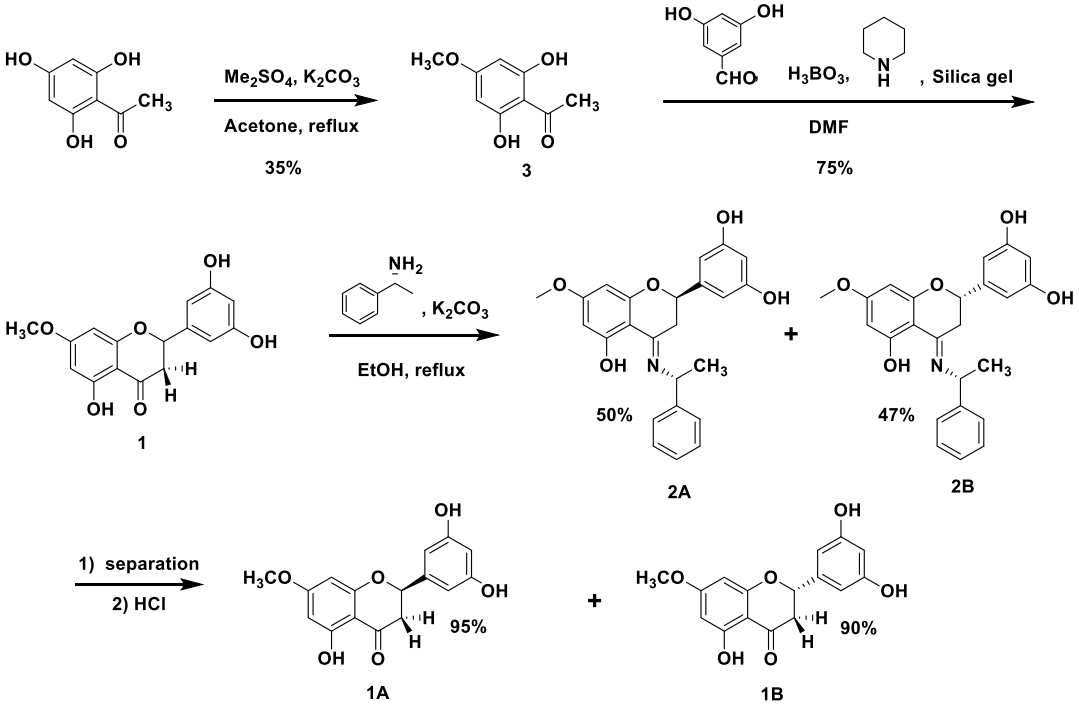

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Table 1. ^{1}H NMR, ^{13}C NMR, and HMBC Data for 1 and 4A in DMSO-d_6

	compound 1			compound 4A		
position	δ_H[a]	δ_C[a]	HMBC[a,b]	δ_H[a]	δ_C[a]	HMBC[a,b]
C2	5.45, dd (12.1, 3.2)	78.9, CH	1′, 2′, 6′, 4, 8a	5.45, dd (12.6, 3.0)	79.1, CH	1′, 2′, 5′, 6′
C3	3.16, dd (17.2, 12.1); 2.77, dd (17.2, 3.2)	42.7, CH_2	1′, 2, 4, 4a	3.22, dd (17.1, 12.6); 2.72, dd (17.1, 3.0)	42.6, CH_2	1, 2, 4, 4a
C4		197.1, C			197.4, C	
C4a		102.9, C			103.1, C	
C5		163.7, C			163.6, C	
C6	6.09, d (2.3)	95.1, CH	5, 7, 8, 8a, 4a	6.08, d (2.2)	96.0, CH	5, 7, 8, 8a, 4a
C7		167.9, C			167.9, C	
C8	6.13, d (2.3)	94.4, CH	5, 7, 8, 8a, 4a	6.10, d (2.2)	94.2, CH	5, 7, 8, 8a, 4a
C8a		163.1, C			163.3, C	
C1′		141.0, C			129.7, C	
C2′	6.34, d (2.0)	104.9, CH	2, 3′, 4′, 5′, 6′	6.89, s	114.8, CH	1′, 2, 3′, 4′, 5′
C3′		158.9, C			145.6, C	
C4′	6.21, t (2.1, 2.1)	103.1, CH	2′, 3′, 5′, 6′		146.2, CH	
C5′		158.9, C		6.73−6.78[c], m	115.8, CH	1′, 2′, 2, 3′, 4′
C6′	6.34, d (2.0)	104.9, CH	2, 2′, 3′, 4′, 5′	6.73−6.78[c], m	118.5, CH	1′, 2, 3′, 4′
C3′-OH	9.37, s			9.09, s		
C4′-OH				9.05, s		
C5′-OH	9.37, s					
C7−OCH_3	3.80, s	56.4, CH_3		3.79, s	56.6, CH_3	

[a]Measured at 400 MHz (25 °C). [b]From proton to stated carbon(s). [c]Signal overlapped.

determined as (*R*)-2-(3,5-dihydroxyphenyl)-5-hydroxy-7-methoxy- chroman-4-one and (*S*)-2-(3,5-dihydroxyphenyl)-5-hydroxy-7-methoxychroman-4-one, respectively. Intriguingly, both the synthetic compounds **1A** and **1B** could not match with the natural blumeatin (**4A**) isolated by our group and others (purchased from Tauto Biotech, Co., Ltd. or Rhawn, Co., Ltd.) in TLC (Figure S37) or HPLC experiments (Figures S1, S8, S13, and S34). Further structural analysis of the natural "blumeatin" (**4A**) was pressing.

2.3.1. 1: Synthetic Blumeatin; 4A: Putative Blumeatin. According to ^{1}H NMR, ^{13}C NMR and HMBC data of racemic compound **1** (Table 1, Figure S5), signals at δ H/C 5.45 (H-2)/78.9 (C-2), 3.16 & 2.77 (H-3)/42.7 (C-3), 197.0 (C-4), 102.9 (C-4a), 12.09 (OH-5)/163.6 (C-5), 6.09 (H-6)/91.6 (C-6), 3.80 (OCH_3-7)/167.9 (C-7), 6.13 (H-2)/94.3 (C-8), 163.1 (C-8a), 141.0 (C-1′), 104.9 (C-2′, C-6′), 158.9 (C-3′, C-5′), 103.1 (C-4′), were consistent with the scaffold of 5-hydroxy-7-methoxychroman-4-one. In addition, two symmetrical signals of ^{1}H NMR at 6.34 ppm (H-2′, H-6′), 9.37 ppm (OH-3′, OH-5′) together with a triplet at 6.21 (J = 2.1 Hz, remote coupling with H-2′ and H-6′, respectively), were corresponding to two hydroxy groups at C3′ and C5′ of ring B, respectively.

Similarly, compound **4A** also had a skeleton of 5-hydroxy-7-methoxychroman-4-one (Table 1). However, ^{13}C NMR signals at 129.7 (C-1′), 114.8 (C-2′), 145.6 (C-3′), 146.2 (C-4′), 115.8 (C-5′), and 118.5 (C-6′) indicated that there were no two carbon atoms with identical chemical environment in the B-ring. Together with ^{1}H NMR signals at 6.89 (H-2′), 9.09 (OH-3′), 9.05 (OH-4′), 6.73−6.78 (H-5′ and H-6′ overlapped accidently), it indicated that two hydroxyl groups were severally located at the C3′ and C4′ in the B-ring of **4A**.

The positions of hydroxy groups in the B-ring of **1** or **4A** could be further validated by HMBC data (Table 1). For compound **4A**, cross-peaks between C2 and H2′, H6′, H5′ were readily observed, while C2 was just correlated with H2′ and H6′ in the HMBC spectrum of compound **1**, for both C3′ and C5′ were substituted with hydroxy groups, and we also could not see the correlation from H4′ to C2 in HMBC. Taken together with the results of HMBC, **1** could be validated as 2-(3,5-dihydroxyphenyl)-5- hydroxy-7-methoxychroman-4-one and **4A** was determined as 2-(3,4-dihydroxyphenyl)-5-hydroxy-7-methoxychroman-4-one (Figure 4).

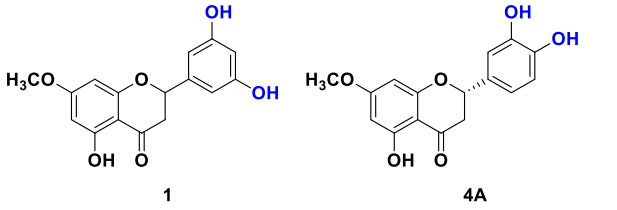

Figure 4. Structural comparison of **1** and **4A**.

The structure of **4A** coincided with the structure of sterubin, namely, (*S*)-2-(3,4-dihydroxyphenyl)-5-hydroxy-7-methoxychroman-4-one.[24] If the reactant of 3,5-dihydroxybenzaldehyde in our synthetic procedure was replaced with 3,4-dihydroxybenzaldehyde, the (±)-2-(3,4-dihydroxyphenyl)-5-hydroxy-7-methoxy-chroman-4-one (**4**, (±)-sterubin) could be obtained (Figure 5). Its ^{1}H NMR and ^{13}C NMR data were highly consistent with those of **4**, so was those of mentioned in literature.[24−28]

In addition, the melting points of newly synthetic compounds were measured many times on two micro melting point apparatuses, and the average values were taken to determine that the melting point of racemate **1** was 181−183 °C. Similarly, the melting points of optically active **1A** and **1B** were 180−181 °C and 179−181 °C, respectively. While the earliest reported melting point of the putative blumeatin was 221−223 °C[8] or 218−220 °C in other literature,[5,29] which were neither consistent with that of **1** nor those of **1A** and **1B**. On the other hand, the melting point of compound **4** was 217−219 °C after testing, which did match with the data reported in the literature.[24] Taken together the consistent results of NMR and HPLC between **4** and **4A** (Figures S8−

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ACS Omega 2023, 8, 14240−14246

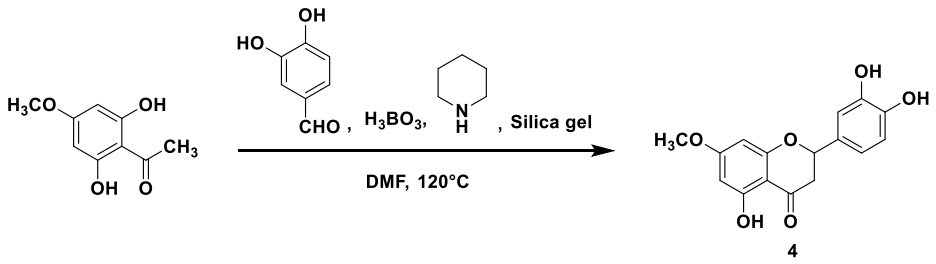

Figure 5. Synthesis of compound racemic 4.

S19), we can unambiguously propose that the structure of the putative blumeatin is corresponding to that of sterubin (mp 215−216 °C).[24]

2.4. Anti-inflammatory Activity of Synthetic Blumeatin. To investigate the anti-inflammatory activities of our synthesized compounds, we performed ear-swelling experiments on mice, with dexamethasone (10 mg/kg) as a positive control, and the results are summarized in Table 2. It could be seen that the preventative anti-inflammatory activity of isolated blumeatin (sterubin) **4A** presented dose-dependent. The swelling inhibitory rate of **4A** at high dose (30 mg/kg) was 73.53%, which was about 1/3 as potent as to positive control (78.39% at 10 mg/kg), and the anti-inflammatory activities of **4A** were also reported in other works.[28,30,31] Similarly, both **1** and **4** inhibited ear swelling in a dose-dependent manner, but **4** was slightly more potent than **1**. It indicated that 3,4-dihydroxy in the B-ring was superior to its 3,5-dihydroxy counterpart. Simultaneously, racemic **4** was slightly less potent than *S*-isomer **4A**, suggesting *S*-isomer might be the eutomer on anti-inflammatory activity.

Table 2. Comparison of Swelling Degree of Mice in Each Group[a]

testing sets	dose (mg/kg)	swelling (mg)	inhibition rate (%)
blank set		8.41 ± 2.28	
solvent set		7.23 ± 1.99	
dexamethasone	10	1.57 ± 1.00**	78.30
1	7.5	4.47 ± 2.78**	38.17
	15	4.29 ± 2.41**	40.66
	30	3.71 ± 1.22**	48.70
4A	7.5	5.23 ± 2.11*	27.74
	15	2.45 ± 2.17**	66.13
	30	1.92 ± 0.85**	73.53
4	7.5	6.58 ± 2.45**	9.79
	15	5.36 ± 2.20**	26.58
	30	3.32 ± 1.60**	54.52

[a]* $P < 0.05$, ** $P < 0.01$. **1**: (±)-2-(3,5-dihydroxyphenyl)-5-hydroxy-7-methoxychroman-4-one; **4A**: putative blumeatin; **4**: (±)-2-(3,4-dihydroxyphenyl)-5-hydroxy-7-methoxychroman-4-one.

3. CONCLUSIONS

The structure of putative blumeatin was proposed as (*S*)-2-(3,5-dihydroxyphenyl)-5-hydroxy-7-methoxychroman-4-one by its discoverer. We here provide a synthetic and resolving procedure for efficiently forming optically pure blumeatin. However, the synthesized blumeatin was not consistent with its isolated counterpart (commercially available "blumeatin"). The data of ^{1}H NMR and ^{13}C NMR, HMBC, and HPLC and the results of our synthetic procedure supported that the isolated blumeatin match with sterubin [(*S*)-2-(3,4-dihydroxyphenyl)-5-hydroxy-7-methoxychroman-4-one]. The results of the ear-swelling test exhibited that synthetic (±)-blumeatin and (±)-sterubin had moderate anti-inflammatory activity, which was less than that of putative blumeatin (sterubin).

4. EXPERIMENTAL SECTION

4.1. Reagents and Instruments. Partial reactions were carried out in flame-dried glassware under a nitrogen atmosphere. Reagents used were purchased from Aladdin, Macklin, Innochem, or TLC unless otherwise noted. Chromatographic separation used silica gel, AR, 200−300 mesh. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker (Avance) 400 MHz NMR instrument using $CDCl_3$ and DMSO-d_6 as solvents. Infrared spectra were acquired with KBr particles on a Shimadzu FT-IR-8400S spectrometer. Optical rotations were determined on an Insmark digital polarimeter using a sodium (589 nm, D-line) lamp and reported as follows: c = 0.20 g/100 mL, ethanol, 20 °C. The melting point of the compound was recorded by the X-4D micro melting point apparatus. An Applied Photophysics Chirascan spectrometer was employed to get ECD spectra. Retention time was obtained in Agilent 1260 HPLC. TLC analysis was visualized using UV, vanillin, and iodine elemental staining. High-resolution mass spectra were obtained using AB SCIEX X500R QTOF.

4.2. Synthetic Procedures. *4.2.1. 2,6-Dihydroxy-4-methoxy-acetophenone (**3**).* 2,4,6-Trihydroxy acetophenone (8 g, 0.048 mol, 1 equiv) and anhydrous potassium carbonate (13.14 g, 0.095 mol, 2 equiv) were dissolved in acetone (100 mL). Dimethyl sulfate (2.7 mL, 0.029 mol, 0.6 equiv) was added dropwise, and the reaction mixture was stirred at 60 °C under nitrogen for 24 h. Then, the mixture was allowed to slowly cool to room temperature. After the reaction was quenched with 50 mL of water and diluted with 50 mL of ethyl acetate (EA). The layers were separated, and the aqueous layer was extracted 2× with 50 mL of EA. The combined organics were washed with brine and dried over Na_2SO_4. Finally, the evaporated residue was purified by column chromatography (3−4% EA in petroleum ether) to 2,6-dihydroxy -4-methoxy-acetophenone (**3**) (3.33 g, 35%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.93 (s, 2H), 3.79 (s, 3H), 2.67 (s, 3H); mp 126−130 °C; HRMS (ESI/TOF) m/z calcd for $C_8H_9O_4$, 183.06573; found, 183.06592 $[M + H]^+$.

*4.2.2. 2-(3,5-Dihydroxy phenyl)-5-hydroxy-7-methoxy-chromanone (**1**).* 2,6-Dihydroxy-4-methoxy-acetophenone (**3**) (500 mg, 2.75 mmol, 0.8 equiv) and 3,5-dihydroxybenzaldehyde (470 mg, 3.41 mmol, 1 equiv) were added to a mixture of H_3BO_3 (337 mg, 5.41 mmol, 1.5 equiv) and silica gel (1.5 g) in DMF (30 mL), and then piperidine (113 μL, 1.4 mmol, 0.25 equiv) was added dropwise under a nitrogen atmosphere. The reaction mixture was stirred and heated at

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ACS Omega 2023, 8, 14240−14246

120 °C under nitrogen for 10−12 h, followed by cooling to room temperature and diluting with acetone (50 mL) and filtering. The silica gel was rinsed with acetone (5 mL) three times. The acetone solution was combined with the filtrate and evaporated at reduced pressure to dryness. The residue was purified by column chromatography (12−16% EA in petroleum ether) to 2-(3,5-dihydroxy phenyl)-5-hydroxy-7-methoxychromanone (**1**) (750 mg, 75%) as a pale yellow solid. IR: 3290, 3140, 2992, 1603, 1354, 1263, 1148, 1090, 974, 825, 709; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.09 (s, 1H),9.37 (s, 2H), 6.34 (d, J = 2.0 Hz, 2H), 6.21 (t, J = 2.1, 2.1 Hz, 1H), 6.13 (d, J = 2.3 Hz, 1H), 6.09 (d, J = 2.3 Hz, 1H), 5.45 (dd, J = 12.1, 3.2 Hz, 1H),3.80 (s, 3H), 3.16 (dd, J = 17.2, 12.1 Hz, 1H), 2.77 (dd, J = 17.2, 3.2 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 197.1 (C4), 167.9 (C7), 163.7 (C5), 163.1 (C9), 158.9 (C3′ and C5′), 141.0 (C1′), 104.9 (C6′), 103.1 (C2′), 102.9 (C4′), 95.1 (C6), 94.4 (C8), 78.9 (C2), 56.4 (7-OCH3), 42.7 (C3); mp 181−183 °C; HRMS (ESI/TOF) m/z calcd for $C_{16}H_{14}O_6Na$, 325.06881; found, 325.06749 [M + Na]$^+$.

*4.2.3. 5-((R)-5-Hydroxy-7-methoxy-4-(((R)-1-phenylethyl)-imino)chroman-2-yl)benzene-1,3-diol (**2A**).* 2-(3,5-Dihydroxy phenyl)-5-hydroxy-7-methoxychromanone (**1**) (500 mg, 1.65 mmol, 1 equiv) and anhydrous potassium carbonate (450 mg, 3.26 mmol, 2 equiv) were dissolved in absolute ethanol (10 mL). (*S*)-(−)-α-methyl benzylamine (840 μL, 6.72 mmol, 4 equiv) was added dropwise, and the reaction mixture was stirred under reflux for 3.5 h. Then the mixture was allowed to slowly cooled to room temperature. After the reaction mixture was quenched with 30 mL of water, the aqueous layer was extracted 3× with 30 mL of EA. The combined organics were washed with brine, dried over Na_2SO_4 and evaporated to dryness and purified by column chromatography (40−50% EA in petroleum ether) to afford 5-((*R*)-5- hydroxy-7-methoxy-4-(((*R*)-1-phenylethyl)imino)chroman-2-yl)benzene-1,3-diol (**2A**) (300 mg, 50%) as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.34 (s, 2H), 7.48−7.28 (m, 5H), 6.33, (d, J = 1.9 Hz, 2H), 6.21 (t, J = 1.8 Hz, 1H), 5.84 (d, J = 2.4 Hz, 1H), 5.80 (d, J = 2.4 Hz, 1H), 5.04 (dd, J = 11.3, 3.2 Hz, 1H), 3.71 (s, 3H), 3.18 (dd, J = 16.6, 3.2 Hz, 1H), 3.07 (dd, J = 17, 11.3 Hz, 1H), 1.45 (m, 3H); mp 237−239 °C; HRMS (ESI/TOF) m/z calcd for $C_{24}H_{24}NO_5$, 406.16545; found, 406.16468 [M + H]$^+$.

*4.2.4. 5-((S)-5-Hydroxy-7-methoxy-4-(((R)-1-phenylethyl)-imino)chroman-2-yl)benz-ene-1,3-diol (**2B**).* 5-((*S*)-5-Hydroxy-7-methoxy-4-(((*R*)-1-phenylethyl)imino)chroman-2-yl)-benz-ene-1,3-diol (**2B**) was obtained by the same process of **2A** (300 mg, 47%) as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.34 (s, 2H), 7.48−7.28 (m, 5H), 6.33 (d, J = 2.1 Hz, 2H), 6.21 (t, J = 1.9 Hz, 1H), 5.84 (d, J = 2.4 Hz, 1H), 5.80 (d, J = 2.4 Hz, 1H), 5.08 (dd, J = 11.1, 3.1 Hz, 1H), 3.71 (s, 3H), 3.18 (dd, J = 17.4, 3.1 Hz, 1H), 3.07 (dd, J = 17.4, 11.1 Hz, 1H), 1.45 (m, 3H); mp 238−240 °C; HRMS (ESI/TOF) m/z calcd for $C_{24}H_{24}NO_5$, 406.16545; found, 406.16471 [M + H]$^+$.

*4.2.5. (R)-2-(3,5-Dihydroxyphenyl)-5-hydroxy-7-methoxy-chroman-4-one (**1A**).* 5-((*R*)-5-Hydroxy-7-methoxy-4-(((*R*)-1-phenylethyl)imino)chroman-2-yl)benz-ene-1,3-diol (**2A**) (200 mg, 0.48 mmol, 1 equiv) was dissolved in EA (10 mL), and 5 N hydrochloric acid was added (15 mL). The reaction mixture was stirred at 100 °C under nitrogen for 10 h. and the mixture was allowed to slowly cooled to room temperature. Then the reaction mixture was neutralized with sodium hydroxide solution and diluted with 20 mL of EA. The layers were separated, and the aqueous layer was extracted 2× with 10 mL of EA. The combined organics were washed with brine, dried over Na_2SO_4, and purified by column chromatography (12−16% EA in petroleum ether) to (*R*)-2-(3,5-dihydroxyphenyl)-5-hydroxy-7-methoxychroman-4-one (**1A**) (160 mg, 95%) as a yellow solid. IR: 3300, 3110,2890, 1600, 1420, 1350, 1260, 1200, 1144, 980, 820, 710; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.09 (s, 1H), 9.37 (s, 2H), 6.34 (d, J = 2.0 Hz, 2H), 6.21 (t, J = 2.1, 2.1 Hz, 1H), 6.13 (d, J = 2.3 Hz, 1H), 6.09 (d, J = 2.3 Hz, 1H), 5.45 (dd, J = 12.1, 3.2 Hz, 1H), 3.80 (s, 3H), 3.16 (dd, J = 17.2, 12.1 Hz, 1H), 2.77 (dd, J = 17.2, 3.2 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 197.1 (C4), 167.9 (C7), 163.7 (C5), 163.1 (C9), 158.9 (C3′ and C5′), 141.03 (C1′), 104.9 (C6′), 103.1 (C2′), 102.9 (C4′), 95.1 (C6), 94.4 (C8), 78.9 (C2), 56.4 (7-OCH3), 42.7 (C3); mp 180−181 °C; $[\alpha]_D^t$ = +7.2 HRMS (ESI/TOF) m/z calcd for $C_{16}H_{14}O_6Na$, 325.06881; found, 325.06790 [M + Na]$^+$.

*4.2.6. (S)-2-(3,5-Dihydroxyphenyl)-5-hydroxy-7-methoxy-chroman-4-one (**1B**).* 5-((*S*)-5-Hydroxy-7-methoxy-4-(((*R*)-1-phenylethyl)imino)chroman-2-yl)benz-ene-1,3-diol (**2B**) was hydrolyzed by the same process of **2A** to afford (*S*)-2-(3,5-dihydroxyphenyl)-5-hydroxy-7- methoxychroman-4-one **1B** (150 mg, 90%) as a yellow solid. IR: 3340, 3163, 2868, 1596, 1430, 1301, 1255, 1190, 1144, 1071, 978, 822, 711; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.09 (s, 1H), 9.37 (s, 2H), 6.34 (d, J = 2.0 Hz, 2H), 6.21 (t, J = 2.1, 2.1 Hz, 1H), 6.13 (d, J = 2.3 Hz, 1H), 6.09 (d, J = 2.3 Hz, 1H),5.45 (dd, J = 12.1, 3.2 Hz, 1H), 3.80 (s, 3H), 3.16 (dd, J = 17.2, 12.1 Hz, 1H), 2.77 (dd, J = 17.2, 3.2 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 197.1 (C4), 167.9 (C7), 163.7 (C5), 163.1 (C9), 158.9 (C3′ and C5′), 141.03 (C1′), 104.9 (C6′), 103.1 (C2′), 102.9 (C4′), 95.1 (C6), 94.4 (C8), 78.9 (C2), 56.4 (7-OCH3), 42.7 (C3); mp 179−181 °C; $[\alpha]_D^t$ = −7.0; HRMS (ESI/TOF) m/z calcd for $C_{16}H_{14}O_6Na$, 325.06881; found, 325.06824 [M + Na]$^+$.

*4.2.7. 2-(3,4-Dihydroxy phenyl)-5-hydroxy-7-methoxy-chromanone (**4**).* Compound **4** (600 mg, 65%) as a white solid was obtained according to the synthetic method of compound **1**. IR: 3331, 3116, 1594, 1437, 1354, 1247, 1189, 1148, 1065, 949, 817, 709, 544; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.1 (s, 1H), 9.16 (s, −OH), 9.08 (s, −OH), 6.85 (d, J = 1.28 Hz, 1H), 6.73−6.78 (m, 2H), 6.10 (d, J = 2.3 Hz, 1H), 6.08 (d, J = 2.3 Hz, 1H), 5.42 (dd, J = 12.6, 3.0 Hz, 1H), 3.79 (s, 3H), 3.24 (dd, J = 17.2, 12.6 Hz), 2.72 (dd, J = 17.2, 3.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 197.4 (C4), 167.9 (C7), 163.6 (C5), 163.3 (C9), 145.7 (C3′), 145.2 (C4′), 129.7 (C1′), 118.4 (C6′), 115.9 (C5′), 114.9 (C2′), 103.1 (C10), 95.0 (C6), 94.2 (C8), 79.1 (C2), 56.4 (7-OCH$_3$), 42.6 (C3); mp 217−219 °C; HRMS (ESI/TOF) m/z calcd for $C_{16}H_{14}O_6Na$, 325.06881; found, 325.06784 [M + Na]$^+$.

4.3. Ear Swelling Test on Mice. The Animal Care Welfare Committee of Guizhou University gave the approval to conduct animal experiments. One hundred and twenty Kunming mice were randomly divided into 12 groups (with 10 mice in each group), comprising a blank group (normal saline), a solvent group ($V_{ethanol}/V_{Tween\ 80}/V_{normal\ saline}$ = 1:1:8), a positive drug group (dexamethasone acetate 10 mg/kg), three isolated blumeatin (**4A**) groups (including low-, medium-, and high-dose sets), three (±) blumeatin (**1**) groups, and three (±) sterubin (**4**) groups. The test compounds were dissolved in a solvent containing 1 mL of ethanol, 1 mL of Tween 80, and 8 mL of normal saline.

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ACS Omega 2023, 8, 14240−14246

All mice were injected intraperitoneally as per group. Five hours later, the animals in each group were smeared with 30 μL of xylene on the inside and outside of the right ear. The left ear was not smeared which served as a control and was given normal saline. One hour after the inflammation was induced, the animals in each group were killed by cervical dislocation, and then the ears were pierced with a puncher of a fixed size. Finally, the tissues cut from the ears were weighed and recorded with a balance, and the degree of swelling and inhibition rate were calculated..

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c01247.

HPLC, TLC, ^{1}H NMR, ^{13}C NMR, HMBC, HSQC, IR, and HRMS data of intermediates and target compounds (PDF)

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Notes

The authors declare no competing financial interest.

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