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Syntheses of Minutuminolate and Related Coumarin Natural Products and Evaluation of Their TNF-α Inhibitory Activities

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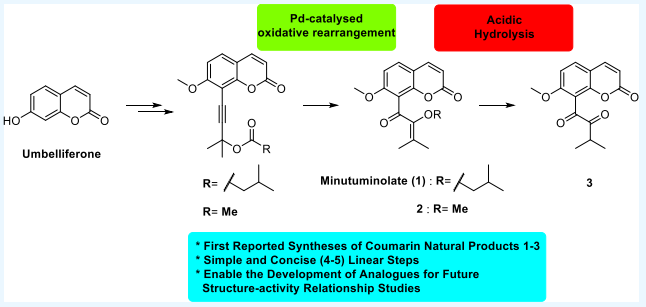
ABSTRACT: The concise syntheses of the coumarin natural product, minutuminolate (**1**), and its related natural products, 7-methoxy-8-(2-acetoxy-3-methyl-1-oxobut-2-enyl) coumarin (**2**) and muralatin I (**3**), were accomplished for the first time in 4−5 steps from the commercially available umbelliferone. The key step involves a palladium-catalyzed oxidative rearrangement reaction to assemble the α-acyloxyenone moiety in **1** and **2**. The incorporation of this functionality enables the successful synthesis of coumarin **3** through an acidic hydrolysis reaction. The anti-inflammatory activities of the compounds were also evaluated against tumor necrosis factor-alpha production in lipopolysaccharides-stimulated RAW264.7 cells. Our developed synthetic route will facilitate the development of analogues and derivatives of **1**−**3** with potent anti-inflammatory activities.



INTRODUCTION

Coumarins, formed from the fusion of a pyrone ring with a benzene moiety, constitute an important class of heterocyclic natural products. Since its isolation from Tonka beans by Vogel in 1820, and its subsequent synthesis by Perkin in 1868,[1] reports on the isolation, characterization, and biological evaluation of natural coumarins and their syntheses have been reported extensively.[2−8] Coumarins have been reported to possess a diverse range of biological activities, *e.g.*, antibacterial,[9] antioxidant,[10] antifungal,[11] anticancer,[12] and anti-inflammatory properties.[13−15] As such, the coumarin scaffold is considered to be privileged in the field of medicinal chemistry.[6,13]

Several naturally occurring and synthetic coumarins have been reported to attenuate inflammation through various mechanisms such as enzymatic inhibition of iNOS, COX, and LOX, as well as inhibition of the production of pro-inflammatory cytokines tumor necrosis factor-alpha (TNF-α) and interleukin 6 (IL-6).[16−21] In 2016, a new C7, C-8 substituted coumarin, minutuminolate (**1**) was isolated from the roots of *Micromelum minutum* (Figure 1).[5] Minutuminolate (**1**) contains a C-7 methoxy group, and its C-8 substituent comprises an α-acyloxyenone moiety (highlighted in green). **1** has been reported to possess anti-inflammatory properties. Specifically, it has been shown to inhibit nitric oxide (NO) production in lipopolysaccharides (LPS)-induced BV-2 cells with IC_{50} values ranging from 26.9 ± 0.8 to 53.2 ± 4.4 μM.[4,22,23] The structural analogue of **1**, 7-methoxy-8-(2-acetoxy-3-methyl-1-oxobut-2-enyl) coumarin (**2**) was isolated from *Phebalium ralstonii* in 1988.[24] However, the biological activities of **2** have not been investigated. Muralatin I (**3**),

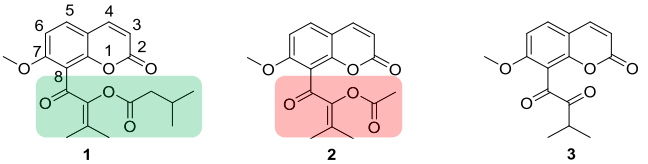


Figure 1. Structures of the naturally occurring coumarins, **1**−**3**. The α-acyloxyenone moiety in **1** is highlighted by the green bubble while the α-acetoxyenone in **2** is enclosed by the red bubble.

isolated from the leaves of *Murraya alata* in 2015, was also found to inhibit nitric oxide production in RAW 264.7 cells with an IC_{50} value of 30.5 ± 1.5 μM.[3]

Surprisingly, despite the potential of these coumarin natural products to be developed as anti-inflammatory agents, no synthetic routes have been reported to date. In this study, we report for the first time the concise syntheses of coumarin natural products **1**−**3** facilitated by a palladium-catalyzed oxidative rearrangement reaction. Additionally, the synthesized compounds were evaluated for their ability to inhibit the production of the pro-inflammatory cytokine TNF-α in LPS-stimulated RAW 264.7 cells. Given the importance of the coumarin scaffold for the development of anti-inflammatory

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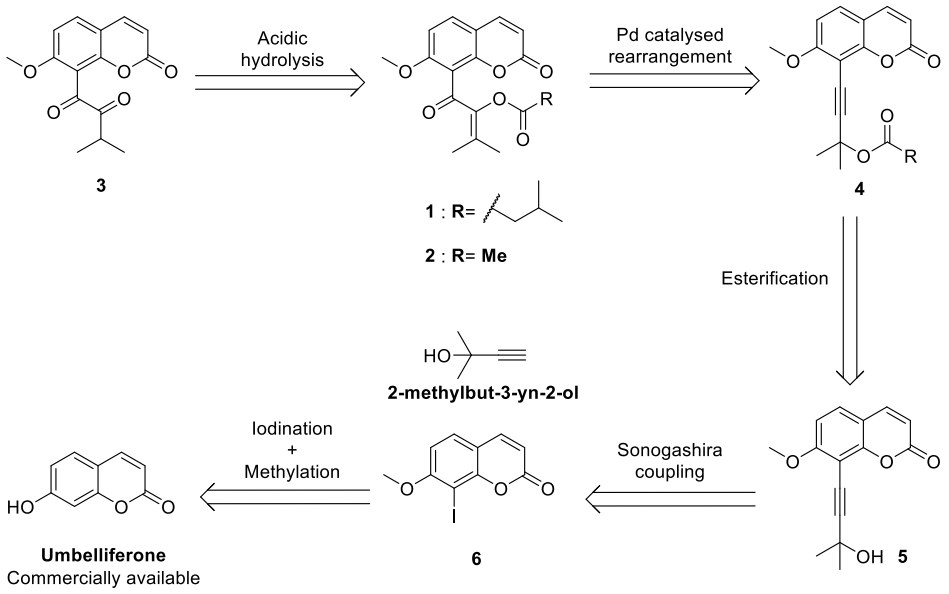
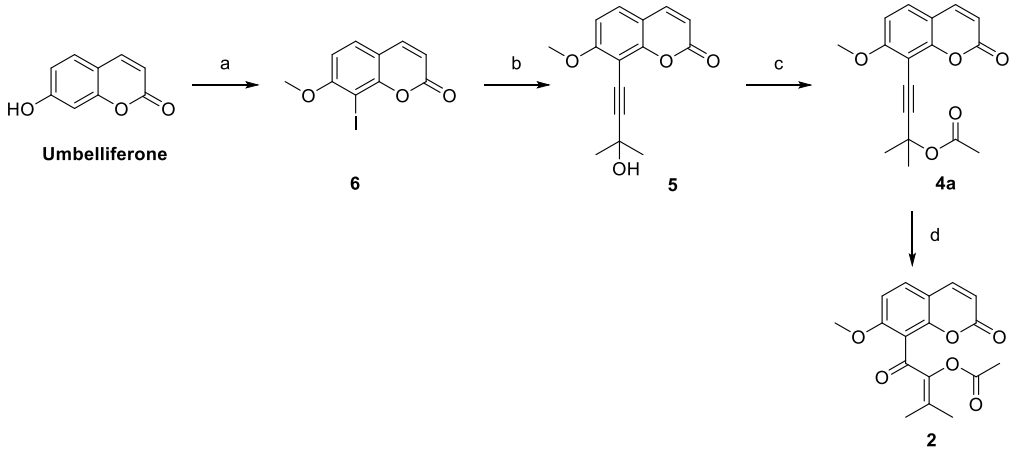
Scheme 1. Retrosynthetic Analysis of Coumarin Natural Products 1−3



Scheme 2. Synthetic Route to Coumarin 2[a]



[a]Reagents and conditions: (a) 1. KI, I_2, NH_3 (aq), 24 h. 2. MeI, K_2CO_3, acetone, reflux, 24 h (35% over 2 steps); (b) Pd $(PPh_3)_2Cl_2$, CuI, PPh_3, 2-methylbut-3-yn-2-ol, Et_3N, DMF, 80 °C, 12 h (88%); (c) Ac_2O, DMAP, pyridine, CH_2Cl_2, 12 h (90%) (d) Pd $(OAc)_2$, PhI $(OAc)_2$, AcOH, 50 °C, 16 h (20%).

Table 1. Optimization of Reaction Conditions for the Oxidative Isomerization

entry	cat	oxidant/additive	solvent	T (°C)	conv	yield (%)	by-products
1	$Pd(OAc)_2$	$PhI(OAc)_2$	AcOH	50	100	20	complex
2	$Pd(OAc)_2$		AcOH	50	100	18	**I,II**
3	$Pd(OAc)_2$		THF/H_2O	60	100	27	**III**
4	$Pd(OAc)_2$		$toluene/H_2O$	60	<5	N.A	N.A
5	$PdCl_2$		THF/H_2O	60	100	30	**III**
6	$IrCl_3$		THF/H_2O	60	100	0	**5**
7	$Ag(SO_2CF_3)$		THF/H_2O	60	100	0	**5**
8	$PdCl_2$	$CuCl_2$	THF/H_2O	60	100	55	**IV**

agents,[25] the developed synthetic routes in this study will allow for the synthesis of the analogues/derivatives of **1**−**3** for systematic structure−activity relationship (SAR) studies and enable further development of compounds with potent anti-inflammatory activities.

RESULTS AND DISCUSSION

A retrosynthesis for compounds **1**−**3** is shown in Scheme 1. We envisioned that **3** could be accessed via acidic hydrolysis of either **1** or **2**. The α-acyloxyenone moieties in **1** and **2** can be introduced via a palladium-catalyzed rearrangement reaction from the corresponding propargylic esters **4**. Such Pd-catalyzed oxidative rearrangements are well established for the trans-

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formation of propargylic esters to α-acetoxyenones,[26−28] but to our knowledge, the generality of the transformation for the installation of various α-acyloxyenones, such as that present in **1**, has not been investigated. Propargylic ester **4** is in turn derived from the esterification of propargylic alcohol **5**, which can be accessed via a Sonogashira coupling reaction of iodo coumarin **6** and 2-methylbut-3-yn-2-ol. Finally, **6** can be furnished from the commercially available umbelliferone via an iodination and methylation reaction.

The synthesis of coumarin **2** began with the commercially available umbelliferone (Scheme 2). From umbelliferone, **6** was obtained in 35% yield through a sequence of iodination followed by *O*-methylation reactions.[29] Subsequent Sonogashira coupling with 2-methylbut-3-yn-2-ol gave propargyl alcohol **5** in an excellent yield of 88%, which was esterified with acetic anhydride to afford the propargylic acetate **4a**. Finally, the desired coumarin **2** was obtained in a low yield of 20% using a combination of $Pd(OAc)_2$ (15% mol), $PhI(OAc)_2$ (1.2 equiv), and acetic acid as a solvent at 50 °C (Table 1, entry 1). Unfortunately, efforts to elucidate the side products formed were unsuccessful, as a complex reaction mixture was obtained.

Given the low yields of **2**, attempts were made to optimize the oxidative rearrangement reaction (Table 1). For all of the conditions attempted, the catalyst loading was kept at 15% mol. We first investigated the role of the oxidant, $PhI(OAc)_2$, in the rearrangement. When the reaction was performed with $Pd(OAc)_2$ in acetic acid and in the absence of the oxidant $PhI(OAc)_2$, the yield of **2** was *ca.* 18% (entry 2). Under these conditions, the reaction mixture was less complex, and side products **I** and **II** were isolated and identified as vinylic diacetates, with **I** and **II** being the (*E*)- and (*Z*)-isomers, respectively (Figure 2). As both **I** and **II** contain an additional

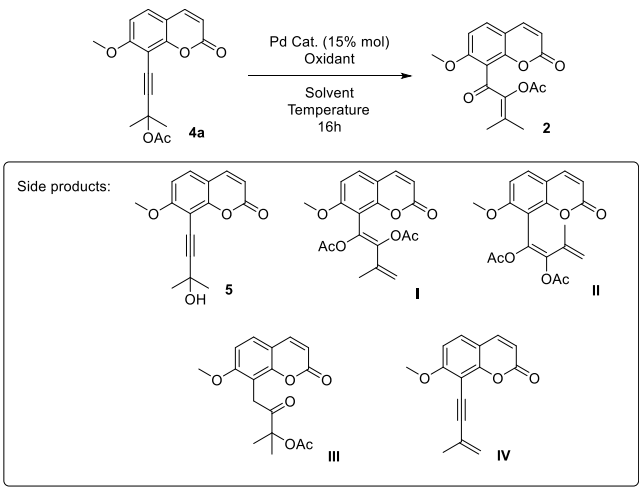

Figure 2. Side products formed from the oxidative isomerization reaction.

acetoxy moiety as compared to the starting material **4a**, it is likely that this arises from the participation of acetic acid, which was used as a solvent in this reaction. Replacement of acetic acid as a solvent with a solvent mixture of THF/H_2O (entry 3) gave a marginally improved yield of **2** of 27%. Under these conditions, a new side product, the α-acetoxy ketone **III** was isolated in a 60% yield (Figure 2). When the solvent was changed to toluene/H_2O, less than 5% conversion of **4a** was obtained (entry 4). Based on the above observations, the presence of the oxidant $PhI(OAc)_2$ is not crucial for the successful transformation of **4a** to the desired product **2**. However, the choice of solvent used is critical, and using THF/H_2O as a solvent mixture along with $Pd(OAc)_2$ gave the best yields of **2** (entry 3).

The effect of the metal catalyst used was next investigated. When the catalyst was changed to $PdCl_2$ (entry 5), comparable yields (30%) were obtained to that of $Pd(OAc)_2$ (entry 3). However, other metal catalysts like $IrCl_3$ (entry 6) or $Ag(SO_2CF_3)$ (entry 7) proved to be ineffective for the reaction as the propargylic alcohol **5** was obtained exclusively (Figure 2),[30−32] presumably through the hydrolysis of the propargylic acetate **4a**. Interestingly, when 15% mol % $CuCl_2$ was added with $PdCl_2$, the yield of **2** improved significantly to 55% (entry 8). The ene-yne side product **IV** was also observed, likely due to the elimination of acetic acid from **4a** (Figure 2). The best yield for the natural product **2** was thus obtained under the conditions of 15% mol $PdCl_2$ and $CuCl_2$, with heating at 60 °C using THF/H2O as the solvent (Table 1, entry 8).

For the synthesis of minutuminolate (**MNT**), propargylic isovalerate **4b** was obtained from alcohol **5** in 60% yield (Scheme 3). Remarkably, the Pd-catalyzed oxidative rearrangement reaction of **4b** under optimized conditions afforded **MNT** (**1**) in 52% yield. Subsequent acidic hydrolysis of the ester moiety in **1** gave diketone coumarin **3** in 55% yield (Scheme 3). As such, the syntheses of coumarin natural products **1**−**3** were accomplished in a concise manner for the first time.

The anti-inflammatory effects of coumarins **1**−**3** were next examined. Although the NO inhibitory activities of the compounds have been previously reported,[3,4,22,23] their inhibitory effects on the production of other pro-inflammatory mediators are not known. Tumor necrosis factor-α (TNF-α) is a multifunctional pro-inflammatory cytokine secreted mainly by activated macrophages. Dysregulation of TNF-α production is associated with a variety of diseases like rheumatoid arthritis, inflammatory bowel disease (IBD), Alzheimer's disease, and cancer. In view of this, the inhibitory effects of coumarins **1**−**3** on LPS-induced (25 ng/mL) TNF-α generation in RAW 264.7 cells were assessed, using dexamethasone (10 μM) as the positive control (Figure 3A). The cytotoxicities of the compounds were also examined in the same cell line using the MTS assay (Figure 3B).

As seen in Figure 3B, none of the compounds exhibited any detectable cytotoxicities up to 200 μM. Coumarin **3** exhibited a dose-dependent decrease in TNF-α production, with about 50% inhibition at 100 μM (Figure 3A). Interestingly, while **1** and **2** could potentially act as the ester prodrugs of **3**, they did not exhibit any TNF-α inhibitory activities, even at high concentrations of 100 μM (Figure 3A). This suggests that coumarins **1** and **2** are resistant to hydrolysis by cellular esterases, thus preventing the formation of the active compound, coumarin **3**.

CONCLUSIONS

In summary, we have accomplished the syntheses of the coumarin natural products **1**−**3** for the first time in a concise manner from the commercially available umbelliferone. The key step of the synthesis includes a palladium-catalyzed oxidative isomerization reaction to furnish the α-acyloxyenone moiety present in **1** and **2**. Subsequent acidic hydrolysis of the ester group in **1** yielded coumarin **3**. The abilities of **1**−**3** to

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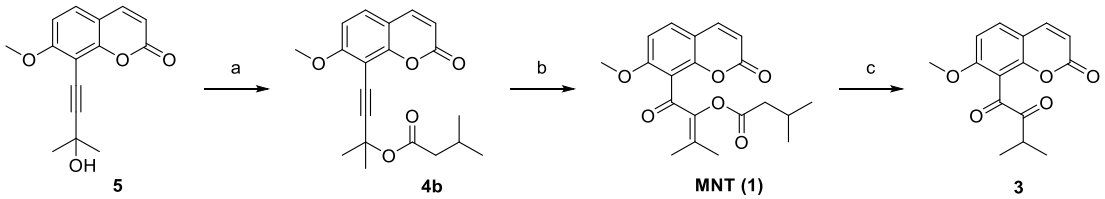
Scheme 3. Synthetic Route to MNT and Coumarin 3[a]



[a](a) Isovaleryl chloride, DMAP, pyridine, CH_2Cl_2, 12 h (60%); (b) $PdCl_2$, $CuCl_2$, THF/H_2O, 60 °C, 16 h (52%); (c) HCl, MeOH, 24 h (55%).

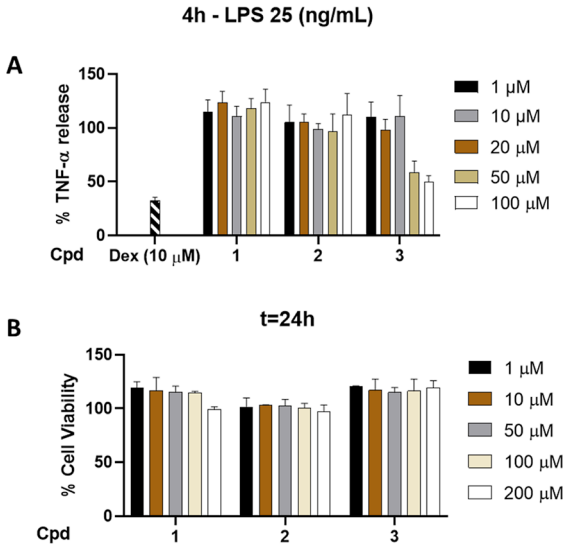


Figure 3. (A) Inhibitory effects of coumarins **1**−**3** on TNF-α production in RAW 264.7 cells under LPS stimulation. (B) Effects of coumarins **1**−**3** on cell viability. Data from LPS-only treated cells were counted as 100%. Data are presented as mean ± SEM of at least n = 3 independent experiments.

inhibit the pro-inflammatory cytokine TNF-α in LPS-activated RAW 264.7 cells were then examined. While coumarins **1** and **2** did not display any TNF-α inhibitory activities, **3** was able to attenuate TNF-α production in a dose−response manner. Given the privileged nature of the coumarin scaffold for the development of anti-inflammatory drugs, the developed synthetic route to these compounds will enable the synthesis of analogues of compounds **1**−**3** to generate comprehensive SARs for anti-inflammatory activities.

EXPERIMENTAL SECTION

General Experimental Procedures. All reagents and solvents (of analytical and HPLC grades) were used as received from commercial sources unless otherwise stated. Analytical thin layer chromatography (TLC) was performed on Merck's precoated silica gel plates and visualized under UV light (254 nm) or by staining with basic $KMnO_4$. Purification of compounds was done using flash chromatography on a glass column using Merck silica gel 60 (230e400 mesh). ^1H and ^{13}C NMR spectra were obtained at 400 and 100 MHz, respectively, on a Bruker Ultra Shield spectrometer. The chemical shifts are recorded in parts per million (ppm) on the δ scale using residual protio-solvent signals as an internal reference. Peak multiplicities are expressed as follows: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; br s, broad singlet; and m, multiplet. MS spectra were obtained with ESI or APCI modes on a Shimadzu single quadrupole liquid chromatograph mass spectrometer (LCMS-2020).

*8-Iodo-7-methoxy-2H-chromen-2-one (**6**).* Umbelliferone (4.00 g, 24.6 mmol) was dissolved in a 20% ammonium hydroxide solution (100 mL). A solution of potassium iodide (10 g, 60 mmol) and iodine (6.5 g, 25.6 mmol) in water (200 mL) was added over 75 min. The mixture was stirred for 24 h at room temperature before sulfuric acid (100 mL, 4 M) was carefully added. The brown precipitate that formed was filtered and suspended in acetone (100 mL). The mixture was stirred at reflux temperature for 20 min before it was filtered; this extraction was repeated once. The filtrates were combined, and the solvent was evaporated in vacuo. The crude material was then dissolved in acetone (30 mL). Potassium carbonate (3.88 g, 28 mmol) and MeI (1.73 mL, 27.8 mmol) were added, and the resulting mixture was stirred under reflux for 24 h, after which it was diluted with EtOAc (50 mL) and filtered. The filtrate was evaporated *in vacuo*, and the crude product was purified by flash chromatography (SiO_2, 30 to 40% EtOAc in hexane) to give **6** (2.61 g, 8.64 mmol, 35%) as a brown solid. The analytical and spectral data of the product were fully consistent with those described in the literature.[29]

*8-(3-Hydroxy-3-methylbut-1-yn-1-yl)-7-methoxy-2H-chromen-2-one (**5**).* $Pd(PPh_3)_2Cl_2$ (8.3 mg, 0.012 mmol), CuI (2 mg, 0.012 mmol), and PPh_3 (6.2 mg, 0.024 mmol) were added to a solution of **6** (179 mg, 0.59 mmol) and 2- methyl-3-butyn-2-ol (69 μL, 0.71 mmol) in Et_3N (4 mL) and DMF (1 mL). N_2 gas was bubbled through the mixture for 15 min, following which the reaction mixture was stirred at 80 °C for 12 h. The reaction was quenched with saturated aqueous NH_4Cl (10 mL), and the aqueous phase was extracted with EtOAc (3 × 15 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, and evaporated *in vacuo*. The crude product was purified by flash chromatography (SiO_2, 40% EtOAc in hexane) to give **5** (137.6 mg, 0.531 mmol, 90% yield) as a white solid. The analytical and spectral data of the product were fully consistent with those described in the literature.[33]

*4-(7-Methoxy-2-oxo-2H-chromen-8-yl)-2-methylbut-3-yn-2-yl Acetate (**4a**).* To a solution of **5** (70 mg, 0.27 mmol) in CH_2Cl_2 (1 mL) were added Et_3N (115 μL, 0.81 mmol), Ac_2O (64 μL, 0.68 mmol), and a catalytic amount of DMAP (3.29 mg, 0.027 mmol). The reaction mixture was stirred at room temperature for 16 h before quenching with water. The mixture was extracted three times with CH_2Cl_2 (3 × 15 mL) and washed with brine. The organic layers were combined, dried in Na_2SO_4, and evaporated *in vacuo*. The crude product was purified by flash chromatography (SiO2, 40% EtOAc in hexane) to give product **4a** (72.9 mg, 0.243 mmol, 90%) as a colorless oil.

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^{1}H NMR (400 MHz, $CDCl_3$): δ 7.58 (d, J = 9.5 Hz, 1H), 7.35 (d, J = 8.7 Hz, 1H), 6.80 (d, J = 8.7 Hz, 1H), 6.25 (d, J = 9.5 Hz, 1H), 3.95 (s, 3H), 2.07 (s, 3H), and 1.83 (s, 6H).

^{13}C NMR (101 MHz, $CDCl_3$): δ 169.45, 163.07, 160.44, 155.67, 143.01, 128.37, 113.80, 112.78, 107.29, 101.37, 101.24, 73.70, 72.70, 56.60, 29.00, and 22.04.

*1-(7-Methoxy-2-oxo-2H-chromen-8-yl)-3-methyl-1-oxobut-2-en-2-yl Acetate (**2**).* To a solution of $PdCl_2$ (7.98 mg, 0.045 mmol) and $CuCl_2$ (6.05 mg, 0.045 mmol) in THF/H_2O (9:1 v/v, 3 mL) was added **4a** (90.0 mg, 0.3 mmol). The resulting mixture was warmed to 60 °C and allowed to stir for 16 h. The mixture was then evaporated *in vacuo* to remove the THF, after which the aqueous solution was extracted thrice with EtOAc (3 × 15 mL) and washed with brine. The organic layers were combined, dried over Na_2SO_4, and evaporated *in vacuo*. The crude product was purified by flash chromatography (SiO_2, 100% CH_2Cl_2 to 10% EtOAc in CH_2Cl_2) to give coumarin **2** (52.1 mg, 0.165 mmol, 55%) as a colorless oil. The analytical and spectral data of the product were fully consistent with those described in the literature.[24]

^{1}H NMR (400 MHz, acetone-d_6): δ 7.93 (d, J = 9.6 Hz, 1H), 7.73 (d, J = 8.7 Hz, 1H), 7.12 (d, J = 8.7 Hz, 1H), 6.24 (d, J = 9.6 Hz, 1H), 3.91 (s, 3H), 2.21 (s, 3H), and 1.80 (d, J = 8.6 Hz, 6H).

^{13}C NMR (101 MHz, acetone-d_6): δ 188.17, 169.72, 161.14, 160.89, 153.52, 145.28, 142.78, 142.66, 132.07, 119.28, 115.16, 114.68, 109.80, 57.90, 31.32, 31.13, 30.94, 30.75, 30.55, 30.36, 30.17, 22.21, 20.88, and 20.84.

*4-(7-Methoxy-2-oxo-2H-chromen-8-yl)-2-methylbut-3-yn-2-yl 3-Methylbutanoate (**4b**).* To a solution of **5** (200 mg, 0.775 mmol) in CH_2Cl_2 (10 mL) were added pyridine (312 μL, 3.87 mmol) and DMAP (94.7 mg, 0.775 mmol) at 0 °C. Isovaleryl chloride (482 μL, 3.87 mmol) was then added dropwise. The reaction mixture was stirred at room temperature for 16 h before quenching with water. The mixture was extracted three times with CH_2Cl_2 (3 × 30 mL) and washed with brine. The organic layers were combined, dried over Na_2SO_4, and evaporated *in vacuo*. The crude product was purified by flash chromatography (SiO_2, 40% EtOAc in hexane) to give **4b** (159 mg, 0.465 mmol, 60%) as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.56 (d, J = 9.5 Hz, 1H), 7.32 (d, J = 8.7 Hz, 1H), 6.76 (d, J = 8.7 Hz, 1H), 6.19 (d, J = 9.5 Hz, 1H), 3.89 (s, 3H), 2.18 (d, J = 6.3 Hz, 2H), 2.15−2.08 (m, 1H), 1.80 (s, 6H), and 0.97 (d, J = 6.3 Hz, 6H).

^{13}C NMR (101 MHz, $CDCl_3$): δ 171.44, 162.99, 160.43, 155.45, 143.12, 128.40, 113.60, 112.69, 107.27, 101.30, 101.15, 73.68, 72.34, 56.47, 44.16, 29.07, 25.91, and 22.34.

*1-(7-Methoxy-2-oxo-2H-chromen-8-yl)-3-methyl-1-oxobut-2-en-2-yl 3-Methylbutanoate (**1**).* To a solution of $PdCl_2$ (7.98 mg, 0.045 mmol) and $CuCl_2$ (6.05 mg, 0.045 mmol) in THF/H_2O (9:1 v/v, 3 mL) was added **4b** (102.6 mg, 0.3 mmol). The resulting mixture was warmed up to 60 °C and allowed to stir for 16 h. The mixture was then evaporated *in vacuo* to remove THF, after which the aqueous layer was extracted thrice with EtOAc (3 × 15 mL) and washed with brine. The organic layers were combined, dried over Na_2SO_4, and evaporated *in vacuo*. The crude product was purified by flash chromatography (reversed phase SiO_2, 3:1H_2O/ACN to 2:1 ACN/H_2O) to give minutuminolate **MNT** (**1**) (55.8 mg, 0.156 mmol, 52%) as a colorless oil. The analytical and spectral data of the product were fully consistent with those described in the literature.[5]

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.63 (d, J = 9.5 Hz, 1H), 7.46 (d, J = 8.7 Hz, 1H), 6.87 (d, J = 8.7 Hz, 1H), 6.28 (d, J = 9.5 Hz, 1H), 3.90 (s, 3H), 2.31 (s, 3H), 1.95 (d, J = 7.0 Hz, 2H), 1.86 (s, 3H), 1.85−1.77 (m, 1H), and 0.76 (d, J = 6.5 Hz, 6H).

^{13}C NMR (101 MHz, $CDCl_3$): δ 186.48, 170.79, 159.85, 159.63, 151.85, 142.86, 142.78, 140.70, 129.57, 117.80, 113.95, 112.57, 107.97, 56.56, 42.47, 25.10, 22.21, 21.69, and 20.48.

*1-(7-Methoxy-2-oxo-2H-chromen-8-yl)-3-methylbutane-1,2-dione (**3**).* **1** (16 mg, 0.050 mmol) was dissolved in MeOH (3 mL), after which 37% HCl solution (100 μL) was added. The reaction mixture was stirred at room temperature for 24 h. The solvent was then evaporated *in vacuo*, and the crude product was purified by flash chromatography (SiO_2, 100% CH_2Cl_2) to give diketone **3** (7.54 mg, 0.0275 mmol, 55%) as a light-yellow oil. The analytical and spectral data of the product were fully consistent with those described in the literature.[3]

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.65 (d, J = 9.6 Hz, 1H), 7.57 (d, J = 8.7 Hz, 1H), 6.91 (d, J = 8.7 Hz, 1H), 6.29 (d, J = 9.6 Hz, 1H), 3.90 (s, 3H), 3.52 (m, 1H), and 1.30 (d, J = 7.0 Hz, 6H).

^{13}C NMR (101 MHz, $CDCl_3$): δ 202.55, 190.39, 161.10, 159.23, 153.58, 142.82, 131.89, 114.19, 113.86, 112.97, 107.91, 56.54, 34.71, and 17.

Cell Culture. RAW264.7 cells were grown in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% FBS, 2 mM L-glutamine, penicillin (100 U/mL), and streptomycin (100 mg/mL). Cells were maintained at 37 °C under a humidified atmosphere containing 5% CO_2.

Cell Viability Assay. RAW264.7 cells (20,000 cells per 100 μL of cell culture media) were seeded into 96-well tissue culture plates and incubated for 12 h. Cells were treated with the test compounds (**1**−**3**) at increasing concentrations (1−200 μM) or vehicle control (DMSO), in triplicates, for another 24 h. Blank/control wells received only the medium but no cells. The number of viable cells was determined by the MTS assay (abcam), according to the manufacturer's instructions. Results were expressed as a percentage of vehicle control group (i.e.,% cell viability).

Determination of LPS-Induced TNF-α Production in RAW264.7 Cells. RAW264.7 cells (20,000 cells per 100 μL cell culture media) were seeded into 96-well tissue culture plates and incubated for 12 h. Cells were pretreated with the test compounds at increasing concentrations (*e.g.*, 1, 5, 10, 20, 50, and 100 μM) or vehicle control (DMSO) for 1 h, followed by exposure to LPS (25 ng/mL) (*E. coli* LPS serotype 0111:B4, Sigma-Aldrich) for 24 h.[34,35] Blank control wells received only the vehicle with no LPS-stimulation. The concentration of DMSO was kept at 0.1% throughout all of the experimental groups. Dexamethasone (10 μM) (Sigma-Aldrich) was used as a positive control. After incubation at 37 °C for 24 h, the plate was centrifuged (1000*g*, 5 min, 4 °C), and the supernatant was collected. TNF-α production in the culture medium was determined using commercially available mouse TNF-α ELISA kits, according to the manufacturer's instructions (Mono/Mono OptEIA ELISA Set, BD Biosciences). Results were expressed as percentage of the vehicle control group (i.e., % cytokine release).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c06361.

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^{1}H and ^{13}C NMR spectra for all synthetic compounds and HPLC chromatograms for compounds **1**−**3** (PDF)

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Notes

The authors declare no competing financial interest.

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