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Synthesis of a novel series of diphenolic chromone derivatives as inhibitors of NO production in LPS-activated RAW264.7 macrophages

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ABSTRACT

A novel series of diphenolic chromone derivatives were synthesized and their inhibitory activity on nitric oxide (NO) production and cytotoxicity were evaluated using LPS-activated murine macrophages RAW264.7 assay and MTT method, respectively. Among these compounds, (5,7-dihydroxy-4-oxo-4*H*-chromen-3-yl) methyl esters (**6b**, **6c**, **6f**, **6g**, and **6h**) showed quite potent inhibitory activities with IC_{50} values of 2.20, 3.48, 0.35, 0.80, and 0.61 μM, respectively. The MTT results showed that all of the active compounds exhibited no cytotoxicity at the effective concentrations. The preliminary mechanism of the most potent compounds (**6b**, **6c**, **6f**, **6g**, and **6h**) was further examined based on the RT-PCR results and the compounds **6f**, **6g**, and **6h** inhibited NO production by suppressing the expression of iNOS mRNA in a dose dependent manner. Furthermore, a computational analysis of physicochemical parameters revealed that the most of the compounds possessed drug-like properties.

1. Introduction

Nitric oxide (NO), an endogenous free radical is an important signaling molecule involved in a wide range of physiological functions, as well as pathophysiological states.[1] NO is generated from L-arginine by a family of nitric oxide synthases (NOSs) including major of isozymes, endothelial NOS (eNOS), neuronal NOS (nNOS), and inducible NOS (iNOS).[2,3] Both eNOS and nNOS are constitutively expressed and produce NO at a low level. However, iNOS is often expressed at high levels and is essentially unregulated once expressed. If activated by many immunological stimuli such as lipopolysaccharide (LPS), interferon (IFN-γ) and a variety of pro-inflammatory cytokines, iNOS produces a high level of NO to exert defense against pathogens.[4–6] It is well known that NO plays a major role in anti-inflammatory and immune reactions, however, an extremely high level of NO induced by iNOS causes inflammatory diseases such as rheumatoid arthritis.[7] Therefore, as drug development targets, inhibitors of NO overproduction and overexpression of iNOS might be beneficial for treatment of inflammatory disorders caused by excessive production of NO.

Many natural and synthetic flavonoids, such as flavones, isoflavones and azaisoflavones with several hydroxyl groups on their skeletons (Fig. 1), possess inhibitory effect on NO production and expression of iNOS.[8–12] Hydroxyl groups might be effective moiety to improve the potency of NO inhibitory activity for this type of compounds. Chromone skeleton, very similar with flavonoid structure, is a class of heterocyclic compounds which possess a variety of biological effects, such as antioxidant,[13,14] anti-inflammatory,[15,16] antibacterial,[17] antiviral,[18] antitumor,[19] and HIV-inhibitory[20,21] activities. Our previous data revealed that eucryphin, a chromone rhamnoside possessed a more potent activity on

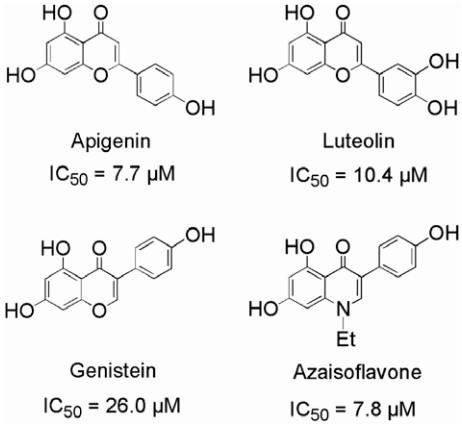

Figure 1. Some natural and synthetic flavonoids.

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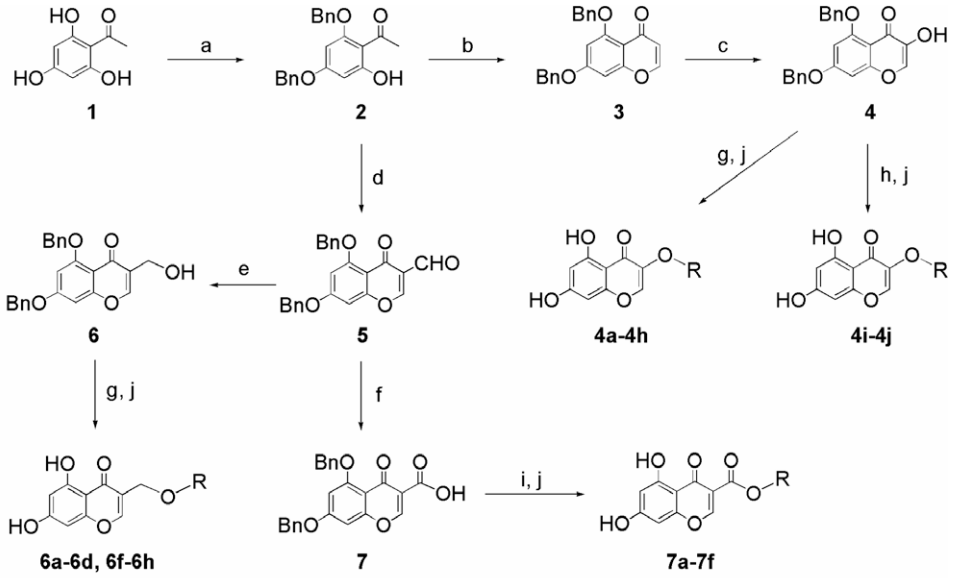
preventing hepatocyte damage caused by inflammatory and immunological reactions compared with several flavonoids.[22] Moreover, it has also been reported that the conjugates of various pharmacophores with fatty acids, aromatic acids or sulfonic acids could provide effective bioactivity, and different fatty chain lengths and different aromatic substituents could also impact the activity.[23–25] These results give us a clue that increasing the number of hydroxyl groups and different substituents in chromone skeleton might improve the NO inhibitory activity. However, chromones, especially multi-hydroxyl chromones together with NO inhibitory activity were reported rarely. With the aim of exploring new chemical pharmacophores with anti-inflammatory activity, we chose to focus on multi-hydroxyl chromone derivatives.

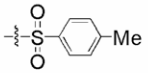
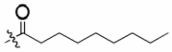
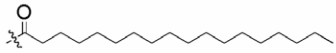
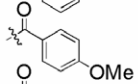
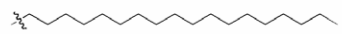
In this paper, a series of diphenolic chromone derivatives were synthesized. The NO inhibitory activity of the synthesized compounds was evaluated with LPS-activated macrophages assay and their cytotoxicity was tested using MTT method. The preliminary mechanism of some compounds with potent activity was examined by reverse transcription-polymerase chain reaction (RT-PCR) analysis. A computational calculation on the physicochemical parameters of all the compounds was also conducted to understand their drug-like properties.

2. Results and discussion

2.1. Chemistry

The syntheses of diphenolic chromone derivatives are outlined in Scheme 1. Selective protection of two hydroxyl groups of the commercially available and cheap starting material 2,4,6-trihy-



Compd.	R	Compd.	R
4a, 6a	H	**4i**	
4b, 6b		**4j**	
4c, 6c		**7a**	H
4d, 6d		**7b**	
4e		**7c**	
4f, 6f		**7d**	
4g, 6g		**7e**	
4h, 6h		**7f**	

Scheme 1. Reagents and conditions: (a) BnCl, K_2CO_3, HMPA, 90 °C, 3 h, 75%; (b) (i) HCOOEt, NaH, −10 °C, 4 h; (ii) concd HCl, rt, 12 h, 72%; (c) (i) KOH, PhI(OAc)$_2$, MeOH, THF, 0 °C, 5 h; (ii) concd HCl, acetone, rt, 3 h, 62%; (d) $POCl_3$, DMF, 0 °C to rt, 12 h, 70%; (e) basic alumina, *i*-propanol, 80 °C, 5 h, 68%; (f) $NaClO_2$, NH_2SO_3H, CH_2Cl_2, H_2O, 0 °C, 1 h, 92%; (g) RCOOH, EDCI, DMAP, CH_2Cl_2, rt, 6 h; (h) RSO_2Cl, Et_3N, CH_2Cl_2, rt, 5 h; (i) oxalyl chloride, CH_2Cl_2, rt, 6 h; (ii) ROH, Et_3N, CH_2Cl_2, rt, 2 h; (j) Pd/C, cyclohexene, MeOH, THF, reflux, 6 h, one step yield 85–92% (for **4a**, **6a**, and **7a**); two steps yield 50–83% (for other compounds).

droxyacetophenone (**1**) was carried out via reaction with benzyl chloride in hexamethylphosphotriamide (HMPA) to give **2** in good yield (75%) following recrystallization from CH_2Cl_2/MeOH.[26] Condensation of compound **2** with ethyl formate in the presence of sodium hydride, followed by dehydration of the resulting chromanol utilizing concentrated hydrochloric acid furnished **3** in an overall yield of 72%.[27] 3-Hydroxychromone can be prepared through the oxidation of an appropriate chromone derivative using an oxidizing agent, such as *m*-chloroperoxybenzoic acid (MCPBA),[28] $NbCl_5$,[29] or $PhI(OAc)_2$.[30] In recent years, the use of hypervalent iodine compounds in synthetic organic chemistry has gained attention because of their high selectivity, therefore, a hypervalent iodine reagent $PhI(OAc)_2$ was used in the present reaction. Mild oxidation in basic solution followed by acidification provided target compound 3-hydroxychromone (**4**). Elucidation of the structure of **4** was facilitated by the presence of a characteristic singlet associated with the 2-H, as opposed to a doublet in compound **3**.

Compound **5** was prepared via Vilsmeier–Haack formylation of compound (**2**) using $POCl_3$/DMF as formylation reagents in a moderate yield (70%).[31] Compound **5** was a key intermediate and could undergo both reduction and oxidation. Reduction of **5** was carried out in a suspension of basic alumina and isopropanol to get **6** in 68% yield. This reaction is the reverse process of Oppenauer oxidation, and increasing of the amount of isopropanol could be beneficial for the reduction yield.[32] Oxidation of **5** using sodium chlorite in the presence of aminosulfonic acid provided **7** in a satisfactory yield (92%) after a recrystallization in MeOH.[33] The structures of compounds **5**, **6**, and **7** could be easily determined by their characteristic proton signals in ^1H NMR spectra, and the singlet peak of CHO at 10.38 ppm for **5**, the two hydrogen atoms of CH_2OH at 4.50 ppm for **6** and the broad peak of COOH at 14.26 ppm for **7** were clearly displayed. The protective groups (Bn) of **4**, **6**, and **7** were removed by catalytic hydrogenolysis using Pd/C as a catalyst, cyclohexene as a hydrogen donor and THF as an auxiliary solvent to yield **4a**, **6a**, and **7a**, respectively.

In order to understand the influences on activity, esterifications of the hydroxyl and carboxyl of chromone were conducted and the corresponding esters of **4a**, **6a**, and **7a** were prepared, respectively. EDC/DMAP-mediated esterification is commonly used in organic synthesis due to its highly reactive performance and the easy removal of the condensing byproduct of EDC.[34,35] Thus, as shown in Scheme 1, esterifications of compounds **4** or **6** with different fatty acids or aromatic acids using 1-ethyl-3-(3-dimethyllaminopropyl)carbodiimide hydrochloride (EDCI) as a condensing agent and 4-dimethylaminopyridine (DMAP) as a catalyst afforded corresponding esters. Compound **4** was also reacted with substituted sulfonyl chlorides to yield the sulfonyl esters. While compound **7** was first converted to acyl chloride using oxalyl chloride which was reacted with different alcohols to yield the corresponding esters. These esters of **4**, **6**, and **7** were deprotected by catalytic hydrogenolysis to obtain the final products **4b**–**4j**, **6b**–**6d**, **6f**–**6h**, and **7b**–**7f**, respectively.

2.2. Biological activity

The inhibitory effect of the synthesized diphenolic chromone derivatives on nitric oxide production was evaluated using LPS-activated murine macrophages-like RAW264.7 cell culture systems. The cytotoxicity of these compounds was checked using MTT assay. The results are summarized in Table 1.

As can been seen from Table 1, compounds **4a**, **6a**, and **7a** did not show obvious inhibitory activity, while most of the esters of **4a**, **6a**, and **7a** exhibited a great improvement of the activity when compared to the corresponding parent compounds. This result revealed that the esterification of diphenolic chromone was beneficial in elevating activity.

Table 1
Inhibitory activities on the NO production in LPS-activated RAW264.7 macrophages and cytotoxicity of diphenolic chromone derivatives

Compd	Activity IC_{50}		Cytotoxicity IC_{50}[a]
	(μM)	(μg/mL)	(μM)
4a	>100	>19.40	>100
4b	60.01	14.16	>100
4c	60.36	16.78	>100
4d	62.25	20.80	>100
4e	>100	>46.03	>100
4f	86.56	25.79	>100
4g	47.20	15.48	>100
4h	16.97	5.29	>100
4i	19.22	6.69	>100
4j	53.71	14.61	>100
6a	>100	>20.80	>100
6b	2.20	0.55	67.38
6c	3.48	1.02	80.21
6d	29.16	10.15	81.58
6f	0.35	0.11	54.69
6g	0.80	0.27	51.76
6h	0.61	0.20	55.61
7a	>100	>22.20	>100
7b	>100	>25.00	>100
7c	>100	>27.81	>100
7d	58.80	16.35	>100
7e	24.91	8.32	>100
7f	>100	>47.43	>100

[a] Cytotoxicity IC_{50}: the concentration of inhibition of 50% cell growth.

The fatty acids esters (**4b**–**4d**) of **4a** showed similar inhibitory activity, with IC_{50} 60.01, 60.36, and 62.25 μM, respectively, but compound **4e** showed no effect on NO production. This result suggested that the appropriate length of the fatty chains almost did not affect the inhibitory activity to some extent (**4b**–**4d**), but the extremely long chain length might decrease the activity (**4e**). Most of the esters of **4a** with aromatic acids showed a better activity than fatty acids derivatives, and the activity of substituted aromatic acids modifiers (**4g** and **4h**, with IC_{50} 47.20 and 16.97 μM, respectively) were higher than that of non-substituted aromatic acid one (**4f**, IC_{50} 86.56 μM). The aromatic sulfonic acid modifier **4i** (IC_{50} 19.22 μM) exhibited better activity than fatty sulfonic acid modifier **4j** (IC_{50} 53.71 μM), which was consistent with the result above.

The esters of **6a** exhibited quite potent inhibitory activity compared with the derivatives of **4a**. Interestingly, unlike the case of **4a** derivatives, the length of fatty acids was closely related to the inhibitory activity for the esters of **6a**. As the chain length increased, the activity of fatty acids modifiers decreased obviously (**6b** > **6c** > **6d**, with IC_{50} 2.20, 3.48, and 29.16 μM, respectively). While the aromatic acids modifiers (**6f**–**6h**) of **6a** displayed very strong inhibitory effect on NO production. In contrast to **4a** derivatives, the non-substituted aromatic acid modifier (**6f**, IC_{50} 0.35 μM) showed better inhibitory effect than the substituted aromatic acids ones (**6g** and **6h**, with IC_{50} 0.80 and 0.61 μM). From all of the derivatives, the three compounds (**6f**, **6g**, and **6h**) displayed much more potent activity than others.

Among the esters of **7a**, only **7d** and **7e** exhibited moderate inhibitory activity, while other compounds were inactive, suggesting that the carboxyl group is inimical to inhibitory activity.

The results cited above revealed that the 3-CH_2OH group of chromone skeleton, an appropriate length of fatty chain and aromatic substituents play a critical role in the enhancement of activity and might be effective moieties to improve the potency of inhibitory activity.

It is worthy to mention that the molecular weight of this series of diphenolic chromone derivatives was low (about 300). As can be seen in Table 1, when expressed in the unit of μg/mL, the IC_{50}

values of the compounds decreased to about 1/3 of the former values. The active compounds **6f**, **6g**, and **6h** displayed noteworthy IC_{50} values of 0.11, 0.27, and 0.20 μg/mL, respectively.

From the MTT results, the cytotoxicity IC_{50} (the concentration of inhibition of 50% cell growth) values of all the synthesized compounds were above 50 μM, some compounds even to 100 μM, which were much larger than those of inhibitory activity IC_{50}. It could be concluded that all the compounds displayed no obvious cytotoxicity at the effective concentrations.

Because the high level of NO is mainly generated by iNOS, iNOS expression is one of the key steps during the process of LPS-activated NO production. To further study the mechanism of the active compounds, the RT-PCR experiment was applied to assess the effect of the active compounds on mRNA expression of iNOS. As can be seen in Figure 2, the aromatic acids derivatives (**6f**, **6g**, and **6h**) of **6a** strongly inhibited the expression of iNOS mRNA in a dose dependent manner. However, the two derivatives (**6b** and **6c**) did not suppress the expression of iNOS, suggesting that the two compounds might inhibit NO production through other mechanisms.

2.3. In silico study

In order to understand the drug-like properties of the compounds, a preliminary assessment of their geometrical and electronic structures and physicochemical parameters was conducted. A density functional theory calculation was performed at DFT:B3LYP/6-31G* level for the determination of geometrical and electronic structures including E_{HOMO}, E_{LUMO}, GAP, and dipole. The final RMS gradient less than 0.01 kcal/mol was achieved for all fully optimized molecules, which was performed using Gaussian 03 software. The physicochemical parameters including miLog *P*, Mw, HBD, HBA, TPSA, and Rotatable bonds were calculated using molinspiration server (http://www.molinspiration.com/cgi-bin/properties). All the calculated results are summarized in Table 2.

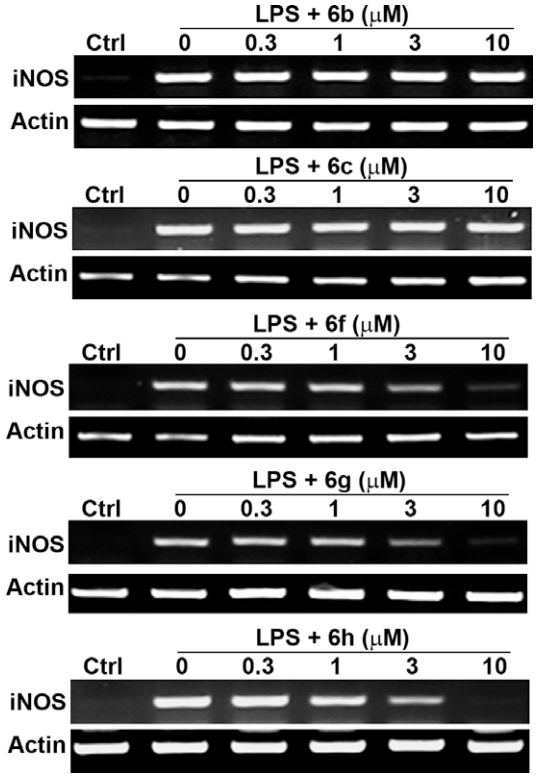


Figure 2. The RT-PCR analysis of iNOS mRNA for compounds **6b**–**6c**, **6f**–**6h**.

The electronic properties such as HOMO, LUMO, and GAP energy values of all the compounds were very similar and no correlation with the activity values was observed. However, the most active compounds (**6f**, **6g**, and **6h**) showed higher dipole values than the rest of the compounds, suggesting that dipole might be an important factor concerned with activity.

'Lipinski rule of 5' is very important in drug design and bioavailability prediction. Molecules violating more than one of these rules may have problems with bioavailability.[36] As shown in Table 2, all parameters of the compounds were consistent with the 'rule of 5' except the miLog *P* values of **4e** and **7f**, which possessed extremely long chain alkyl substituent. It was reported that a molecule with ten or fewer rotatable bonds and PSA less than 140 $Å^2$ might have a high probability of good oral bioavailability.[37] As can be seen in Table 2, all the compounds fulfilled both of the criteria except compounds **4e** and **7f**. From the calculation results and the biological activity above, it could be concluded that extremely long alkyl chain is not suitable for improving the activity, and miLog *P* at the range of 2.7–2.9 and a big dipole value might relate with a potent activity.

3. Conclusion

We have synthesized a series of diphenolic chromone derivatives with inhibitory effect on overproduction of NO and overexpression of iNOS in LPS-activated RAW264.7 macrophages. Esterification of diphenolic chromone could be beneficial to elevate the activity. Most of the esters of **6a**, including fatty acids esters (**6b** and **6c**) and aromatic acids esters (**6f**, **6g**, and **6h**), showed quite potent inhibitory activity and no cytotoxicity at effective concentrations. The most active compounds (**6f**, **6g**, and **6h**) inhibited NO production by suppressing the expression of iNOS in a dose dependent manner. The physicochemical parameters suggested that most of the compounds possessed drug-like properties. The present results revealed that the diphenolic chromone derivatives as a new class of anti-inflammatory leads warrant further studies. Further design and synthesis of related compounds, and the in vivo activity together with detailed mechanism of **6f**, **6g**, and **6h** are in progress in our lab.

4. Experimental

4.1. Synthesis

4.1.1. General

All reagents and solvents were commercially available and used without further purification. Melting points were determined on a Taike X-4 digital micromelting point apparatus and uncorrected. ^1H and ^{13}C NMR spectra were taken on a Bruker DPX-300 spectrometer, using TMS as an internal standard (chemical shifts in δ). ESI-MS were obtained on ThermoFisher LCQ Fleet mass spectrometer. ESI-HR-MS were obtained on Esquire 4000 mass spectrometer.

4.1.2. 1-(2,4-Bis(benzyloxy)-6-hydroxyphenyl)ethanone (2)

To a solution of **1** (60 g, 0.36 mol) in HMPA (300 mL) was added K_2CO_3 (148 g, 1.07 mol) and BnCl (86.3 mL, 0.75 mol), and the suspension was stirred at 90 °C for 3 h. The solid was filtered, and the filtrate was poured into ice-water. The pH of the solution was adjusted to 2 by adding diluted hydrochloric acid and the resulting yellow solid was filtered and recrystallized in CH_2Cl_2/MeOH to give **2**. Yield: 75%; ^1H NMR ($CDCl_3$, 300 MHz) δ: 2.56 (3H, s), 5.06 (4H, s), 6.11 (1H, s), 6.17 (1H, s), 7.41 (10H, m), 14.04 (1H, s); HRMS-ESI (*m*/*z*): calcd for $C_{22}H_{21}O_4$ $[M+H]^+$: 349.1440 found: 349.1446.

4.1.3. 5,7-Bis(benzyloxy)-4*H*-chromen-4-one (3)

To a solution of **2** (8.0 g, 23.0 mmol) in HCOOEt (70 mL) at −10 °C was added NaH (8.0 g, 60%, 200 mmol) in batches, and

Table 2
Calculated physicochemical properties of diphenolic chromone derivatives

Compd	E_{HOMO} (eV)	E_{LUMO} (eV)	GAP[a] (eV)	Dipole (debye)	Lipinski rule of 5				TPSA[e] (Å^2)	Rotatable bonds
					miLog *P*[b]	Mw	HBD[c]	HBA[d]		
4a	−0.215	−0.035	0.180	5.139	0.732	194.142	3	5	90.895	0
4b	−0.225	−0.040	0.185	3.148	0.504	236.179	2	6	96.972	2
4c	−0.224	−0.039	0.185	2.996	2.237	278.260	2	6	96.972	5
4d	−0.224	−0.039	0.185	2.948	4.258	334.368	2	6	96.972	9
4e	−0.222	−0.039	0.183	2.968	8.590	460.611	2	6	96.972	18
4f	−0.228	−0.042	0.186	3.729	2.820	298.250	2	6	96.972	3
4g	−0.220	−0.041	0.179	1.787	2.876	328.276	2	7	106.206	4
4h	−0.227	−0.040	0.187	3.625	2.882	312.277	2	6	96.972	3
4i	−0.235	−0.050	0.186	3.655	2.365	348.332	2	7	114.043	3
4j	−0.234	−0.047	0.187	5.637	0.383	272.234	2	7	114.043	2
6a	−0.231	−0.045	0.186	5.863	0.289	208.169	3	5	90.895	1
6b	−0.231	−0.043	0.188	4.372	0.993	250.206	2	6	96.972	3
6c	−0.231	−0.043	0.188	4.028	2.418	292.287	2	6	96.972	6
6d	−0.231	−0.042	0.188	3.982	4.439	348.395	2	6	96.972	10
6f	−0.232	−0.043	0.190	6.026	2.717	312.277	2	6	96.972	4
6g	−0.222	−0.041	0.181	6.910	2.774	342.303	2	7	106.206	5
6h	−0.233	−0.044	0.189	5.723	2.779	326.304	2	6	96.972	4
7a	-0.233	−0.051	0.182	4.992	0.524	222.152	3	6	107.966	1
7b	−0.229	−0.047	0.182	4.117	1.160	250.206	2	6	96.972	3
7c	−0.229	−0.046	0.182	4.018	2.222	278.260	2	6	96.972	5
7d	−0.221	−0.047	0.175	5.929	1.969	278.260	2	6	96.972	3
7e	−0.228	−0.046	0.182	3.979	4.242	334.368	2	6	96.972	9
7f	−0.228	−0.046	0.182	3.977	8.830	474.638	2	6	96.972	19

[a] GAP = $E_{LUMO} - E_{HOMO}$.
[b] miLog *P*: the log *P* value calculated using molinspiration server.
[c] HBD: hydrogen bond donor (expressed as the sum of OH and NH).
[d] HBA: hydrogen bond acceptor (expressed as the sum of O and N atoms).
[e] TPSA: topological polar surface area (defined as a sum of surfaces of polar atoms in a molecule).

the suspension was stirred for 4 h. The reaction was quenched by the addition of methanol. Subsequently concentrated hydrochloric acid (20 mL) was added, and the suspension was stirred overnight. Water (50 mL) was added, and the aqueous layer was extracted with CH_2Cl_2 (3 × 100 mL), the combined organic layer was washed with brine and dried over anhydrous Na_2SO_4. Solvent was evaporated in vacuo and the residue was recrystallized in methanol to provide **3**. Yield: 72%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 5.09 (2H, s), 5.21 (2H, s), 6.19 (1H, d, *J* = 6.0 Hz), 6.49 (1H, d, *J* = 2.4 Hz), 6.52 (1H, d, *J* = 2.4 Hz), 7.30–7.42 (8H, m), 7.60 (1H, d, *J* = 6.0 Hz), 7.61 (2H, d, *J* = 7.8 Hz); ESI-MS (*m/z*): 381 $[M+Na]^+$; HRMS-ESI (*m/z*): calcd for $C_{23}H_{19}O_4$ $[M+H]^+$: 359.1283 found: 359.1288.

4.1.4. 5,7-Bis(benzyloxy)-3-hydroxy-4*H*-chromen-4-one (4)

To a solution of KOH (2.4 g, 42.8 mmol) in anhydrous methanol (60 mL) was added a solution of **3** (3.2 g, 8.9 mmol) in anhydrous THF (25 mL) dropwise and the solution was stirred for 1 h. $PhI(OAc)_2$ (4.0 g, 12.4 mmol) was added in batches, and the suspension was stirred for 5 h. The reaction mixture was concentrated in vacuo, and water (50 mL) was added. The aqueous layer was extracted with CH_2Cl_2 (3 × 100 mL). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated in vacuo. The residue was dissolved in acetone (5 mL), and concentrated hydrochloric acid (2 mL) was added. The suspension was stirred for 3 h at rt, and the resulting solid was filtered, washed with cold acetone and dried to get **4**. Yield: 62%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 5.10 (2H, s), 5.23 (2H, s), 6.49 (1H, d, *J* = 2.4 Hz), 6.52 (1H, d, *J* = 2.4 Hz), 7.32–7.42 (8H, m), 7.60 (2H, d, *J* = 7.5 Hz), 7.78 (1H, s); ESI-MS (*m/z*): 397 $[M+Na]^+$; HRMS-ESI (*m/z*): calcd for $C_{23}H_{19}O_5$ $[M+H]^+$: 375.1232 found: 375.1239.

4.1.5. 5,7-Bis(benzyloxy)-4-oxo-4*H*-chromene-3-carbaldehyde (5)

To a solution of **2** (15.0 g, 43 mmol) in DMF (100 mL) was added $POCl_3$ (19.5 mL, 213 mmol) dropwise at 0 °C and the solution was stirred at rt for 12 h. The reaction mixture was poured into ice-water, and resulting solid was dissolved in CH_2Cl_2. The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified through chromatography eluting with CH_2Cl_2/EtOAc (25/1) to give **5**. Yield: 70%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 5.11 (2H, s), 5.22 (2H, s), 6.56 (1H, d, *J* = 2.1 Hz), 6.58 (1H, d, *J* = 2.1 Hz), 7.42 (8H, m), 7.59 (2H, d, *J* = 7.5 Hz), 8.32 (1H, s), 10.38 (1H, s); ESI-MS (*m/z*): 387 $[M+H]^+$; HRMS-ESI (*m/z*): calcd for $C_{24}H_{19}O_5$ $[M+H]^+$: 387.1232 found: 387.1237.

4.1.6. 5,7-Bis(benzyloxy)-3-(hydroxymethyl)-4*H*-chromen-4-one (6)

To a solution of **5** (3.0 g, 7.8 mmol) in isopropanol (150 mL) under N_2 atmosphere was added basic Al_2O_3 (30.0 g, 294 mmol). The suspension was stirred at 80 °C for 4 h. The solid was filtered, washed with hot isopropanol (3 × 50 mL) and the filtrate was concentrated in vacuo to afford **6**. Yield: 68%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 4.50 (2H, s), 5.07 (2H, s), 5.21 (2H, s), 6.49 (1H, d, *J* = 2.1 Hz), 6.51 (1H, d, *J* = 2.1 Hz), 7.41 (8H, m), 7.58 (2H, d, *J* = 7.5 Hz), 7.70 (1H, s); ESI-MS (*m/z*): 389 $[M+H]^+$, 411 $[M+Na]^+$; HRMS-ESI (*m/z*): calcd for $C_{24}H_{21}O_5$ $[M+H]^+$: 389.1389 found: 389.1396.

4.1.7. 5,7-Bis(benzyloxy)-4-oxo-4*H*-chromene-3-carboxylic acid (7)

To a solution of **5** (4.0 g, 10.4 mmol) in CH_2Cl_2 (160 mL) at 0 °C was added an aqueous solution (120 mL) of NH_2SO_3H (5.0 g, 51.5 mmol). An aqueous solution (80 mL) of $NaClO_2$ (4.7 g, 80%, 41.6 mmol) was added dropwise. The suspension was stirred for 30 min. The aqueous layer was extracted with CH_2Cl_2 (3 × 200 mL). The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was recrystallized in MeOH to afford **7**. Yield: 92%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 5.14 (2H, s), 5.24 (2H, s), 6.61 (1H, d, *J* = 2.1 Hz), 6.65 (1H, d, *J* = 2.1 Hz), 7.42 (8H, m), 7.56 (2H, d, *J* = 7.2 Hz), 8.78 (1H, s), 14.26 (1H, br); ESI-MS (*m/z*): 403 $[M+H]^+$, 425 $[M+Na]^+$; HRMS-ESI (*m/z*): calcd for $C_{24}H_{19}O_6$ $[M+H]^+$: 403.1181 found: 403.1186.

4.1.8. General procedure for compounds 4a, 6a, and 7a

To a solution of **4** (**6** or **7**, 0.5 mmol) in a mixed solution of MeOH (5 mL), THF (5 mL) and cyclohexene (2 mL) was added Pd/C (212 mg, 10%, 0.2 mmol). The suspension was refluxed for 8 h. Pd/C was filtered, and the filtrate was concentrated in vacuo. The crude product was purified through chromatography to give **4a**, **6a**, and **7a**.

4.1.8.1. 3,5,7-Trihydroxy-4*H*-chromen-4-one (4a). Yield: 85%; mp 258–260 °C; ^{1}H NMR (C_5D_5N, 300 MHz) δ: 6.66 (1H, d, J = 2.1 Hz), 6.73 (1H, d, J = 2.1 Hz), 8.17 (1H, s), 13.24 (1H, br); ^{13}C NMR (C_5D_5N, 75 MHz) δ: 96.09, 101.04, 107.16, 142.61, 143.69, 160.09, 164.41, 167.17, 179.63; HRMS-ESI (m/z): calcd for $C_9H_7O_5$ $[M+H]^+$: 195.0293 found: 195.0287.

4.1.8.2. 5,7-Dihydroxy-3-(hydroxymethyl)-4*H*-chromen-4-one (6a). Yield: 88%; mp 208–210 °C; ^{1}H NMR (CD_3OD, 300 MHz) δ: 4.46 (2H, s), 6.19 (1H, d, J = 2.0 Hz), 6.31 (1H, d, J = 2.0 Hz), 7.99 (1H, s); ^{13}C NMR (CD_3OD, 75 MHz) δ: 56.64, 94.91, 100.08, 105.95, 123.36, 155.36, 159.90, 163.50, 166.33, 182.60; HRMS-ESI (m/z): calcd for $C_{10}H_9O_5$ $[M+H]^+$: 209.0450 found: 209.0449.

4.1.8.3. 5,7-Dihydroxy-4-oxo-4*H*-chromene-3-carboxylic acid (7a). Yield: 92%; mp 293–294 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 6.27 (1H, d, J = 2.1 Hz), 6.45 (1H, d, J = 2.1 Hz), 8.88 (1H, s), 11.15 (1H, br), 12.35 (1H, br); ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 94.69, 100.05, 104.36, 113.43, 157.51, 161.89, 163.29, 163.49, 165.10, 178.96; HRMS-ESI (m/z): calcd for $C_{10}H_7O_6$ $[M+H]^+$: 223.0243 found: 223.0240.

4.1.9. General procedure for compounds 4b–4h, 6b–6d, and 6f–6h

To a solution of **4** or **6** (1.0 mmol) in CH_2Cl_2 (15 mL) was added RCOOH (1.0 mmol), EDCI (230 mg, 1.2 mmol) and a few crystals of DMAP. The solution was stirred at rt for 6 h. Diluted hydrochloric acid (10 mL) was added, and the aqueous layer was extracted with CH_2Cl_2 (3 × 20 mL). The organic layer was washed with diluted hydrochloric acid, saturated $NaHCO_3$, and brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified through chromatography eluting with PE/EtOAc to give the corresponding compound which was deprotected using the same procedure as described for the preparation of **4a** to give **4b–4h, 6b–6d**, and **6f–6h**.

4.1.9.1. 5,7-Dihydroxy-4-oxo-4*H*-chromen-3-yl acetate (4b). Yield: 68%; mp 296–297 °C; ^{1}H NMR (CD_3COCD_3, 300 MHz) δ: 2.28 (3H, s), 6.30 (1H, d, J = 2.0 Hz), 6.44 (1H, d, J = 2.0 Hz), 8.25 (1H, s), 12.18 (1H, br); ^{13}C NMR (CD_3COCD_3, 75 MHz) δ: 19.17, 94.22, 99.19, 105.04, 135.09, 149.41, 157.99, 164.75, 167.79, 175.58; HRMS-ESI (m/z): calcd for $C_{11}H_9O_6$ $[M+H]^+$: 237.0399 found: 237.0401.

4.1.9.2. 5,7-Dihydroxy-4-oxo-4*H*-chromen-3-yl pentanoate (4c). Yield: 50%; mp 174–177 °C; ^{1}H NMR (CD_3COCD_3, 300 MHz) δ: 0.91 (3H, t, J = 8.0 Hz), 1.45 (2H, m), 1.66 (2H, m), 2.59 (2H, t, J = 6.0 Hz), 6.30 (1H, d, J = 2.0 Hz), 6.44 (1H, d, J = 2.0 Hz), 8.26 (1H, s), 12.20 (1H, s); ^{13}C NMR (CD_3COCD_3, 75 MHz) δ: 13.09, 21.77, 26.71, 32.68, 94.21, 99.13, 105.17, 149.36, 157.99, 162.13, 162.38, 164.51, 170.52; HRMS-ESI (m/z): calcd for $C_{14}H_{15}O_6$ $[M+H]^+$: 279.0869 found: 279.0863.

4.1.9.3. 5,7-Dihydroxy-4-oxo-4*H*-chromen-3-yl nonanoate (4d). Yield 54%; mp 119–121 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0.88 (3H, m), 1.28 (10H, m), 1.75 (2H, m), 2.64 (2H, t, J = 6.5 Hz), 6.26 (1H, s), 6.30 (1H, s), 7.85 (1H, s), 12.00 (1H, s); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 14.12, 22.65, 24.71, 29.00, 29.09, 29.71, 31.79, 33.65, 94.57, 99.80, 106.07, 134.97, 148.51, 157.66, 162.17, 163.06, 172.16, 175.62; HRMS-ESI (m/z): calcd for $C_{18}H_{23}O_6$ $[M+H]^+$: 335.1495 found: 335.1491.

4.1.9.4. 5,7-Dihydroxy-4-oxo-4*H*-chromen-3-yl stearate (4e). Yield 72%; mp 127–128 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0.88 (3H, t, J = 6.0 Hz), 1.26 (28H, m), 1.75 (2H, m), 2.63 (2H, t, J = 7.2 Hz), 6.24 (1H, s), 6.28 (1H, s), 7.84 (1H, s), 11.95 (1H, s); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 14.26, 22.84, 24.87, 29.16, 29.85, 32.07, 33.82, 94.72, 99.96, 106.24, 135.13, 148.69, 157.80, 162.32, 163.20, 172.42, 175.77; HRMS-ESI (m/z): calcd for $C_{27}H_{41}O_6$ $[M+H]^+$: 461.2903 found: 461.2904.

4.1.9.5. 5,7-Dihydroxy-4-oxo-4*H*-chromen-3-yl benzoate (4f). Yield 68%; mp 191–193 °C; ^{1}H NMR (CD_3COCD_3, 300 MHz) δ: 6.33 (1H, d, J = 2.1 Hz), 6.49 (1H, d, J = 2.1 Hz), 7.60 (2H, t, J = 7.5 Hz), 7.75 (1H, t, J = 7.5 Hz), 8.16 (2H, d, J = 7.5 Hz), 8.45 (1H, s), 12.17 (1H, s); ^{13}C NMR (CD_3COCD_3, 75 MHz) δ: 95.21, 100.13, 106.00, 129.29, 129.74, 130.94, 135.00, 136.18, 150.55, 158.94, 163.02, 163.26, 164.53, 165.58, 176.39; HRMS-ESI (m/z): calcd for $C_{16}H_{11}O_6$ $[M+H]^+$: 299.0556 found: 299.0563.

4.1.9.6. 5,7-Dihydroxy-4-oxo-4*H*-chromen-3-yl 4-methoxybenzoate (4g). Yield 60%; mp 196–197 °C; ^{1}H NMR (CD_3COCD_3, 300 MHz) δ: 3.92 (3H, s), 6.32 (1H, d, J = 1.8 Hz), 6.48 (1H, d, J = 1.8 Hz), 7.11 (2H, d, J = 9.0 Hz), 8.11 (2H, d, J = 9.0 Hz), 8.40 (1H, s), 12.21 (1H, br); ^{13}C NMR (CD_3COCD_3, 75 MHz) δ: 56.08, 95.15, 100.07, 106.04, 115.00, 121.34, 133.17, 136.23 150.51, 158.94, 163.04, 164.16, 165.34, 165.54, 176.59; HRMS-ESI (m/z): calcd for $C_{17}H_{13}O_7$ $[M+H]^+$: 329.0661 found: 329.0659.

4.1.9.7. 5,7-Dihydroxy-4-oxo-4*H*-chromen-3-yl 2-methylbenzoate (4h). Yield: 64%; mp 190–192 °C; ^{1}H NMR (CD_3OD, 300 MHz) δ: 2.63 (3H, s), 6.26 (1H, d, J = 2.0 Hz), 6.40 (1H, d, J = 2.0 Hz), 7.35–7.38 (2H, m), 7.53 (1H, td, J = 8.1, 1.5 Hz), 8.12 (1H, dd, J = 7.2, 1.5 Hz), 8.29 (1H, s); ^{13}C NMR (CD_3OD, 75 MHz) δ: 22.71, 96.25, 101.31, 127.97, 129.71, 133.27, 133.81, 135.17, 137.47, 143.39, 151.79, 160.56, 164.33, 167.00, 167.39, 178.70; HRMS-ESI (m/z): calcd for $C_{17}H_{12}O_6Na$ $[M+Na]^+$: 335.0532 found: 335.0536.

4.1.9.8. (5,7-Dihydroxy-4-oxo-4*H*-chromen-3-yl)methyl acetate (6b). Yield: 66%; mp 189–191 °C; ^{1}H NMR (C_5D_5N, 300 MHz) δ: 2.04 (3H, s), 5.16 (2H, s), 6.63 (1H, d, J = 1.8 Hz), 6.72 (1H, d, J = 1.8 Hz), 8.22 (1H, s), 13.18 (1H, br); ^{13}C NMR (C_5D_5N, 75 MHz) δ: 21.11, 58.08, 95.39, 100.76, 105.74, 118.46, 156.98, 159.11, 163.52, 166.77, 171.26, 181.44; HRMS-ESI (m/z): calcd for $C_{12}H_{11}O_6$ $[M+H]^+$: 251.0555 found: 251.0555.

4.1.9.9. (5,7-Dihydroxy-4-oxo-4*H*-chromen-3-yl)methyl pentanoate (6c). Yield: 63%; oil; ^{1}H NMR (C_5D_5N, 300 MHz) δ: 0.77 (3H, t, J = 7.4 Hz), 1.24 (2H, sxt, J = 7.4 Hz), 1.58 (2H, quint, J = 7.4 Hz), 2.36 (2H, t, J = 7.4 Hz), 5.20 (2H, s), 6.64 (1H, d, J = 2.1 Hz), 6.73 (1H, d, J = 2.1 Hz), 8.27 (1H, s), 13.20 (1H, br); ^{13}C NMR (C_5D_5N, 75 MHz) δ: 14.17, 22.74, 27.53, 34.31, 57.99, 95.36, 100.72, 105.53, 118.53, 156.98, 159.14, 163.48, 166.73, 173.87, 181.34; HRMS-ESI (m/z): calcd for $C_{15}H_{17}O_6$ $[M+H]^+$: 293.1025 found: 293.1014.

4.1.9.10. (5,7-Dihydroxy-4-oxo-4*H*-chromen-3-yl)methyl nonanoate (6d). Yield: 69%; oil; ^{1}H NMR (C_5D_5N, 300 MHz) δ: 0.83 (3H, t, J = 6.8 Hz), 1.15–1.24 (10H, m), 1.64 (2H, quint, J = 7.5 Hz), 2.40 (2H, t, J = 7.5 Hz), 5.23 (2H, s), 6.64 (1H, d, J = 2.1 Hz), 6.73 (1H, d, J = 2.1 Hz), 8.29 (1H, s), 13.22 (1H, br); ^{13}C NMR (C_5D_5N, 75 MHz) δ: 14.61, 23.24, 25.60, 29.66, 29.73, 29.84, 32.34, 34.66, 57.99, 95.37, 100.74, 105.74, 118.57, 157.03, 159.11, 163.52,

166.78, 173.93, 181.45; HRMS-ESI (*m/z*): calcd for $C_{19}H_{25}O_6$ $[M+H]^+$: 349.1651 found: 349.1637.

4.1.9.11. (5,7-Dihydroxy-4-oxo-4*H*-chromen-3-yl)methyl benzoate (6f). Yield: 64%; mp 208–209 °C; ^{1}H NMR (C_5D_5N, 300 MHz) δ: 5.43 (2H, s), 6.66 (1H, d, J = 1.9 Hz), 6.74 (1H, d, J = 1.9 Hz), 7.40 (2H, t, J = 7.3 Hz), 7.52 (1H, t, J = 7.3 Hz), 8.21 (2H, d, J = 7.3 Hz), 8.38 (1H, s), 13.21 (1H, br); ^{13}C NMR (C_5D_5N, 75 MHz) δ: 58.81, 95.44, 100.78, 105.72, 118.38, 129.24, 130.40, 130.88, 133.88, 157.30, 159.28, 163.54, 166.77, 166.88, 181.46; HRMS-ESI (*m/z*): calcd for $C_{17}H_{13}O_6$ $[M+H]^+$: 313.0712 found: 313.0708.

4.1.9.12. (5,7-Dihydroxy-4-oxo-4*H*-chromen-3-yl)methyl 4-methoxybenzoate (6g). Yield: 64%; mp 186–188 °C; ^{1}H NMR (C_5D_5N, 300 MHz) δ: 3.68 (3H, s), 5.43 (2H, s), 6.66 (1H, d, J = 2.2 Hz), 6.74 (1H, d, J = 2.2 Hz), 7.01 (2H, d, J = 9.1 Hz), 8.23 (2H, d, J = 9.1 Hz), 8.36 (1H, s), 13.23 (1H, br); ^{13}C NMR (C_5D_5N, 75 MHz) δ: 55.81, 58.48, 95.40, 100.74, 105.72, 114.62, 118.57, 123.15, 132.52, 157.21, 159.17, 163.53, 164.37, 166.65, 166.72, 181.40; HRMS-ESI (*m/z*): calcd for $C_{18}H_{15}O_7$ $[M+H]^+$: 343.0818 found: 343.0812.

4.1.9.13. (5,7-Dihydroxy-4-oxo-4*H*-chromen-3-yl)methyl 2-methylbenzoate (6h). Yield: 67%; mp 212–214 °C; ^{1}H NMR (C_5D_5N, 300 MHz) δ: 2.66 (3H, s), 5.40 (2H, s), 6.66 (1H, d, J = 2.0 Hz), 6.73 (1H, d, J = 2.0 Hz), 7.20–7.26 (2H, m), 7.39 (1H, t, J = 7.5 Hz), 8.11 (1H, d, J = 7.5 Hz), 8.36 (1H, s), 13.20 (1H, br); ^{13}C NMR (C_5D_5N, 75 MHz) δ: 22.12, 58.65, 95.48, 100.82, 105.70, 118.54, 126.59, 131.51, 132.48, 132.93, 141.15, 157.24, 159.32, 163.59, 166.80, 167.83, 174.84, 181.46; HRMS-ESI (*m/z*): calcd for $C_{18}H_{15}O_6$ $[M+H]^+$: 327.0868 found: 327.0865.

4.1.10. General procedure for compounds 4i and 4j

To a solution of **4** (374 mg, 1.0 mmol) in CH_2Cl_2 (10 mL) was added RSO_2Cl (1.5 mmol) and Et_3N (0.42 mL, 3.0 mmol). The solution was stirred at rt for 4 h. CH_2Cl_2 (20 mL) was added, and the organic phase was washed with diluted hydrochloric acid, saturated $NaHCO_3$ and brine, and dried over anhydrous Na_2SO_4. The solvent was evaporated in vacuo and the residue was purified through chromatography eluting with PE/EtOAc to give the compound which was deprotected using the same procedure as described for the preparation of **4a** to give **4i** and **4j**.

4.1.10.1. 5,7-Dihydroxy-4-oxo-4*H*-chromen-3-yl 4-methylbenzenesulfonate (4i). Yield: 83%; mp 232–234 °C; ^{1}H NMR (CD_3OD, 300 MHz) δ: 2.46 (3H, s), 6.20 (1H, d, J = 2.1 Hz), 6.35 (1H, d, J = 2.1 Hz), 7.43 (2H, d, J = 8.2 Hz), 7.86 (2H, d, J = 8.2 Hz), 8.19 (1H, s); ^{13}C NMR (CD_3OD, 75 MHz) δ: 22.54, 96.35, 101.48, 111.91, 117.60, 130.88, 131.87, 134.24, 135.98, 147.30, 148.64, 153.98, 159.82, 164.34, 167.55; HRMS-ESI (*m/z*): calcd for $C_{16}H_{13}O_7S$ $[M+H]^+$: 349.0382 found: 349.0384.

4.1.10.2. 5,7-Dihydroxy-4-oxo-4*H*-chromen-3-yl methanesulfonate (4j). Yield: 80%; mp 226–228 °C; ^{1}H NMR (CD_3OD, 300 MHz) δ: 3.44 (3H, s), 6.26 (1H, d, J = 2.2 Hz), 6.39 (1H, d, J = 2.2 Hz), 8.37 (1H, s); ^{13}C NMR (CD_3OD, 75 MHz) δ: 39.43, 95.63, 100.73, 106.55, 135.75, 153.56, 159.59, 163.59, 166.79, 170.01, 177.39; HRMS-ESI (*m/z*): calcd for $C_{10}H_9O_7S$ $[M+H]^+$: 273.0069 found: 273.0057.

4.1.11. General procedure for compounds 7b–7f

To a solution of **7** (301 mg, 0.75 mmol) in CH_2Cl_2 (10 mL) was added oxalyl chloride (1 mL), and the solution was stirred at rt for 6 h. Then the mixture was concentrated in vacuo, and cyclohexane (3 × 20 mL) was added to the residue and the solution was concentrated in vacuo (this procedure was repeated three times) to eliminate the excess oxalyl chloride. To the solution of the above acid chloride in CH_2Cl_2 (10 mL) was added corresponding ROH (1.5 mmol) and Et_3N (0.22 mL, 1.5 mmol). The reaction mixture was stirred at rt for 2 h, and then concentrated and chromatographed to yield the compound which was deprotected using the same procedure as described for the preparation of **4a** to give the final product **7b–7f**.

4.1.11.1. Ethyl 5,7-dihydroxy-4-oxo-4*H*-chromene-3-carboxylate (7b). Yield: 72%; mp 224–226 °C; ^{1}H NMR (C_5D_5N, 300 MHz) δ: 1.24 (3H, t, J = 7.1 Hz), 4.31 (2H, q, J = 7.1 Hz), 6.30 (1H, d, J = 2.1 Hz), 6.70 (1H, d, J = 2.1 Hz), 8.81 (1H, s), 13.41 (1H, br); ^{13}C NMR (C_5D_5N, 75 MHz) δ: 15.54, 62.47, 96.61, 102.26, 106.81, 116.31, 159.18, 163.94, 164.90, 167.71, 179.97; HRMS-ESI (*m/z*): calcd for $C_{12}H_{11}O_6$ $[M+H]^+$: 251.0556 found: 251.0544.

4.1.11.2. Butyl 5,7-dihydroxy-4-oxo-4*H*-chromene-3-carboxylate (7c). Yield: 68%; mp 204–206 °C; ^{1}H NMR (C_5D_5N, 300 MHz) δ: 0.85 (3H, t, J = 7.4 Hz), 1.40 (2H, sxt, J = 7.4 Hz), 1.64 (2H, quint, J = 6.7 Hz), 4.42 (2H, t, J = 6.7 Hz), 6.64 (1H, d, J = 2.1 Hz), 6.70 (1H, d, J = 2.1 Hz), 8.82 (1H, s), 13.40 (1H, br); ^{13}C NMR (C_5D_5N, 75 MHz) δ: 14.18, 19.79, 31.27, 65.43, 95.75, 101.41, 105.94, 115.61, 158.34, 162.97, 163.00, 164.06, 166.86, 179.11; HRMS-ESI (*m/z*): calcd for $C_{14}H_{14}O_6Na$ $[M+Na]^+$: 301.0688 found: 301.0685.

4.1.11.3. *tert*-Butyl 5,7-dihydroxy-4-oxo-4*H*-chromene-3-carboxylate (7d). Yield: 65%; mp 252–254 °C; ^{1}H NMR (C_5D_5N, 300 MHz) δ: 1.59 (9H, s), 6.63 (1H, d, J = 1.9 Hz), 6.69 (1H, d, J = 1.9 Hz), 8.71 (1H, s), 13.44 (1H, br); ^{13}C NMR (C_5D_5N, 75 MHz) δ: 28.51, 82.23, 95.65, 101.27, 105.94, 116.92, 158.36, 162.39, 164.22, 166.75, 179.38; HRMS-ESI (*m/z*): calcd for $C_{14}H_{14}O_6Na$ $[M+Na]^+$: 301.0688 found: 301.0686.

4.1.11.4. Octyl 5,7-dihydroxy-4-oxo-4*H*-chromene-3-carboxylate (7e). Yield: 76%; mp 158–160 °C; ^{1}H NMR (C_5D_5N, 300 MHz) δ: 0.85 (3H, t, J = 6.6 Hz), 1.21 (8H, m), 1.39 (2H, quint, J = 7.7 Hz), 1.71 (2H, quint, J = 6.9 Hz), 4.36 (2H, t, J = 6.9 Hz), 6.64 (1H, d, J = 1.9 Hz), 6.70 (1H, d, J = 1.9 Hz), 8.85 (1H, s), 13.40 (1H, br); ^{13}C NMR (C_5D_5N, 75 MHz) δ: 14.64, 23.26, 26.59, 29.32, 29.80, 29.83, 32.37, 65.80, 95.73, 101.38, 105.94, 115.64, 158.35, 162.99, 163.34, 164.06, 166.82, 179.17; HRMS-ESI (*m/z*): calcd for $C_{18}H_{23}O_6$ $[M+H]^+$: 335.1495 found: 335.1490.

4.1.11.5. Octadecyl 5,7-dihydroxy-4-oxo-4*H*-chromene-3-carboxylate (7f). Yield: 70%; mp 144–146 °C; ^{1}H NMR (C_5D_5N, 300 MHz) δ: 0.87 (3H, t, J = 6.6 Hz), 1.28–1.43 (30H, m), 1.74 (2H, quint, J = 6.8 Hz), 4.37 (2H, t, J = 6.8 Hz), 6.50 (1H, d, J = 2.2 Hz), 6.71 (1H, d, J = 2.2 Hz), 8.88 (1H, s), 13.43 (1H, br); ^{13}C NMR (C_5D_5N, 75 MHz) δ: 15.58, 24.24, 27.51, 30.21, 30.81, 30.91, 31.10, 31.18, 31.22, 31.25, 31.29, 33.41, 66.70, 96.61, 102.26, 106.81, 116.47, 159.22, 163.94, 164.35, 164.94, 167.74, 180.06; HRMS-ESI (*m/z*): calcd for $C_{28}H_{42}O_6Na$ $[M+Na]^+$: 497.2879 found: 497.2875.

4.2. Biological assay

4.2.1. Materials

Murine macrophages-like RAW264.7 cell line was obtained from Institute of Biochemistry and Cell Biology (Shanghai, China). Stock solutions of compounds were prepared with 100% dimethylsulfoxide (DMSO, Sigma) and diluted with RPMI 1640 medium containing 10% fetal bovine serum (FBS, Life Technologies Inc., Grand Island, NY). MTT (3-(4,5-dimethylthylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) and lipopolysaccharide (LPS, *Escherichia coli*) were purchased from Sigma (St. Louis, Mo). Griess reagent was obtained from Promega (Madison, USA). Trizol reagent

was purchased from Invitrogen (Carlsbad, USA). M-MLV reverse transcriptase was obtained from Toyobo (Osaka, Japan).

4.2.2. Cell culture

RAW264.7 cells were cultured in RPMI 1640 medium supplemented with penicillin (100 U/mL), streptomycin (100 μg/mL), and 10% FBS at 37 °C in a humidified 5% CO_2 and 95% air. Cell concentration was adjusted to 4×10^5 cells/mL, and 200 μL of cell suspension was seeded in each well of a 96-well flat-bottomed plate. After a 2 h incubation, medium was replaced with a fresh 2% FBS-RPMI 1640 medium containing 2 μg/mL of LPS plus 0.1% DMSO or 2 μg/mL LPS plus the test compounds at various concentrations (0.01–100 μM) and cells was then incubated for 24 h. Cells incubated in fresh 2% FBS-RPMI 1640 medium containing 0.1% DMSO were used as control. The levels of NO in the culture supernatant were analyzed. When determining the mRNA expressions of iNOS in RAW264.7 cells, cells were treated with LPS and compounds for 8 h.

4.2.3. Detection of NO levels and cytotoxicity

NO production was determined by measuring the accumulation of nitrite in the culture supernatant using the Griess reagent according to manufacturer's instructions. Briefly, 100 μL culture supernatant was transferred into a new 96-well plate and mixed with 100 μL of Griess reagent. After 10 min, the absorbance value at 540 nm was collected by microplate reader. The inhibition rate (%) of NO release was determined using the following formula:

$$\begin{aligned}\text{Inhibition } (\%) = [&\text{LPS } (OD_{540}) \\ &- \text{Compounds } (OD_{540})]/[\text{LPS } (OD_{540}) \\ &- \text{Control } (OD_{540})] \times 100\end{aligned}$$

Cytotoxicity was determined using the MTT colorimetric method. Cells cultured in 96-well plated were left untreated or treated with compounds at various concentrations for 24 h. Twenty-microliter MTT (5 mg/mL) reagent was added 4 h before the end of culture. Then 90 μL of lysis buffer (10% SDS, 50% DMF, pH 7.2) was added to each well for 6 h and the absorbance value at 570 nm was collected by microplate reader. Cytotoxicity (%) was determined using the following formula:

$$\begin{aligned}\text{Cytotoxicity } (\%) = (1 - [&\text{Compounds } (OD_{570}) \\ &- \text{Background } (OD_{570})]/[\text{Control } (OD_{570}) \\ &- \text{Background } (OD_{570})]) \times 100\end{aligned}$$

The IC_{50} values for both inhibition and cytotoxicity were determined graphically using software Origin v7.5.

4.2.4. Examination of iNOS mRNA by RT-PCR assay

To determine iNOS mRNA expression levels in RAW264.7 cells, total RNA was extracted using the Trizol reagent and cDNA was synthesized from 1 μg RNA using M-MLV reverse transcriptase according to manufacturer's instructions. The sense primer of iNOS was 5′-GGAGCGAGTTGTGGATTGTC-3′, and the antisense primer was 5′-CTCTGCCTATCCGTCTCGTC-3′. The sense primer of β-Actin was 5′-TGCTGTCCCTGTATGCCTCT-3′, and the antisense primer was 5′-TTTGATGTCACGCACGATTT-3′. The PCR reaction was performed under the following conditions: 94 °C for 5 min and 30 cycles at 94 °C for 30 s, 58 °C for 30 s and 72 °C for 30 s with a final elongation step of 72 °C for 5 min. The PCR products were run on a 2% agarose gel and visualized by ethidium bromide staining. The bands in the gel were then photographed.

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Supplementary data

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