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Full Paper

Synthesis of New Tricyclic and Tetracyclic Fused Coumarin Sulfonate Derivatives and Their Inhibitory Effects on LPS-Induced Nitric Oxide and PGE_2 Productions in RAW 264.7 Macrophages: Part 2

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The synthesis of a new series of 21 fused coumarin derivatives is described, and the biological evaluation of their *in vitro* antiinflammatory effects as inhibitors of lipopolysaccharide (LPS)-induced nitric oxide (NO) and prostaglandin E_2 (PGE_2) production in RAW 264.7 macrophages. The target compounds **1a–u** were first tested for cytotoxicity to determine a non-toxic concentration for antiinflammatory screening, so that the inhibitory effects against NO and PGE_2 production would not be caused by cytotoxicity. Compounds **1f** and **1p** were the most active PGE_2 inhibitors with IC_{50} values of 0.89 and 0.95 μM, respectively. Western blot and cell-free COX-2 screening showed that their effects were due to inhibition of both COX-2 protein expression and COX-2 enzyme activity. Their IC_{50} values against the COX-2 enzyme were 0.67 and 0.85 μM, respectively, which is more potent than etoricoxib. The selectivity indexes of compounds **1f** and **1p** against COX-2 compared to COX-1 were 41.1 and 42.5, respectively. Compound **1f** showed strong inhibitory effects at 5 μM concentration on COX-2 mRNA expression in LPS-induced RAW 264.7 macrophages. Moreover, the tricyclic compounds **1l** and **1n** as well as the tetracyclic analog **1u** were the most potent NO inhibitors, with one-digit micromolar IC_{50} values. They showed dose-dependent inhibition of inducible nitric oxide synthase (iNOS) protein expression. The tetracyclic derivative **1u** was the most potent inhibitor of NO production. It also exhibited a strong inhibitory effect on iNOS mRNA expression in LPS-induced RAW 264.7 macrophages.

Keywords: Antiinflammatory / Coumarin sulfonate / COX-2 / NO / PGE_2

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Introduction

Inflammation is a biological symptom that occurs as a normal response to some external factors such as edema or microbial infection [1]. Mild and short-term inflammation is not

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considered as a pathological state. But exaggerated and chronic inflammation requires treatment with antiinflammatory therapeutic agents. The chronic inflammation is concomitant to several human diseases such as Alzheimer's disease [2], cancer [3], arthritis [4], inflammatory bowel syndrome [5], and arteriosclerosis [6].

Nitric oxide (NO) is an endothelial-derived relaxation factor that causes vasodilation. Recent reports have highlighted the significance of NO-releasing antiinflammatory agents as cardioprotective and hypotensive agents [7–9]. However, NO contributes to localized inflammation. During the chronic inflammatory condition, the inducible nitric oxide synthase (iNOS) secretes NO as an inflammatory mediator. It causes vasodilation at the location of inflammation, and this causes edema [10]. The cyclooxygenase-2 (COX-2) converts the arachidonic acid into another inflammatory mediator, PGE_2 [11]. So inhibition of NO production through inhibition of iNOS enzyme activity and/or protein expression can be a useful avenue for treatment of inflammation. Similarly, inhibition of PGE_2 production via inhibition of COX-2 protein expression and/or enzyme activity is another potential approach for inflammation therapy.

Several coumarin derivatives have been recently reported as antiinflammatory agents [12–15]. Our group has published the NO and PGE_2 production inhibition effects of a series of tricyclic cycloalkane-fused coumarin derivatives. A cycloheptane-fused tricyclic coumarin compound possessing 4-(trifluoromethyl) benzene sulfonate moiety showed high potency as inhibitor of NO and PGE_2 productions (Fig. 1). Its IC_{50} values as inhibitor of NO and PGE_2 productions in lipopolysaccharide (LPS)-induced RAW 264.7 macrophages were 3.84 μM and 6.45 nM, respectively [16]. Its promising activity encouraged us to modify the structure to produce new derivatives with modified ring fused to the coumarin nucleus, with substitutions on the coumarin nucleus, positional isomer, or a tetracyclic instead of tricyclic structure as illustrated in Fig. 1, to explore the effects of these structural modifications on the activity. Most of these new derivatives (compounds **1a–n** and **1q–t**) were tested for anticancer activity [17, 18]. Herein, the 21 derivatives were tested for their inhibitory effects against NO and PGE_2 productions in LPS-induced RAW 264.7 macrophage cells. The most promising compounds were further considered at cellular and molecular levels for more understanding of their redundancy mechanisms of action.

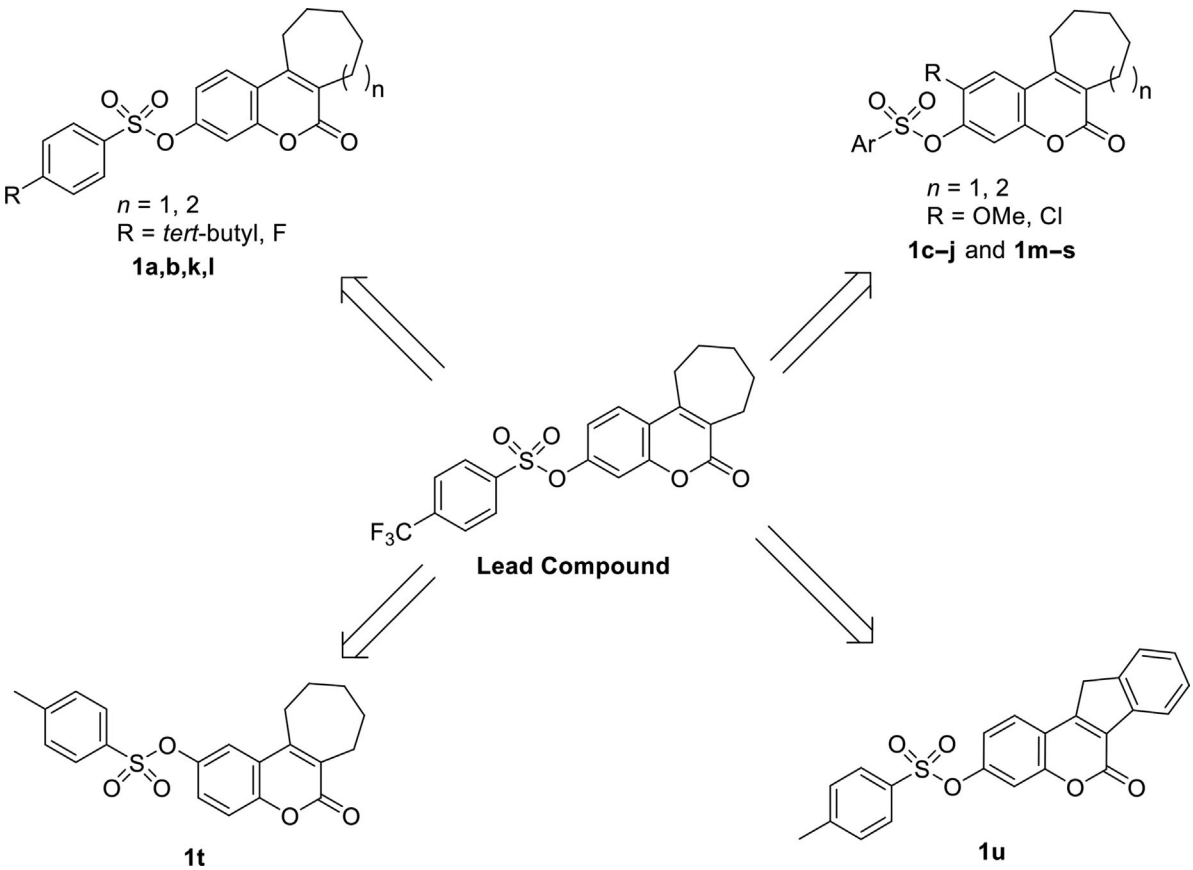

Figure 1. Structure of the lead compound [16] and structural modifications to the new derivatives.

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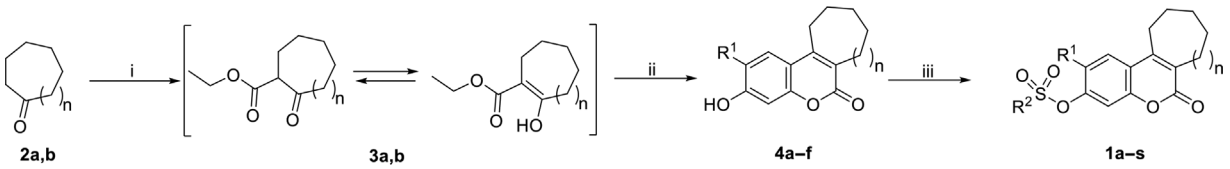


n = 1, 2
R^1 = H, OMe, Cl
R^2 = Me, *n*-Pr, Ph, *p*-tolyl, *p*-$(CF_3)C_6H_4$, *p*-$(F)C_6H_4$, *p*-(*tert*-butyl)C_6H_4

Scheme 1. Reagents and conditions: (i) diethyl carbonate, NaH, benzene, reflux, 90% (**3a**, n = 1), 85% (**3b**, n = 2); (ii) (substituted) resorcinol, CF_3COOH, conc. H_2SO_4, 0°C; rt, 3 h; (iii) appropriate sulfonyl chloride derivative, triethylamine, CH_2Cl_2, 0°C; rt, 1 h.

Results and discussion

Chemistry

The target compounds **1a–u** could be successfully prepared utilizing the synthetic pathways illustrated in Schemes 1–3. Refluxing cycloheptanone (**2a**) or cyclooctanone (**2b**) with diethyl carbonate and sodium hydride in benzene yielded the β-keto esters **3a,b** as a mixture of keto and enol tautomers [19]. Compounds **3a,b** were interacted with (substituted) resorcinol derivatives in the presence of conc. sulfuric and trifluoroacetic acids to produce the fused cyclized phenolic intermediates **4a–f** [20]. They were subsequently reacted with the appropriate sulfonyl chloride analog in the presence of triethylamine to give the corresponding sulfonate target compounds **1a–s**. Compound **1t** was synthesized by the same procedure but using hydroquinone instead of resorcinol to form the phenolic intermediate **5** (Scheme 2). The tetracyclic derivative **1u** was also synthesized by the same method but starting with 2-indanone (**6**) at the first step to get the β-keto ester **7** (Scheme 3). All the target compounds were purified by normal phase column chromatography, and their structures were confirmed by NMR and LC-MS analyses. Their purities were confirmed by elemental microanalysis.

Biological screening

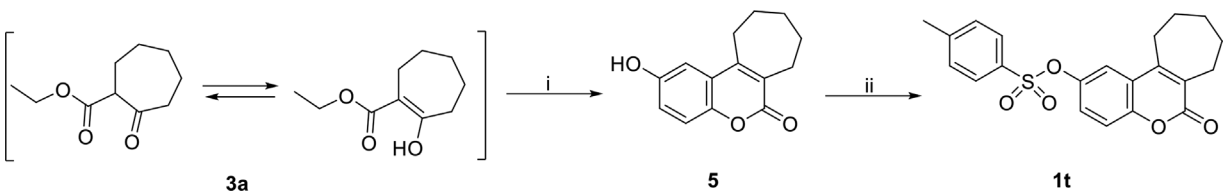
Cytotoxicity testing

Before conducting the NO and PGE_2 production inhibition screening, it was essential to carry out a cytotoxicity testing in order to determine a safe and non-toxic concentration of each compound. Every tested compound will be examined at this safe dose level for NO and PGE_2 production inhibitory effect, and any inhibition recorded will not be due to cytotoxicity against RAW 264.7 cells. The concentrations at which the cell viability decreased by 20 or 50% (IC_{80} and IC_{50}, respectively) are depicted in Table 1. The results showed that most of the compounds did not decrease the cell viability to 50% by using up to 100 μM concentration. The IC_{80} values of the target compounds were ≥10.97 μM, except for compound **1q**. So it was decided to exclude that compound from the next NO and PGE_2 production inhibition screening due to the recorded cytotoxicity.

The target compounds inhibited LPS-induced NO and PGE_2 production in RAW 264.7 macrophages

Based on the MTT cell viability assay, a single-dose concentration was selected for each compound so that any inhibitory effects on NO and/or PGE_2 production would be due to direct effect(s) on the NO and/or PGE_2 releasing pathways, not because of cytotoxicity. Every compound was tested at this single-dose concentration for inhibitory effect of various tested compounds on LPS-induced NO and PGE_2 production in RAW 264.7 macrophages. The inhibition percentage values are summarized in Table 2.

The tricyclic *para*-fluorobenzenesulfonate compound **1l** and the tetracyclic analog **1u** were the most active as NO production inhibitors. Compound **1l** possesses a cyclooctane ring fused to the coumarin nucleus, and it was more active than the corresponding less bulky cycloheptane analog. Moreover,



Scheme 2. Reagents and conditions: (i) hydroquinone, CF_3COOH, conc. H_2SO_4, 0°C; rt, 60 h; (ii) *p*-toluenesulfonyl chloride, triethylamine, CH_2Cl_2, 0°C; rt, 1 h, 65%.

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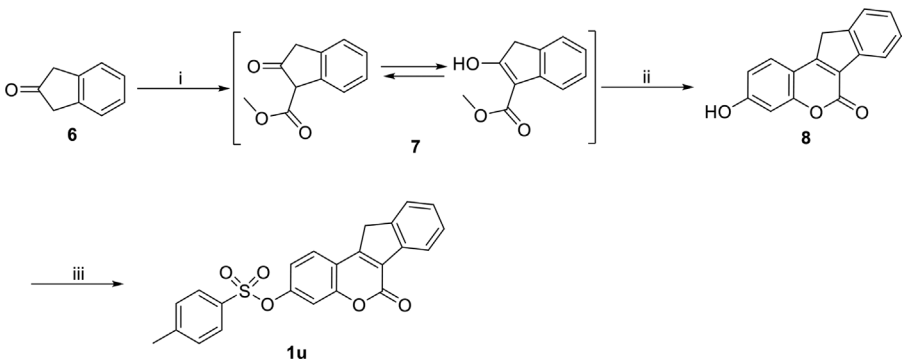
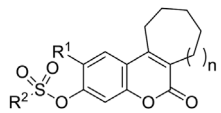
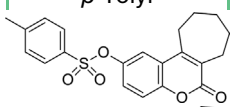
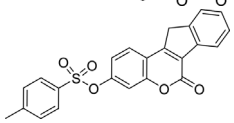


Scheme 3. Reagents and conditions: (i) dimethyl carbonate, NaH, benzene, reflux, 3 h, 54%; (ii) resorcinol, CF_3COOH, conc. H_2SO_4, 0°C; rt, 3h; (iii) *p*-toluenesulfonyl chloride, triethylamine, CH_2Cl_2, 0°C; rt, 1 h, 35%.

Table 1. Structures of the target compounds 1a–u and their IC_{50} and IC_{80} values against RAW 264.7 macrophage cell viability (50 and 80% viability, respectively).



Compound no.	R^1	R^2	n	IC_{50} (μM)[a)]	IC_{80} (μM)[b)]
1a	H	*p*-(*tert*-Butyl) C_6H_4	1	>100	19.89
1b	H	*p*-(F)C_6H_4	1	>100	20.60
1c	OMe	Me	1	>100	>100
1d	OMe	*n*-Pr	1	>100	50.91
1e	OMe	Ph	1	>100	12.06
1f	OMe	*p*-Tolyl	1	>100	35.65
1g	OMe	*p*-(CF_3)C_6H_4	1	>100	20.55
1h	Cl	Me	1	89.63	30.31
1i	Cl	Ph	1	>100	42.95
1j	Cl	*p*-Tolyl	1	>100	13.39
1k	H	*p*-(*tert*-Butyl) C_6H_4	2	>100	26.93
1l	H	*p*-(F)C_6H_4	2	>100	11.00
1m	OMe	Me	2	>100	>100
1n	OMe	*n*-Pr	2	>100	>100
1o	OMe	Ph	2	>100	86.92
1p	OMe	*p*-Tolyl	2	>100	35.02
1q	OMe	*p*-(CF_3)C_6H_4	2	>100	3.12
1r	Cl	Ph	2	>100	15.33
1s	Cl	*p*-Tolyl	2	>100	10.97
1t				>100	40.56
1u				53.59	24.25

[a)] The concentration required to inhibit 50% of the cell viability. [b)] The concentration required to inhibit 20% of the cell viability.

Table 2. Inhibitory effects of the target compounds 1a–u on LPS-induced NO and PGE_2 in RAW 264.7 macrophages.

Compound no. (tested concentration)	% Inhibition[a)]	
	NO production	PGE_2 production
1a (10 μM)	23.24 ± 0.89	23.33 ± 0.28
1b (10 μM)	34.10 ± 0.86	98.28 ± 0.82
1c (10 μM)	74.17 ± 1.08	91.44 ± 1.08
1d (10 μM)	45.83 ± 0.33	98.35 ± 0.12
1e (5 μM)	53.32 ± 1.73	97.00 ± 0.07
1f (25 μM)	70.99 ± 1.72	99.48 ± 0.30
1g (10 μM)	78.78 ± 0.62	90.70 ± 0.11
1h (10 μM)	48.33 ± 0.77	84.53 ± 0.18
1i (25 μM)	59.96 ± 1.32	98.50 ± 0.15
1j (5 μM)	59.32 ± 0.98	81.44 ± 0.89
1k (10 μM)	26.97 ± 0.68	41.11 ± 0.98
1l (10 μM)	91.18 ± 1.45	94.94 ± 1.95
1m (50 μM)	63.66 ± 0.99	88.39 ± 1.23
1n (100 μM)	86.93 ± 0.48	98.80 ± 0.07
1o (10 μM)	46.12 ± 0.56	81.32 ± 0.85
1p (25 μM)	65.65 ± 1.08	98.65 ± 0.01
1q	NT[b)]	NT[b)]
1r (5 μM)	56.69 ± 2.17	90.07 ± 1.95
1s (5 μM)	61.25 ± 1.35	82.44 ± 0.72
1t (10 μM)	28.40 ± 0.89	38.90 ± 0.68
1u (10 μM)	79.69 ± 0.27	98.20 ± 0.09
L-NIL (20 μM)	46.73	–
NS398 (3 μM)	–	94.34

[a)] Values represent means ± SD of three independent experiments. [b)] Not tested because of low IC_{80} value (Table 1).

compound **1u** contains an indene motif fused with the coumarin ring, so the bulkier ring fused to the coumarin nucleus might be more favorable for NO inhibitory effect.

Compound **1e** with methoxy group on the coumarin nucleus was more active than the corresponding unsubstituted derivative previously reported by our group (0% NO production inhibition at 20 μM concentration) [16]. Furthermore, the chlorocoumarin derivatives **1j**, **1r**, and **1s** exhibited higher activity than the corresponding unsubstituted coumarin analogs (37.45, 15.86, and 32.25% NO inhibitions at 20 μM concentrations) [16]. So the chloro or methoxy groups on the coumarin nucleus might contribute to higher affinity to the receptor site through electronic and/or steric effects. Moreover, compound **1t** was less active as NO production inhibitor than the corresponding positional isomer reported in part 1 of this study (37.45% NO production inhibition at 20 μM concentration) [16]. So the site of substitution in compound **1t** might not provide the proper orientation at the receptor site, and hence deteriorated the affinity and potency. This non-promising result of compound **1t** discouraged us to prepare more similar derivatives.

Regarding the effects on PGE_2 production in LPS-induced RAW 264.7 macrophages, several compounds showed near 100% inhibition at the tested concentration. The methoxycoumarin derivative **1e** was more active than the corresponding unsubstituted coumarin analog (81.85% PGE_2 production inhibition at 5 μM concentration) [16]. And the chlorocoumarin compounds **1j** and **1r** were also more active than the corresponding unsubstituted derivatives (37.86 and 50.44% PGE_2 inhibitions at 5 μM concentrations) [16]. So these chloro or methoxy derivatives could be optimal for PGE_2 production inhibitory activity of the compounds, and their effects can be rationalized in the same way discussed above in case of NO production inhibition. Some cycloheptane fused derivatives such as **1b**, **1d**, and **1e** showed higher activity than the corresponding cyclooctane fused analogs **1l**, **1n**, and **1o**, while opposite result was noticed upon comparing the activities of compounds **1a** and **1k**.

The most active compounds against both NO and PGE_2 productions were selected for further investigation at lower doses. The six selected compounds **1f**, **1i**, **1l**, **1n**, **1p**, and **1u** were tested at 1, 5, or 10 μM concentrations against NO production, and at 0.01, 0.1, or 1 μM concentrations against PGE_2 production.

In case of LPS-induced NO production inhibition, the cyclooctane-fused compounds **1l** and **1n**, as well as the indene-fused tetracyclic derivative **1u** showed the best results. Compound **1p** was more active than the corresponding cycloheptane fused analog **1f**. These reinforce the previously mentioned assumption that the bulkier the ring fused to the coumarin nucleus, the higher the NO inhibitory effect.

Concerning LPS-induced PGE_2 production inhibition, the highest activity was encountered with compounds **1f** and **1p**. Both of them possess methoxycoumarin nucleus and *para*-toluenesulfonate side chain. So both moieties could be optimal for appropriate affinity at the receptor site.

The IC_{50} values of compounds **1l**, **1n**, and **1u** as inhibitors of NO production, and the IC_{50} values of compounds **1f** and **1p** as PGE_2 production inhibitors were further calculated and summarized in Table 3. The bulkiest tetracyclic compound **1u** was the most potent NO production inhibitor. It showed higher

Table 3. IC_{50} values of the most active compounds as inhibitors of NO and PGE_2 productions in LPS-induced RAW 264.7 macrophages.

Compound no.	IC_{50} (μM)	
	NO production	PGE_2 production
1f	ND[a)]	0.89
1l	3.11	ND[a)]
1n	4.76	ND[a)]
1p	ND[a)]	0.95
1u	2.95	ND[a)]

[a)] Not determined.

potency than the previously reported tricyclic coumarin derivatives (its IC_{50} value was found as 2.95 μM while that of the most potent previously reported lead compound was 3.84 μM) [16]. In addition, the methoxycoumarin derivative **1n** was more potent than the corresponding unsubstituted coumarin derivative which showed IC_{50} value of 10 μM [16]. The *para*-fluorobenzenesulfonate compound **1l** exhibited superior potency compared to the corresponding *para*-(trifluoromethyl)benzenesulfonate and *para*-toluenesulfonate analogs (IC_{50} = 75.32 and 40.69 μM, respectively). In addition, compound **1f** possessing cycloheptane-fused ring was slightly more potent than the corresponding cyclooctane analog **1p** as PGE_2 production inhibitors.

Western blotting

For the purpose of deep understanding of the molecular mechanism of action of the target compounds, the most promising compounds were tested for inhibitory effects on COX-2 and iNOS protein expressions in LPS-induced RAW 264.7 macrophages by Western blot (Fig. 2). Compounds **1f** and **1p** showed dose-dependent inhibition of the COX-2 expression, and compound **1p** was more active. Moreover, compounds **1l**, **1n**, and **1u** inhibited the iNOS protein expression in a dose-dependent way. So the PGE_2 inhibitors and NO inhibitors could show their effects through inhibition of COX-2 and iNOS protein expressions in a dose-dependent manner.

COX enzyme inhibition

Cell-free COX-2 enzyme assay was conducted for compounds **1f** and **1p**. The IC_{50} values are summarized in Table 4. The obtained results were compared to those of etoricoxib, a selective COX-2 inhibitor [21]. Both compounds showed submicromolar IC_{50} results, and they were more potent than etoricoxib. So both compounds **1f** and **1p** could inhibit the LPS-induced PGE_2 production due to dual effects: inhibition of COX-2 protein expression and COX-2 enzyme inhibition. The methoxycoumarin analog **1f** was more potent than the corresponding unsubstituted coumarin derivative as COX-2 inhibitor [17]. Both compounds were further tested against COX-1 enzyme to investigate their selectivity, the IC_{50} values are also shown in Table 4. Compounds **1f** and **1p** were 41.1 and 42.5 times, respectively, more selective toward COX-2 than COX-1. So PGE_2 production inhibition could be due to selective inhibition of COX-2 isozyme.

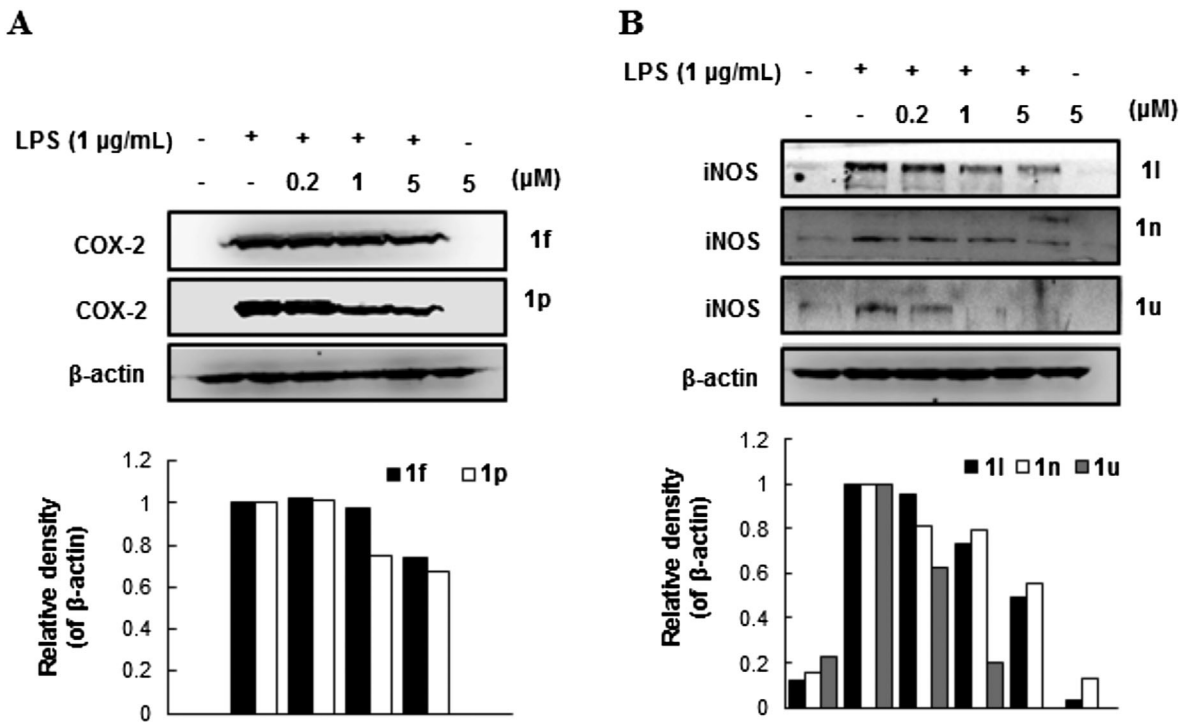


Figure 2. Inhibitory effects of compounds **1f** and **1p** on LPS-induced COX-2 protein expression in RAW 264.7 macrophages (A), and inhibitory effects of compounds **1l**, **1n**, and **1u** on LPS-induced iNOS protein expression in RAW 264.7 macrophages (B). The cellular lysates were prepared from the pretreated with/without the tested compounds for 1 h and then with LPS (10 ng/mL) for 24 h. Total cellular proteins were resolved by SDS-PAGE, transferred to PVDF membranes, and detected with specific iNOS and COX-2 antibodies. β-Actin was used as an internal control.

Table 4. IC_{50} **values of compounds 1f, 1p, and etoricoxib against COX-2 and COX-1 enzymes in cell-free enzymatic assays.**

Compound	IC_{50} (μM)[a]	
	COX-2	COX-1
1f	0.67 ± 0.08	27.52 ± 0.76
1p	0.85 ± 0.12	36.15 ± 1.17
Etoricoxib	1.10 ± 0.09	–

[a] Values represent means ± SD of three independent experiments.

mRNA expression assay

The most potent NO production inhibitor **1u** was tested for its effect on iNOS mRNA expression in LPS-induced RAW 264.7 macrophages. Similarly, the most potent COX-2 inhibitor **1f** was tested for COX-2 mRNA expression inhibition. The results are illustrated in Figs. 3 and 4. Compound **1u** strongly inhibited the iNOS mRNA expression, and compound **1f** inhibited COX-2 mRNA expression only at 5 μM.

Conclusion

We have designed and synthesized a new series of (substituted) fused coumarin derivatives as extension of our previously reported series, and tested their biological effects as inhibitors of LPS-induced NO and PGE_2 productions in RAW 264.7 macrophages. We could obtain three promising NO production inhibitors, **1l**, **1n**, and **1u**, which worked through inhibition of iNOS protein expression. Compound **1u** exerted an additional iNOS mRNA expression inhibition. It

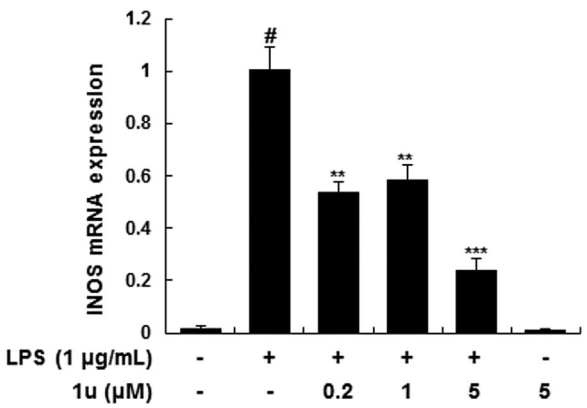


Figure 3. Effect of compound **1u** on iNOS mRNA protein expression in LPS-induced RAW 264.7 macrophages.

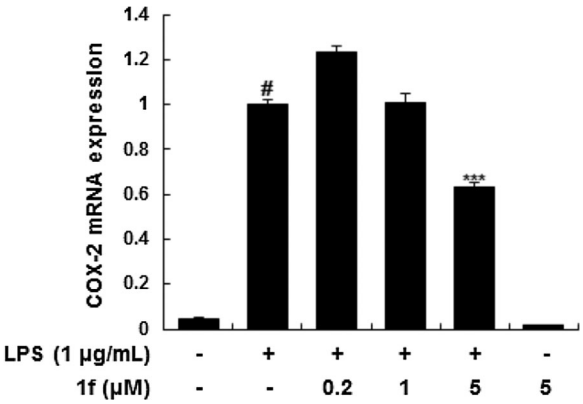


Figure 4. Effect of compound **1f** on COX-2 mRNA protein expression in LPS-induced RAW 264.7 macrophages.

was found that bulkier rings fused to the coumarin nucleus were more favorable for activity. The three compounds, especially the tetracyclic analog **1u**, was more potent than the previously reported tricyclic coumarin derivatives [16]. This study led to discovery of another two derivatives, **1f** and **1p**, as potential inhibitors of PGE_2 production through inhibition of both COX-2 protein expression and COX-2 enzymatic activity. Compound **1f** also showed inhibitory effect on COX-2 mRNA expression at 5 μM concentration. Both compounds were more potent than etoricoxib. They showed selective inhibition of COX-2 enzyme than COX-1. And both of them possess methoxycoumarin and *para*-toluenesulfonate moieties which could be favorable for appropriate affinity and potency. The cycloheptane ring was more suitable for PGE_2 production inhibition than the bulkier cyclooctane or indene rings. Compound **1t** was less active than the corresponding positional isomers, so its site of substitution with the aryl sulfonate was improper. And the chloro and methoxy substituents on the coumarin nucleus generally enhanced the activity compared with the corresponding unsubstituted coumarin derivatives.

Experimental

Chemistry

General

The target compounds were purified by column chromatography using silica gel (230–400 mesh, 0.040–0.063 mm) and hexane/ethyl acetate (technical grade). After purification, they were analyzed by ^1H NMR and CMR using a Bruker Avance 400 or 300 spectrometer. Mass spectra (MS) were obtained by LC-MS analysis. All solvents and reagents were commercially available and used as such.

Representative ^1H NMR, ^{13}C NMR, and MS spectra and the InChI codes of the investigated compounds are provided as Supporting Information.

*Synthesis of ethyl 2-oxocycloheptanecarboxylate (**3a**) and ethyl 2-oxocyclooctanecarboxylate (**3b**)*
They were synthesized through the procedure reported in the literature [16, 17, 19].

*Synthesis of the phenolic intermediates **4a–f***
They were prepared through the procedure reported in the literature [16–19].

*Synthesis of the target sulfonate compounds **1a–s***
To a solution of the appropriate hydroxyl compound **4a–f** (0.2 mmol) in dry methylene chloride (10 mL), triethylamine (0.033 mL, 0.4 mmol) was added at 0°C. A solution of the appropriate sulfonyl chloride derivative (0.22 mmol) in dry methylene chloride (5 mL) was added dropwise to the reaction mixture at the same temperature. The reaction mixture was stirred at room temperature for 1 h. The reaction mixture was washed with saline (3 × 10 mL) and dried using anhydrous magnesium sulfate.

*6-Oxo-6,7,8,9,10,11-hexahydrocyclohepta[c]chromen-3-yl 4-(tert-butyl)benzenesulfonate (**1a**)*
It was purified by flash column chromatography (silica gel, hexane/ethyl acetate 20:1 v/v, then switching to hexane/ethyl acetate 15:1 v/v); yield: 94%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.79 (d, 2H, J = 8.5 Hz), 7.64 (d, 1H, J = 8.9 Hz), 7.56 (d, 2H, J = 8.5 Hz), 7.08 (dd, 1H, J = 8.8 Hz, 2.1 Hz), 6.90 (d, 1H, J = 2.2 Hz), 2.91 (dt, 4H, J = 10.6 Hz, 9.8 Hz), 1.90 (q, 2H, J = 6.0 Hz), 1.69–1.59 (m, 4H), 1.36 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 161.5, 158.8, 152.9, 152.8, 150.7, 132.2, 128.9, 128.3, 126.4, 125.1, 118.8, 118.5, 110.7, 35.4, 31.9, 31.0, 28.2, 26.8, 25.5, 24.9; LC-MS: 427.2 (M^++1); elemental analysis ($C_{24}H_{26}O_5S$): calculated C: 67.58%, H: 6.14%, S: 7.52%; found: C: 67.80%, H: 6.03%, S: 7.57%.

*6-Oxo-6,7,8,9,10,11-hexahydrocyclohepta[c]chromen-3-yl 4-fluorobenzenesulfonate (**1b**)*
It was purified by flash column chromatography (silica gel, hexane/ethyl acetate 30:1 v/v, then switching to hexane/ethyl acetate 25:1 v/v); yield: 90%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.91–7.88 (m, 2H), 7.64 (d, 1H, J = 8.8 Hz), 7.26–7.22 (m, 2H), 7.03 (dd, 1H, J = 8.8 Hz, 2.3 Hz), 6.89 (d, 1H, J = 2.2 Hz), 2.94–2.88 (m, 4H), 1.90 (q, 2H, J = 6.0 Hz), 1.69–1.60 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 161.4, 152.8, 152.7, 150.4, 131.4, 131.3, 129.1 (d, J_{CF} = 9.12 Hz), 125.3, 119.0, 118.3, 117.0, 116.8, 110.7, 31.8, 28.2, 26.8, 25.4, 24.8; elemental analysis ($C_{20}H_{17}FO_5S$): calculated C: 61.85%, H: 4.41%, S: 8.25%; found: C: 61.60%, H: 4.50%, S: 8.35%.

*2-Methoxy-6-oxo-6,7,8,9,10,11-hexahydrocyclohepta[c]-chromen-3-yl methanesulfonate (**1c**)*
It was purified by flash column chromatography (silica gel, hexane/ethyl acetate 10:1 v/v, then switching to hexane/ethyl acetate 3.5:1 v/v); yield: 85%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.30 (s, 1H), 7.17 (s, 1H), 3.79 (s, 3H), 3.25 (s, 3H), 2.94–2.90 (m, 4H), 1.95–1.90 (m, 2H), 1.73–1.62 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 161.5, 152.3, 148.4, 146.5, 139.7, 129.6, 119.2, 113.3, 107.0, 56.7, 38.8, 31.8, 28.4, 26.9, 25.5, 24.9; LC-MS: 339.0 (M^++1); elemental analysis ($C_{16}H_{18}O_6S$): calculated C: 56.79%, H: 5.36%, S: 9.47%; found: C: 56.83%, H: 5.30%, S: 9.60%.

*2-Methoxy-6-oxo-6,7,8,9,10,11-hexahydrocyclohepta[c]-chromen-3-yl propane-1-sulfonate (**1d**)*
It was purified by flash column chromatography (silica gel, hexane/ethyl acetate 10:1 v/v, then switching to hexane/ethyl acetate 6:1 v/v); yield: 95%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.29 (s, 1H), 7.16 (s, 1H), 3.96 (s, 3H), 3.35–3.31 (m, 2H), 2.94–2.89 (m, 4H), 2.08–2.00 (m, 2H), 1.95–1.91 (m, 2H), 1.73–1.67 (m, 2H), 1.64–1.58 (m, 2H), 1.13 (t, 3H, J = 7.5 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 161.6, 152.5, 148.5, 146.5, 139.8, 129.4, 119.0, 113.1, 107.0, 56.7, 31.8, 28.4, 26.9, 25.5, 24.9, 17.4, 12.9; LC-MS: 367.0 (M^++1); elemental analysis ($C_{18}H_{22}O_6S$): calculated C: 59.00%, H: 6.05%, S: 8.75%; found: C: 58.65%, H: 6.31%, S: 8.53%.

*2-Methoxy-6-oxo-6,7,8,9,10,11-hexahydrocyclohepta[c]-chromen-3-yl benzenesulfonate (**1e**)*
It was purified by flash column chromatography (silica gel, hexane/ethyl acetate 7:1 v/v, then switching to hexane/ethyl acetate 5:1 v/v); yield: 92%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.92–7.89 (m, 2H), 7.73–7.68 (m, 1H), 7.58–7.53 (m, 3H), 7.04 (d, 2H, J = 3.0 Hz), 3.70 (s, 3H), 2.91–2.88 (m, 4H), 1.90 (q, 2H, J = 6.0 Hz), 1.70–1.59 (m, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 161.7, 152.5, 148.9, 146.2, 140.0, 136.0, 134.4, 129.4, 129.1, 128.5, 119.1, 112.6, 106.8, 56.4, 31.8, 28.4, 26.9, 25.5, 24.9; LC-MS: 401.0 (M^++1); elemental analysis ($C_{21}H_{20}O_6S$): calculated C: 62.99%, H: 5.03%, S: 8.01%; found: C: 62.71%, H: 4.90%, S: 8.12%.

*2-Methoxy-6-oxo-6,7,8,9,10,11-hexahydrocyclohepta[c]-chromen-3-yl 4-methylbenzenesulfonate (**1f**)*
It was purified by flash column chromatography (silica gel, hexane/ethyl acetate 10:1 v/v, then switching to hexane/ethyl acetate 6:1 v/v); yield: 96%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.80–7.74 (m, 2H), 7.36–7.25 (m, 2H), 7.06–6.94 (m, 2H), 3.76 (s, 3H), 2.91–2.88 (m, 4H), 2.48 (s, 3H), 1.89 (t, 2H, J = 6.0 Hz), 1.68–1.61 (m, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 162.3, 161.7, 152.6, 149.0, 146.2, 145.7, 140.2, 133.0, 129.8, 129.4, 128.5, 119.0, 112.4, 106.9, 56.5, 31.9, 28.4, 26.9, 25.5, 24.9, 21.8; LC-MS: 415.0 (M^++1); elemental analysis ($C_{22}H_{22}O_6S$): calculated C: 63.75%, H: 5.35%, S: 7.74%; found: C: 63.54%, H: 5.43%, S: 7.81%.

*2-Methoxy-6-oxo-6,7,8,9,10,11-hexahydrocyclohepta[c]-chromen-3-yl 4-(trifluoromethyl)benzenesulfonate (**1g**)*
It was purified by flash column chromatography (silica gel, hexane/ethyl acetate 10:1 v/v, then switching to hexane/ethyl acetate 6:1 v/v); yield: 87%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.05 (d, 2H, J = 7.7 Hz), 7.83 (d, 2H, J = 7.6 Hz), 7.16 (s, 1H), 7.04 (s, 1H), 3.64 (s, 3H), 2.89 (brs, 4H), 1.91 (d, 2H, J = 4.0 Hz), 1.69–1.61 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 161.5, 152.3, 148.4, 146.3, 139.4, 136.0, 135.7, 129.7, 129.1, 126.2, 126.1, 119.3, 112.8,

106.7, 56.1, 31.8, 28.4, 26.9, 25.4, 24.8; LC-MS: 469.1 (M^++1); elemental analysis ($C_{22}H_{19}F_3O_6S$): calculated C: 56.41%, H: 4.09%, S: 6.84%; found: C: 56.27%, H: 4.22%, S: 6.60%.

*2-Chloro-6-oxo-6,7,8,9,10,11-hexahydrocyclohepta[c]-chromen-3-yl methanesulfonate (**1h**)*
It was purified by flash column chromatography (silica gel, hexane/ethyl acetate 10:1 v/v, then switching to hexane/ethyl acetate 3.5:1 v/v); yield: 85%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.76 (s, 1H), 7.43 (s, 1H), 3.30 (s, 3H), 2.92–2.90 (m, 4H), 1.93–1.91 (m, 2H), 1.70–1.61 (m, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 162.3, 151.6, 151.3, 145.7, 130.4, 125.6, 122.6, 120.0, 113.0, 39.1, 31.8, 28.3, 27.0, 25.4, 24.8; LC-MS: 343.1 (M^++1); elemental analysis ($C_{15}H_{15}ClO_5S$): calculated C: 52.56%, H: 4.41%, S: 9.35%; found: C: 52.18%, H: 4.62%, S: 9.57%.

*2-Chloro-6-oxo-6,7,8,9,10,11-hexahydrocyclohepta[c]-chromen-3-yl benzenesulfonate (**1i**)*
It was purified by flash column chromatography (silica gel, hexane/ethyl acetate 13:1 v/v, then switching to hexane/ethyl acetate 10:1 v/v); yield: 85%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.94 (d, 2H, J = 6.0 Hz), 7.86–7.66 (m, 4H), 7.18 (s, 1H), 2.91–2.89 (m, 4H), 1.92–1.90 (m, 2H), 1.66–1.62 (m, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 161.0, 151.8, 151.1, 146.2, 135.4, 134.9, 130.2, 129.5, 129.4, 128.6, 125.5, 123.4, 119.7, 112.5, 31.8, 28.3, 27.0, 25.4, 24.8; LC-MS: 405.0 (M^++1); elemental analysis ($C_{20}H_{17}ClO_5S$): calculated C: 59.33%, H: 4.23%, S: 7.92%; found: C: 59.20%, H: 4.40%, S: 7.74%.

*2-Chloro-6-oxo-6,7,8,9,10,11-hexahydrocyclohepta[c]-chromen-3-yl 4-methylbenzenesulfonate (**1j**)*
It was purified by flash column chromatography (silica gel, hexane/ethyl acetate 13:1 v/v, then switching to hexane/ethyl acetate 10:1 v/v); yield: 86%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.81 (d, 2H, J = 9.0 Hz), 7.66 (s, 1H), 7.37 (d, 2H, J = 9.0 Hz), 7.15 (s, 1H), 2.89 (q, 4H, J = 6.0 Hz), 2.48 (s, 3H), 1.95–1.87 (m, 2H), 1.71–1.57 (m, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 161.0, 151.8, 151.0, 146.4, 146.3, 132.4, 130.1, 129.9, 128.6, 128.5, 125.5, 123.5, 119.6, 112.4, 31.8, 28.3, 27.0, 25.4, 24.8, 21.8; LC-MS: 418.9 (M^++1); elemental analysis ($C_{21}H_{19}ClO_5S$): calculated C: 60.21%, H: 4.57%, S: 7.65%; found: C: 60.14%, H: 4.82%, S: 7.48%.

*6-Oxo-7,8,9,10,11,12-hexahydro-6H-cycloocta[c]chromen-3-yl 4-(tert-butyl)benzenesulfonate (**1k**)*
It was purified by flash column chromatography (silica gel, hexane/ethyl acetate 20:1 v/v, then switching to hexane/ethyl acetate 15:1 v/v); yield: 95%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.81 (d, 2H, J = 8.5 Hz), 7.59–7.56 (m, 3H), 7.07 (dd, 1H, J = 8.8 Hz, 2.2 Hz), 6.91 (d, 1H, J = 2.2 Hz), 2.96 (t, 2H, J = 6.4 Hz), 2.79 (t, 2H, J = 6.0 Hz), 1.83–1.69 (m, 4H), 1.52–1.43 (m, 4H), 1.36 (s, 9H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 160.9, 158.8, 153.0, 150.5, 149.7, 132.2, 128.3, 127.1, 126.5, 125.4, 118.6, 118.3, 110.7, 35.4, 31.0, 29.7, 29.1, 26.9, 26.6, 26.4, 25.9; elemental analysis ($C_{25}H_{28}O_5S$): calculated C: 68.16%, H: 6.41%, S: 7.28%; found: C: 67.84%, H: 6.60%, S: 7.13%.

*6-Oxo-7,8,9,10,11,12-hexahydro-6H-cycloocta[c]chromen-3-yl 4-fluorobenzenesulfonate (**1l**)*
It was purified by flash column chromatography (silica gel, hexane/ethyl acetate 30:1 v/v); yield: 89%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.92–7.89 (m, 2H), 7.58 (d, 1H, J = 8.8 Hz), 7.24–7.22 (m, 2H), 7.05 (dd, 1H, J = 2.2 Hz, 8.7 Hz), 6.88 (d, 1H, J = 2.2 Hz), 2.97 (t, 2H, J = 6.5 Hz), 2.81 (t, 2H, J = 6.1 Hz), 1.81–1.73 (m, 4H), 1.53–1.44 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 160.8, 153.0, 150.2, 149.5, 131.4, 131.3, 127.3 (d, J_{CF} = 9.6 Hz), 125.5, 118.5, 118.4, 117.0, 116.8, 110.8, 29.6, 29.1, 26.9, 26.6, 26.4, 25.9; elemental analysis ($C_{21}H_{19}FO_5S$): calculated C: 62.68%, H: 4.76%, S: 7.97%; found: C: 62.73%, H: 4.93%, S: 7.67%.

*2-Methoxy-6-oxo-7,8,9,10,11,12-hexahydro-6H-cycloocta[c]chromen-3-yl methanesulfonate (**1m**)*
It was purified by flash column chromatography (silica gel, hexane/ethyl acetate 10:1 v/v, then switching to hexane/ethyl acetate 3.5:1 v/v); yield: 85%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.30 (s, 1H), 7.11 (s, 1H), 3.96 (s, 3H), 3.26 (s, 3H), 2.98 (t, 2H, J = 6.0 Hz), 2.82 (t, 2H, J = 6.0 Hz), 1.84–1.74 (m, 4H), 1.53–1.44 (m, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 161.0, 149.0, 148.5, 146.7, 139.6, 127.9, 118.7, 113.4, 107.1, 56.7, 38.8, 29.7, 29.1, 27.0, 26.8, 26.6, 25.9; LC-MS: 353.0 (M^++1); elemental analysis ($C_{17}H_{20}O_6S$): calculated C: 57.94%, H: 5.72%, S: 9.10%; found: C: 57.69%, H: 5.87%, S: 8.90%.

*2-Methoxy-6-oxo-7,8,9,10,11,12-hexahydro-6H-cycloocta[c]chromen-3-yl propane-1-sulfonate (**1n**)*
It was purified by flash column chromatography (silica gel, hexane/ethyl acetate 10:1 v/v, then switching to hexane/ethyl acetate 6:1 v/v); yield: 91%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.29 (s, 1H), 7.10 (s, 1H), 3.95 (s, 3H), 3.34 (t, 2H, J = 7.7 Hz), 2.97 (t, 2H, J = 6.4 Hz), 2.80 (t, 2H, J = 6.0 Hz), 2.10–1.97 (m, 2H), 1.86–1.73 (m, 4H), 1.53–1.44 (m, 4H), 1.12 (t, 3H, J = 6.9 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 161.0, 149.2, 146.6, 139.6, 127.6, 121.8, 118.4, 113.1, 107.1, 56.7, 53.6, 29.7, 29.0, 26.9, 26.7, 26.5, 25.8, 17.4, 12.9; LC-MS: 381.0 (M^++1); elemental analysis ($C_{19}H_{24}O_6S$): calculated C: 59.98%, H: 6.36%, S: 8.43%; found: C: 59.67%, H: 6.62%, S: 8.30%.

*2-Methoxy-6-oxo-7,8,9,10,11,12-hexahydro-6H-cycloocta[c]chromen-3-yl benzenesulfonate (**1o**)*
It was purified by flash column chromatography (silica gel, hexane/ethyl acetate 10:1 v/v, then switching to hexane/ethyl acetate 5:1 v/v); yield: 87%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.93 (d, 2H, J = 7.5 Hz), 7.71 (t, 1H, J = 7.5 Hz), 7.59–7.55 (m, 2H), 7.00 (brs, 2H), 3.72 (s, 3H), 2.94 (t, 2H, J = 6.3 Hz), 2.81 (t, 2H, J = 5.9 Hz), 1.81–1.73 (m, 4H), 1.53–1.44 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 161.1, 149.2, 149.0, 146.4, 139.9, 134.4, 129.3, 129.1, 128.9, 128.5, 127.7, 118.5, 112.6, 107.0, 56.4, 29.7, 29.0, 26.9, 26.7, 26.5, 25.9; LC-MS: 415.0 (M^++1); elemental analysis ($C_{22}H_{22}O_6S$): calculated C: 63.75%, H: 5.35%, S: 7.74%; found: C: 63.52%, H: 5.58%, S: 7.47%.

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*2-Methoxy-6-oxo-7,8,9,10,11,12-hexahydro-6H-cycloocta[c]chromen-3-yl 4-methylbenzenesulfonate (**1p**)*
It was purified by flash column chromatography (silica gel, hexane/ethyl acetate 10:1 v/v, then switching to hexane/ethyl acetate 5:1 v/v); yield: 90%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.80 (d, 2H, J = 8.0 Hz), 7.68–7.66 (m, 1H), 7.36–7.33 (m, 2H), 7.02 (s, 1H), 6.94 (s, 1H), 3.76 (s, 3H), 2.95 (t, 2H, J = 6.0 Hz), 2.81 (t, 2H, J = 5.6 Hz), 2.48 (s, 3H), 1.81–1.73 (m, 4H), 1.53–1.44 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 161.1, 149.3, 145.6, 140.0, 133.1, 129.8, 129.5, 127.5, 122.0, 118.4, 112.4, 107.0, 56.5, 29.7, 29.0, 26.9, 26.7, 26.5, 25.9, 21.8; LC-MS: 429.2 (M^++1); elemental analysis ($C_{23}H_{24}O_6S$): calculated C: 64.47%, H: 5.65%, S: 7.48%; found: C: 64.35%, H: 5.76%, S: 7.61%.

*2-Methoxy-6-oxo-7,8,9,10,11,12-hexahydro-6H-cycloocta[c]chromen-3-yl 4-(trifluoromethyl)-benzenesulfonate (**1q**)*
It was purified by flash column chromatography (silica gel, hexane/ethyl acetate 10:1 v/v, then switching to hexane/ethyl acetate 6:1 v/v); yield: 85%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.07 (d, 2H, J = 8.1 Hz), 7.84 (d, 2H, J = 8.2 Hz), 7.14 (s, 1H), 6.99 (s, 1H), 3.65 (s, 3H), 2.94 (t, 2H, J = 6.0 Hz), 2.81 (t, 2H, J = 5.8 Hz), 1.81–1.74 (m, 4H), 1.53–1.44 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 160.9, 149.0, 148.5, 146.4, 139.7, 139.4, 136.1, 135.7, 129.1, 128.0, 126.2, 118.8, 112.8, 106.8, 56.2, 29.6, 29.0, 26.9, 26.7, 26.6, 25.8; LC-MS: 483.1 (M^++1); elemental analysis ($C_{23}H_{21}F_3O_6S$): calculated C: 57.26%, H: 4.39%, S: 6.64%; found: C: 56.98%, H: 4.61%, S: 6.47%.

*2-Chloro-6-oxo-7,8,9,10,11,12-hexahydro-6H-cycloocta[c]-chromen-3-yl benzenesulfonate (**1r**)*
It was purified by flash column chromatography (silica gel, hexane/ethyl acetate 13:1 v/v, then switching to hexane/ethyl acetate 6:1 v/v); yield: 81%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.39 (d, 2H, J = 7.5 Hz), 7.97 (t, 1H, J = 7.4 Hz), 7.75–7.70 (m, 2H), 7.05 (brs, 2H), 2.92 (t, 2H, J = 6.3 Hz), 2.79 (t, 2H, J = 5.9 Hz), 1.79–1.71 (m, 4H), 1.50–1.41 (m, 4H); LC-MS: 419.0 (M^++1); elemental analysis ($C_{21}H_{19}ClO_5S$): calculated C: 60.21%, H: 4.57%, S: 7.65%; found: C: 59.90%, H: 4.86%, S: 7.55%.

*2-Chloro-6-oxo-7,8,9,10,11,12-hexahydro-6H-cycloocta[c]-chromen-3-yl 4-methylbenzenesulfonate (**1s**)*
It was purified by flash column chromatography (silica gel, hexane/ethyl acetate 13:1 v/v, then switching to hexane/ethyl acetate 7:1 v/v); yield: 80%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.26 (d, 2H, J = 7.9 Hz), 7.94–7.92 (m, 1H), 7.56–7.53 (m, 2H), 7.28 (s, 1H), 7.03 (s, 1H), 2.93 (t, 2H, J = 5.9 Hz), 2.79 (t, 2H, J = 5.6 Hz), 2.48 (s, 3H), 1.78–1.70 (m, 4H), 1.50–1.41 (m, 4H); LC-MS: 433.0 (M^++1); elemental analysis ($C_{22}H_{21}ClO_5S$): calculated C: 61.04%, H: 4.89%, S: 7.41%; found: C: 60.87%, H: 5.10%, S: 7.24%.

*Synthesis of 2-hydroxy-8,9,10,11-tetrahydrocyclohepta[c]-chromen-6(7H)-one (**5**)*
It was prepared utilizing the same method reported in the literature, similar to synthesis of compounds **4a–f** starting with hydroquinone [20].

*6-Oxo-6,7,8,9,10,11-hexahydrocyclohepta[c]chromen-2-yl 4-methylbenzenesulfonate (**1t**)*
It was synthesized by the same method used for synthesis of compounds **1a–s** starting with compound **5** and *p*-toluenesulfonyl chloride. Compound **1t** was purified by column chromatography (silica gel, hexane/ethyl acetate 10:1 v/v, then switching to hexane/ethyl acetate 7:1 v/v); yield: 65%; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.93 (d, 1H, J = 18.4 Hz), 7.79 (d, 2H, J = 15.0 Hz), 7.49 (d, 2H, J = 15.0 Hz), 7.10 (s, 1H), 6.99 (d, 1H, J = 9.0 Hz), 2.98–2.76 (m, 4H), 2.44 (s, 3H), 1.86–1.79 (m, 2H), 1.61–1.46 (m, 4H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 161.0, 153.6, 152.7, 150.4, 146.7, 131.5, 130.8, 128.9, 127.0, 126.8, 119.1, 119.0, 110.8, 31.8, 27.8, 26.6, 25.4, 21.8, 21.5; elemental analysis ($C_{21}H_{20}O_5S$): calculated C: 65.61%, H: 5.24%, S: 8.34%; found: C: 65.43%, H: 5.45%, S: 8.20%.

*Methyl 2-oxo-2,3-dihydro-1H-indene-1-carboxylate (**7**)*
It was synthesized by the same procedure utilized for the synthesis of compounds **2a**,**b** starting with 2-indanone. It was purified by column chromatography using hexane/dichloromethane 15:1 v/v, then 10:1 v/v. It exists as a mixture of keto and enol tautomers. Yield: 54%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 11.00 (brs, 1H), 7.59 (d, 1H, J = 9.0 Hz), 7.30–7.24 (m, 2H), 7.10 (dt, 1H, J = 15.0 Hz, 9.0 Hz, 3.0 Hz), 3.96 (s, 3H), 3.57 (s, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 180.8, 169.3, 139.5, 133.1, 127.1, 123.8, 123.6, 120.2, 105.2, 105.2, 51.5, 37.7.

*3-Hydroxyindeno[1,2-c]chromen-6(11H)-one (**8**)*
It was synthesized by the same procedure utilized for the synthesis of compounds **4a–f** starting with compound **7** and resorcinol. It was used in the next step as such with no further purification.

*6-Oxo-6,11-dihydroindeno[1,2-c]chromen-3-yl 4-methylbenzenesulfonate (**1u**)*
It was synthesized through the same procedure utilized for synthesis of compounds **1a–t** through reaction of compound **8** and tosyl chloride in the presence of triethylamine. It was purified by column chromatography using hexane/ethyl acetate 10:1 v/v. Yield: 35%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.77 (dd, 3H, J = 19.6 Hz, 8.0 Hz), 7.66 (d, 1H, J = 8.2 Hz), 7.40–7.36 (m, 3H), 7.33–7.28 (m, 3H), 6.81 (d, 1H, J = 8.0 Hz), 4.28 (s, 2H), 2.46 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 150.0, 147.7, 145.5, 142.0, 134.6, 132.0, 131.8, 130.1, 129.9, 129.7, 129.4, 129.1, 128.5, 128.1, 127.1, 123.3, 121.2, 121.0, 118.5, 40.1, 21.8; LC-MS: 405.0 (M^++1); elemental analysis ($C_{23}H_{16}O_5S$): calculated C: 68.31%, H: 3.99%, S: 7.93%; found: C: 68.05%, H: 4.07%, S: 8.02%.

Biological evaluation

Cell culture, sample treatment, nitrite determination, PGE_2 assay, and MTT assay
Cell culture and sample treatment, nitrite determination, PGE_2 assay, and MTT assay for cell viability have been carried out following the reported procedures [16, 22].

Western blot

RAW 264.7 macrophage cells were re-suspended in PRO-PREP™ protein extraction solution (Intron Biotechnology, Seoul, Korea) and incubated with 20 min at 4°C. The cell debris was removed by microcentrifugation, and the supernatants were quickly frozen. The protein concentration was determined using the Bio-Rad protein assay reagent according to the manufacture's instruction. Cellular proteins were electroblotted onto a PVDF membrane following separation on 8–12% SDS-polyacrylamide gel electrophoresis. The immunoblot was incubated with blocking solution (5% skimmed milk) at room temperature for 1 h, followed by incubation overnight with a primary antibody at 4°C. The blots were washed four times with a Tween 20/Tris-buffered saline (T/TBS) and incubated with a 1:2000 dilution of horseradish peroxidase-conjugated secondary antibody for 2 h at room temperature. The blots were again washed three times with T/TBS, and then developed by enhanced chemiluminescence (GE Healthcare, WI, USA).

COX enzymatic cell-free assay

Compounds **1f**, **1p**, and etoricoxib were tested against bovine COX-2 and COX-1 isozymes using an enzyme immunoassay (EIA) (kit catalog number 560101, Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's instructions [23].

Quantitative real-time RT-PCR (qRT-PCR)

Total RNA was isolated from macrophages using Easy Blue® kits (Intron Biotechnology, Seoul, Korea). RNA (1 μg) was reverse-transcribed (RT) using MuLV reverse transcriptase, 1 mM deoxyribonucleotide triphosphate (dNTP) and 0.5 mg/mL random primer. PCR amplification was performed using the incorporation of SYBR green using SYBR Premix Ex Taq (Takara, Shiga, Japan). The PCR primers used in this study are listed below and were purchased from Bioneer (Seoul, Korea): for iNOS designed from mouse were CAT GCT ACT GGA GGT GGG TG (forward) and CAT TGA TCT CCG TGA CAG CC (reverse); for COX-2 designed from mouse were GGA GAG ACT ATC AAG ATA GT (forward) and ATG GTC AGT AGA CTT TTA CA (reverse). The oligonucleotide primers for β-actin used as a house-keeping gene designed from mouse were ATC ACT ATT GGC AAC GAG CG (forward) and TCA GCA ATG CCT GGG TAC AT (reverse). Steady-state mRNA levels were determined by real time qPCR using the Takara thermal cycler device. A dissociation curve analysis of iNOS, COX-2, and β-actin showed a single peak for each. Mean Ct values of genes of interest were calculated from triplicate measurements and normalized versus the mean Ct of β-actin [24, 25].

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