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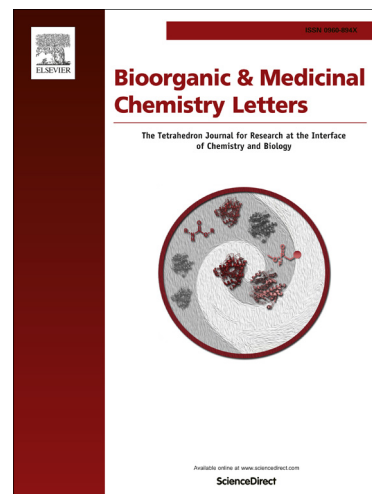
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### 3-Arylcoumarins: Synthesis and potent anti-inflammatory activity

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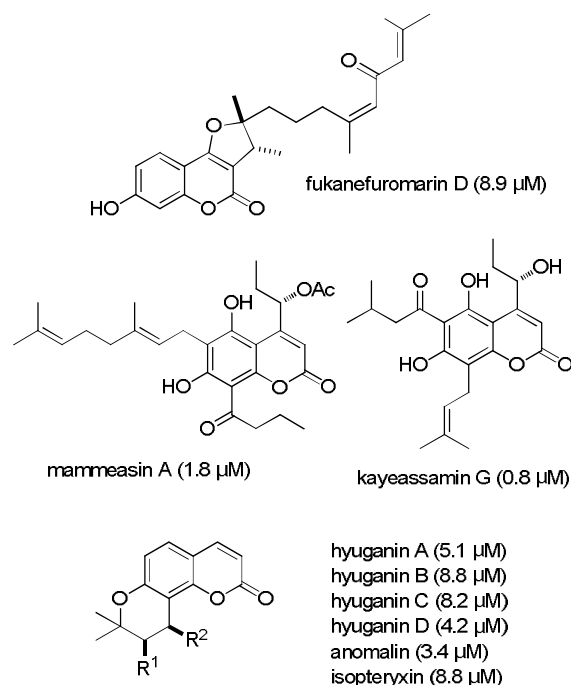
#### ABSTRACT

Chronic inflammation is the persistent and excessive immune response and can lead to a variety of diseases. Aiming to discover new compounds with anti-inflammatory activity, we report herein the synthesis and biological evaluation of 3-arylcoumarins. Thirty five 3-arylcoumarins were prepared through Perkin condensation and further acid-promoted hydrolysis if necessary. In lipopolysaccharide-activated mouse macrophage RAW264.7 cells, 6,8-dichloro-3-(2-methoxyphenyl)coumarin (**16**) and 6-bromo-8-methoxy-3-(3-methoxyphenyl)coumarin (**25**) exhibited nitric oxide production inhibitory activity with the IC<sub>50</sub> values of 8.5  $\mu$ M and 6.9  $\mu$ M, respectively, providing a pharmacological potential as anti-inflammatory agents.

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Inflammation is one of the protective immune responses which occurs as a result of chemical, physical, immunological, and/or biological stimuli to human tissue.<sup>1</sup> Persistent and excessive immune response can promote tissue damage, resulting in chronic inflammation and generating a vicious cycle between inflammation and the accompanying pathological state, such as arteriosclerosis, periodontitis, allergic rhinitis, inflammatory bowel disease, rheumatoid arthritis, Alzheimer's disease and cancer.<sup>2</sup> The most widely explored targets for controlling inflammation are inflammatory mediators.<sup>3</sup> Nitric oxide (NO), one of the important inflammatory mediators, was secreted by activated immune cells (such as macrophages). High levels of NO in chronic inflammation state can result in various pathological conditions.<sup>4</sup> Therefore, the development of new anti-inflammatory agents for the control of NO production in immune cells is the subject of interest in recent years.

Coumarins and their natural and/or synthetic derivatives are biologically interesting compounds because of their biological activities and pharmacological potentials. They have been reported with anticancer, antioxidant, anti-inflammatory, antimicrobial, antiviral and enzyme-inhibitory activities.<sup>5</sup> Hyuganin A-D, anomalin and isoterpyxin from the roots of *Angelica furcijuga* KITAGAWA,<sup>6</sup> fukanefuromarin D from the roots of *Ferula fukanensis*<sup>7</sup> and a series of coumarins from the flowers of *Mammea siamensis* (Calophyllaceae)<sup>8</sup> demonstrated potent inhibitory activity on lipopolysaccharide (LPS)-induced NO production in mouse macrophages (Figure 1). Recently,



**Figure 1.** Coumarins with potent inhibitory activity on LPS-induced NO production in mouse macrophages

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**Table 1.** Synthesis of 3-arylcoumarin via Perkin reaction<sup>a</sup>

**1-30,32,34,35**

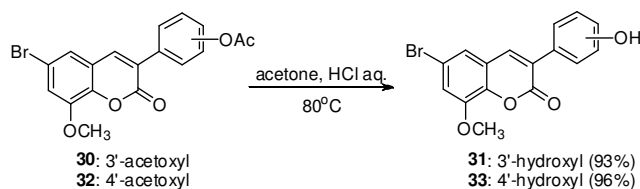
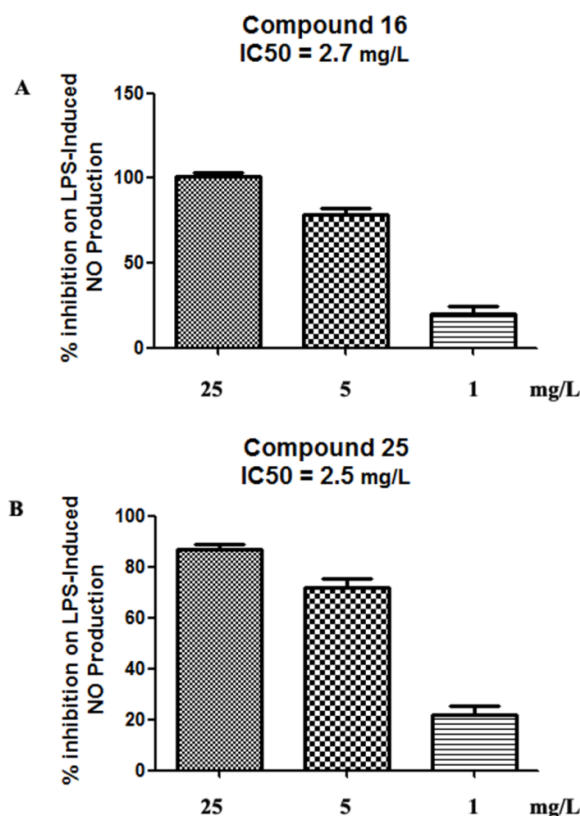
Compound	R <sup>1</sup>	R <sup>2</sup>	Ar (Phenyl) <sup>c</sup>	Yield (%) <sup>b</sup>	Compound	R <sup>1</sup>	R <sup>2</sup>	Ar (Phenyl) <sup>c</sup>	Yield (%) <sup>b</sup>
<b>1</b>	H	OCH <sub>3</sub>	3,4-dimethoxyl	65	<b>18</b>	H	H	3,4-dimethoxyl	59
<b>2</b>	H	OCH <sub>3</sub>	4-methoxyl	58	<b>19</b>	H	H	4-methoxyl	60
<b>3</b>	H	OCH <sub>3</sub>	3-methoxyl	73	<b>20</b>	H	H	3-methoxyl	64
<b>4</b>	H	OCH <sub>3</sub>	3,4,5-trimethoxyl	47	<b>21</b>	H	H	- <sup>d</sup>	63
<b>5</b>	H	OCH <sub>3</sub>	- <sup>d</sup>	63	<b>22</b>	H	H	4-fluoro	56
<b>6</b>	H	OCH <sub>3</sub>	4-chloro	60	<b>23</b>	Br	OCH <sub>3</sub>	3,4-dimethoxyl	66
<b>7</b>	H	OCH <sub>3</sub>	4-fluoro	56	<b>24</b>	Br	OCH <sub>3</sub>	4-methoxyl	64
<b>8</b>	Cl	H	3,4-dimethoxyl	72	<b>25</b>	Br	OCH <sub>3</sub>	3-methoxyl	59
<b>9</b>	Cl	H	4-methoxyl	54	<b>26</b>	Br	OCH <sub>3</sub>	2-methoxyl	52
<b>10</b>	Cl	H	2-methoxyl	47	<b>27</b>	Br	OCH <sub>3</sub>	3,4,5-trimethoxyl	58
<b>11</b>	Cl	H	3,4,5-trimethoxyl	51	<b>28</b>	Br	OCH <sub>3</sub>	- <sup>d</sup>	62
<b>12</b>	Cl	H	4-chloro	59	<b>29</b>	Br	OCH <sub>3</sub>	4-fluoro	53
<b>13</b>	Cl	Cl	3,4-dimethoxyl	73	<b>30</b>	Br	OCH <sub>3</sub>	3-acetoxyl	72
<b>14</b>	Cl	Cl	4-methoxyl	70	<b>32</b>	Br	OCH <sub>3</sub>	4-acetoxyl	74
<b>15</b>	Cl	Cl	3-methoxyl	67	<b>34</b>	Br	OCH <sub>3</sub>	2,5-dimethoxyl	69
<b>16</b>	Cl	Cl	2-methoxyl	46	<b>35</b>	Br	OCH <sub>3</sub>	pyridin-3-yl	55
<b>17</b>	Cl	Cl	- <sup>d</sup>	54					

<sup>a</sup>See supplementary data for the general procedure of 3-arylcoumarin preparation. <sup>b</sup>Isolated yield. <sup>c</sup>3-Aryl of compound **35** is pyridine-3-yl. <sup>d</sup>No substituents.

prenylated coumarins, columbianadin<sup>9</sup> and glycycomarin<sup>10</sup>, showed moderate inhibitory activity and suggested the potential to be developed into anti-inflammatory agents. However, considering the pharmaceutical applications, these inhibitors were not favorable as a result of limited natural abundance, complicated structure and/or poor activity. Therefore, efforts are still needed to develop new coumarin derivatives with both high activity and production feasibility.

With considerable efforts to develop synthetic strategies, coumarins can be prepared by Perkin, Pechmann, Knoevenagel, Wittig, Kostanecki-Robinson or Reformatsky reactions.<sup>5</sup> The most widely used method was Perkin reaction starting from salicylaldehyde in the presence of base and acid anhydride.<sup>11</sup> In this work, we present the synthesis of manifold 3-aryl substituted coumarins and their inhibitory activity on LPS-induced NO production in RAW 264.7 cells.

The preparation of 3-arylcoumarin was performed via Perkin reaction using salicylaldehydes and phenylacetic acids as starting materials. In the presence of acetic anhydride and triethylamine, the reaction mixture was stirred at 120°C overnight and quenched with ice water. After filtration, 3-arylcoumarins were obtained

**Scheme 1.** Acid-promoted hydrolysis of compounds **30** and **32****Figure 2.** Inhibitory Effect of Compounds **16** and **25** on NO Production Stimulated by LPS<sup>13</sup>

**Table 2.** Inhibitory effects on LPS-Induced NO production and cytotoxicity of compounds **1-35** in RAW264.7 cells at 5 mg/L<sup>13</sup>

Compounds	Inhibition on LPS-induced NO production (%)	Cell viability (%)	Compounds	Inhibition on LPS-induced NO production (%)	Cell viability (%)
Curcumin <sup>a</sup>	77.04 ± 2.22		<b>18</b>	47.36 ± 8.26	47.03 ± 6.17
<b>1</b>	32.33 ± 5.41	87.71 ± 0.25	<b>19</b>	59.64 ± 5.72	93.44 ± 5.1
<b>2</b>	44.58 ± 10.28	81.29 ± 1.92	<b>20</b>	49.23 ± 1.24	48.44 ± 2.47
<b>3</b>	91.36 ± 2.78	34.19 ± 10.37	<b>21</b>	69.45 ± 2.18	103.88 ± 2.87
<b>4</b>	52.01 ± 9.96	54.4 ± 3.04	<b>22</b>	9.98 ± 4.06	110.45 ± 6.93
<b>5</b>	78.4 ± 3.09	64.46 ± 5.95	<b>23</b>	53.52 ± 2.6	98.03 ± 0.06
<b>6</b>	61.74 ± 5.39	93.54 ± 0.52	<b>24</b>	44.25 ± 14.66	99.88 ± 2.36
<b>7</b>	47.61 ± 11.18	126.61 ± 1.1	<b>25</b>	<b>72.28 ± 2.08</b>	<b>116.42 ± 6.7</b>
<b>8</b>	31.25 ± 2.55	105.34 ± 3.8	<b>26</b>	47.65 ± 13.86	95.72 ± 6.06
<b>9</b>	47.53 ± 0.85	101.85 ± 14.14	<b>27</b>	47.28 ± 12.69	115.19 ± 2.09
<b>10</b>	65.73 ± 8.18	115.38 ± 9.99	<b>28</b>	111.38 ± 0.2	29.62 ± 2.16
<b>11</b>	57.62 ± 2.77	78.41 ± 13.06	<b>29</b>	33.88 ± 9.61	102.58 ± 1.06
<b>12</b>	12.11 ± 20.16	113.58 ± 0.79	<b>30</b>	84.13 ± 2.7	66.48 ± 1.71
<b>13</b>	27.27 ± 15.12	117.83 ± 7.63	<b>31</b>	115.72 ± 0.4	28.46 ± 0.45
<b>14</b>	20.79 ± 10.12	99.31 ± 0.63	<b>32</b>	77.78 ± 1.22	61.72 ± 5.68
<b>15</b>	35.99 ± 4.71	105.26 ± 24.2	<b>33</b>	91.33 ± 2.06	24.45 ± 5.14
<b>16</b>	<b>76.9 ± 1.52</b>	<b>119.17 ± 1.67</b>	<b>34</b>	45.86 ± 9.08	93.53 ± 3.01
<b>17</b>	53.38 ± 8.19	103.52 ± 1.07	<b>35</b>	58.65 ± 11	84.87 ± 1.62

The values are mean ± SEM (n = 3). <sup>a</sup>Curcumin was used as a positive control.

after purification with the yields from 46% to 74% (Table 1). 3-Arylcoumarins **31** and **33** were further synthesized via acid-promoted hydrolysis of **30** and **32**, respectively (Scheme 1).

Compounds **1-35** were subsequently evaluated for their inhibitory activity on LPS-induced NO production in RAW 264.7 cells. As shown in Table 2, compounds **3**, **5**, **16**, **21**, **25**, **28** and **30-33** caused low percentage of NO production at 5 mg/L. Simultaneously, cytotoxic effects of compounds **1-35** were examined in mouse macrophage RAW264.7 cells. The results revealed that, compounds **1**, **2**, **6-10**, **12-17**, **19**, **21-27**, **29**, **34** and **35** had no cytotoxic activity in these cells at 5 mg/L, while compounds **3**, **18**, **20**, **28**, **31** and **33** showed strong cytotoxicity. With regard to these proofs, we believed that the low percentage of NO production in the presence of compounds **3**, **5**, **28** and **30-33** may be as a result of low cell viability of RAW 264.7 cells. As shown in Figure 2, IC<sub>50</sub> values of compounds **16** and **25** were 2.7 mg/L (8.5 μM) and 2.5 mg/L (6.9 μM), respectively, which were comparable with the value of curcumin (6.2 μM, positive control<sup>12</sup>). Considering reported anti-inflammatory coumarins (Figure 1), these results demonstrated that anti-inflammatory activity of coumarins could be maintained by introducing aromatic groups into 3-position and the replacement of long and/or asymmetric alkyl side chains with simple functional groups. Low cell viability of compounds **31** and **33** (28.46% and 24.45%) indicated that the presence of hydroxyl on 3-aryl group could cause intense cytotoxicity. 4'-Halogen substituted 3-arylcoumarins resulted in poor inhibitory activities, as can be seen from **12**, **22** and **29** (12.11%, 9.98% and 33.88%, respectively). The inhibitory activities of compounds **16** and **25** were 76.9% and 72.28%. Our results indicated that the introduction of halogen into 6-position, substituents (other than hydrogen) at 8-position as well as Ar group together could favor the activity, which is in accord with Bansal's speculation.<sup>3</sup>

In conclusion, a series of 3-arylcoumarins were prepared via low-cost Perkin reaction (and further hydrolysis if necessary) and

evaluated for their inhibitory effects on LPS-Induced NO production and cytotoxic effects on RAW264.7 cells. Compounds **16** and **25** showed potent inhibitory activity with the IC<sub>50</sub> values of 8.5 μM and 6.9 μM, respectively. Our results suggested that it is possible to develop synthetic coumarins as anti-inflammatory agents for related diseases of chronic inflammation.

## Acknowledgments

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## Supplementary Material

General synthetic procedures and spectral data (<sup>1</sup>H-NMR, <sup>13</sup>C-NMR and HRMS) associated with this article can be found in the online version.

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$$\% \text{Inhibition} = (A - B) / (A - C) \times 100$$
A: LPS (+), sample (-); B: LPS (+), sample (+); C: LPS (-), sample (-)
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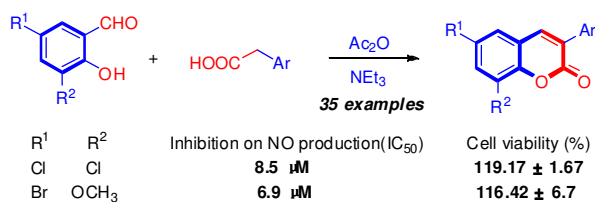
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