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ARTICLE *in* BIOORGANIC & MEDICINAL CHEMISTRY LETTERS · OCTOBER 2014

Impact Factor: 2.42 · DOI: 10.1016/j.bmcl.2014.10.033

Bioorganic & Medicinal Chemistry Letters 24 (2014) 5432–5434

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

3-Arylcoumarins: Synthesis and potent anti-inflammatory activity

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ARTICLE INFO

Article history:
Received 23 December 2013
Revised 19 February 2014
Accepted 9 October 2014
Available online 16 October 2014

Keywords:
Anti-inflammatory activity
Nitric oxide production
Coumarin
Perkin reaction

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_{50} values of 8.5 μM and 6.9 μM, respectively, providing a pharmacological potential as anti-inflammatory agents.

Inflammation is one of the protective immune responses which occurs as a result of chemical, physical, immunological, and/or biological stimuli to human tissue.[1] 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.[2] The most widely explored targets for controlling inflammation are inflammatory mediators.[3] 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.[4] 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.[5] Hyuganin A–D, anomalin and isopteryxin from the roots of *Angelica furcijuga* KITAGAWA,[6] fukanefuromarin D from the roots of *Ferula fukanensis*[7] and a series of coumarins from the flowers of *Mammea siamensis* (Calophyllaceae)[8] demonstrated potent inhibitory activity on lipopolysaccharide (LPS)-induced NO production in mouse macrophages (Fig. 1). Recently, prenylated coumarins, columbianadin[9] and glycycoumarin,[10] showed moderate inhibitory activity and suggested the potential to be developed into anti-inflammatory agents. However,

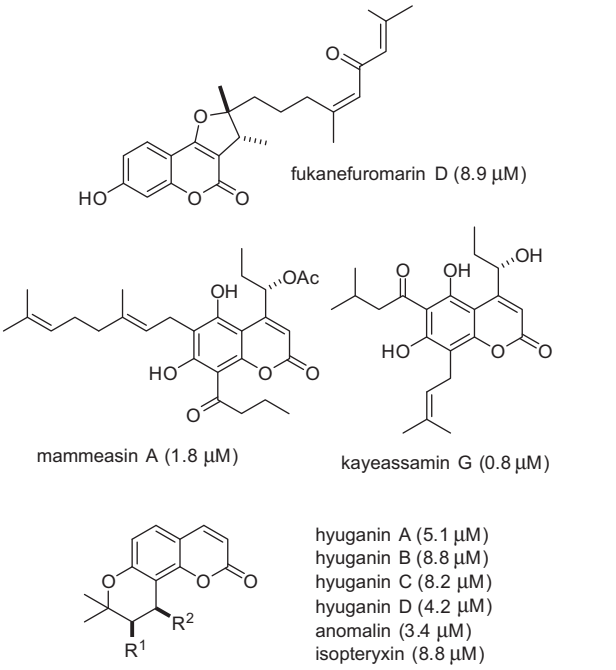

Figure 1. Coumarins with potent inhibitory activity on LPS-induced NO production in mouse macrophages.

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http://dx.doi.org/10.1016/j.bmcl.2014.10.033
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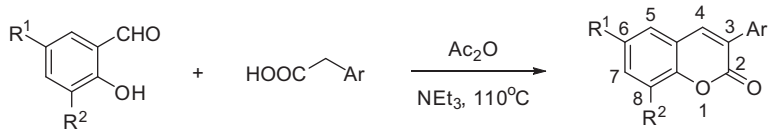
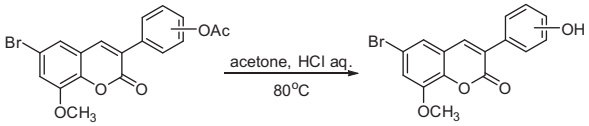
Table 1
Synthesis of 3-arylcoumarin via Perkin reaction[a]



Compound	R^1	R^2	Ar (Phenyl)[c]	Yield[b] (%)	Compound	R^1	R^2	Ar (Phenyl)[c]	Yield[b] (%)
1	H	OCH_3	3,4-Dimethoxyl	65	**18**	H	H	3,4-Dimethoxyl	59
2	H	OCH_3	4-Methoxyl	58	**19**	H	H	4-Methoxyl	60
3	H	OCH_3	3-Methoxyl	73	**20**	H	H	3-Methoxyl	64
4	H	OCH_3	3,4,5-Trimethoxyl	47	**21**	H	H	—[d]	63
5	H	OCH_3	—[d]	63	**22**	H	H	4-Fluoro	56
6	H	OCH_3	4-Chloro	60	**23**	Br	OCH_3	3,4-Dimenthoxyl	66
7	H	OCH_3	4-Fluoro	56	**24**	Br	OCH_3	4-Methoxyl	64
8	Cl	H	3,4-Dimethoxyl	72	**25**	Br	OCH_3	3-Methoxyl	59
9	Cl	H	4-Methoxyl	54	**26**	Br	OCH_3	2-Methoxyl	52
10	Cl	H	2-Methoxyl	47	**27**	Br	OCH_3	3,4,5-Trimethoxyl	58
11	Cl	H	3,4,5-Trimethoxyl	51	**28**	Br	OCH_3	—[d]	62
12	Cl	H	4-Chloro	59	**29**	Br	OCH_3	4-Fluoro	53
13	Cl	Cl	3,4-Dimethoxyl	73	**30**	Br	OCH_3	3-Acetoxyl	72
14	Cl	Cl	4-Methoxyl	70	**32**	Br	OCH_3	4-Acetoxyl	74
15	Cl	Cl	3-Methoxyl	67	**34**	Br	OCH_3	2,5-Dimethoxyl	69
16	Cl	Cl	2-Methoxyl	46	**35**	Br	OCH_3	Pyridin-3-yl	55
17	Cl	Cl	—[d]	54					

[a] See Supplementary data for the general procedure of 3-arylcoumarin preparation.
[b] Isolated yield.
[c] 3-Aryl of compound **35** is pyridine-3-yl.
[d] No substituents.



Scheme 1. Acid-promoted hydrolysis of compounds **30** and **32**.

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.[5] The most wildly used method was Perkin reaction staring from salicylaldehyde in the presence of base and acid anhydride.[11] 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

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

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

The values are mean ± SEM ($n = 3$).
[a] Curcumin was used as a positive control.

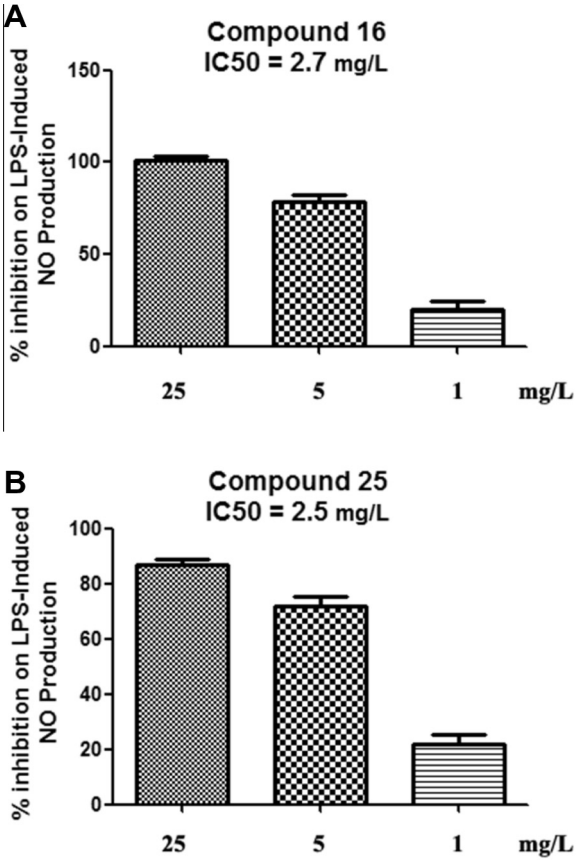


Figure 2. Inhibitory effect of compounds **16** and **25** on NO Production Stimulated by LPS.[13]

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 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_{50} 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[12]). Considering reported anti-inflammatory coumarins (Fig. 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.[3]

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_{50} 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.

Acknowledgment

This work was financially supported by National Natural Science Foundation of China (Grants 20932007 and 21372213).

Supplementary data

General synthetic procedures and spectral data (^{1}H NMR, ^{13}C NMR and HRMS) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.10.033.

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13. *Cell culture:* RAW264.7 cells were grown in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen, Carlsbad, CA, USA) supplemented with 1% penicillin/streptomycin and 10% fetal calf serum (Gibco-Invitrogen). For experiments of nitric oxide content, RAW264.7 cells were maintained in medium devoid of Phenol Red (Invitrogen). Cell proliferation assay: Cell proliferation was assayed as described previously.[14] In brief, 1×10^4 RAW264.7 cells was seeded in 96-well plates, and treated with the compounds for 24 h. After AlamarBlue reagent was added to each well, and fluorescence intensity was measured with excitation at 544 nm and excitation at 590 nm using Thermo Scientific Varioskan Flash Multimode Reader. Cytotoxicity was defined as the ratio of the fluorescence intensity in test wells compared to solvent control wells (0.1% DMSO). The assay was conducted 3 times in triplicate. Measurement of nitric oxide content: The RAW264.7 macrophages were seeded in 96-well plates at 1×10^4 cells/well, and pretreated with various concentrations of compounds for 2 h, followed by 1 μg/mL LPS for an additional 24 h. After that the cell culture medium was used for NO measurements using a commercial available kit (Beyotime, Haimen, China). Nitrite production was measured at OD_{550}. The assay was conducted 3 times in triplicate. Percentage inhibition was calculated using the following equation: % Inhibition = (A − B)/(A − C) × 100 A: LPS (+), sample (−); B: LPS (+), sample (+); C: LPS (−), sample (−).
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