Bioorganic & Medicinal Chemistry Letters 24 (2014) 181–185

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Synthesis of licochalcone analogues with increased *anti*-inflammatory activity

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ARTICLE INFO

Article history:
Received 1 October 2013
Revised 31 October 2013
Accepted 18 November 2013
Available online 26 November 2013

Keywords:
Licohalcone B
Licohalcone D
[3,3]-Sigmatropic rearrangement
anti-Inflammatory effects
IC_{50} values of NO production

ABSTRACT

Licohalcones have been reported to have various biological activities. However, most of licochalcones also showed cytotoxicity even though their versitile utilities. Licochalcones B and D, which have common substituents at aromatic ring B, are targeted to modify the structure at aromatic ring A for inflammatory studies. Licochalcone derivatives (**1**–**6**) thus prepared are compared for their suppression ability of nitric oxide (NO) production and showed 9.94, 4.72, 10.1, 4.85, 2.37 and 4.95 μM of IC_{50} values, respectively.

Licochalcone A (LicoA), licochalcone B (LicoB), licochalcone C (LicoC), licochalcone D (LicoD), echinatin and lsoliquiritigenin are major active components in licorice which is a traditional medicine used in the Northeast Asia for the treatment of ulcer, asthma, inflammation and other diseases.[1] Licochalcones have been isolated and characterized from the root of *Glycyrrhiza inflata*, and have been reported to show various biological properties, including antibacterial,[2] antitumor,[3] *anti*-inflammatory,[4] and antioxidative[5] activities. *G. inflata* is the main species in licorice and contains about 40 kinds of flavonoids of which having no hydroxyl at the position 2′ (or 6′) which is different from usual flavonoid have been found to show the major contribution on biological activities.[6] These unusual chalcones are called retrochalcone or 'reversely constructed chalcone' in which the ring A would be derived from shikimate and the ring B from polyketide of malonate orgin.[7]

The *anti*-inflammatory activities of licochalcones as shown in Scheme 1 were compared by the inhibition effect of the mast cell degranulation which play a key role in allergic inflammation in RBL (rat basophilic leukemia)-2H3 cells.[8] Also, the *anti*-inflammatory activities were compared by the inhibition effect of NO production in inflammatory regions.[9] The 50% inhibitory concentration (IC_{50}) against degranulation, 30% cytotoxicity (CC_{30}), and IC_{50} of LPS-induced NO production of each licochalcones are listed in Scheme 1. Since LicoA, LicoC and LicoD exhibited similar inhibitory effects on the degranulation with the IC_{50} at 17, 24 and 21 μM. Also, LicoB and LicoD showed IC_{50} of LPS-induced NO production at 2.3 and 2.2 μM, respectively. From these inhibitory results, we found that LicoB and LicoD having common substituents at aromatic ring B showed higher activity with relatively lower cytotoxicity than the others.

The structure of LicoB is similar to echinatin except the presence or absence of 3-hydroxy substituent at ring B, however, the *anti*-inflammatory activities are quite different and only LicoB showed the inhibition activity of 2.3 μM for IC_{50} of NO production, but both compounds showed lower cytotoxicity. Also the structure of LicoB is exactly same with LicoD on ring B which has 3,4-dihydroxy-2-methoxy substituents, and both showed the higher inhibition activities for IC_{50} of NO production. In order to find a highly active *anti*-inflammatory licochalcone derivative having lower cytotoxicity, we designed the structures same as LicoB and LicoD at ring B, which have 3,4-dihydroxy-2-methoxy substituents, but modified at ring A to resorcinol type (**2**), catechol type (**3**) and rearranged isopropenyl analogues **5** and **6** (Scheme 2).

The natural LicoB (**1**) was first identified in 1975 from the roots of *Glycyrrhiza glabra* Linn. (licorice from Sinkiang, China)[7] and has been reported many biological activities,[8–10] however, only single report for synthesis has been known.[11] Recently, we reported the first total synthesis of LicoD[12] and we used the similar route for the synthesis of LicoB as shown in Scheme 3 since the ring B of LicoB is exactly same as LicoD. The aldehyde portion (**9**) of protected ring B is condensed with THP protected acetophenone (**11**) using 3 M NaOH in MeOH to produce the chalcone (**12**), and following deprotection using Dowex 50X2 resin afforded the LicoB

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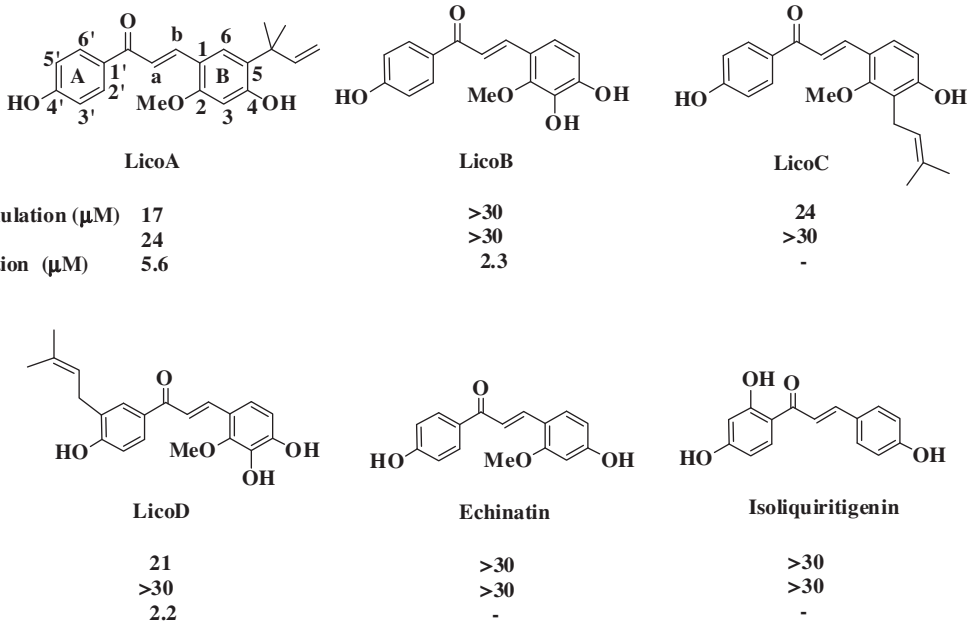
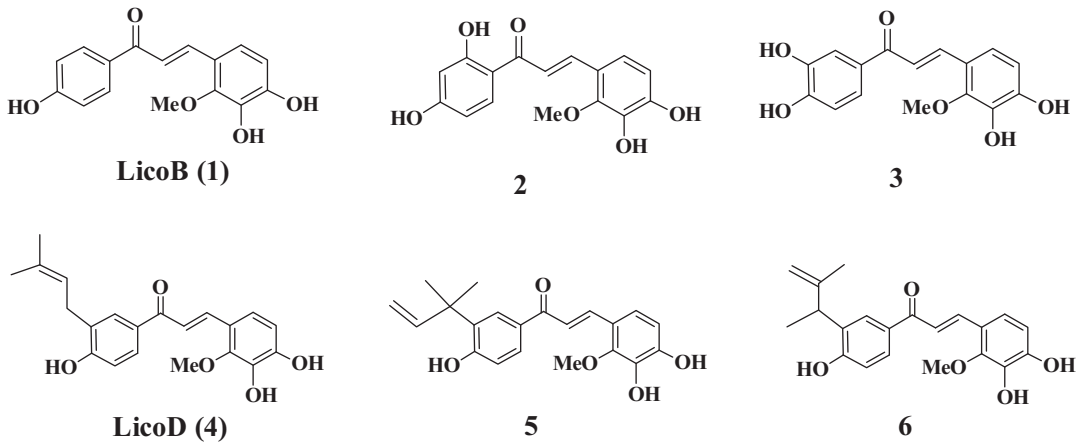
http://dx.doi.org/10.1016/j.bmcl.2013.11.044



Scheme 1. *anti*-Inflammatory activity of licochalcones.



Scheme 2. The designed structures of licochalcone B and D derivatives.

in total 25% yield of 5 steps. The spectral data for LicoB agreed well with the literature values.[7,10,11]

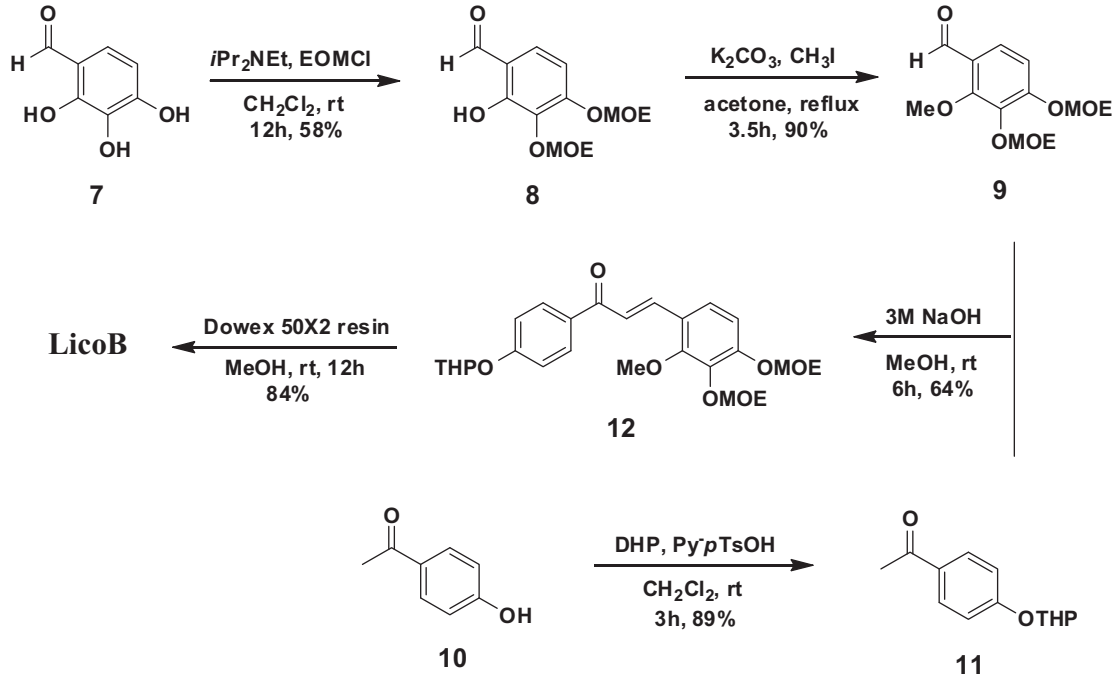
Resorcinol (**2**) analogue of LicoB, which is a new compound, was prepared by conventional Claisen–Schmidt condensation of 2,4-diethoxymethoxyacetophenone (**14**) with the benzaldehyde (**9**) using basic condition followed by deprotection in moderate yields (Scheme 4).

3,4-Diethoxymethoxyacetophenone (**19**) was prepared from commercially available 3,4-dihydroxybenzaldehyde (**16**) by protection using diisopropylethylamine with EOMCl followed by CH_3-MgCl reaction and PDC oxidation (Scheme 5). It is noteworthy that the deprotonation of 2-hydroxyacetophenone (**13**) requires stronger base than 3-hydroxy analogue (**16**) because of intramolecular hydrogen bonding. The acetophenone **19** afforded the catechol analogue (**3**), which is a known natural licochalcone as tetrahydroxymethoxychalcone,[13] by condensation and deprotection.

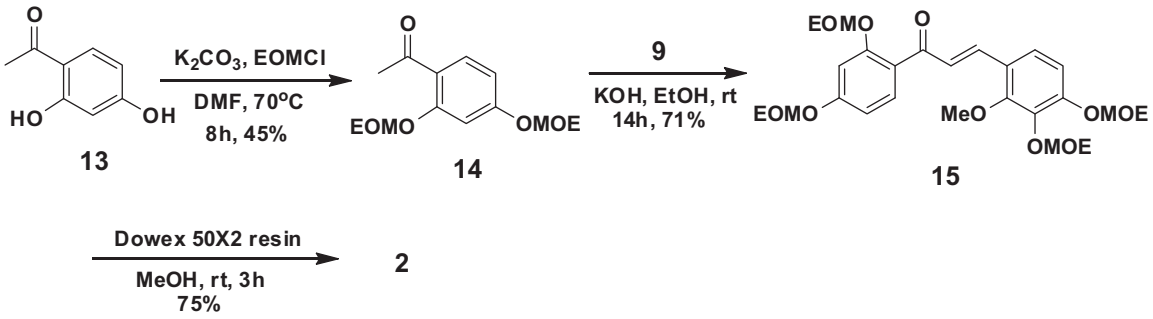
Compound **5**, a new compound, is an anologue of LicoD, which has a similar isopropenyl substituent to LicoA. [3,3]-Sigmatropic rearrangement in LicoD synthesis has been applied for this synthesis.[12] 4-Hydroxyacetophenone (**10**) is *O*-prenylated using 1-bromo-3-methyl-2-butene in K_2CO_3 to give **21** in 98% yield, which is then condensed with the aldehyde **9** to give chalcone **22** in 86% yield, and following water-accelerated Claisen rearrangement[14] produced the analogue **5** in 52% yield (Scheme 6).

Another isopropenyl analogue **6**, which is also a new compound, is prepared from **10** using same methododlogy via *O*-prenylated acetophenone **23** and chalcone **24** as shown in Scheme 7.

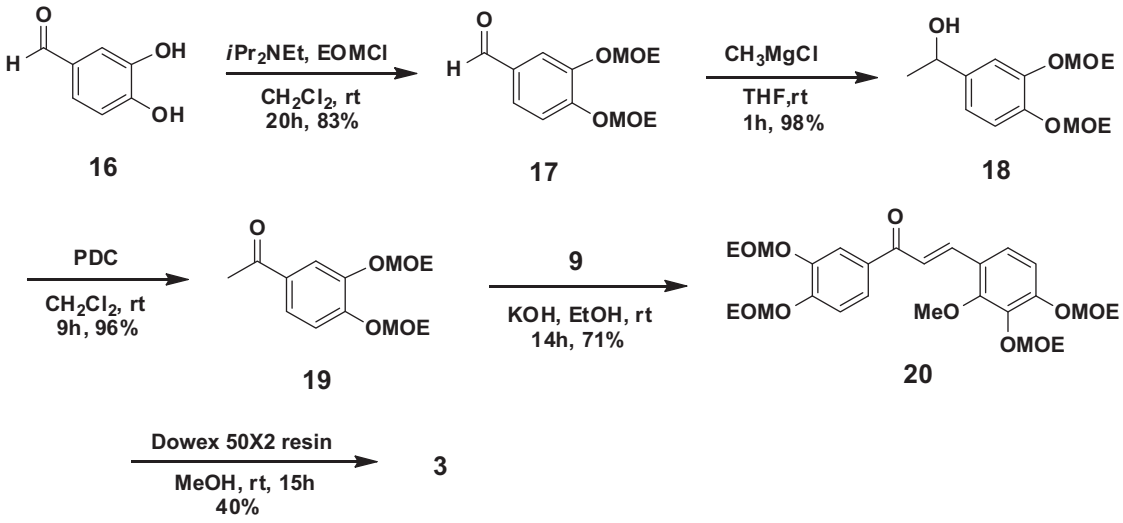
The synthetic licochalcones **1**–**6** are subjected to comparison of their *anti*-inflammatory activities. The suppression results of nitric oxide (NO) production in lipopolysaccharide (LPS)-stimulated RAW264.7 machrophages for the licochalcones **1**–**6** are compared in Table 1. Isopropenyl analogue **2** and resorcinol analogue **5** showed 75.9% and 71.1% suppression of NO production at 2 μM, respectively. LicoD (**4**) and its analogue **6** also showed moderate% inhibition at same concentration, however, LicoB (**1**) and its catechol analogue **3** showed very low or no inhibition activities. Also, Resorcinol **2**, isopropenyl **5**, LicoD (**4**) and its analogue **6** showed 96.5%, 96.1%, 95.4% and 94.3% inhibition, respectively, at 20 μM. Interestingly, catechol analogue showed 92.5% inhibition, however, LicoB (**1**) still showed only 69.6% inhibition at this concentration.



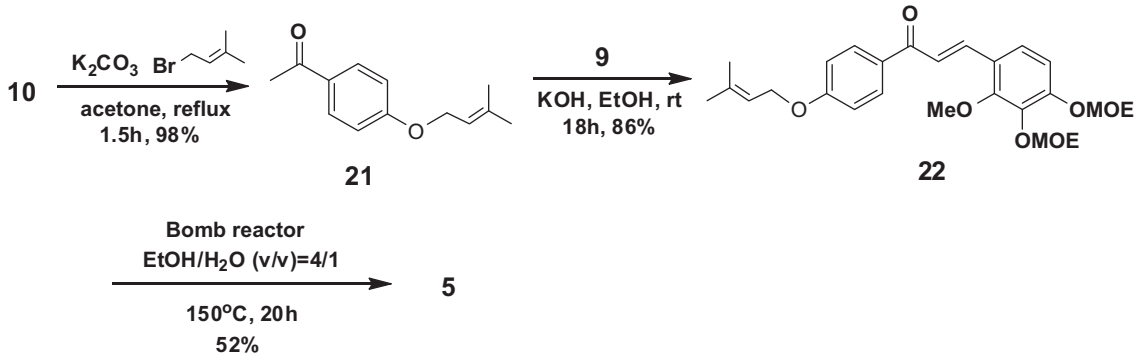
Scheme 3. Synthesis of licochalcone B (**1**).



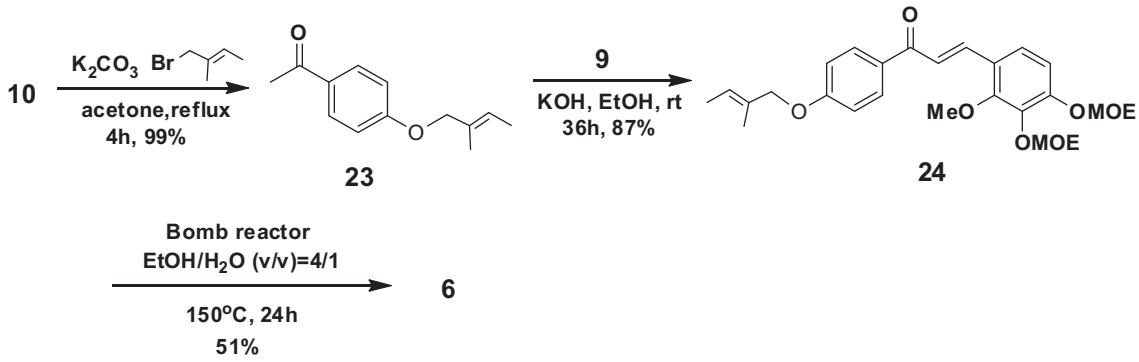
Scheme 4. Resorcinol analogue (**2**) of licochalcone B.



Scheme 5. Catechol analogue (**3**) of licochalcone B.



Scheme 6. Synthesis of licochalcone analogue **5**.



Scheme 7. Synthesis of licochalcone analogue **6**.

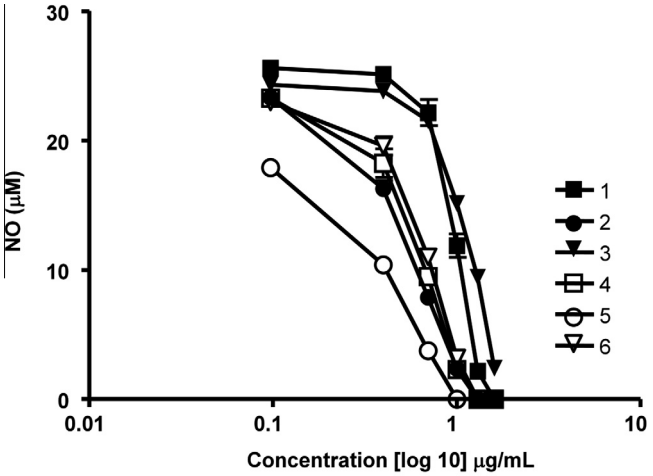
Table 1
anti-Inflammatory activities of licochalcones **1**–**6**

Compound	NO production (% inhibition)	
	2 μM	20 μM
Medium (MED)	5.4 ± 0.3 (94.6)	5.4 ± 0.3 (94.6)
1	82.8 ± 0.3 (17.2)	30.4 ± 0.3 (69.6)
2	28.9 ± 0.3 (71.1)	3.5 ± 0.3 (96.5)
3	106.0 ± 6.0 (−6.0)	7.5 ± 0.8 (92.5)
4	42.2 ± 0.6 (57.8)	4.6 ± 0.2 (95.4)
5	24.1 ± 1.0 (75.9)	3.9 ± 0.1 (96.1)
6	43.5 ± 0.6 (56.5)	5.7 ± 0.3 (94.3)
LPS	100.0 ± 0.8 (0.0)	100.0 ± 0.8 (0.0)

The results are reported as mean value ± SEM for $n = 3$.
% Inhibition is based on LPS as shown in parenthesis.

Table 2
Proliferation effect of licochalcones **1**–**6**

	MED	**1**	**2**	**3**	**4**	**5**	**6**
2 μM	1.9	2.0	2.3	2.0	1.8	1.8	1.9
20 μM	1.9	2.1	2.3	2.4	0.5	0.6	0.6



IC_{50} values 1 : 9.94 μM, **2** : 4.72 μM, **3** : 10.1 μM, **4** : 4.85 μM, **5** : 2.37 μM, **6** : 4.95 μM

Figure 1. IC_{50} values for NO production of synthetic licochalcones **1**–**6**.

The cell viability assay at 20 μM concentration was not affected by the licochalcones **1**–**3** indicating no cytotoxicity as shown in Table 2. LicoD (**4**) and its isopropenyl analogues **5**–**6**, unfortunately, showed significant cytotoxicities, and the inhibition activities of 4–**6** considered due to cytotoxicities. IC_{50} values for NO production of synthetic licochalcones 1–**6** were evaluated by using GraphPad Prism 4.0 software and showed 9.94, 4.72, 10.1, 4.85, 2.37 and 4.95 μM, respectively (Fig. 1).

Previously reported data[15–17] showed that various licochalcones significantly inhibited LPS-induced nuclear factor (NF)-κB activation. Based on these data, inhibitory effect of NO production may due to the blocking of NF-κB activation.

In summary, we prepared licochalcone analogues **1**–**6** by conventional Claisen–Schmidt condensation in basic condition. In *anti*-inflammatory studies, all the compounds tested (**1**–**6**) showed 69.6%, 96.5%, 92.5%, 95.4%, 96.1% and 94.3% suppression of NO production, respectively, at 20 μM and also showed 9.94, 4.72, 10.1, 4.85, 2.37 and 4.95 μM of IC_{50} values, respectively. However, LicoD (**4**) and its analogues **5**–**6** showed significant cytotoxicities, but the

resorcinol analogue (**2**) showed the prominent% inhibition activity even at 2 μM without any cytotoxicities.

Acknowledgments

This research was financially supported by the Ministry of Education, Science Technology (MEST) and Korea Institute for Advancement of Technology (KIAT) through the Human Resource Training Project for Regional Innovation (2012H1B8A2026036), and by Priority Research Centers Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (NRF-2009-0094071).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.11.044.

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