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Synthesis of coumarin derivatives and their cytoprotective effects on *t*-BHP-induced oxidative damage in HepG2 cells

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ABSTRACT

Coumarins are ubiquitous in higher plants and exhibit various biological actions. The aim of this study was to investigate the structure-activity relationships of coumarin derivatives on *tert*-butyl hydroperoxide (*t*-BHP)-induced oxidative damage in human hepatoma HepG2 cells. A series of coumarin derivatives were prepared and assessed for their cytoprotective effects. Among these, a caffeoyl acid-conjugated dihydropyranocoumarin derivative, caffeoyllomatin, efficiently protected against cell damage elicited by *t*-BHP. Our findings suggest that caffeoyllomatin appears to be a potent cytoprotective agent.

Coumarins constitute a class of polyphenols that occur naturally in higher plants. Their framework is comprised of fused benzene and α-pyrone rings, which are produced from the shikimic acid pathway.[1] Coumarins assume prominent functions in plant physiology, chemical defense, and interactions between plants and their environment. During winter, some coumarins inhibit seed germination by suppressing mitosis until growth conditions are favorable.[2] In addition, coumarins act as phytoalexins against invading microbial pathogens and as defensive compounds against insect herbivores.[3–6] Thus, coumarin constituents are of primary importance in plants.

In the view of the therapeutic use of coumarins in human health, coumarins have been tested as anticoagulant, anti-inflammatory, cardiovascular, and neuroprotective agents.[7,8] Dicoumarol, a fermentation product in *Medicago sativa* is a lead compound of warfarin, which is the most frequently used oral anticoagulant worldwide.[9] A linear-type furanocoumarin, methoxsalen (also called ammoidin and xanthotoxin), in *Ammi majus* is a drug used to treat psoriasis, eczema, and vitiligo in clinical practice.[10] It has been reported that simple plant coumarins, esculetin and scoparone play a critical part in the prevention of oxidative stress.[11,12]

Linear-type and angular-type *p*-coumaric acid-conjugated dihydrofuranocoumarin and dihydropyranocoumarin derivatives (angelmarin, coumaroyllomatin, and secorin) have been isolated from *Angelica pubescens*,[13] *Lomatium columbianum*,[14] and *Seseli coronatum*, respectively.[15] While diversely modified coumarins have been identified from plants, only a few phenylpropanoid-conjugated coumarin derivatives have been isolated.[1] This study focuses on exploring structure-activity relationships of these rare coumarin derivatives with respect to their protective effects on *tert*-butyl hydroperoxide (*t*-BHP)-induced hepatic damage.

An isoprenyl unit and an *ortho*-substituted hydroxy group participate in the formation of either five-membered dihydrofuran or six-membered dihydropyran rings, commonly encountered in natural products. The cyclization has been postulated to involve an epoxide intermediate, so that nucleophilic attack of the neighboring hydroxy group on to the epoxide group can lead to formation of those heterocycles. We therefore embarked on the preparation of osthenol and demethylsuberosin as synthetic precursors from readily available umbelliferone. The synthetic route is shown in Scheme 1. Because direct and selective isoprenylation at the C6 or C8 positions of umbelliferone is difficult to achieve, we employed an approach using a Claisen rearrangement.[16] The *O*-1,1-dimethyl-2-propynylation of umbelliferone using 3-chloro-3-methyl-1-butyne, DBU, and a catalytic amount of $CuCl_2{\cdot}2H_2O$ furnished compound **1** (60% yield), which upon Lindlar reduction afforded 1,1-dimethyl-2-propenyl product **2** in 76% yield. The Claisen rearrangement of **2** in water at 75 °C offered ready access to the desired osthenol **3** and demethylsuberosin **4** in 65% and 12% yields, respectively.

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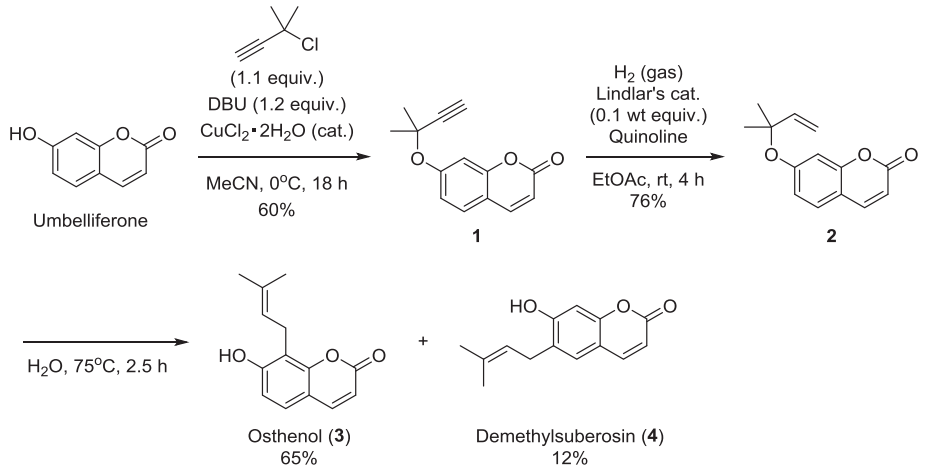
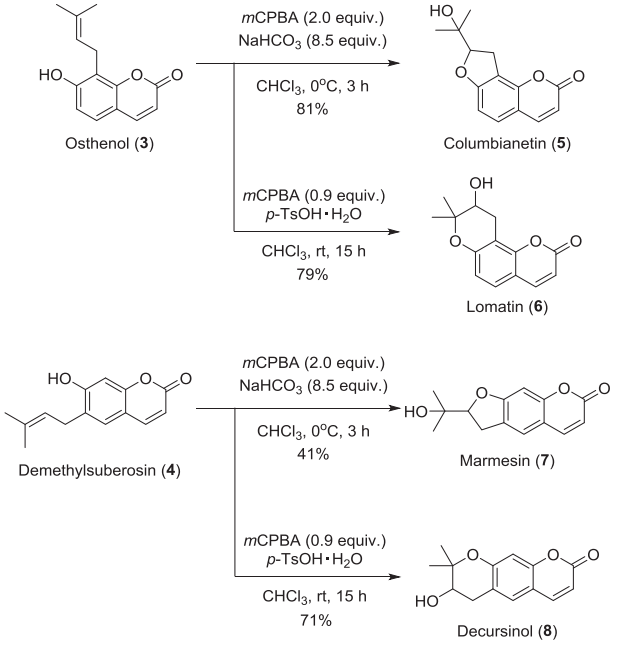
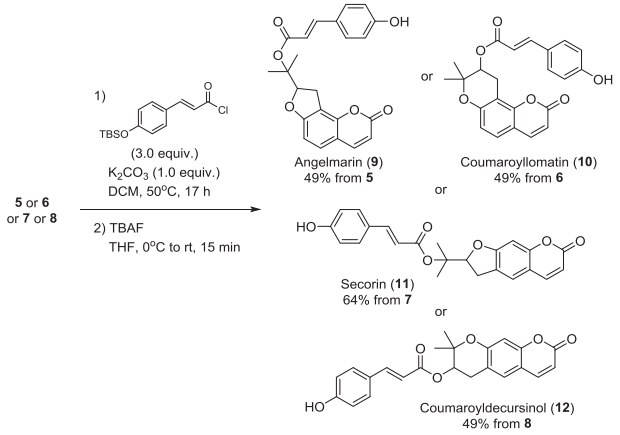


Scheme 1. Synthesis of osthenol and demethylsuberosin.



Scheme 2. Synthesis of various dihydrofurano- and dihydropyranocoumarins.



Scheme 3. Synthesis of *p*-coumaroyl derivatives.

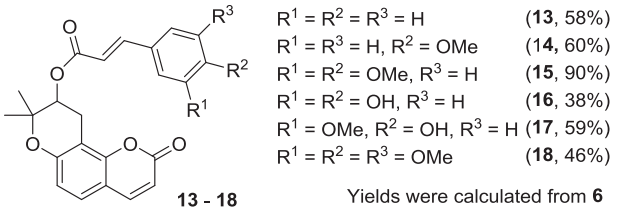


Fig. 1. Chemical structures of phenylpropanoid-conjugated coumarins.

The cyclization started with the *m*CPBA-mediated epoxidation of the isoprenyl group in **3** and **4** to form the epoxide intermediates, which were immediately converted under basic conditions into dihydrofurano products, columbianetin **5** in 81% yield and marmesin **7** in 41% yield (Scheme 2).[17] In contrast, epoxide opening under acidic conditions happened at the more substituted end, to generate the dihydropyrano products, lomatin **6** in 79% yield and decursinol **8** in 71% yield.

After the successful formation of dihydrofurano-fused and dihydropyrano-fused coumarins, the stage was set for the synthesis of *p*-coumaric acid-conjugated derivatives (Scheme 3). Esterification of compounds **5–8** with *O*-TBS-protected *p*-coumaric acid chloride in the presence of K_2CO_3 and subsequent deprotection by means of TBAF afforded the *p*-coumaroyl derivatives, angelmarin (**9**, 49% yield), coumaroyllomatin (**10**, 49% yield), secorin (**11**, 64% yield), and coumaroyldecursinol (**12**, 49% yield). In addition, from lomatin (**6**), a series of phenylpropanoid-conjugated derivatives **13–18** were similarly prepared in 38–90% yields by various esterification reactions with acid chlorides and deprotection (if needed). Their chemical structures are presented in Fig. 1.

Because all compounds (**5–18**) were non-toxic to HepG2 cells at a concentration of 10 μM (data not shown), we examined their possible protective effects against *t*-BHP-induced cytotoxicity on human hepatoma HepG2 cells, by preincubating the cells in the presence or absence of these compounds.[18–20] The viability of HepG2 cells was measured by CCK-8 assay.[21] A large decrease in cell viability (22%) was observed upon treatment of HepG2 cells with 100 μM *t*-BHP (Fig. 2A). Pretreatment with compound **16** (10 μM) displayed a powerful protection (cell viability: 86%) against cell death resulting from *t*-BHP exposure. The compound **16** was more effective than quercetin (cell viability: 62%), which served as a positive control. In contrast, other synthetic derivatives tested were ineffective in this concentration. Methyl caffeate (**19**), a substructure of **16** showed a weak effect (cell viability: 31%). Even with 1 mM *t*-BHP exposure to the cells, **16** exhibited an excellent protective effect (cell viability: 39%, Fig. 2B). For assessment of mitochondrial membrane potential changes, we stained the cells with Rhodamine 123 (Rh123).[22] The Rh123-staining images observed under fluorescence microscopy are shown in Fig. 3. Mitochondrial membrane potential decreased after treatment with *t*-BHP (100 μM) compared to the untreated control, which may be due to the depolarization of the

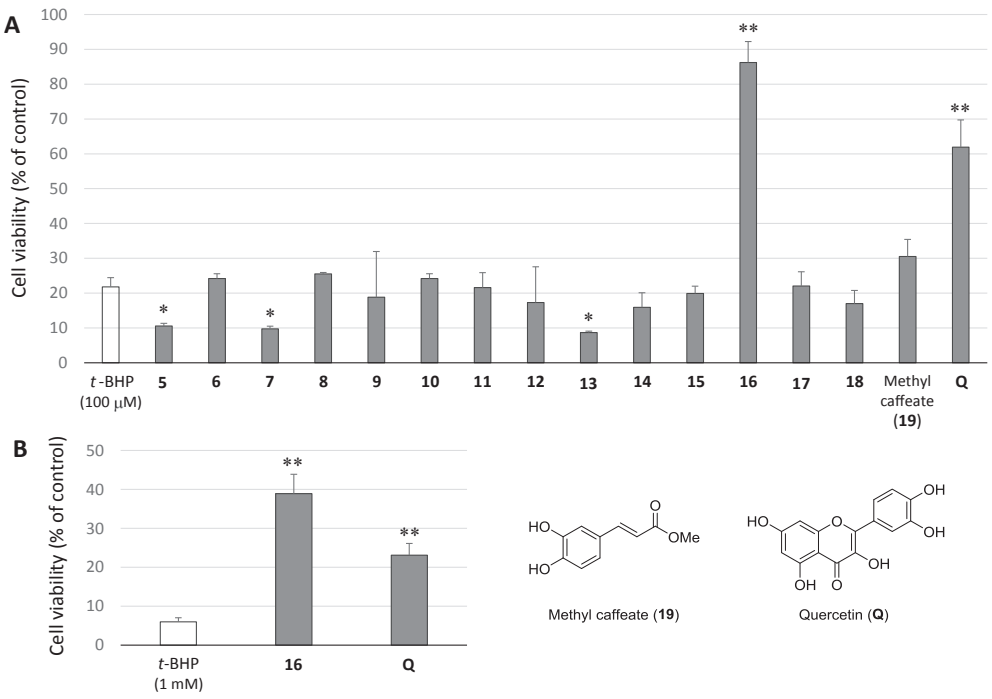


Fig. 2. Cytoprotective effects of coumarin derivatives (10 μM) on *t*-BHP-induced cytotoxicity in HepG2 cells. Cells were pretreated with samples for 1 h, and were subsequently exposed to *t*-BHP for 3 h. Values (mean ± S.D., $n = 3$) are expressed as a percentage relative to viability of cells treated with *t*-BHP alone. $^{*}p < 0.05$ and $^{**}p < 0.01$, respectively (vs. cells treated with *t*-BHP alone). A: 100 μM *t*-BHP exposure; B: 1 mM *t*-BHP exposure.

membrane. The fluorescence intensity of compound (**16**)-pretreated cells was no different from that of untreated control, indicating the mitochondrial protective nature of the compound.

A catechol group is known to possess antioxidant activity and is a common substructure in caffeoyllomatin (**16**), methyl caffeate (**19**), and quercetin (Q). DPPH radical scavenging activity of the three compounds was evaluated to confirm whether the catechol group makes a major contribution to the cytoprotective effects of the compounds.[23] These compounds exerted the antioxidant capacity and their EC_{50} values were 3.06 μM for **16**, 1.50 μM for **19**, and 0.17 μM for quercetin. Detoxification of harmful oxygen species by the catechol group of **16** contributed to the cytoprotective effect.

Nuclear factor erythroid 2-related factor 2 (Nrf2) has emerged as a transcription factor that maintains cellular homeostasis. The Keap1-Nrf2 signaling pathway evokes an adaptive response to oxidative stress that serves to enhance cell survival.[24–26] Previous studies have shown that several plant phenolics regulate the Keap1/Nrf2 complex.[27,28] Our computational molecular simulations demonstrated that **16** favorably docked to the Kelch domain of Keap1 protein (PDB ID: 4L7B)[29] with free binding energy of −7.08 kcal/mol (see Supplementary data).

In conclusion, we have presented the practical synthesis of naturally occurring coumarin derivatives and assessment of their cytoprotective

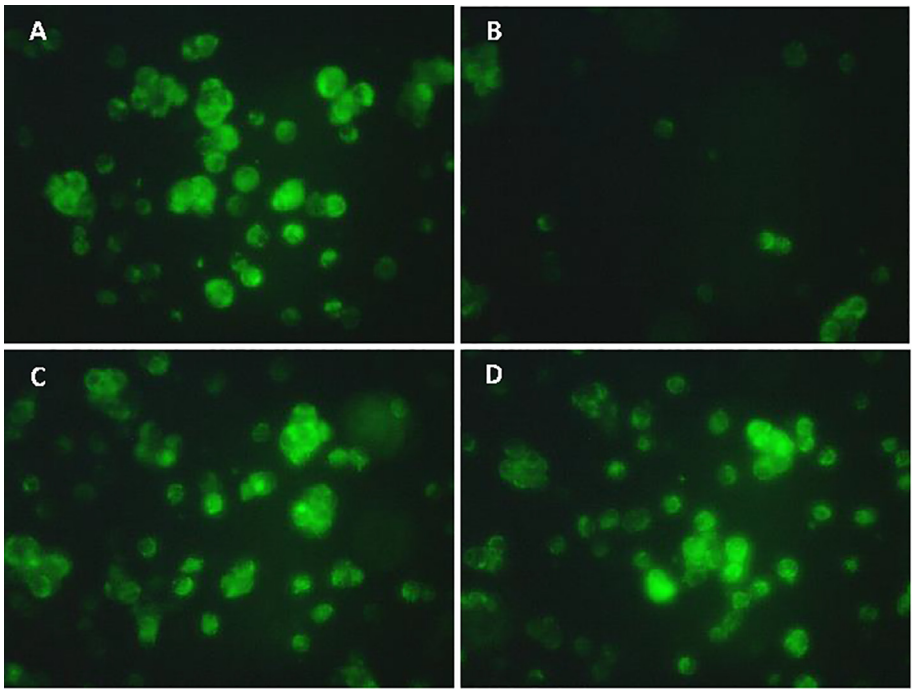


Fig. 3. Mitochondrial membrane potential changes observed with Rhodamine 123 staining. A: untreated control; B: 100 μM *t*-BHP exposure; C: pretreatment with caffeoyllomatin **16** before 100 μM *t*-BHP exposure; D: pretreatment with quercetin (Q) before 100 μM *t*-BHP exposure.

effects. Notably, caffeoyllomatin (**16**) attenuated *t*-BHP-induced HepG2 cell injury. The discovery of **16** as a hepatoprotective agent may lead to further development of natural product-derived chemotherapeutic drugs for treatment of liver disorders.

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2018.06.018.

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