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

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Keywords: Coumarin
Cytoprotection
Human hepatoma HepG2 cell

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.
The cyclization started with the mCPBA-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). In contrast, epoxide opening under acidic conditions happened at the more substituted end, to generate the dihydropyrano products, lomatian 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$_2$CO$_3$ and subsequent deprotection by means of TBAF afforded the p-coumaroyl derivatives, angelmarin (9, 49% yield), coumaroyldescursinol (12, 49% yield). In addition, from lomatian (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. The viability of HepG2 cells was measured by CCK-8 assay. 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 (Rho123). 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

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Scheme 1. Synthesis of ostheno and demethylsuberosin.

Scheme 2. Synthesis of various dihydrofurano- and dihydropyranocoumarins.


Fig. 1. Chemical structures of phenylpropanoid-conjugated coumarins.
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. 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. Previous studies have shown that several plant phenolics regulate the Keap1/Nrf2 complex. Our computational molecular simulations demonstrated that 16 favorably docked to the Kelch domain of Keap1 protein (PDB ID: 4L7B) 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 effects.

![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.]

![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.]

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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.

Acknowledgment

We gratefully thank Assistant Prof. You Fukka (Division of Antioxidant Research, Life Science Research Center, Gifu University) for providing HepG2 cells.

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.

References