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Structural modification of luteolin from *Flos Chrysanthemi* leads to increased tumor cell growth inhibitory activity

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ABSTRACT

The luteolin from *Flos Chrysanthemi* was found to directly bind to the Bcl-2 protein and inhibit the tumor cell growth in our previous study. However, it has been shown to possess wide and week biological activities. In this study, a series of derivatives of luteolin were designed and synthesized, and their tumor cell growth inhibitory activities were evaluated against human leukemia cell line HL-60. The results showed that compounds **1B-2**, **2A-3**, and **2B-5**, with hydrophobic substituted benzyl groups introduced to B ring and hydrogen or methyl introduced to 7-OH group of luteolin, exhibited the strongest inhibitory activity with the IC_{50} lower than 10 μM, which were significantly more potent than luteolin. The studies presented here offer a good example for modifications of flavones to improve their tumor cell growth inhibitory activities.

In China, the *Flos Chrysanthemi* is both a common health food as chrysanthemum tea and a traditional Chinese medicine included in the Chinese Pharmacopoeia.[1,2] Luteolin (Fig. 1), a main substance found in *Flos Chrysanthemi*, is a flavonoid contained in many plants, and has been shown to possess a variety of pharmacological activities, including antioxidant, anti-inflammatory and anticancer activities. Several possible mechanisms involved in the biological activities of luteolin were reported.[3–5] In our previous study, that luteolin directly binds to and shows inhibitory activity against the activity of Bcl-2 protein, and its anti-tumor activity is related to the effect were reported.[6]

Flavones are a class of natural products widely distributed in different plants with a wide range of biological activities. However, many natural flavones including luteolin have been shown to possess week biological activities and low specificity.[7–10] It is possible to improve their specificity by rational designing their analogues based on a particular mechanism. In our previous study, the derivatives of luteolin with a benzyl group introduced to the B ring (3′-benzyl luteolin and 2′-benzyl luteolin) (Fig. 1) were designed, and showed increased inhibitory activity against the activity of Bcl-2 protein and tumor cell growth inhibitory activity.[6] In addition, the benzyl-substituted flavone compounds are rare in nature,[11–14] which are a structural type worthy of further study.

The oxidative cyclization of 2′-hydroxy-chalcone and the acidic rearrangement of benzyl groups in aryl benzyl ethers were used to totally synthesize benzyl-substituted flavone compounds based on our previous study (Scheme 1A).[6,15] In the re-synthesis process of 3′-benzyl luteolin and 2′-benzyl luteolin, two new byproducts of the last demethylation reaction were found. Through exhaustive isolation, purification and structural characterization, they were confirmed to be the products of partial demethylation (3′-benzyl-7-methoxy luteolin and 2′-benzyl-7-methoxy luteolin) (Fig. 1). The bioactivities of these derivatives in inhibiting the growth of human leukemia cell line HL-60 were evaluated by the MTT assay (Table 1). In order to further improve its tumor cell growth inhibitory activity, a series of derivatives of luteolin were designed and synthesized with different substituted benzyl groups introduced to B ring and hydrogen or methyl introduced to 7-OH group (Fig. 1). The structures of the target compounds are listed in Table 1.

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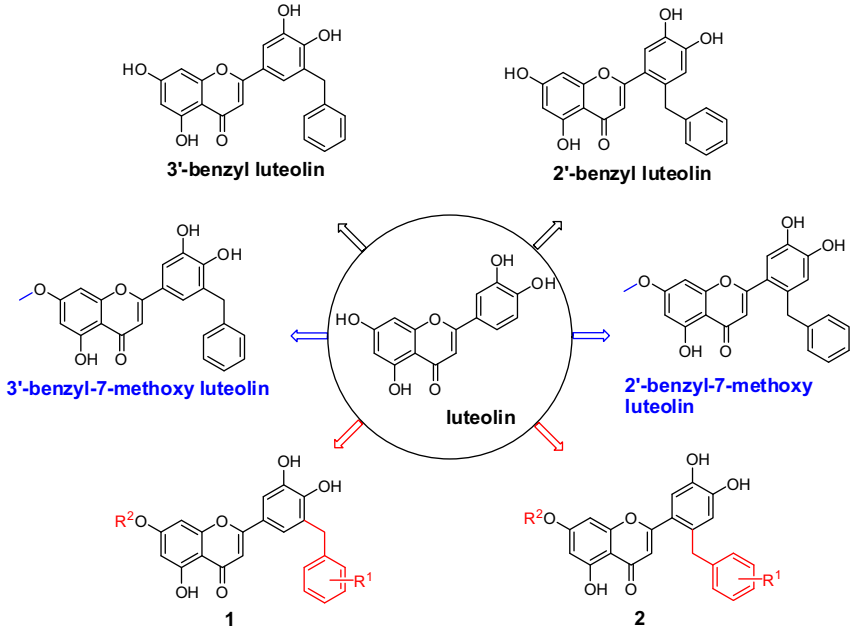

Figure 1. The structures of luteolin and its derivatives.

The target derivatives of luteolin were synthesized by the modification of our previous procedure of benzyl-substituted flavone compounds (Scheme 1B), which was reduced two steps to improve the synthesis efficiency. Starting from key intermediate **11**, a serial of flavone benzyl ether compounds **12** were synthesized by the benzylation reaction in the presence of different substituted benzyl chlorides with yields higher than 80%. Using microwave, the intermediate **12** underwent a rearrangement reaction in the presence of methylsulfonic acid. This reaction yielded rearrangement products **13** and **14** with the benzyl group introduced to the 2′ or 3′ on the B ring with moderate yields respectively, and debenzylated product **11**. Finally, intermediates **13** and **14** were treated with boron tribromide to obtain the products of completely demethylation **1A** and **2A**, and the products of partial demethylation **1B** and **2B** with moderate yields respectively. Although the yield of the single reaction via this route was not high, the intermediate **11** could be re-used to improve the overall yield of the multiple reactions.

The growth inhibitory activities of the target compounds on HL-60 were tested (Table 1). AT-101 which also derivated from the natural product and has been investigated in clinical research was used as a positive control. Among them, compounds **1B-2**, **2A-3**, and **2B-5** showed the strongest inhibitory activity with the IC_{50} lower than 10 μM, which were significantly more potent than luteolin and even equivalent to AT-101. The main objective of this study was achieved. In addition, the preliminary structure–activity relationships (SAR) of these compounds were further discussed. Most of the compounds showed stronger inhibitory activity than luteolin, and half of them showed stronger inhibitory activity than 3′-benzyl luteolin and 2′-benzyl luteolin. It indicated that the hydrophobic substituted benzyl groups introduced to B ring of luteolin were good for improving its activities. Moreover, most of compounds **2B** and **1B** were more potent than compounds **2A** and **1A**, respectively. It suggested that the methyl introduced to 7-OH group of luteolin were also good for improving its activities. However, the more detailed SAR of them was not clear, which should be with the help of more derivatives synthesized in the future study.

To test whether the target compounds in this study would bind to Bcl-2 protein and inhibit it activity, representative compound 2′-benzyl-7-methoxy was subjected to evaluation by fluorescence polarization-based binding assay (FP assay) (Table 2).[6,16,17] Luteolin, 2′-benzyl luteolin and AT-101 were also evaluated by this assay as controls. The IC_{50} of this compound was 1.3 μM, lower than luteolin, 2′-benzyl luteolin and slight higher than AT-101. Considering that it was consistent with their tumor cell growth inhibitory activities, it seems that Bcl-2 protein represents an important potential target for these compounds. In addition, the toxicity of these compounds was evaluated by testing the growth inhibitory activities against the normal human HEK-293 cell line. The results indicated their low toxicity (Table 1S).

In summary, the luteolin from *Flos Chrysanthemi* was found to directly bind to the Bcl-2 protein and inhibit the tumor cell growth in our previous study. However, it has been shown to possess wide and week biological activities. In this study, a series of derivatives of luteolin were designed and synthesized, and their tumor cell growth inhibitory activities were evaluated on human leukemia cell line HL-60. The results showed that compounds **1B-2**, **2A-3**, and **2B-5**, with hydrophobic substituted benzyl groups introduced to B ring and hydrogen or methyl introduced to 7-OH group of luteolin, exhibited the strongest inhibitory activity with the IC_{50} lower than 10 μM, which were significantly more potent than luteolin. The studies presented here provide a new structural type for the development of novel antitumor agents, and also offer a good example for future modifications of flavones to improve their tumor cell growth inhibitory activities.