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Original article

Synthesis and antitumor activity evaluation of chrysin derivatives

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ABSTRACT

A series of 5,7-disubstituted chrysin, 7-monosubstituted chrysin, 5-monosubstituted chrysin derivatives were synthesized by alkylation, acetylation, benzoylation, carboxymethylation, and evaluated on their antitumor activity of H22 cells in the search for potential antitumor agents. Among them, compound **3** (5,7-diacetyl chrysin) displayed the most potent antitumor activity with IC_{50} value of 141 μM. Moreover, there is significant up-regulation of G2 in cell cycle of H22.

1. Introduction

Chrysin (5,7-dihydroxyflavone), a natural product of flavonoid, has a wide range of biological activity. Recently its antioxidant, antimellanogenic, antivirus, antiallergy, anti-inflammatory, anti-anxiolytic, and antidiabetogenic effects have been extensively investigated [1–5]. Being a natural product, chrysin presents only a small side effect. In order to improve the pharmaceutical activity of chrysin, a lot of studies have been done by synthesis of its derivatives. Zhou et al. reported that the 5-OH of chrysin is the active site [6], Babu et al. reported that the aromatic ring is the active site [7] and others reported that increasing the water solubility could strengthen its activity. As anticancer activity is particularly important for clinic application, the aim of present study was to verify the antitumor activity of the different types of derivatives, in order to explore the optimal derivatization way for chrysin, and to infer the active sites of chrysin.

From the previous studies we find that chrysin needs to enter the cells, such as inhibiting the metabolism of the carcinogen benzo [α]pyrene [8], to markedly augment the cytotoxicity of TNF (tumor necrosis factor-α) [9]. It displays the tyrosinase inhibitory activity [10], and moderate aromatase inhibitory activity [11], also it is able to inhibit the estradiol-induced DNA synthesis [12]. So in our study the 5-OH, 7-OH or both of them of chrysin were modified by adding some hydrophobic groups, such as alkyl, acetyl, benzoyl, carboxymethyl, in order to find out which positions are the active sites, and which substituent is the best one.

2. Results and discussion

The MTT results were summarized in Table 1 for anti-H22 cells. Among the synthesized chrysin derivatives, the 5,7-diacetyl chrysin has a significant (enhancement of activity is 91.56% than chrysin) growth in anti-H22 cells. On the one hand other activity of the compounds decreased, but the decline of disubstituted compounds were less than the monosubstituted ones. On the other hand small substituent groups show more activity than benzene ring. Furthermore, there is a significant change when 5-position was modified.

In summary, the present results indicated that hydrophobic modification of 5- and 7-positions would change the activity. Increase or decrease of the activity depends on the substituents. Chrysin is still active when 5- and 7-positions are modified. It shows that these two positions have no direct relationship with the activity. They may be the binding sites from drug to protein. Although the drug can enter cells easily, the benzene ring has a larger steric hindrance which could reduce the activity. It may be

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Table 1
In vitro cytotoxicity against the H22 cells.

Compound	H22 (IC_{50} μM)
1	1671
2	4290
3	141
4	4034
5	3123
6	1709
7	3659
8	3223
9	2466

postulated that the oxygen atom of 9-carbonyl plays important roles on anticancer activity, making it an active site. Hydrogen bond between the hydrogen atom of 7-hydroxyl and the oxygen atom of 9-carbonyl disappeared when the hydrogen atom of 7-hydroxyl was replaced, thus the oxygen atom of 9-carbonyl is easier to bond with the other hydrogens, such as arginine, aspartic acid, glutamic acid, lysine, threonine, tyrosine, asparagine, glutamine, serine in protein, atom of hydroxyl by competitive inhibition. In conclusion, the small size of hydrophobic substitution at both 5- and 7-posiotions is a good way of derivatization to increase the chrysin bioactivity. Our present results are quite different from the previous results that 5-hydroxyl is the active site and hydrophilic modification can increase activity.

Besides, as shown from the cell cycle detection (Fig. 1), after treatment with compounds **3** (5,7-diacetyl chrysin), there is significant up-regulation of G2 phase. The change of percentage of each cell cycle was provided in Table 2. We speculate that the compounds **3** has interaction with protein which affects the cell mitosis, making cells stay in G2 phase. However which protein was affected by 5,7-diacetyl chrysin is not clear, further study is currently in progress.

3. Chemistry

The synthetic procedures and reaction conditions are shown in Scheme 1. Reaction of chrysin (**1**) in pyridine with benzoyl chloride or acetic anhydride gave compounds **2**–**5**. Reaction of chrysin (**1**) in acetone with bromoacetate in the presence of K_2CO_3 provided compound **6**, which, after benzoylation with benzoyl chloride, gave compound **7**. Compound **6** reacted with bromoacetate in the presence of K_2CO_3 provided compound **8**, which was hydrolyzed under alkaline conditions to give compound **9**. The structures of all the synthetic compounds were fully characterized by spectroscopic data (NMR, MS).

4. Antitumor activity

H22 cells (Liver Cancer of murine H22 tumor cell line), purchased from Shanghai Institute for Biological Sciences (Chinese Academy of Sciences, Shanghai, China). The cells are grown in RPMI 1640 (Gibco, Grand Island, USA) medium containing 10% fetal bovine serum (Gibco, Grand Island, USA) and 2 mM L-glutamine. Cells are inoculated into 96-well microtiter plates in 100 μl at plating densities ranging from 20,000 cells/well. After cell inoculation, the microtiter plates are incubated at 37 °C, 5% CO_2, 95% air and 100% relative humidity for 24 h prior to addition of experimental drugs.

All the compounds were tested for their anticancer activities, against H22 cells, by MTT-based assay in vitro. The assays were performed in 96-well plates essentially as described by Mosmann. IC_{50} is a characteristic constant means that the minimum

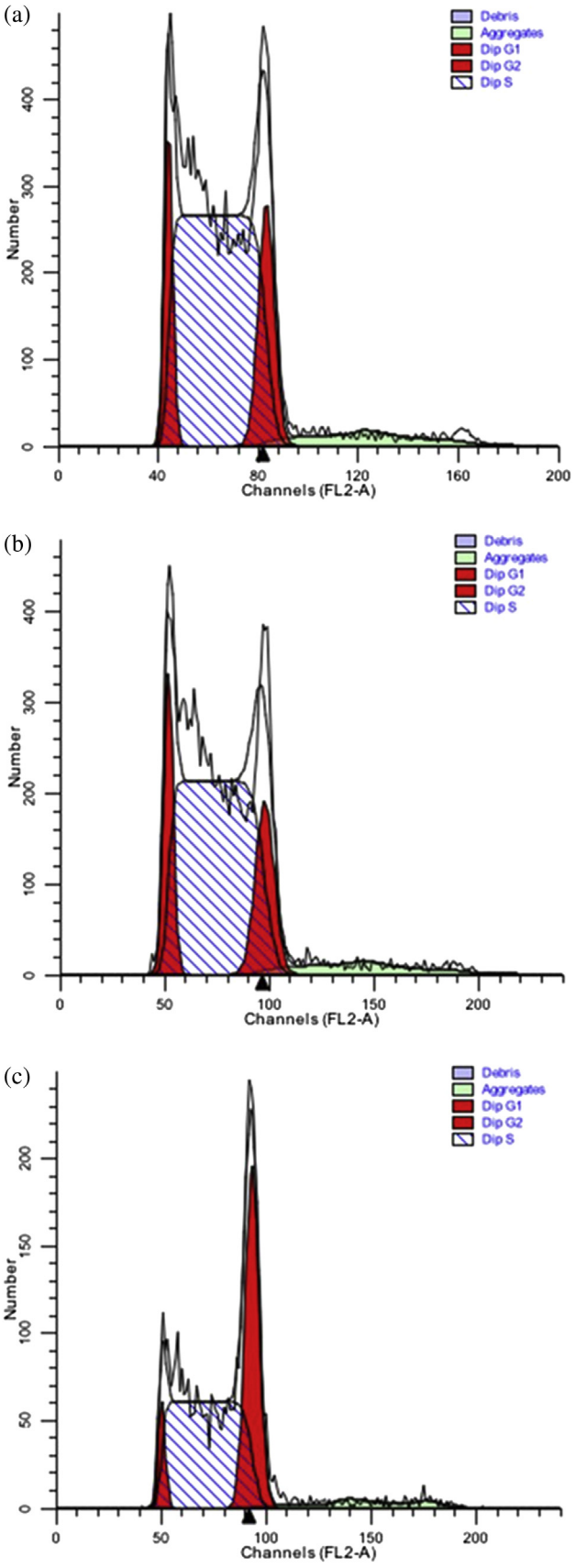

Fig. 1. Cell cycle phases of H22 was analyzed by FCM. Cells were collected from different groups and dyed by PI. Histograms H22 cells showed distributions of G1, S and G2/M-phases, which were measured by FCM in the chrysin and compounds **3** of 1 mmol/l (a: H22 control; b: chrysin; c: compound **3**).