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Synthesis and Comparison of Anti-inflammatory Activity of Chrysin Derivatives

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Abstract: A series of five chrysin derivatives was synthesized and examinated for their anti-inflammatory activities. The *in vivo* anti-inflammatory activity of synthetic compounds was carried out using the model of carrageenan induced mice paw edema. The results showed that methylation of 5,7-dihydroxyl groups of chrysin resulted to increase the *in vivo* bioactivity in comparison with the corresponding chrysin derivatives having two free hydroxyl groups. The introduction two halide groups into B ring at 6 and 8 positions of chrysin did not to improve any significant increase positive effect on the *in vivo* bioactivity.

Keywords: anti-inflammatory activity, chrysin derivatives

INTRODUCTION

Chrysin is a naturally occurring flavone chemically extracted from the blue passion flower (Passiflora caerulea). Chrysin is a flavone widely distributed in plants which was reported to have many biological activities such as anti-oxidant, anti-microbial, anti-spasmodic, anxiolytic and anti-inflammatory activities…[1,2,3,4,5] Chrysin has been shown to induce an anti-inflammatory effect, most likely by inhibition of COX-2 expression and via IL-6 signaling[6]. Chrysin demonstrated cell toxicity and inhibition of DNA synthesis at very low concentrations in a normal trout liver cell line.[7]

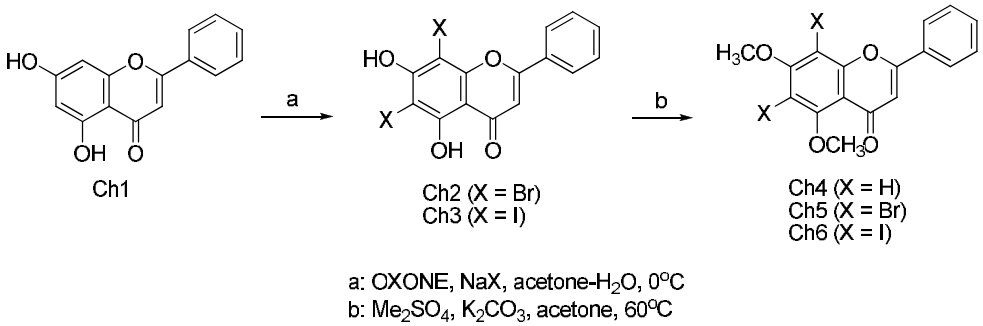
It is well known that halogenated compounds are also strongly biological activitives[8] but to our knowledge no natural chrysin derivatives have reported with halogen as substituents. In the previous report we have found that 6,8-dihalogen substituted chrysin derivatives had stronger inhibiton of production of PGE_2 from the RAW 264.7 cells than that of chrysin[9]. Additionally, we were also interested to check the effect of halogen substituents at 6 and 8 positions of chrysin on their biological activity. Therefore, in order to search for new compounds that can be used for treatment of inflammatory diseases, this paper describes the processes for synthesis of some 6,8-dihalogenated chrysin derivatives and their anti-inflammatory activities.

MATERIAL AND METHODS

Chemistry: All chemicals were obtained from commercial suppliers, and used without further purification. NMR spectra were recorded on a Varian Gemini 2000 instrument (200 MHz) spectrometer. Chemical shifts are reported in parts per million (ppm) downfield relative to

tetramethylsilane as an internal standard. Analytical thin-layer chromatography (TLC) was performed using commercial glass plate with silica gel 60 F_{254} purchased from Merck. Chromatographic purification was carried out by flash chromatography using Kieselgel 60 (230~400 mesh, Merck).

The chemical processes used to prepare the chrysin derivatives were summarized in the following Scheme 1.



Scheme 1. Synthesis of chrysin derivatives

***In vivo* anti-inflammatory activity test**

The method of Winter et al. was employed in this experiment [10]. ddY Mice weighing 22 ± 2g of either sex were obtained from Pasteur Institute, HCM city, were used this study. Animals were housed in groups of eight in PVC cages, under 12-h light/ 12-h dark cycle with hard food pellets and tap water ad libitum. Before the experimentation, mice were acclimated to the animal care facility for at least two days and fasted for 12 h but allowed free access to tap water.

Carrageenan was purchased from Sigma Aldrich and 1% carrageenan suspension freshly prepared in physiological saline (w//v) to use. Dihalogenated chrysin derivatives were prepared as cream at the doses of 2.5 % and 5 % to use. Ketoprofen 2.5% cream was used as reference sample.

Briefly, the acute paw edema was induced in the right hind paw of mice by subplantar injection of 0.025 ml/mouse of 1 % freshly prepared carrageenan suspension. The thickness of the paw was measured before and 3 h after carrageenan injection using a plethysmometer (Model 7140, Ugo Basile). The mice having the paw edema volume increase more than 50 % normal paw volume were chosen into the experiments. The mice were applied vehicle or creams twice a day, in the morning after measurement the paw edema and in the afternoon. The mean increase of paw volume at each time interval was compared with that of control group treated with carrageenan, but without test compounds at the same time intervals. The percentage of edema volume were calculated according to the formula:

$$X\% = \frac{Vn - Vo}{Vo} x100$$

X%: the percentage of increase volume of paw edema

V_o: the volume of paw edema before carrageenan-induced paw edema (1/100 ml)

V_n: the volume of paw edema after carrageenan-induced paw edema (1/100 ml)

Statistical analysis

All data were expressed as mean ± SEM. The data was evaluated by Kruskal-Wallis test and Mann Withney test using Minitab 14. Differences between groups were considered significant when $p < 0.05$.

RESULTS AND DISCUSSION

All of creams containing chrysin or chrysin derivatives have anti-inflammatory effects which are similar or stronger than that of ketoprofen cream 2.5%. Almost of results in the *in vivo* carrageenan-induced paw edema test in mice are correlated to the results *in vitro* test. However, in the *in vitro* test, anti-inflammatory activities of **Ch2**, **Ch3** and chrysin are similar, but in the *in vitro* test, **Ch2** and **Ch3** cream at the dose of 5% are better than that of chrysin. On the other hand, in the *in vitro* test, chrysin has anti-inflammatory activity better than **Ch4** but in the *in vivo* test **Ch4** cream at the dose of 5% has anti-inflammatory activity better than that of chrysin cream.

Anti-inflammatory activity of dihalogenated chrysin derivatives is similar that of chrysin. There was no significant difference between chrysin and dibromochrysin or diiodochrysin in *in vivo* anti-inflammatory activity, although the dihalogenation of chrysin increased six to sevenfold the *in vitro* anti-inflammatory activity. The bulky structure of dihalogenated chrysin derivatives probably restricted their permeability through the skin to the target inflammation sites.

The methylation of chrysin in **Ch4** derivatives minimized the polarization of chrysin, supported for suspension of **Ch4** in cream vehicle and increased in permeability through the skin.

Most 6,8-dihalogenated chrysin derivatives possessing free hydroxyl groups at 5 and 7 positions showed better inhibitory activity of PGE_2 production by comparison with chrysin. The 6,8-dibromochrysin displayed stronger bio-activity by comparison with 6,8-diiodochrysin. This result may be thought due to the present of withdrawing electron groups (bromide versus iodine).

Also, the process for methylation of hydroxyl groups at 5 and 7 positions in A-ring of chrysin resulted to decrease the *in vitro* bio-activity of chrysin derivatives, regardless of halide substituents in A-ring.

The cream formulations of chrysin and its derivatives at the dose of 2.5% exhibited the rapid- onset effect while those cream formulations at the dose of 5% have a slower onset effect. Dihalogenation of chrysin does make no changes in the *in vivo* anti-inflammatory activity. In contrast, the dimethylation of chrysin lead to increase *in vivo* anti-inflammatory activity of chrysin derivatives.

In summary, chrysin and five other dihalogenated/methylated chrysin derivatives were synthesized and evaluated their anti-inflammatory activities *in vivo* tests. The results of *in vitro* test showed that all synthetic chrysin derivatives have the anti-inflammatory activity better than that of chrysin, except the compound 5,7-*O*-dimethylchrysin. The results also were confirmed in the *in vivo* carrageenan-induced inflammatory activity test in mice. All cream formulations containing chrysin or derivatives at both doses of 2.5% and 5% show the *in vivo* anti-inflammatory activity similar or better than that of ketoprofen cream formulation at the dose of 2.5%.

EXPERIMENTAL SECTION

Process for halogenation of chrysin

To the flask dissolved chrysin (5 mmol) and sodium halide (NaBr or NaI, 11 mmol) with 30 ml of mixture of acetone - water (5-1). After cooling, a solution of OXONE® (11 mmol) in 20 ml of water was added slowly with magnetic stirring over 2 h. At the end of reaction (monitoring by TLC with the solvent system of chloroform and methanol 20:1), the reaction mixture was treated with saturated

$Na_2S_2O_3$ solution to remove trace of free halogen. The solvent was removed by evaporation in vacuum. The solid was washed with water and crystallized from absolute methanol to obtain the title compound.

Ch2: 6,8-dibromochrysin (6,7-dibromo-5,7-dihydroxyflavone)
Yield: 85%. mp 291 ^oC. ^1H-NMR (200 MHz, DMSO-d_6): δ 13.75 (s, 1H, OH), 11.77 (s, 1H, OH), 8.12-8.16 (d, 2H, H2', H6'), 7.60-7.64 (t, 3H, H3', H4', H5'), 7.21 (s, 1H, H3). ^{13}C- NMR (50 MHz, DMSO-d_6): δ 181.5 (C-4), 163.4 (C-7), 157.9 (C-2), 157.1 (C-10), 152.4 (C-5), 132.5 (C-1'), 130.3 (C-3' and C-5'), 129.3 (C-4'), 126.5 (C-2', C-6'), 105.1 (C-9), 104.8 (C-3), 94.7 (C-6), 88.6 (C-8).

Ch3: 6,8-diiodochrysin (6,8-diiodo-5,7-dihydroxyflavone)
Yield: 72%. mp 282 ^oC. ^1H-NMR (200 MHz, DMSO-d_6): δ 13.89 (s, 1H, OH), 8.08-8.12 (m, 2H, 6.8 Hz, H2', H6'), 7.48-7.53 (m, 3H, H3', H4'), 7.08 (s, 1H, H3). ^{13}C- NMR (50 MHz, DMSO-d_6): δ 181.5 (C-4), 178.5 (C-7), 163.8 (C-2), 162.0 (C-5), 161.1 (C-10), 132.6 (C-1'), 130.4 (C-3' and C5'), 129.4 (C-4'), 126.8 (C-2' and C-6'), 105.1 (C-9), 104.7 (C-3), 63.4 (C-6 and C-8).

Methylation of chrysin and dihalogenated chrysin derivatives

To a mixture of 2 mmol of chrysin or it's derivatives in 20 ml of anhydrous acetone, anhydrous potassium carbonate (5.0 mmol) and dimethylsulfate (2.5 mmol) added slowly under stirring. The reaction mixture was then put under nitrogen atmosphere and refluxed for 22-23 h. Potassium carbonate was removed by suction filtration, the filtrate was evaporated and then the residues was extracted with dichloromethane. The crude materials were re-crystallized with methanol.

Ch4: 5,7-*O*-dimethylchrysin (5,7-dimethoxyflavone)
Yield: 87 %, mp 132 ^oC. ^1H-NMR (200 MHz, $CDCl_3$): δ 7,75-7,98 (d, 2H, *J* = 8,2 Hz, H2', H6'), 7.49-7.53 (m, 3H, H3', H4', H5'), 6.69 (s, 1H, H3), 6,39 (ds, 1H, J = 2,2Hz, H8); 3.97-3.92 (s, 6H, 2xOCH_3).

Ch5: 6,8-dibromo-5,7-*O*-dimethylchrysin (6,8-dibromo-5,7-dimethoxyflavone)
Yield: 89%; mp > 300 ^oC (decomposed). ^1H-NMR (200 MHz, $CDCl_3$): δ 8.18-8.24 (d, 2H, H2', H6'); 7.68-7.75 (m, 3H, H3', H4', H5'); 7.17 (s, 1H, H3); 3.94-4.03 (s, 6H, 2xOCH_3). ^{13}C- NMR (50 MHz, DMSO-d_6): δ 175.8 (C-7), 166.5 (C-4), 161.7 (C-5), 160.8 (C-2), 153.5 (C-9), 132.2 (C-1'), 130.4 (C-3' and C-5'), 129.4 (C-4'), 126.3 (C-2' and C-6'), 115.6 (C-10), 108.1 (C-3), 99.0 (C-8), 77.6 (C-6), 61.7 and 61.0 (2xOCH_3).

Ch6: 6,8-diiodo-5,7-*O*-dimethylchrysin (6,8-diiodo-5,7-dimethoxyflavone)
Yield: 82%. mp 296 ^oC (decomposed.). ^1H-NMR (200 MHz, $CDCl_3$): δ 8.04-8.09 (d, 2H, *J* = 7.4 Hz, 2 Hz, H2', H6'), 7.54-7.58 (m, 3H, *J* = 7.2 Hz, 2.2 Hz, H3', H4', H5'), 6.79 (s, 1H, H3), 3.96-3.99 (s, 6H, 2OCH_3). ^{13}C- NMR (50 MHz, DMSO-d_6): δ 175.02 (C-7), 163.41 (C-4), 161.07 (C-5), 160.18 (C-2), 157.41 (C-10), 132.09 (C-1'), 130.52 (C-3' and C-5'), 129.28 (C-4'), 126.52 (C-2' and C-6'), 115.74 (C-9), 107.83 (C-3), 89.02 (C-8), 79.43 (C-6), 61.57 and 60.73 (5-OCH_3 and 7-OCH_3).

***In vivo* anti-inflammatory activity**

Carrageenan induced paw edema in mice and the increase in volume of paw is greatest 3 hour after

carrageenan injection are displayed in the **Table 1** and **Figure 1**.

Table 1. Percentage of paw edema volume (X%) by chrysin derivatives

N^{o}	Samples (concentration %)	3 hrs	1st day	2^{sd} day	3rd day	4th day	5th day	6th day
1	Control (-)	67.06 ±13.79	69.26 ±11.83	65.47 ±14.49	58.43 ±16.99	49.36 ±21.56	41.62 ±20.67	35.97 ±17.21
2	Vehicle (VEH)	69.92 ±10.30	65.08 ±12.92	59.32 ±15.14	49.02 ±11.88	48.33 ±13.35	41.06 ±11.15	33.79 ±12.41
3	Ketoprofen (2.5%)	67.39 ±12.24	52.99 ±12.15	49.26 ±14.31	40.68 ±14.50	27.89 ±13.13	19.12 ±12.20	12.28 ±10.24
4	Ch.1.1 (2.5%)	64.49 ±9.15	45.56 ±11.79	47.83 ±11.51	36.83 ±14.58	19.72 ±9.15	13 ±11.33	10.07 ±11.95
5	Ch.1.2 (5%)	68.6 ±14.50	55.66 ±16.18	44.84 ±15.00	29.64 ±11.07	24.62 ±7.43	20.8 ±6.71	11.51 ±9.12
6	Ch.2.1 (2.5%)	62.27 ±9.34	56.73 ±11.59	42.64 ±16.46	29.82 ±15.32	22.09 ±12.12	16.64 ±13.00	9.18 ±11.34
7	Ch.2.2 (5%)	74.62 ±9.53	62.35 ±17.29	45.94 ±23.35	24.9 ±19.85	18.75 ±16.03	16.84 ±15.32	11.6 ±10.74
8	Ch.3.1 (2.5%)	68.32 ±11.83	56.16 ±17.18	51.9 ±18.75	27.08 ±11.61	20.59 ±12.63	14.99 ±8.86	12.97 ±10.03
9	Ch.3.2 (5%)	68.6 ±14.50	69.15 ±28.37	53.09 ±22.27	45.05 ±23.78	33.08 ±15.83	27.84 ±13.59	17.3 ±11.21
10	Ch.4.1 (2.5%)	74.5 ±11.50	41.64 ±17.56	36.72 ±15.92	25.39 ±12.52	16.68 ±9.31	9.97 ±12.47	8.93 ±9.39
11	Ch.4.2 (5%)	63.86 ±11.61	43.18 ±15.33	32.65 ±9.00	22.5 ±10.12	13.18 ±13.07	9.09 ±9.00	5.76 ±6.64
12	Ch.5.1 (2.5%)	76.85 ±13.63	45.26 ±24.75	26.96 ±12.40	18.1 ±8.86	15.3 ±10.06	11.17 ±8.08	8.44 ±9.86
13	Ch.5.2 (5%)	67.02 ±8.85	56.28 ±8.99	49.92 ±7.36	43.8 ±8.27	35.79 ±9.38	26.94 ±10.38	11.74 ±8.76
14	Ch.6.1 (2.5%)	71.54 ±14.33	52.15 ±16.04	39.54 ±19.70	31.7 ±16.90	20.84 ±20.45	15.37 ±17.28	10.15 ±11.28
15	Ch.6.2 (5%)	67.19 ±8.76	62.07 ±12.71	50.25 ±7.94	43.47 ±8.88	32.31 ±11.67	24.79 ±10.26	7.77 ±5.91

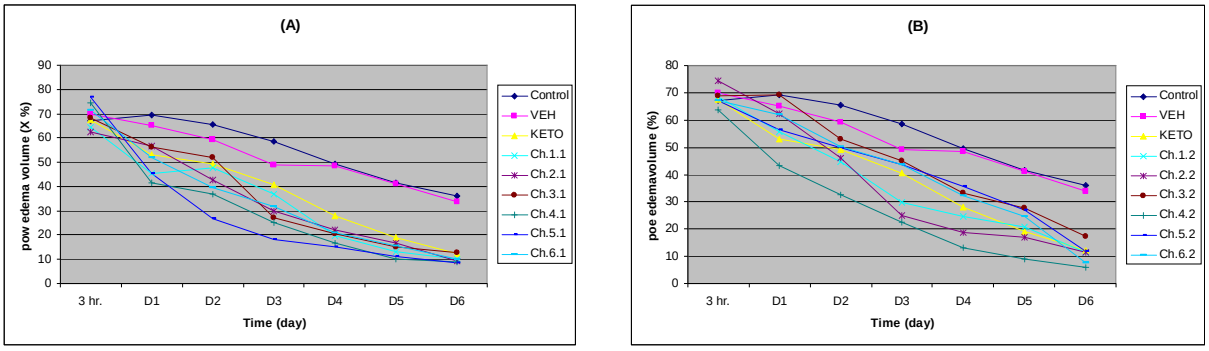


Figure 1. Anti-inflammatory activity of dihalogenated chrysin derivatives in carrageenan-induced inflammation in at the doses of 2.5% (A) and 5% (B). The creams were applied to edema paw 3 hours after carrageenan injection. Ketoprofen cream 2.5% used as positive control. Data are expressed as the mean of percentage of paw edema volume (X%) ± SEM.

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