Synthesis and Preliminary Biological Evaluation of Chrysin Derivatives as Potential Anticancer Drugs

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Abstract: A series of chrysin derivatives were prepared from 2-hydroxyacetophenone, 2,4-dihydroxyacetophenone, 2,4,6-trihydroxy- acetophenone, using modified Baker-Venkataraman transformation. Their anticancer activities *in vitro* were evaluated by the standard MTT method. The results of biological test showed that some of chrysin derivatives showed stronger anticancer activity than 5-fluorouracil.

INTRODUCTION

Chrysin, a natural flavonoid widely distributed in plants, has been reported to have various biological activities such as anticancer [1], anti-inflammatory [2], anti-oxidant [3], anti-anxiolytic [4], vasodilatory actions [5]. In anticancer area, chrysin has been found to possess anti-proliferative activity through inhibiting malignant cell growth by down-regulated expression of PCNA in Hela cells [6], and induced apoptosis through caspase activation and Akt inactivation in U937 cells [7]. However, the anticancer activity of chrysin *in vivo* studies on animals and humans has been disappointing mainly due to extremely poor oral bioavailability [8]. The crucial feature of chrysin in this respect is their unprotected hydroxyl groups, which are rapidly and efficiently metabolized *via* glucuronidation and sulfation [9]. As a part of our current work on the search for anticancer substances with high efficacy, low toxicity and minimum of side effects [10-12], herein, we describe the synthesis of chrysin derivatives and their anticancer activities against human gastric carcinoma SGC-7901, human colon cancer HT-29, and human promyelocytic leukemia HL-60 cell lines.

SYNTHESIS CHRYSIN DERIVATIVES

Among the reported methods to synthesize flavonoids, the Baker-Venkataraman transformation is a largely applied one. The synthesis of compounds **4a-4m** were carried out according to modified Baker-Venkataraman transformation (Fig. **1**). Commercially available 2,4-dihydroxyacetophenone **1a** and 2,4,6-trihydroxyaceto- phenone **1b** were treated with anhydrous potassium carbonate and dimethyl sulfate in acetone to give **2a** and **2b**, respectively. **2a** and **2b** were reacted with substituted benzoyl chlorides in the anhydrous potassium carbonate in acetone to afford the corresponding 1,3-diketones **3a-3c** and **3d-3h**, respectively. Treatment of **3a-3c** and **3d-3h** with glacial acetic acid and anhydrous sodium acetate gave 5-methoxy-flavones **4a-4c** and 5,7-dimethoxy-flavones **4d-4h**, respectively. Reaction of 2-hydroxy-acetophenone **2c** with substituted benzoyl chlorides followed by the flavone ring formation in same conditions gave the flavone analogues **4i-4m**.

All the new compounds were characterized by detailed spectroscopic analysis. **4a** White powder, mp 142-143 ^{o}C. HRFABMS m/z 321.0733 $[M+H]^+$ ($C_{17}H_{11}F_3O_3$, calc. 321.0738). IR (cm^{-1}, KBr): 1365, 1438, 1501, 1589, 1613, 1642(C=O), 2948, 2976; ^{1}H NMR (300MHz, $CDCl_3$): 3.967(3H, s), 6.815(1H, s), 7.007-7.047(2H, m), 7.648-7.701(1H, m), 7.791-7.817(1H, m), 8.065-8.091(1H, m), 8.139-8.171(1H, m), 8.194(1H, s). **4d** White powder, mp 156-157 ^{o}C. HRFABMS m/z 351.0839 $[M+H]^+$ ($C_{18}H_{13}F_3O_4$, calc. 351.0844). IR (cm^{-1}, KBr): 1390, 1437, 1460, 1489, 1602, 1614, 1653(C=O), 2945, 2971; ^{1}H NMR (300MHz, $CDCl_3$): 3.942(3H, s), 3.973(3H, s), 6.308(1H, d, J = 2.1Hz), 6.718(1H, d, J = 2.1Hz), 6.745(1H, s), 7.617-7.670(1H, m), 7.760-7.788(1H, m), 8.023-8.050(1H, m), 8.150(1H, s). **4f** White powder, mp 186-187 ^{o}C. HRFABMS m/z 317.0586 $[M+H]^+$ ($C_{17}H_{13}O_4Cl$, calc. 317.0580). IR (cm^{-1}, KBr): 1417, 1459, 1487, 1584, 1605, 1660(C=O), 2847, 2944; ^{1}H NMR (300MHz, $CDCl_3$): 3.920(3H, s), 3.964(3H, s), 6.392(1H, d, J = 2.4Hz), 6.566(1H, d, J = 2.4Hz), 6.653(1H, s), 7.454-7.500(2H, m), 7.788-7.834(2H, m). **4h** White powder, mp 177-178 ^{o}C. HRFABMS m/z 313.1076 $[M+H]^+$ ($C_{18}H_{16}O_5$, calc. 313.1075). IR (cm^{-1}, KBr): 1429, 1458, 1493, 1574, 1592, 1601, 1634(C=O), 2954; ^{1}H NMR (300MHz, $CDCl_3$): 3.900(3H, s), 3.933(3H, s), 3.958(3H, s), 6.367(1H, d, J = 2.1Hz), 6.535(1H, d, J = 2.1Hz), 7.012-7.060(2H, m), 7.082-7.110(1H, m), 7.423-7.481 (1H, m), 7.853-7.885(1H, m) **4i** White needles, mp 146-147 ^{o}C. HRFABMS m/z 291.0633 $[M+H]^+$ ($C_{16}H_9F_3O_2$, calc. 291.0632). IR (cm^{-1}, KBr): 1382, 1445, 1466, 1577, 1605, 1625, 1667(C=O), 3139; ^{1}H NMR (300MHz, $CDCl_3$): 6.879(1H, s), 7.437-7.491(1H, m), 7.609-7.831(4H, m), 8.090-8.120(1H, m), 8.202-8.268(2H, m).

BIOLOGICAL ACTIVITY

All of the chrysin derivatives were tested for their anticancer activity *in vitro* against HL-60, HT-29 and SGC-7901 cells by MTT-Based Assay, using 5-Fluorouracil as control.

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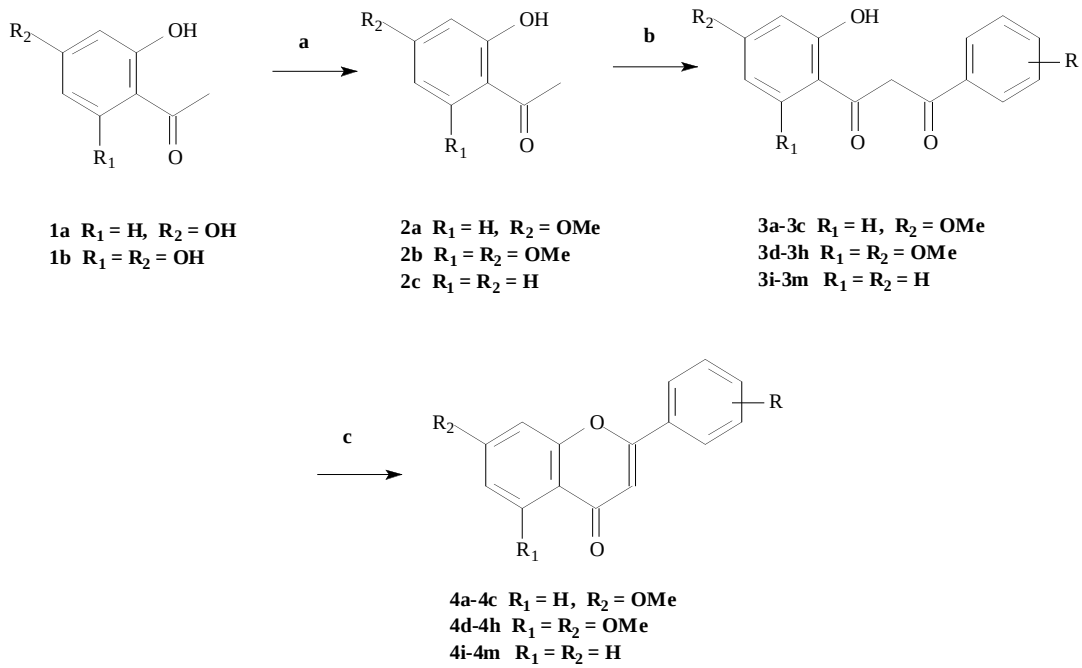

1a R_1 = H, R_2 = OH
1b $R_1 = R_2$ = OH

2a R_1 = H, R_2 = OMe
2b $R_1 = R_2$ = OMe
2c $R_1 = R_2$ = H

3a-3c R_1 = H, R_2 = OMe
3d-3h $R_1 = R_2$ = OMe
3i-3m $R_1 = R_2$ = H

4a-4c R_1 = H, R_2 = OMe
4d-4h $R_1 = R_2$ = OMe
4i-4m $R_1 = R_2$ = H

R : = 3'-CF_3, 4'-NO_2, 4'-Cl, 4'-OMe, 2'-OMe

Fig. (1). Reagents and conditions: (a) dimethyl sulfate, K_2CO_3, acetone, rt; (b) substituted benzoyl chlorides, K_2CO_3, acetone, reflux; (c) NaOAc, HAc, reflux.

Table 1. The Structures and Anticancer Activities of the Target Compounds *In Vitro*

÷	R_1	R_2	R	IC_{50} (μmol/L)		
				HL-60	HT-29	SGC-7901
4a	H	OMe	3'-CF_3	5.38	6.19	5.78
4b	H	OMe	4'-NO_2	5.56	5.86	3.57
4c	H	OMe	4'-Cl	6.92	4.37	6.89
4d	OMe	OMe	3'-CF_3	3.03	3.80	2.46
4e	OMe	OMe	4'-NO_2	5.29	5.81	4.86
4f	OMe	OMe	4'-Cl	5.00	5.22	6.08
4g	OMe	OMe	4'-OMe	11.37	10.16	12.05
4h	OMe	OMe	2'-OMe	16.57	14.90	12.24
4i	H	H	3'-CF_3	13.03	14.00	13.28
4j	H	H	4'-NO_2	8.84	11.79	6.25
4k	H	H	4'-Cl	6.34	8.33	6.15
4l	H	H	4'-OMe	18.45	15.56	14.92
4m	H	H	2'-OMe	11.98	9.88	7.98
5-Fluorouracil				12.92	9.56	5.28

The assays were performed in 96-well plates essentially as described by Mosmann [13].

As shown in Table **1**, although general structure-activity relationship of these compounds was not elucidated from these data, the following points were noteworthy: (1) compound **4d** was the most active one, showing a significant activity toward all the tested cell lines. This is probably due to the presence of CF_3 at 3'-position which is the most elec-

tronegative group. (2) Compounds **4g**, **4h**, **4l**, **4m** with a methoxy group as a substituent on B-ring were found to be inactive against all the tested cell lines, which may imply that B-ring substituted with electron-donating groups would diminish the anticancer activity. (3) Analyses of compounds **4e** and **4f** against HL-60 and HT-29, showed that a Chlorine atom in position 4' had higher activity than a nitro group. (4) Only compounds **4b**, **4d**, **4e** showed better inhibitory activity towards SGC-7901 cell than 5-fluorouracil.

In conclusion, we have synthesized a series of chrysin derivatives. The preliminary biological activity screening tests indicated that **4d** was the most active compound against all the tested cell lines.

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