ISSN 1070-3632, Russian Journal of General Chemistry, 2018, Vol. 88, No. 5, pp. 1036–1041. © Pleiades Publishing, Ltd., 2018.

An Efficient One-Pot Synthesis and Anticancer Activity of 4'-Substituted Flavonoids[1]

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Received April 8, 2018

Abstract—A number of 4'-substituted (R = H, Me, Cl, F) flavone derivatives is synthesized from 2-hydroxyacetophenones using the modified Baker–Venkataraman reaction. Compound [3-(4-fluorobenzoyl)-5-hydroxy-4'-fluoroflavone] was synthesized for the first time with the yield of 12%. Antiproliferative assays indicate that the synthesized flavones with F substituent at the 4' position demonstrate higher activity than the other flavone derivatives, particularly against HeLa and MCF-7 with the IC_{50} 9.5 and 2.7 μM, respectively.

Keywords: flavone derivatives, synthesis, Baker–Venkataraman, antiproliferative activity

DOI: 10.1134/S1070363218050328

INTRODUCTION

Flavones are well known to possess a wide range of biological activities, including antioxidant, anticancer, antiviral, and many more [1–4]. An extensive investigation of flavones activity is limited usually by harsh reactions conditions.

According to the Baker–Venkataraman rearrangement [5], flavonoids are synthesized by a three-step process involving esterification, rearrangement and cyclization (Scheme 1). Later the method has been improved [6, 7]. Heating of 2,6-dihydroxyacetophenone with acyl chloride in the presence of potassium carbonate in dry acetone gave 5-hydroxyflavone and a small amount of the corresponding phenolic ester [8] (Scheme 2). The approach was modified by treating 2-hydroxyacetophenones with 3 equiv. of aroyl chloride in wet K_2CO_3/acetone (1% w/w water) medium, which led to a suitable yield of a flavone and a small amount of 3-aroylflavone (Scheme 3) [9].

In the current study we synthesized a series of 4'-substituted flavonoids by repeating the earlier methods and the modified one-pot synthesis. Anti-cancer potency of the synthesized compounds was evaluated by the MTT assay against two human tumor cell lines (HeLa and MCF-7).

[1] The text was submitted by the authors in English.

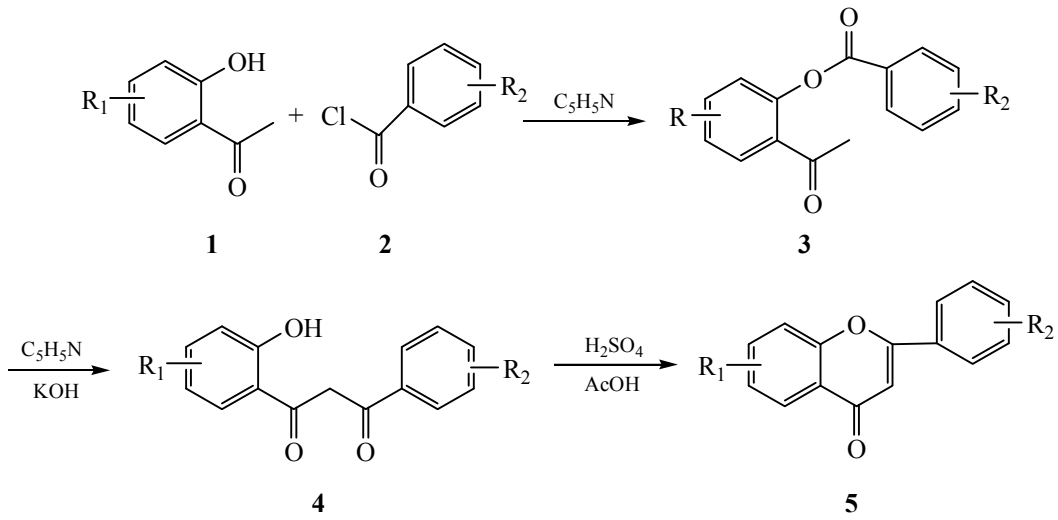
RESULTS AND DISCUSSION

Synthesis of flavone derivatives. Initial approach to the target compounds included heating of 2-hydroxy (**13**), 2,4-dihydroxy (**17**), and 2,5-dihydroxyacetophenones (**20**) with 4-substituted benzoyl chloride in the presence of wet potassium carbonate (1% w/w water) in acetone. This led to β-diketone according to melting points and ^{1}H NMR spectra. Then β-diketone underwent cyclization by treatment with concentrated sulfuric acid in glacial acetic acid. The corresponding flavonoids were produced with high yields.

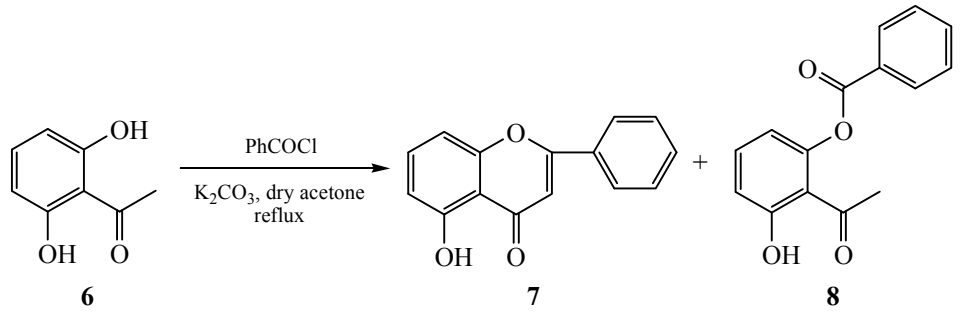
Flavonoids and 3-aroylflavones were synthesized also under the similar conditions in the presence of the additional OH group in the 6-position of 2-hydroxyacetophenone, different combinations of 2,6-dihydroxy acetophenones and aroyl chlorides (Scheme 4). The yield of products was low and their purification involved time consuming column chromatography.

According to the improved method 2-hydroxyacetophenone was treated with 2 equiv. of benzoyl chloride in wet K_2CO_3/acetone (contains 1% w/w water) medium in the presence of 4 equiv. of pyridine (Scheme 5). Under such conditions different combinations of 2-hydroxy, 2,4-dihydroxy, 2,5-dihydroxy, and 2,6-dihydroxyacetophenones with 4-substituted aroyl chlorides **14a–14e** gave flavones directly. The target compound **16a–16e, 19a–19e, 22a–22e**, and

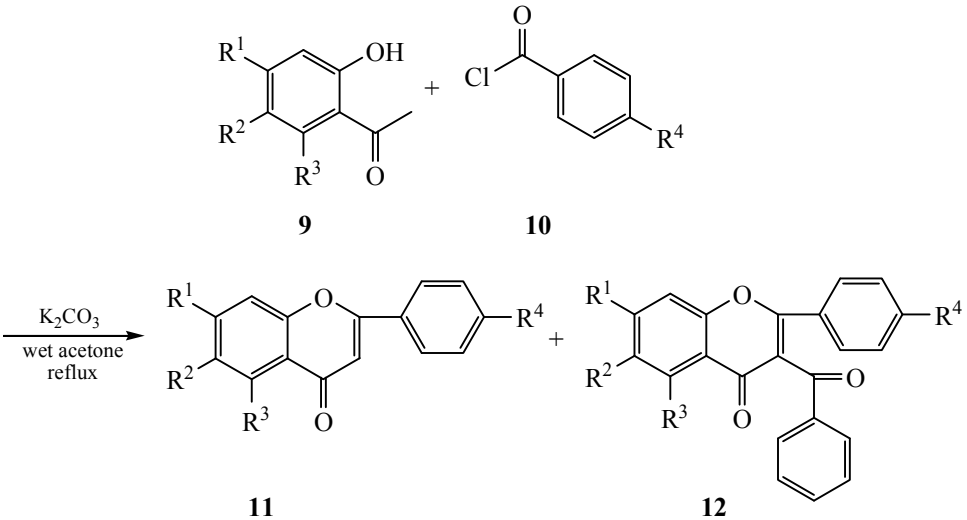
Scheme 1.



Scheme 2.



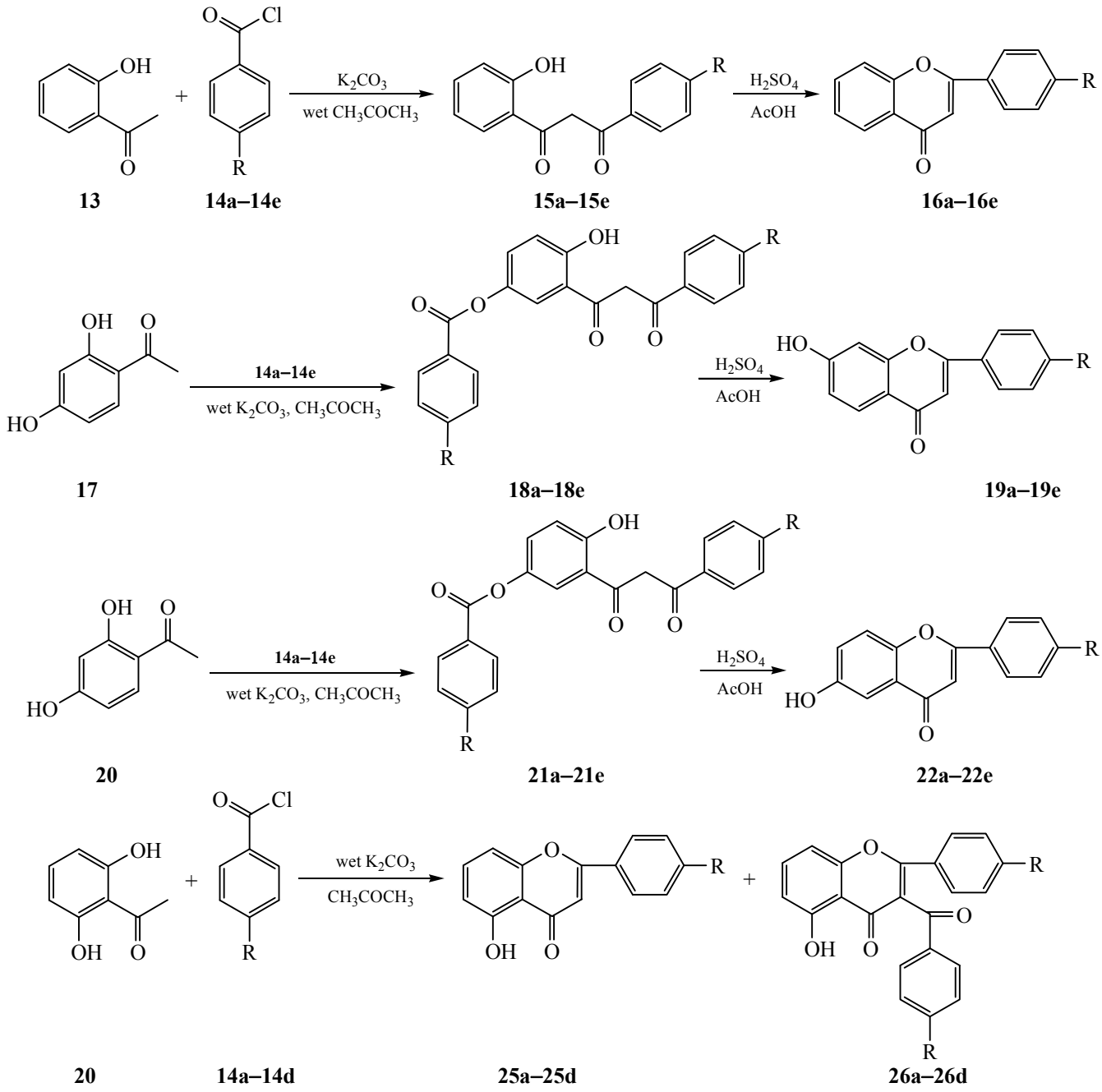
Scheme 3.



25a–25d were purified by recrystallization from acetone without chromatographic separation.

Antiproliferative activity of flavone derivatives. Antiproliferative activity of all synthesized flavone derivatives was evaluated with HeLa (human cervical cancer) and MCF-7 (human breast cancer) *in vitro*. Viable cells were determined using the MTT [3-(4,5-dimethylthiazol–2-yl)–2,5-diphenyltetrazolium bromide] assay after exposure for 72 h (see the table). The com-

Scheme 4.



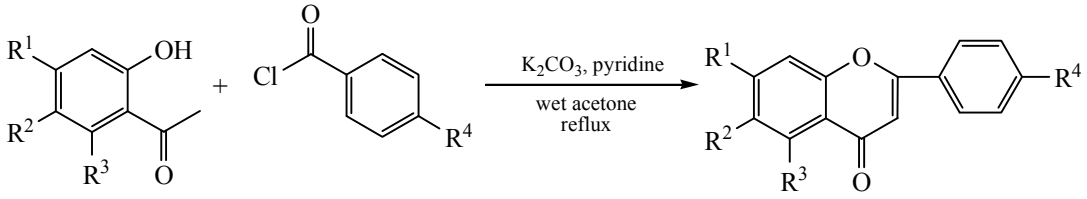
R = H (**a**), F (**b**), Cl (**c**), CH_3 (**d**), OCH_3 (**e**).

pounds **16b, 19b, 22b, 25b**, and **26b** that contained the F substituent at the 4' position demonstrated higher activity than the other products. The compound **26b** showed most promising antiproliferative activity against HeLa and MCF-7 with IC_{50} equal to 9.5 and 2.7 μM, respectively.

EXPERIMENTAL

Melting points were determined on a XT5A melting point instrument. NMR spectra were measured on a VARIAN INOVA (400 MHz) spectrometers using TMS as the internal standard. MS spectra were measured

Scheme 5.



13, 17, 20, 23 **14a–14e** **16a–16e, 19a–19e, 22a–22e, 25a–25d**

$R^1 = R^2 = R^3 = H$ (**13**); $R^1 = OH$, $R^2 = R^3 = H$ (**17**); $R^1 = R^3 = H$, $R^2 = OH$ (**20**); $R^1 = R^2 = H$, $R^3 = OH$ (**23**); $R^4 = H$ (**14a**); F (**14b**); Cl (**14c**); CH_3 (**14d**); OCH_3 (**14e**); $R^1 = R^2 = R^3 = H$, R^4 = H, F, Cl, CH_3, OCH_3 (**16a–16e**); $R^1 = OH$, $R^2 = R^3 = H$, R^4 = H, F, Cl, CH_3, OCH_3 (**19a–19e**); $R^1 = R^3 = H$, $R^2 = OH$, R^4 = H, F, Cl, CH_3, OCH_3 (**22a–22e**); $R^1 = R^2 = H$, $R^3 = OH$, R^4 = H, F, Cl, CH_3 (**25a–25d**).

on a LTQ-XL Electrospray ionization mass spectrometer (American Thermo Finnigan company). Analytical TLC was carried out on Merck precoated aluminum silica gel sheets (Kieselgel 60 F–254). Column chromatography was carried out with silica gel 60 (230-400 mesh) from Merck.

General procedure for preparation of flavones 16a–16e, 19a–19e, 22a–22e, 25a–25d. To a solution of acetophenone (1 mmol) in wet acetone (containing 1% w/w water) (20 mL/mmol) was added potassium carbonate (8 equiv.) and then pyridine (4 equiv.). The solution was stirred at 60°C for 15 min and aroyl chloride (2 equiv.) was slowly added. The reaction mixture was stirred upon refluxing for 24–48 h. After cooling down to room temperature, acetone was evaporated. Acetic acid (10%) was added, the solution was stirred till it stopped bubbling. The residue was filtered off, washed with water (2×50 mL) to make it neutral, vacuum-dried, and then crystallized from acetone (20 mL) to produce pure products.

2-Phenyl-4*H*-chromen-4-one (16a). Yield 83%, mp 96–99°C [9].

4'-Fluoroflavone (16b). Yield 80%, mp 133–135°C [10].

4'-Chloroflavone (16c). Yield 78%, mp 186–187°C [11].

4'-Methylflavone (16d). Yield 70%, mp 227–229°C [10].

4'-Methoxylflavone (16e). Yield 70%, mp 158–160°C [11].

7-Hydroxyflavone (19a). Yield 80%, mp 241–242°C [9].

7-Hydroxy-4'-fluoroflavone (19b). Yield 77%, mp 246–248°C [12].

7-Hydroxy-4'-chloroflavone (19c). Yield 75%, mp 280–282°C [9].

7-Hydroxy-4'-methylflavone (19d). Yield 71%, mp 278–280°C [13].

7-Hydroxy-4'-methoxylflavone (19e). Yield 70%, mp 263–265°C [9].

6-Hydroxyflavone (22a). Yield 79%, mp 232–235°C [9].

6-Hydroxy-4'-fluoroflavone (22b). Yield 75%, mp 261–263°C [12].

6-Hydroxy-4'-chloroflavone (22c). Yield 78%, mp 283–286°C [13].

6-Hydroxy-4'-methylflavone (22d). Yield 72%, mp 282–285°C [13].

6-Hydroxy-4'-methoxylflavone (22e). Yield 70%, mp 254–257°C [13].

5-Hydroxyflavone (25a). Yield 75%, mp 160–162°C [9].

5-Hydroxy-4'-fluoroflavone (25b). Yield 73%, mp 158–160°C [14].

5-Hydroxy-4'-chloroflavone (25c). Yield 72%, mp 192–194°C [11].

5-Hydroxy-4'-methylflavone (25d). Yield 17.2%, mp 184–186°C [15].

General procedure for synthesis of 3-aroylflavones 26a–26d. To a solution of acetophenone (1 mmol) in wet acetone (containing 1% w/w water)

Cytotoxic activity of flavone derivatives against HeLa and MCF-7 cells

Comp. no.	R_1	R_2	R_3	R_4	IC_{50}, μM	
					HeLa	MCF-7
16a	H	H	H	H	>150	>150
16b	H	H	H	F	>100	>100
16c	H	H	H	Cl	>150	>150
16d	H	H	H	CH_3	>150	>150
16e	H	H	H	OCH_3	>150	>150
19a	OH	H	H	H	>150	>150
19b	OH	H	H	F	>100	>100
19c	OH	H	H	Cl	>150	>150
19d	OH	H	H	CH_3	>150	>150
19e	OH	H	H	OCH_3	>150	>150
22a	H	OH	H	H	>150	>150
22b	H	OH	H	F	>100	>100
22c	H	OH	H	Cl	>150	>150
22d	H	OH	H	CH_3	>150	>150
22e	H	OH	H	OCH_3	>150	>150
25a	H	H	OH	H	>150	>150
25b	H	H	OH	F	>100	>100
25c	H	H	OH	Cl	>150	>150
25d	H	H	OH	CH_3	>150	>150
26a	–	–	–	R=H	>150	>150
26b	–	–	–	R=F	9.5±3.82[a]	2.7±0.81[a]
26c	–	–	–	R=Cl	>150	>150
26d	–	–	–	$R=CH_3$	>150	>150

[a] The data are mean ±S.D. of three independent measurements.

(20 mL/mmol) was added potassium carbonate (8 equiv.). The solution was stirred at 60°C for 15 min, then aroyl chloride (3 equiv) was slowly added. The mixture was stirred upon refluxing for 24–48 h. After cooling down to room temperature, acetone was evaporated and 10% acetic acid was added. The solution was stirred until it stopped bubbling. The solid residue was filtered off, washed with water (2×50 mL) to make it neutral and dried in vacuum. 3-Aroylflavones **26a–26d** were purified by column chromatography using petroleum ether : ethyl acetate (10 : 1) as an eluent.

3-Benzoyl-5-hydroxyflavone (26a). Yield 15%, mp 164–166°C [9].

3-(4-Fluorobenzoyl)-5-hydroxy-4'-fluoroflavone (26b). Yield 12%, mp 133–136°C. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 6.88 d (J = 8.3 Hz, 1H, 6-H), 7.13–7.01 m (5H, Ar-H), 7.64 m (3H, Ar-H), 7.90–8.00 m (2H, 2',6'-H), 12.09 s (1H, OH). ^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 102.69, 115.32, 115.81, 121.58, 125.27, 126.89, 128.52, 130.31, 130.73, 131.18, 132.02, 132.20, 135.66, 157.72, 160.60, 163.57, 174.86, 192.73.

$C_{22}H_{12}O_4F_2$. Found, %: 379.0768. Calculated, %: 379.0776. HRMS $[M + H]^+$.

3-(4-Chlorobenzoyl)-5-hydroxy-4'-chloroflavone (26c). Yield 12%, mp 164–166°C [16].

3-(4-Methylbenzoyl)-5-hydroxy-4'-methylflavone (26d). Yield 15%, mp 223–225°C [15].

Anticancer activity. The cancerous cell lines were maintained in RPMI medium supplemented with 10% foetal bovine serum in a CO_2 incubator. Cytotoxicity of the compounds was determined according to the MTT assay. Two different kinds of cancerous cell lines, HeLa (human cervical cancer) and MCF-7 (human breast cancer), were plated in a 96-well plate at the density of 10000 cells per well. After 24 h, the cells were treated with various concentrations of flavones. The cells were further incubated for 72 h. Cytotoxicity was measured upon adding 5 mg/mL of MTT to each well and incubation for another 3 h. The absorbance was determined at 490 nm. The cell death was calculated as follows: Cell death = (control absorbance-test absorbance)/control absorbance×100%.

CONCLUSIONS

Flavone and 3-aroylflavone were produced efficiently in a one-step process by treating 2,6-dihydroxyacetophenone with 2 equiv. of aroyl chloride in a wet K_2CO_3/acetone system. The approach was successfully applied to the synthesis of 23 flavones containing various substituents, one of which was synthesized for the first time. The *in-vitro* tests of the synthesized flavone derivatives against two human tumour cell lines (HeLa and MCF-7) using the MTT assay indicated high antiproliferative activity of flavones containing the F substituent in 4' position. The compound **26b** may be a potent lead compound for further optimization as an anticancer agent.

ACKNOWLEDGEMENTS

The present work was supported by the National Natural Science Foundation of China (no. 20872118, 30070905) and the Natural Science Foundation of the Science and Technology Department of Shaanxi Province (2014JM4097).

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