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Concise Synthesis and Cellular Evaluation of 3'-Formyl-4',6'-dihydroxy-2'-methoxy-5'-methylchalcone (FMC) and Its Analogues

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CONCISE SYNTHESIS AND CELLULAR EVALUATION OF 3'-FORMYL-4',6'-DIHYDROXY-2'-METHOXY-5'-METHYLCHALCONE (FMC) AND ITS ANALOGUES

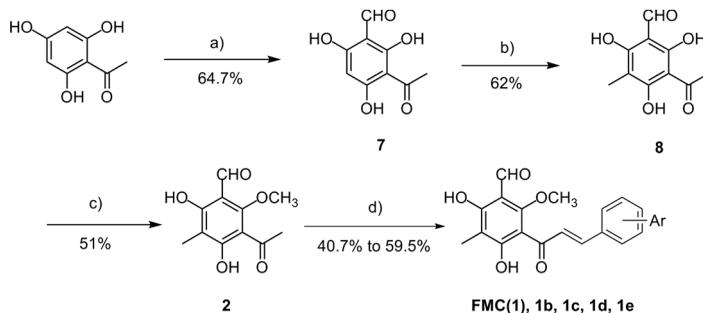
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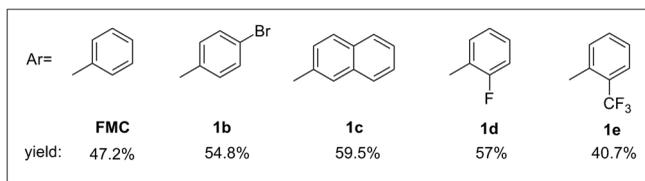
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³School of Pharmacy, Anhui Medical University, Hefei, China

GRAPHICAL ABSTRACT



a) DMF, POCl₃, rt, 1 h; b) Mel, KOH, reflux, 2 h; c) TMSCHN₂, -40°C, 6 h; d) ArCHO, 30% KOH, rt, 36 h;



Abstract 3'-Formyl-4',6'-dihydroxy-2'-methoxy-5'-methylchalcone (FMC) was a natural product isolated from *Cleistocalyx operculatus*. A four-step synthetic strategy toward FMC and its four analogues (**1b–1e**) was first developed. All compounds were synthesized from commercially available 2,4,6-trihydroxyacetophenone; formylation at 3' position under Vilsmeier–Haack conditions was followed by the introduction of a methyl group at 5' position. The key step of selective methylation at 2' position was achieved by trimethylsilyldiazomethane (TMSCHN₂). Then substituted aromatic aldehydes were condensed

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through Claisen–Schmidt reaction in the presence of potassium hydroxide. All structures were confirmed by ^1H NMR, ^{13}C NMR, and high-resolution mass spectra. FMC and analogues were screened for their antiproliferative activity.

Keywords Antiproliferative activity; chalcones; FMC; synthesis

INTRODUCTION

Chalcones, also known as chalconoids, are polyketide natural products containing an aromatic ketone and an enone moiety, which represent the central core for a variety of important biological compounds. The closure of chalcones leads to the formation of the flavonoids, which are substances in the plant secondary metabolism with an array of biological activities. Chalcone and its derivatives have become one of the most pursued classes of natural product in recent years for their various biological activities, including antidiabetic,^[1] anticancer,^[2–5] anti-inflammatory,^[4] antioxidant,^[4,5] antifungal,^[5,7] antimalarial,^[8] and anti-HIV^[9,10] properties.

Cleistocalyx operculatus (Roxb.) Merr. et Perry (Myrtaceae), distributed in southern China, has buds widely used in folk medicines and tonic drinks.^[11] In 2004, Ye et al.^[12] isolated two new natural products, 3'-formyl-4',6'-dihydroxy-2'-methoxy-5'-methylchalcone (FMC) and (2S)-8-formyl-5-hydroxy-7-methoxy-6-methylflavanone (FMF), from the buds of *Cleistocalyx operculatus* (Fig. 1). Biological evaluation of FMC (compound **1**) showed good antioxidant activity and antiproliferative activity against different cancer cell lines.^[13] To date, neither synthesis nor structure–activity relationship (SAR) studies have been done on FMC (**1**) and its pharmacological profile and therapeutic potentials remain largely unknown. Based on previous studies on chalcones and flavonoids,^[14,15] modification of the terminal phenyl ring could enhance the cytotoxicity. We proposed that its anticancer activity can be possibly improved with modifications on the ring B. Therefore, as part of our studies on the discovery of anticancer chalcone derivatives, we initiated the synthesis and biological evaluation of FMC (**1**) and its analogues bearing different substituents at chalcone terminal. These aromatic aldehydes with substituents at different positions included 4-bromobenzaldehyde and 2-fluorobenzaldehyde and with different steric hindrance included benzaldehyde, 2-(trifluoromethyl)benzaldehyde, and 2-naphthaldehyde.

FMC (**1**) has a common chalcone skeleton with a unique fully substituted ring A: a methoxyl group at 2' position, dihydroxy groups at 4' and 6' position, a formyl group at 3' position, and methyl group at 5' position. The preliminary retrosynthesis

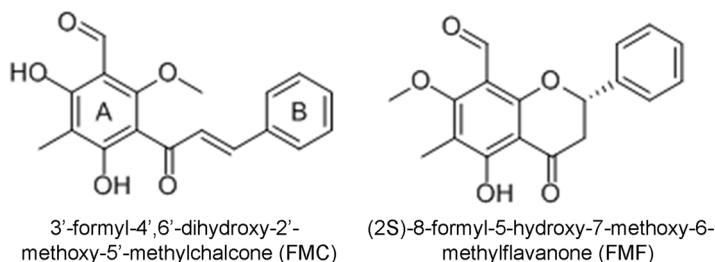


Figure 1. Structures of FMC and FMF.

analysis showed that the selective methylation of the hydroxy group at 2' position has been the most challenging step (limiting synthetic factor). Accordingly, our group recently established a synthetic methodology that was able to selectively methylate the hydroxyl group of 3-acetyl-2,4,6-trihydroxy-5-methylbenzaldehyde (**8**) in good yield.^[16,17] Based on this finding, we designed and accomplished a four-step concise synthesis of **1** and its derivatives. In general, starting from commercially available 2,4,6-trihydroxyacetophenone, formylation at 3' position under Vilsmeier–Haack condition was followed by the introduction of methyl group at 5' position. The key step of selective methylation at 2' position was achieved by trimethylsilyldiazomethane (TMSCHN₂) and afforded the common intermediate (**2**) with good regioselectivity and yield (51%) after optimization. Then various substituted aromatic aldehydes were condensed through Claisen–Schmidt reaction in the presence of potassium hydroxide. As the first reported synthesis of FMC and its analogues, our synthesis enabled efficient and concise access to the key intermediate and made the parallel synthesis of its analogues feasible, which could potentially enhance the development of this compound class into clinical use.

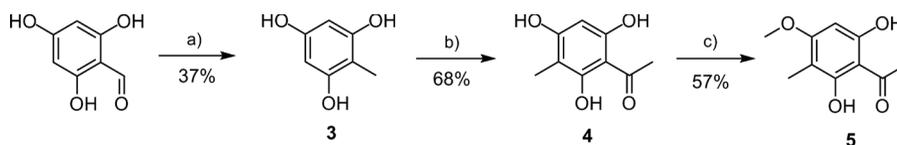
RESULTS AND DISCUSSION

Chemistry: Synthesis of FMC (**1**) and Its Analogues

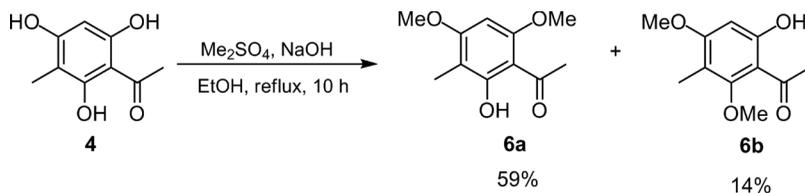
Claisen–Schmidt condensation between benzaldehyde and acetophenone was one of the most popular methods for the synthesis of chalcones. Given the significant amount of substituted benzaldehydes that are commercially available, this method was applied in our synthesis of FMC (**1**) and its derivatives. The synthesis of the key intermediate **2**, 3-acetyl-4,6-dihydroxy-2-methoxy-5-methyl benzaldehyde, was investigated.

In our initial synthesis, starting from 2,4,6-trihydroxybenzaldehyde, the formyl group was reduced to methyl group by sodium cyanoborohydride, and then the acetyl group was introduced to provide compound **4** with a decent overall yield (about 68%) (Scheme 1). The first effort to synthesis of the key intermediate, compound **2**, was to directly methylate the hydroxyl group at 6' position with an adjacent acetyl as proximal directing group. Unfortunately, treatment of compound **4** with methyl iodide and bases (potassium hydroxide/potassium carbonate) mostly afforded compound **5**, with the methylation occurring at 4' position instead of 6' position.

Additional efforts to methylate compound **4** with dimethyl sulfate^[18] in the presence of sodium hydroxide led to two dimethoxy positional isomers, compounds **6a** and **6b**, in 59% and 14% yields respectively. However, selective removal of the



Scheme 1. Methylation of compound **3**. Reagents and conditions: (a) NaBH₃CN, HCl, rt, 20 h; (b) BF₃·Et₂O, HOAc·Ac₂O; and (c) CH₃I, KOH, or CH₃I, K₂CO₃, reflux, 4 h.

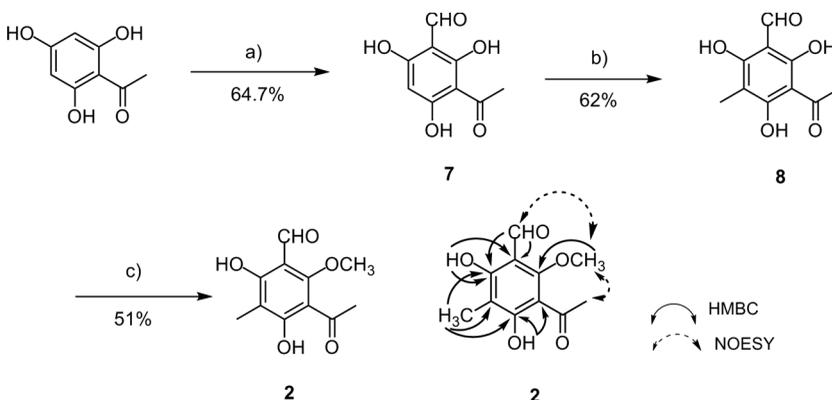


Scheme 2. Dimethylation of compound **4**.

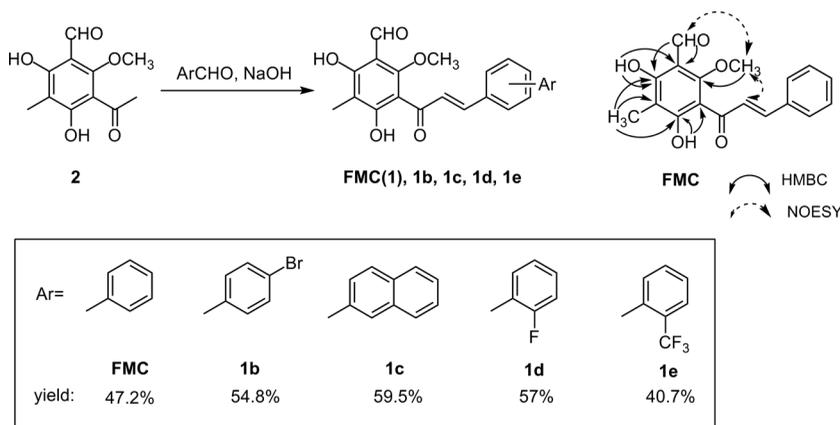
4-hydroxyl group of **6a** by sodium ethanethiolate^[19] failed to afford the desired compound **2** (Scheme 2).

The methylation reaction can be directed by the adjacent proximal groups, which can facilitate the reaction by guiding the methyl group to the site of reaction. Additional carbonyl groups may stabilize the intermediate anion formed with the methylating agents at the desired position, and so an alternative strategy was sought to introduce the formyl group at an adjacent place before methylation. As shown in Scheme 3, compound **7**, 3-acetyl-2,4,6-trihydroxybenzaldehyde, was prepared in 64.7% yield from the commercially available 2,4,6-trihydroxyacetophenone by Vilsmeier–Haack reaction.^[16] Treatment of compound **7** with 3 equivalents of methyl iodide^[16] in the presence of potassium hydroxide afforded compound **8** in 62% yield.

With the installation of the formyl group, better selectivity for the monomethylation at 6' position was observed when treating compound **8** at room temperature with equivalent trimethylsilyldiazomethane (TMSCHN₂, 2 M, solution in Et₂O), an alternative to diazomethane. The desired monoalkylated product, compound **8**, was obtained in 8% yield accompanied with 21% of dialkylated species. This observation was quite exciting; the selective monomethylation was facilitated by the adjacent acetyl group when TMSCHN₂ was used as methylating agent.^[17] Further optimizations were conducted toward the stoichiometric amount of TMSCHN₂ and reaction temperature. When treating compound **8** with 2 M TMSCHN₂ at –40 °C, compound **2** was obtained in 51% yield and 26% of starting



Scheme 3. Synthesis of compound **2**. Reagents and conditions: (a) DMF, POCl₃, rt, 1 h; (b) MeI, KOH, reflux, 2 h; and (c) TMSCHN₂, –40 °C, 6 h.



Scheme 4. Synthesis of compound FMC and its derivative.

materials were recovered, which could be reused after purification. The structure of compound **2** was confirmed by two-dimensional (2D) NMR analysis, nuclear Overhauser effect spectrometry (NOESY) and heteronuclear multiple bond correlation (HMBC), as shown in Scheme 4. The NOE cross peaks between the formyl proton and ene proton were observed.

As shown in Scheme 4, compound **1** and four derivatives were finally prepared via Claisen–Schmidt reaction. Treatment of compound **2** with the corresponding substituted benzaldehydes in the presence of 30% KOH aqueous solution afforded the desired compounds in good yields (40.7–59.5%). The structure of **1** was confirmed by MS, NMR, HMBC, and NOESY studies, and the results are consistent with prior findings in the literature.^[12]

Biological Evaluation

FMC (**1**) has been reported to exhibit cytotoxicity on five human tumor cell lines with IC_{50} values ranged from $79.8 \pm 2.6 \mu\text{M}$ to $165.7 \pm 6.1 \mu\text{M}$,^[13] showing an enhanced inhibitory effect compared to several potential antitumor flavonoids that isolated from Chinese herbal medicines.^[20–22] All synthesized FMC (**1**) and analogues **1b–1e** were evaluated for their in vitro anticancer activity against a panel of human cancer cell lines, including A549 lung carcinoma cells, DU-145 prostate cancer cells, HCT-8 colon adenocarcinoma cells, HL-60 acute leukemia cells, GNM lymph node metastasis of cancer cells, and human endothelial HUVEC cells, a normal cell useful for assessing anti-angiogenic potential. As the results show in Table S1 (available online in the Supporting Information), compound **1** and its analogues **1c**, **1d**, and **1e** showed no overt cell killing or growth inhibition with IC_{50} values greater than $50 \mu\text{M}$, which was consistent with prior results in the literature ($79.8 \pm 2.6 \mu\text{M}$ to $165.7 \pm 6.1 \mu\text{M}$). The analogue **1b** with a bromine at 4' position on ring B exhibited the moderate activity against HCT-8, A549, GNM, DU-145, HL-60, and HUVEC cancer cell lines with IC_{50} values of 17.9 ± 0.6 , 15.5 ± 0.8 , 18.1 ± 1.0 , 20.2 ± 1.6 , 10.8 ± 0.9 , and $16.3 \pm 1.2 \mu\text{M}$ respectively, which were up to 10 times more potent than the parent compound **1**.

CONCLUSION

In summary, the first total synthesis of FMC (**1**) was accomplished. A facile synthetic route was reported and all compounds were synthesized from 2,4,6-trihydroxyacetophenone through a four-step sequence, which could be used to rapidly produce analogues for SAR studies toward to activity and pharmacological properties. The analogue with bromide at C-4 on ring B was optimal for tumor cell growth inhibition with IC_{50} values ranging from 10.8 ± 0.9 to $20.2 \pm 1.6 \mu\text{M}$, showing a 10-fold improved activity compared to the lead compound **1**. Further modifications on this compound series and investigations of their mechanism of action are undergoing and results will be reported in due course.

EXPERIMENTAL

All melting points were measured on a YuHua X-4 melting-point apparatus without correction. Progress of the reactions was monitored with thin-layer chromatography (TLC) using aluminium sheets with silica gel 60 F254 from Merck. The spectra of ^1H NMR and ^{13}C NMR were recorded in solution in CDCl_3 or dimethylsulfoxide (DMSO-d_6) on a Bruker ARX 500-MHz spectrometer. The chemical shifts are expressed in parts per million (ppm) using tetramethylsilane (TMS) as internal reference. HRMS was recorded on Bruker microTOF-Q II mass spectrometers. 4-Bromobenzaldehyde, 2-naphthaldehyde, 2-fluorobenzaldehyde, and 2-trifluoromethylbenzaldehyde were purchased from Acros Organics (New Jersey, USA). Other chemicals were purchased from SCRC (Beijing, China). All reagents and solvents were analytical grade.

All experiments, which were sensitive to moisture or air, were carried out under N_2 atmosphere. Commercial reagents were used as received without further purification unless otherwise noted. EtOAc was washed with an equal volume of 5% Na_2CO_3 and dried over CaCl_2 .

3-Acetyl-2,4,6-trihydroxybenzaldehyde (**7**)

DMF (0.4 mL, 5 mmol) and phosphoryl chloride (0.5 mL, 5.5 mmol) to the solution of 2,4,6-trihydroxyacetophenone (841 mg, 5 mmol) and EtOAc (10 mL) in an ice bath. Then the reaction mixture was further stirred for 60 min at room temperature. Ice water was added to the reaction mixture and extracted with EtOAc. The EtOAc layer was washed with brine solution, dried over Na_2SO_4 , and concentrated to afford the crude product. The residue was purified by column chromatography on silica gel with petroleum ether–EtOAc (5:1 to 7:3 v/v) as an eluent to obtain **7** (635 mg, 64.7%) as colorless solid, which was used directly in the next reaction without recrystallization. Mp: 162–163 °C. ^1H NMR (500 MHz, DMSO-d_6) δ : 14.84 (1H, s, chelated-OH-2), 13.65 (1H, s, chelated-OH-4), 12.22 (1H, s, chelated-OH-6), 9.99 (1H, s, CHO), 5.88 (1H, s, Ph-5), 2.52 [3H, s, $\text{C}(\text{O})\text{CH}_3$]. ^{13}C NMR (125 MHz, DMSO-d_6) δ : 203.8 (CO), 192.3 (CHO), 171.2 (C-6), 170.3 (C-2), 168.1 (C-4), 104.3 (C-5), 103.9 (C-3), 94.9 (C-1), 32.8 [$\text{C}(\text{O})\text{CH}_3$]. HRMS m/z : calcd. for $\text{C}_9\text{H}_7\text{O}_5$: 195.0293 [$\text{M} - \text{H}$] $^+$; found: 195.0306.

3-Acetyl-2,4,6-trihydroxy-5-methylbenzaldehyde (**8**)

KOH (225 mg, 4 mmol) was added to the solution of **7** (392 mg, 2 mmol) in methanol (10 mL) and stirred in an ice bath. Then methyl iodide (0.5 mL) was slowly added to the mixture. The reaction mixture was refluxed for 2 h. Solvent was evaporated and the residue was purified by column chromatography on silica gel with petroleum ether–EtOAc (5:1 to 2:1 v/v) to obtain **8** (261 mg, 62%) as colorless solid, which was used directly in the next reaction without recrystallization. Mp: 153–154 °C. ¹H NMR (500 MHz, CDCl₃) δ: 15.23 (1H, s, chelated-OH-2), 14.25 (1H, s, chelated-OH-4), 10.13 (1H, s, CHO), 5.77 (1H, s, chelated-OH-6), 2.73 [3H, s, C(O)CH₃], 2.04 (3H, s, CH₃-5). ¹³C NMR (125 MHz, DMSO-d₆) δ: 204.0 (CO), 193.1 (CHO), 170.0 (C-6), 167.9 (C-2), 165.3 (C-4), 104.7 (C-5), 103.9 (C-3), 103.0 (C-1), 33.0 [C(O)CH₃], 7.6 (5-CH₃). HRMS *m/z*: calcd for C₁₀H₉O₅: 209.0450 [M – H]⁺; found: 209.0462.

3-Acetyl-4,6-dihydroxy-2-methoxy-5-methylbenzaldehyde (**2**)

Compound **8** (2.1 g, 10 mmol) was dissolved in anhydrous EtOAc–MeOH (5:1, 30 mL), TMSCHN₂ (10 mL, 20 mmol, 2 M solution in Et₂O) was added slowly at –40 °C under N₂, and the mixture was stirred for 4 h. Then additional TMSCHN₂ (5 mL, 10 mmol, 2 M solution in Et₂O) was added and stirred at –40 °C for another 2 h. The reaction mixture was quenched with acetic acid with stirring at room temperature. The solvents were removed under reduced pressure, and the residue was purified by silica gel using petroleum ether–EtOAc (20:1 to 5:1 v/v) to obtain **2** (1.14 g, 51%) as colorless prisms. Mp: 106–107 °C. ¹H NMR (500 MHz, CDCl₃) δ: 14.07 (1H, s, chelated-OH-6), 12.60 (1H, s, chelated-OH-4), 10.10 (1H, s, CHO), 3.99 (3H, s, OCH₃), 2.75 [3H, s, C(O)CH₃], 2.08 (3H, s, CH₃-5). ¹³C NMR (125 MHz, CDCl₃) δ: 203.2 (CO), 192.4 (CHO), 168.8 (C-6), 166.0 (C-2), 165.9 (C-4), 108.1 (C-5), 100.1 (C-3), 90.8 (C-1), 66.7 (OCH₃), 31.4 [C(O)CH₃], 6.7 (5-CH₃). HRMS *m/z*: calcd. for C₁₁H₁₁O₅: 223.0606 [M – H]⁺; found: 223.0623.

General Procedure for the Claisen–Schmidt Reactions

A solution of **2** (2 mmol) in EtOH (5 mL) and 30% KOH (5 mL) in water containing an appropriate aldehyde (3 mmol) was stirred at room temperature for 36 h. After the reaction was complete by TLC analysis, the mixture was poured into ice-cold 1 N HCl and then extracted with CH₂Cl₂. The extract was washed with brine, dried over Na₂SO₄, and concentrated under vacuum. The residue was chromatographed on silica gel with petroleum ether–EtOAc (20:1 to 3:1 v/v) to obtain the target compounds **1**, **1b**, **1c**, **1d**, and **1e**.

Cytotoxic Activity Assay

The cytotoxic activity assay was performed.^[3] All cells stock cultures were grown in 96-well microtiter plates at densities of 5 × 10³ cells per well with test compounds added from DMSO-diluted stock. After 36 h in culture, attached cells were fixed with cold 50% trichloroacetic acid and then stained with 0.4% sulforhodamine

B (SRB). The absorbency at 562 nm was measured using a microplate reader after solubilizing the bound dye. IC₅₀ values were calculated from five different concentrations and data were reported as mean \pm standard deviation (SD). The following human tumor cell lines were used in the assay: A549 (human lung carcinoma), DU145 (human prostate cancer cell), HCT-8 (colon adenocarcinoma), HL-60 (human leukemia cell), GNM (lymph node metastasis of cancer cell), and HUVEC (human endothelial cell). All cell lines were purchased from the American Type Culture Collection (ATCC) and were cultured at 37 °C in a humidified incubator with 5% CO₂. Culture medium was RPMI-1640 supplemented with 25 nM HEPES, 0.25% sodium bicarbonate, and 10% fetal bovine serum.

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SUPPORTING INFORMATION

Supplemental data for this article can be accessed on the publisher's website.

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