SUPPORTING INFORMATION

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

Xiang Zhuo^{1,2}, **Li En-Zhen**², **Liang Hai**^{2,3}, **Guo Hong-Ju**², **Shi Ning**², **Zhang Xue-Hui**²,

Qian Qi-Fang² and **Wu Jiu-Hong**^{* 1,2}

¹ *School of Pharmacy, Fourth Military Medical University; Xi'an 710032, P. R. China;*

² *Department of Pharmacy, 306 Hospital of PLA; Beijing 100101, P. R. China;*

³ *College of Pharmacy, Anhui Medical University; Hefei 230032, P. R. China*

Email: 321xz123@163.com

EXPERIMENTAL

Synthesis

2,4,6-Trihydroxytoluene (3)

To a solution of 2,4,6-trihydroxy benzaldehyde (1.54 g, 10 mmol) in THF (30 ml) and sodium cyanoborohydride (1.9 g, 30 mmol). The solution was stirred for 20 h (keeping the pH 4.0) by adding 1N HCl. The reaction mixture was extracted with EtOAc and were washed with brine, dried over Na₂SO₄ and concentrated under vacuum. The residue was chromatographed on silica gel with petroleum ether-EtOAc (3:1 to 1:1 v/v) to provide **3** (520 mg, 37%), colorless prisms. Compound was characterized by comparing its data with literature values.^[1] Mp: 212-213 °C. ¹H-NMR

(500 MHz, DMSO- d_6) δ : 5.89 (2H, s, Ph-3,5), 2.01 (3H, s, CH₃). ¹³C-NMR (125 MHz, DMSO- d_6) δ : 157.9, 156.8, 103.8, 95.7, and 8.3. HRMS m/z : calcd for C₇H₇O₃: 139.0395 [M – H]⁺, found: 139.0408.

2,4,6-Trihydroxy-3-methylacetophenone (4)

To a solution of trihydroxytoluene **3** (1.54g, 10 mmol) in HOAc (30.0 mL) and Ac₂O (3.0 mL) at room temperature, then BF₃·OEt₂ (1.7 mL, 13 mmol) was added. The mixture was heated at 100°C for 5 h. The mixture was adjusted to pH 4 by 2 N NaOH at 0 °C then the mixture was extracted with EtOAc and 5% MeOH/EtOAc. The combined organic phases were concentrated. The residue was dissolved in MeOH (30 mL) and 2 N NaOH (30 mL). After stirring at room temperature for 12 h, the mixture was adjusted to pH 4 by 3 N HCl at 0 °C, then the volatile solvent was removed in vacuo. The residue was extracted with EtOAc, washed with water, brine, dried over Na₂SO₄ and concentrated under vacuum. The residue was chromatographed on silica gel with petroleum ether-EtOAc (3:1 to 1:2 v/v) to provide **4** (1.24 g, 68%), colorless prisms. Compound was characterized by comparing its data with literature values.^[2] Mp: 209-210°C. ¹H-NMR (500 MHz, DMSO- d_6) δ : 6.05 (1H, s, Ph-5), 2.67 (3H, s, COCH₃), 1.88 (3H, s, CH₃). HRMS m/z : calcd for C₉H₉O₄: 181.0501 [M – H]⁺, found: 181.0513.

2,6-Dihydroxy-4-methoxy-3-methylacetophenone (5)

Compound **4** (1.54g, 10 mmol) was dissolved in anhydrous acetone (50 mL),

then KOH or K₂CO₃ (30 mmol) was added at room temperature. Then methyl iodide (1 mL) was slowly added to the mixture. The reaction mixture was refluxed for 4 h. The reaction mixture was adjusted to pH 4 by 1 N HCl at 0 °C. The volatile solvent was removed in vacuo. The residue was extracted with EtOAc and were washed with brine, dried over Na₂SO₄ and concentrated under vacuum. The residue was purified by silica gel using petroleum ether-EtOAc (20:1 to 5:1 v/v) to obtain **5** (878 mg, 57%), colorless prisms. Compound was characterized by comparing its data with literature values.^[3] Mp: 186-187 °C. ¹H-NMR (500 MHz, DMSO-d₆) δ: 6.01 (1H, s, Ph-5), 3.84 (3H, s, OCH₃-4), 2.66 (3H, s, COCH₃), 1.94 (3H, s, CH₃-3). HRMS m/z: calcd for C₁₀H₁₁O₄: 195.0657 [M – H]⁺, found: 195.0675.

4,6-Dimethoxy-2-hydroxy-3-methylacetophenone (6a) and

2,4-dimethoxy-6-hydroxy-3-methylacetophenone (6b)

Compound **4** (1.82g, 10 mmol) was dissolved in anhydrous ethanol (50 mL), then NaOH (1.66 g, 12 mmol) was added at room temperature. Then dimethyl sulfate (1.5 mL, 12 mmol) was slowly added to the mixture. The reaction mixture was refluxed for 10 h. Then most volatile solvent was removed in vacuo. The residue was extracted with EtOAc and washed with water, brine, dried over Na₂SO₄ and concentrated under vacuum. The residue was purified by silica gel using petroleum ether-EtOAc (5:1 to 1:1 v/v) to obtain **6a** (1.24 g, 59%) and **6b** (294 mg, 14%). Compound was characterized by comparing its data with literature values.^[3] **6a**: colorless prisms, Mp:143-144 °C. ¹H-NMR (500 MHz, DMSO-d₆) δ: 13.86 (1H, s,

OH), 6.20 (1H, s, Ph-5), 3.91 and 3.90 (each s, 3H, OCH₃-4,6), 2.56 (3H, s, COCH₃), 1.88 (3H, s, CH₃-3). HRMS m/z: calcd for C₁₁H₁₃O₄: 209.0814 [M – H]⁺, found: 209.0832. **6b**: colorless oil, ¹H-NMR (500 MHz, DMSO-d₆) δ: 12.98 (1H, s, OH), 6.33 (1H, s, Ph-5), 3.81 and 3.74 (each s, 3H, OCH₃-2,4), 2.59 (3H, s, COCH₃), 1.96 (3H, s, CH₃-3). HRMS m/z: calcd for C₁₁H₁₃O₄: 209.0814 [M – H]⁺, found: 209.0821.

General procedures 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, 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 compound.

3'-Formyl-4',6'-dihydroxy-2'-methoxy-5'-methylchalcone (FMC) (1). 47.2% yield. Compound was characterized by comparing its data with literature values.^[4] Mp: 123-124°C. ¹H-NMR (500 MHz, CDCl₃) δ: 14.01 (1H, s, chelated-OH-6'), 12.67 (1H, s, chelated-OH-4'), 10.17 (1H, s, CHO), 7.96 (1H, d, *J* = 16.0 Hz, H-β), 7.87 (1H, d, *J* = 16.0 Hz, H-α), 7.69 (2H, m, H-2, 6), 7.47 (3H, m, H-3, 4, 5), 3.92 (3H, s, OCH₃), 2.12 (3H, s, CH₃). ¹³C-NMR (125 MHz, CDCl₃) δ: 192.9[C(O)], 192.6 (CHO), 169.1 (C-6'), 167.2 (C-2'), 166.7 (C-4'), 145.0 (C-β), 134.7 (C-1), 131.0 (C-4), 129.1 (C-3,

5), 128.7 (C-2, 6), 125.4 (C- α), 108.6 (C-5'), 103.4 (C-3'), 100.14 (C-1'), 66.3 (OCH₃), 6.8 (CH₃). HRMS m/z: calcd for C₁₈H₁₅O₅: 311.0919[M - H]⁺, found: 311.0934.

3'-Formyl-4',6'-dihydroxy-2'-methoxy-5'-methyl-4-bromo-chalcone (1b).

54.8% yield. Yellow needles, Mp: 116-117°C. ¹H-NMR (500 MHz, CDCl₃) δ : 13.91 (1H, s, chelated-OH-6'), 12.66 (1H, s, chelated-OH-4'), 10.15 (1H, s, CHO), 7.87 (2H, s, H- α , β), 7.61 (2H, m, H-3,5), 7.55 (2H, m, H-2, 6), 3.90 (3H, s, OCH₃), 2.1 (3H, s, CH₃). ¹³C-NMR (125 MHz, CDCl₃) δ : 193.7 (CO), 192.3 (CHO), 166.5 (C-6'), 166.0 (C-2'), 164.4 (C-4'), 138.2 (C- β), 136.5 (C-1), 132.7 (C-3), 132.3 (C-5), 132.1 (C-6), 131.4 (C-2), 131.1 (C-4), 128.8 (C- α), 124.6 (C-5'), 110.2 (C-3'), 108.2 (C-1'), 65.5 (OCH₃), 7.0 (CH₃). HRMS m/z: calcd for C₁₈H₁₆BrO₅: 391.0181 [M + H]⁺, found: 391.0145.

3'-Formyl-4',6'-dihydroxy-2'-methoxy-5'-methyl-3,4-benzchalcone (1c).

59.5% yield. Yellow needles, Mp: 106-108°C. ¹H-NMR (500 MHz, CDCl₃) δ : 14.57 (1H, s, chelated-OH-6'), 12.68 (1H, s, chelated-OH-4'), 10.06 (1H, s, CHO), 8.16 (1H, d, J = 16.0 Hz, H- β), 8.02 (1H, d, J = 16.0 Hz, H- α), 7.93-7.83 (4H, m, Ar-3, 4, 7, 8), 7.58-7.57 (3H, m, Ar-2, 5,6), 3.94 (3H, s, OCH₃), 2.13 (3H, s, CH₃). ¹³C-NMR (125 MHz, CDCl₃) δ : 192.6 (CO), 191.8 (CHO), 169.1 (C-6'), 167.2 (C-2'), 166.1 (C-4'), 145.1 (C- β), 133.4 (C-8a), 133.1 (C-1), 131.1 (C-4a), 130.9 (C-4), 129.0 (C-7), 128.2 (C-3), 127.9 (C-8), 126.9 (C- α), 126.7 (C-5), 125.6 (C-6), 123.6 (C-2), 109.4 (C-5'),

108.6 (C-3'), 91.6 (C-1'), 66.3 (OCH₃), 7.3 (CH₃). HRMS m/z: calcd for C₂₂H₁₇O₅: 361.1076[M – H]⁺, found: 361.1099.

3'-Formyl-4',6'-dihydroxy-2'-methoxy-5'-methyl-2-fluorochalcone (1d). 57% yield. Yellow needles, Mp: 103-104 °C. ¹H-NMR (500 MHz, CDCl₃) δ: 14.01 (1H, s, chelated-OH-6'), 12.67 (1H, s, chelated-OH-4'), 10.16 (1H, s, CHO), 8.05 (1H, d, *J* = 16.0 Hz, H-β), 8.01 (1H, d, *J* = 16.0 Hz, H-α), 7.68 (1H, m, H-4), 7.45 (1H, m, H-6), 7.26-7.17 (2H, m, H-3, 5), 3.93 (3H, s, OCH₃), 2.11 (3H, s, CH₃). ¹³C-NMR (125 MHz, CDCl₃) δ: 192.8 (CO), 192.5 (CHO), 171.1 (C-6'), 168.8 (C-2'), 168.6 (C-4'), 161.9 (C-2), 137.5 (C-β), 132.4 (C-4), 130.9 (C-6), 129.8 (C-5), 128.8 (C-1), 127.1 (C-3), 124.7 (C-α), 116.4 (C-5'), 110.1 (C-3'), 109.5 (C-1'), 66.3 (OCH₃), 6.7 (CH₃). HRMS m/z: calcd for C₁₈H₁₅FO₅Na: 353.0801 [M + Na]⁺, found: 353.0828.

3'-Formyl-4',6'-dihydroxy-2'-methoxy-5'-methyl-2-trifluoromethylchalcone (1e). 40.7% yield. Yellow needles, Mp: 110-112 °C. ¹H-NMR (500 MHz, CDCl₃) δ: 14.96 (1H, s, chelated-OH-6'), 14.57 (1H, s, chelated-OH-4'), 10.06 (1H, s, CHO), 8.54 (1H, d, *J* = 16.0 Hz, H-β), 8.46 (1H, d, *J* = 16.0 Hz, H-α), 7.92 (1H, m, H-3), 7.83 (1H, m, H-5), 7.74 (2H, m, H-5,6), 3.95 (3H, s, OCH₃), 2.75 (3H, s, CH₃). ¹³C-NMR (125 MHz, CDCl₃) δ: 203.9 (CO), 191.8 (CHO), 174.0 (C-6'), 169.9 (C-2'), 167.2 (C-4'), 137.9 (C-β), 129.9 (C-1), 129.9 (C-5), 125.0 (C-4), 121.2 (C-6), 121.0 (C-α), 107.2 (C-3), 105.9 (C-2), 104.9 (CF₃), 102.4 (C-5'), 102.3 (C-3'), 91.6 (C-1'), 56.3 (OCH₃), 7.3 (CH₃). HRMS m/z: calcd for C₁₉H₁₄F₃O₅: 379.0793[M – H]⁺, found:

379.0832.

Cytotoxic Activity Assay.

Table S1. Activities against human cancer cell lines

Compounds	IC ₅₀ (μM)					
	HCT-8	A549	GNM	DU-145	HL-60	HUVEC
1	>50	>50	>50	>50	>50	>50
1b	17.9±0.6	15.5±0.8	18.1±1.0	20.2±1.6	10.8±0.9	16.3±1.2
1c	>50	>50	>50	>50	>50	>50
1d	>50	>50	>50	>50	>50	>50
1e	>50	>50	>50	>50	>50	>50
Paclitaxel (nM)	>50	5.1±0.3	16.0±1.2	9.2±0.7	>50	6.6±0.5

REFERENCES

1. Umehara, K.; Singh, I. P.; Etoh, H.; Takasaki, M.; Konoshima, T. *Phytochemistry*. **1998**, *49*, 1699-1704.
2. Sato, S.; Obara, H.; Onodera, J.; Endo, A.; Matsuba, S. *Bull. Chem. Soc. Jpn.* **1992**, *65*, 452-457.
3. Nakagawa-Goto, K.; Lee, K. H. *Tetrahedron. lett.* **2006**, *47*, 8263-8266.
4. Ye, C. L.; Lu, Y. H.; Wei, D. Z. *Phytochemistry*. **2004**, *65*, 445-447.

Generic Display Report

Analysis Info		Acquisition Date	4/11/2014 1:39:40 PM
Analysis Name	D:\Data\306xiang zhao\2014041002.d	Operator	Q-TOF
Method	tune_low_neg20120216.m	Instrument	micrOTOF-Q
Sample Name	2		
Comment

Bruker Compass DataAnalysis 4.0

printed: 4/11/2014 1:46:28 PM

Page 1 of 1

HRMS spectra of compound 7

¹H NMR spectra of compound 7

¹³C NMR spectra of compound 7

HRMS spectra of compound 8

¹H NMR spectra of compound 8

¹³C NMR spectra of compound 8

Display Report

Analysis Info		Acquisition Date	3/5/2012 1:21:51 PM
Analysis Name	D:\Data\sample\2012\20120305\leg20101230.d	Operator	BDAL@DE
Method	lhl-tune_general.m	Instrument	micrOTOF-Q II
Sample Name	leg20101230		10260
Comment

Bruker Compass DataAnalysis 4.0
printed: 3/5/2012 1:30:56 PM
Page 1 of 1

HRMS spectra of compound 2

¹H NMR spectra of compound 2

¹³C NMR spectra of compound 2

NOE spectra of compound 2

HRMS spectra of compound 1

¹H NMR spectra of compound 1

NOE spectra of compound 1

¹³C NMR spectra of compound 1

Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name D:\Data\sample\2012\20120516\leg20110322Br-n.d
Method lhl-tune_GENERAL.m
Sample Name leg20110322Br-n
Comment

Acquisition Date 5/16/2012 2:30:15 PM

Operator BDAL@DE
Instrument / Ser# microTOF-Q II 10260

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	3000 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	6.0 l/min
Scan End	1000 m/z	Set Collision Cell RF	150.0 Vpp	Set Divert Valve	Source

HRMS spectra of compound 1b

¹H NMR spectra of compound 1b

¹³C NMR spectra of compound 1b

HRMS spectra of compound 1c

^1H NMR spectra of compound 1c

^{13}C NMR spectra of compound 1c

HRMS spectra of compound 1d

¹H NMR spectra of compound 1d

¹³C NMR spectra of compound 1d

HRMS spectra of compound 1e

¹H NMR spectra of compound 1e

¹³C NMR spectra of compound 1e