Synthesis and Biological Evaluation of Curcuminoid Derivatives

Ling Feng,a Yang Li,a Zhi-fang Song,a Hui-jing Li,*b and Qi-yong Huai*a

*aMarine College, Shandong University; Weihai 264209, China; and bSchool of Marine Science and Technology, Harbin Institute of Technology at Weihai; Weihai 264209, China.

Received June 5, 2015; accepted July 22, 2015

Many curcuminoid derivatives have been reported to have multiple biological activities. The aim of this study was to improve the biological activity of curcuminoids by synthesizing 16 new derivatives which combined cinnamic acids with curcuminoids, and we also analyzed the structure–activity relationship of the new compounds. Almost all the new compounds showed encouraging activity, especially compound 7g. It had much better antioxidant activity than curcuminoids and Vitamin C (VC), and also had the most significant antibacterial activity, which was 5-folder better than ampicillin (one of the best marketed antibiotics) with a minimum inhibitory concentration (MIC) of 0.5 µg/mL against Gram-positive cocci (Staphylococcus aureus and Streptococcus viridans) as well as Escherichia coli and 0.6 µg/mL against Enterobacter cloacae. Compound 7g also showed the greatest anticancer activity with a much lower IC50, which was 0.51 µM against MCF-7, 0.58 µM against HepG-2, 0.63 µM against LX-2, and 0.79 µM against 3T3. The results suggest that these compounds have promising potential as candidates for the treatment of cancer and thus further studies are warranted.

Key words curcuminoid derivative; cinnamic acid; anticancer; antibacterial; antioxidant; structure–activity relationship

Results and Discussion

Chemistry To analyze the impact on the biological activities of hydroxyl and methoxy of curcuminoids, we took cinnamic acids as examples to react with CCM which have both hydroxyl and methoxy as well as react with BCM which has no methoxy. The curcuminoid derivatives of cinnamic acid (compounds 3a–d) were obtained as outlined in Chart 1. We used acetic anhydride (Ac2O) to protect hydroxyl with the method described in Chart 2 to obtain compounds 5a–c. To decorate the parent curcuminoids, acetylated compounds were reacted with CCM or BCM to get new compounds 6a–I, to get the final product (7a–I), CH3Ona/methanol (MeOH) was used to hydrolyze the acetylated curcuminoid derivatives (compounds 6a–I). All the newly synthetic compounds were purified by column chromatography and their structures were established by (1H-NMR, 13C-NMR and MS) analysis (shown in Experimental).

Antioxidant Activity As well-known mechanisms, the hydrogen atom or electron donation abilities of some pure compounds were measured by the bleaching of a purple colored methanol solution of the stable 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical, this spectrophotometric assay uses the stable radical, DPPH, as a reagent. This method is quite simple and rapid for screening specific compounds. Thus, the antioxidant activity of all the test compounds (3a–d, 7a–I, CCM, BCM, VC) was evaluated for their radical scavenging ability using the stable DPPH radical method. The assay was conducted at six different concentrations of test compounds (2.5, 5, 10, 20, 40, 80 µM) in a polar, homogenous medium. The antioxidant potency (DPPH FRSA %) was presented in µM concentrations as shown in Table 1.

The radical-scavenging assay obviously showed that compounds 7a–I had much higher DPPH FRSA than curcuminoids. Besides, all of the test compounds, the best radical scavenger was compound 7g (98.3%), whose scavenging abil-

© 2015 The Pharmaceutical Society of Japan
ity was about 1.65-folder higher than curcumin (59.1%) and was 1.7-folder higher than VC (57.6%) which was the standard compound. However, compounds 3a–d which their active ingredients had no hydroxy or methoxy had lower antioxidant activity with much lower DPPH FRSA. Besides, curcuminoid derivatives with two active ingredients had better antioxidant activity than the derivatives with only one active ingredient. Hence, the test compounds 7a–I had great potential to be used as antioxidants.

**Antibacterial Activity** The antibacterial activities of all the target compounds were tested against Gram-positive cocci (Staphylococcus (S.) aureus and Streptococcus viridans) and Gram-negative bacilli (Escherichia (E.) coli and Enterobacter cloacae). The lowest concentration of the tested compounds in µg/mL which prevented in vitro growth of microorganism has been represented with minimum inhibitory concentration (MIC) shown in Table 2.

All the compounds 7a–I showed better antibacterial activities than curcuminoids. The most encouraging results were obtained in the case of compound 7g having an MIC of 0.5 µg/mL against S. aureus, 0.5 µg/mL against S. viridans, 0.5 µg/mL against E. coli and 0.6 µg/mL against E. cloacae respectively, while ampicillin, the best marked antibiotic, shows an MIC of 2.5 µg/mL. It is obviously that compound 7g which had 2-hydroxyl and 4-methoxy was five times more effective than the ampicillin at similar concentrations and nearly twenty-one times more effective than CCM (10.3 µg/mL). On the other hand, the antibacterial activities of curcumin derivatives (3a, c, 7a, c, e, g, i, k) were better than those of demethoxycurcumin derivatives (3b, d, 7b, d, f, h, j, l). Besides, compounds 7g–I which had two active ingredients had much better effect than 7a–f which had only one active ingredient.

However the curcuminoid derivatives of cinnamic acid (3a–d) exhibited low antibacterial activities. In this group, compounds 3a and b which had hydroxyl had relative good activity and the best activity was against E. cloacae with an MIC of 11 µg/mL. However, all this group compounds showed lower activity than curcuminoids (CCM and BCM). Besides, compound 3d which had no hydroxy or methoxy had the lowest activity with an MIC of 24 µg/mL while the MIC of CCM was only 10.3 µg/mL.

**Anticancer Activity** The inhibitory effects of curcuminoid derivatives on the growth of three lines of cultured tumor cells (LX-2, HepG-2 and MCF-7) and one line of normal cells (3T3) were evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. In each experiment, the drug doxorubicin (ADR) was used as positive control and the IC₅₀ of curcuminoids (CCM and BCM) were also presented to compare the anticancer activities. The anticancer activity of curcuminoid derivatives were evaluated at varying concentrations which were from 1 to 50 µM. IC₅₀ values of all the test compounds were presented in µM as shown in Table 3.

Almost all the newly synthetic curcuminoid derivatives exhibited much stronger inhibitory activity against LX-2, HepG-2, MCF-7 and relatively lower inhibitory activity against normal cells (3T3). As shown in Table 3, compound 7g which had 2-hydroxyl and 4-methoxy exhibited an IC₅₀ of 0.61 µM in MCF-7, 0.68 µM in HepG-2, 0.73 µM in LX-2 and 0.89 µM in 3T3, which was about thirteen three fold higher than curcuminoids (9.14–12.11 µM). On the other hand, the IC₅₀ value of compound 7g was almost the same as the positive control drug ADR.

The results shown in Table 3 indicated that compounds 3a–d which their active ingredients had no hydroxy or methoxy had no anticancer activity, while compounds 7a–I which their active ingredients had hydroxyl and methoxy had much better anticancer activity than curcuminoids (CCM and BCM) and they also had lower activity against normal cells (3T3). What’s more, the antitumor activity of CCM derivatives was much better than BCM derivatives. Curcuminoid derivatives with two active ingredients had much better activity than curcuminoid derivatives with one active ingredient. Hence, compounds having both hydroxyl and methoxy have great advantages and hold promise as prodrugs.

**Structure–Activity Relationship** Curcumin has two phenyl rings and substitutions at the 3 and 4 positions with methoxy and hydroxy groups respectively. BCM has two phenyl rings and substitutions at the 4 position with hydroxyl. Research has shown that the biological activities are affected by 4-hydroxyl groups present on both phenyl rings of curcuminoids and 3-methoxy on phenyl rings of CCM.

In this study, cinnamic acids were used as the active ingredients to prepare new compounds which may hold promise as prodrugs with curcuminoids. Among them, the curcuminoid
derivatives of cinnamic acid had no hydroxyl, what’s more, their biological activities were lower than curcuminoids. But curcuminoid derivatives of isoferulic acid, trans-3-hydroxyl cinnamic acid and trans-4-hydroxyl cinnamic acid had hydroxyl, their biological activities were much better than curcuminoids. On the other hand, the derivatives (7b, d, f, h, j, l) which had no methoxy had relatively lower biological activities.

Hence, we attempt to summarize the structure–activity relationships:

1) Hydroxyl group is the major active group in tested compounds. To some extent, the more hydroxyl groups the substituent contains, the better biological activity the derivatives will have. In other words, the hydroxyl groups incorporated are helpful for the improvement of biological activity.

2) The absence of the methoxy group in curcuminoid derivatives leads to a decrease in the biological activity.

3) Positions of hydroxyl can cause difference in the biological activity.

Experimental

Materials and Methods  Used reagents were bought from Aldrich Chemical Co. (Beijing, China) and used without further purification. The products were purified by column chromatography using silica gel (200–300 mesh). 1H- and
a micro melting point apparatus and are uncorrected. The spectrometer (Finnigan). Melting points were measured with a solution of tetramethylsilane (TMS) as an internal standard.

13C-NMR spectra were recorded on Bruker-400 at room temperature with tetramethylsilane (TMS) as an internal standard.

### Table 1. MIC Correlation Diagram of Curcuminoid Derivatives against Bacterial Strains

| Compound | Gram-positive | Gram-negative |
|----------|---------------|---------------|
|          | S. aureus | S. viridans | E. coli | E. cloacae |
| 3a       | 13      | 12       | 14      | 11       |
| 3b       | 17      | 17       | 15      | 15       |
| 3c       | 20      | 18       | 18      | 18       |
| 3d       | 24      | 21       | 21      | 23       |
| 7a       | 0.9     | 0.9      | 0.8     | 0.9      |
| 7b       | 2.1     | 2.1      | 2.3     | 2.5      |
| 7c       | 1.2     | 1.3      | 1.2     | 1.2      |
| 7d       | 2.7     | 2.8      | 2.7     | 2.7      |
| 7e       | 1.1     | 1.2      | 0.9     | 0.9      |
| 7f       | 2.3     | 2.3      | 2.3     | 2.3      |
| 7g       | 0.5     | 0.5      | 0.5     | 0.6      |
| 7h       | 1.4     | 1.4      | 1.4     | 1.4      |
| 7i       | 0.6     | 0.6      | 0.8     | 0.8      |
| 7j       | 1.9     | 1.9      | 1.8     | 1.7      |
| 7k       | 0.6     | 0.7      | 0.7     | 0.7      |
| 7l       | 1.7     | 1.6      | 1.6     | 1.6      |
| CCM      | 10.3    | 10.3     | 13.1    | 13.1     |
| BCM      | 16.1    | 16.1     | 14.3    | 14.3     |
| Amipicillin | 2.5   | 2.5      | 3.2     | 3.2      |

### Table 3. IC50 Values of Test Compounds against 3T3, LX-2, HepG-2 and MCF-7

| Compound | 3T3 | LX-2 | HepG-2 | MCF-7 |
|----------|-----|------|--------|-------|
| 3a       | 40  | 35   | 37     | 29    |
| 3b       | >40 | >40  | >40    | >40   |
| 3c       | >40 | >40  | >40    | >40   |
| 3d       | >40 | >40  | >40    | >40   |
| 7a       | 1.92| 1.58 | 1.61   | 1.53  |
| 7b       | 5.88| 4.86 | 4.72   | 4.75  |
| 7c       | 2.83| 2.62 | 2.55   | 4.51  |
| 7d       | 5.75| 5.46 | 5.51   | 5.44  |
| 7e       | 2.51| 2.31 | 2.19   | 2.28  |
| 7f       | 5.31| 5.21 | 5.33   | 5.19  |
| 7g       | 0.79| 0.63 | 0.58   | 0.51  |
| 7h       | 3.61| 3.32 | 3.39   | 3.38  |
| 7i       | 1.87| 1.62 | 1.59   | 1.55  |
| 7j       | 4.84| 4.61 | 4.59   | 4.52  |
| 7k       | 1.71| 1.42 | 1.38   | 1.41  |
| 7l       | 4.35| 4.12 | 4.27   | 4.33  |
| CCM      | 9.51| 9.14 | 9.44   | 9.28  |
| BCM      | 12.11| 11.93| 11.63 | 11.64 |
| ADRb     | 0.53| 0.51 | 0.49   | 0.47  |

a) Vitamin C (VC) was used in this study as reference.

b) The drug doxorubicin (ADR) was used as positive control in this study.

Reactions were monitored by analytical thin-layer chromatography TLC and the TLC was carried out on silica gel GF254. The anhydrous solvents were dried and purified according to standard techniques before use. Gram-positive cocci (S. aureus and S. viridans), Gram-negative bacilli (E. coli and E. cloacae), cancer cells (LX-2, HepG-2 and MCF-7) and normal cells (3T3) were obtained from Expression Systems (Beijing, China).

**Isolation and Purification of Curcuminoids**

As curcuminoid powder contain three components named curcumin, demethoxycurcumin and bisdemethoxycurcumin. We separated them using silica gel (100 mesh). Briefly, putting dried silica gel (100 mesh) weigh 50 g dissolved in chloroform and filling them in a glass column of 5 cm internal diameter and 60 cm height. Then curcuminoid powder weighing 0.5 g was dissolved in 3 mL chloroform and was put on the silica gel column. Lastly, it was successively eluted with 500 mL of eluent (methanol–carbon tetrachloride–acetic acid=1:10:1). They were analyzed by TLC on silica gel plates and the pure fractions combined and solvents were removed to give pure components which were curcumin, demethoxycurcumin and bisdemethoxycurcumin, respectively.

**General Procedure for the Synthesis of 3a and b**

To a solution of cinnamic acid (5 mmol) in chloroform (20 mL), HOBt (5 mmol) and DIEA (2 mL) were added and were stirred for 0.5 h at 0°C. Then, EDCI (5 mmol) was added and was still stirred at 0°C for 2 h. Afterwards, a chloroform solution (15 mL) of 1a or b (3.5 mmol) was added to the mixture, which was allowed to be stirred at 0°C for 1 h, then was still stirred for another 18 h at room temperature. The reaction was monitored by analytical thin-layer chromatography TLC and the TLC was carried out on silica gel GF254. The drug doxorubicin (ADR) was used as positive control in this study.
to yield pure product (compounds 3a, b).

Curcumino-Mono-cinnamic Acid (3a)

Yield 80%, yellow solid, mp 160–163°C; 1H-NMR (400 MHz, DMSO-d6) δ: 3.83 (3H, s, OCH3), 3.86 (3H, s, OCH3), 4.57 (2H, s), 6.29 (1H, d, J = 14.0 Hz), 6.89 (2H, d, J = 13.5 Hz), 7.03 (2H, s), 7.21 (2H, d, J = 6.9, 1.3 Hz), 7.33 (2H, m), 7.38 (4H, m), 7.41 (2H, d, J = 6.9, 1.3 Hz), 7.45 (2H, m), 7.48 (2H, d, J = 14.0 Hz), 7.54 (4H, ddd, J = 7.1, 1.5, 0.9 Hz), 7.60 (2H, d, J = 7.6 Hz), 7.13 (1H, s), 7.19 (1H, d, J = 6.9 Hz), 7.25 (1H, s), 7.35 (1H, m), 7.42 (2H, m), 7.48 (1H, m, J = 14.0 Hz), 7.54 (2H, ddd, J = 7.1, 1.5, 0.9 Hz), 7.60 (2H, d, J = 13.5 Hz), 7.21 (1H, dd, J = 6.9, 1.3 Hz), 7.31 (1H, m), 7.33 (1H, m), 7.38 (2H, m), 7.41 (1H, dd, J = 6.9, 1.3 Hz), 7.45 (1H, m), 7.48 (1H, m, J = 14.0 Hz), 7.54 (2H, ddd, J = 7.1, 1.5, 0.9 Hz), 7.60 (2H, d, J = 13.5 Hz); 13C-NMR (100 MHz, CDC13) δ: 198.71, 164.27, 151.73, 147.93, 142.67, 135.15, 134.43, 130.8, 129.05, 128.52, 128.46, 127.91, 125.21, 121.16, 120.73, 115.47, 51.63; HR-ESI-MS m/z: 568.6247 [M + H]+ (Calcd for C37H28O6 568.6250).

General Procedure for the Synthesis of 5a–c Three milliliters AC2O was added to a pyridine solution (5 mL) of 4a, b or c (5mmol) and then the mixture was refluxed for 8h. Afterwards, the solution was washed with dilute aqueous sodium hydroxide and then extraction. The organic layer was dried with anhydrous magnesium sulfate (MgSO4) and evaporated to dryness under vacuum. Lastly, the product was recrystallized from ethanol and then dried in vacuum to get 5a–c which were white solids.

Acetylated Isoferulic Acid (5a)

Yield 92%, white solid, mp 120–123°C; 1H-NMR (400 MHz, DMSO-d6) δ: 2.31 (3H, s), 3.83 (3H, s, OCH3), 6.27 (1H, d, J = 13.4 Hz), 6.93 (1H, d, J = 7.2 Hz), 7.03 (1H, s), 7.33 (1H, d, J = 7.2 Hz), 7.45 (1H, d, J = 13.4 Hz), 12.05 (1H, s, OH); 13C-NMR (100 MHz, CDCl3) δ: 171.47, 169.06, 150.58, 144.85, 141.43, 127.04, 126.68, 123.41, 116.49, 111.08, 55.78, 20.22; HRESI-MS m/z: 236.2231 [M+H]+ (Calcd for C19H18O4 236.2230).

Acetylated trans-3-Hydroxycinnamic Acid (5b)

Yield 87%, white solid, mp 122–125°C; 1H-NMR (400 MHz, DMSO-d6) δ: 2.31 (3H, s), 6.27 (1H, d, J = 13.4 Hz), 7.03 (1H, s), 7.21 (1H, dd, J = 6.8, 1.7 Hz), 7.45 (1H, d, J = 13.4 Hz), 7.48 (1H, m), 7.55 (1H, dd, J = 6.8, 1.7 Hz), 12.05 (1H, s, OH); 13C-NMR (100 MHz, CDCl3) δ: 171.5, 169.07, 151.13, 144.93, 134.45, 129.03, 125.26, 121.24, 120.75, 116.43, 20.22; HRESI-MS m/z: 206.1971 [M+H]+ (Calcd for C19H19O5 206.1970).

Acetylated trans-4-Hydroxycinnamic Acid (5e)

Yield 88%, white solid, mp 119–122°C; 1H-NMR (400 MHz, DMSO-d6) δ: 2.31 (3H, s), 6.27 (1H, d, J = 13.4 Hz), 7.28 (2H, d, J = 7.8 Hz), 7.45 (1H, d, J = 13.4 Hz), 7.62 (2H, d, J = 7.8 Hz), 12.05 (1H, s, OH); 13C-NMR (100 MHz, CDCl3) δ: 171.47, 169.06, 150.58, 144.13, 132.02, 129.69, 121.47, 121.16, 20.22; HRESI-MS m/z: 206.1971 [M+H]+ (Calcd for C19H19O5 206.1970).

General Procedure for the Synthesis of 6a–f To a solution of 5a, b or c (5mmol) in chloroform (20 mL), DMAP (0.1mmol) was added and was stirred for 0.5h at 0°C. Then, 4.2mmol EDCl was added and was still stirred at 0°C for 2h. Afterwards, a chloroform solution (20mL) of 1a or b (3.5mmol) was added to the mixture, which was allowed to be stirred 0°C for 1h, then was stirred for another 18h at room temperature. The reaction was indicated by TLC and after it completed, the mixture was washed with aqueous hydrochloric acid and then extraction. The organic layer was dried with anhydrous Na2SO4 and evaporated to dryness under vacuum. Purification by chromatography on silica gel (methanol–carbon tetrachloride–acetic acid=1:10:1.5) to give 6a–f which are yellow solids.

Curcumino-Monoacetylated Isofeural Acid (6a)

Yield 69%, yellow solid, mp 163–166°C; 1H-NMR (400 MHz, DMSO-d6) δ: 2.31 (3H, s, COCH3), 3.75 (6H, s, OCH3), 3.86 (3H, s, OCH3), 4.57 (2H, s), 6.31 (1H, d, J = 14.0 Hz), 6.90 (1H, d, J = 7.3 Hz), 6.89 (2H, d, J = 13.5 Hz), 6.93 (1H, d, J = 7.2 Hz), 6.94 (1H, d, J = 7.2 Hz), 12.02 (1H, d, J = 7.2 Hz), 7.03 (1H, s), 7.19 (1H, d, J = 7.3 Hz), 7.23 (1H, s), 7.33 (1H, d, J = 7.2 Hz), 7.48 (1H, d, J = 14.0 Hz), 7.60 (2H, d, J = 13.5 Hz); 13C-NMR (100 MHz, CDCl3) δ: 198.71, 169.05,
respectively. Then the resulting solution was stirred for 30 min at 0°C. Then dried the solution and unreacted thionyl chloride to give a white solid. Afterwards, a chloroform solution of 1a or b (2.5 mmol) was mixed with the product as well as Et3N at 0°C and then was stirred for 24 h at room temperature. The mixture was washed with aqueous hydrochloric acid and then extraction. The organic layer was dried with anhydrous MgSO4 and evaporated to dryness under vacuum. Purification by chromatography on silica gel (methanol–carbon tetrachloride–acetic acid = 1:10:1.5) to give 6g–I which were yellow solids.

Cucurmin-Diacetylated Isofuranic Acid (6g)

Yield 56%, yellow solid, mp 157–160°C; 1H-NMR (400 MHz, DMSO-d6) δ: 2.31 (3H, s, COCH3), 4.57 (2H, s), 6.31 (1H, d, J = 14.0 Hz), 6.80 (2H, d, J = 13.5 Hz), 7.03 (2H, s), 7.21 (2H, d, J = 7.2 Hz), 7.41 (1H, dd, J = 6.9, 1.3 Hz), 7.45 (4H, d, J = 7.2 Hz), 7.60 (2H, d, J = 7.2 Hz), 7.70 (4H, s), 7.19 (2H, d, J = 7.3 Hz), 7.33 (2H, d, J = 7.2 Hz), 7.48 (2H, d, J = 14.0 Hz), 7.60 (2H, d, J = 13.5 Hz); 13C-NMR (100 MHz, CDCl3) δ: 198.71, 169.05, 164.80, 158.46, 151.17, 150.84, 149.72, 142.72, 135.34, 134.46, 132.02, 130.38, 129.05, 125.80, 124.72, 121.18, 121.01, 120.73, 115.43, 115.13, 51.63, 20.22; HR-ESI-MS m/z: 496.5151 [M+H]+ (Caled for C26H26O14 496.5150).

General Procedure for the Synthesis of 6g–I Ten milliliters thionyl chloride was added to a stirred, ice-cooled chloroform solution (20 mL) rapidly, which contains 5.3 mmol 5a, b or c respectively. Then the resulting solution was stirred for 30 min at 0°C. Then dried the solution and unreacted thionyl chloride to give a yellow solid. Afterwards, a chloroform solution of 1a or b (2.5 mmol) was mixed with the product as well as Et3N at 0°C and then was stirred for 24 h at room temperature. The mixture was washed with aqueous hydrochloric acid and then extraction. The organic layer was dried with anhydrous MgSO4 and evaporated to dryness under vacuum. Purification by chromatography on silica gel (methanol–carbon tetrachloride–acetic acid = 1:10:1.5) to give 6g–I which were yellow solids.

Cucurmin-Diacetylated Isofuranic Acid (6h)

Yield 53%, yellow solid, mp 156–159°C; 1H-NMR (400 MHz, DMSO-d6) δ: 2.31 (3H, s, COCH3), 4.57 (2H, s), 6.31 (1H, d, J = 14.0 Hz), 6.80 (2H, d, J = 13.5 Hz), 7.03 (2H, s), 7.21 (2H, d, J = 7.2 Hz), 7.41 (2H, dd, J = 6.9, 1.3 Hz), 7.33 (1H, d, J = 7.2 Hz), 7.40 (2H, d, J = 14.0 Hz), 7.60 (2H, d, J = 13.5 Hz); 13C-NMR (100 MHz, CDCl3) δ: 198.71, 169.05, 164.80, 158.46, 151.17, 150.84, 149.72, 142.72, 135.34, 134.46, 132.02, 130.38, 129.05, 125.80, 124.72, 121.18, 121.01, 120.73, 115.43, 115.13, 51.63, 20.22; HR-ESI-MS m/z: 496.5151 [M+H]+ (Caled for C26H26O14 496.5150).
13.08, 129.05, 129.28, 129.72, 129.14, 128.32, 128.05, 123.48, 123.47, 115.43, 115.05, 55.73, 51.63, 20.22; HR-ESI-MS m/z: 684.6964 [M+H]^+ (Caled for C_{29}H_{24}O_{7}) 684.6970.

Curcumin-Diacetylated trans-4-Hydroxycinnamic Acid (6k)

Yield 57%, yellow solid, mp 163–166°C; 1H-NMR (400 MHz, DMSO-d_6): δ: 2.31 (6H, s, COCH_3), 4.57 (2H, s), 6.31 (2H, dd, J = 14.01 Hz), 6.89 (2H, d, J = 13.5Hz), 7.03 (2H, s), 7.21 (2H, dd, J = 7.5, 2.3Hz), 7.28 (4H, d, J = 7.8Hz), 7.48 (2H, d, J = 14.0Hz), 7.60 (2H, d, J = 13.5Hz), 7.62 (4H, d, J = 7.8Hz); 13C-NMR (100 MHz, CDCl_3): δ: 198.71, 198.07, 164.28, 158.67, 151.05, 147.92, 147.22, 134.62, 134.02, 123.38, 129.69, 127.03, 126.73, 123.48, 121.47, 115.43, 115.05, 55.73, 51.63, 20.22; HR-ESI-MS m/z: 684.7483 [M+H]^+ (Caled for C_{29}H_{24}O_{7}) 684.7490.

General Procedure for the Synthesis of 7a–I A solution of 6a–I (2mmol) in MeOH (10 mL) and sodium hydroxide solution (10mL, 1m) was stirred at room temperature overnight. The solvent was washed with aqueous hydrochloric and then was extracted with ethyl acetate. Lastly, the ethyl acetate was dried with anhydrous Na_2SO_4 and evaporated to dryness under vacuum to give 7a–I which were yellow solids.

Curcumin-Monoisofuroileric Acid (7a)

Yield 95%, yellow solid, mp 178–181°C; 1H-NMR (400 MHz, DMSO-d_6): δ: 3.75 (3H, s, OCH_3), 3.86 (6H, s, OCH_3), 4.57 (2H, s), 6.31 (1H, d, J = 14.0Hz), 6.90 (1H, dd, J = 13.5Hz), 6.89 (2H, d, J = 13.5Hz), 6.94 (2H, d, J = 13.5Hz), 7.02 (2H, d, J = 7.2Hz), 7.03 (1H, s), 7.19 (1H, d, J = 7.3Hz), 7.23 (2H, s), 7.48 (1H, d, J = 14.0Hz), 7.60 (2H, d, J = 13.5Hz), 9.21 (2H, s, OH); 13C-NMR (100 MHz, CDCl_3): δ: 198.71, 164.28, 150.92, 149.33, 147.85, 147.07, 132.87, 130.38, 127.69, 127.07, 126.63, 123.48, 122.72, 115.43, 114.75, 112.18, 111.05, 56.15, 55.73, 51.63; HR-ESI-MS m/z: 544.5562 [M+H]^+ (Caled for C_{28}H_{32}O_{6}) 544.5560.

Bisdemethoxycurcumin-Mono-trans-4-hydroxycinnamic Acid (7f)

Yield 97%, yellow solid, mp 173–176°C; 1H-NMR (400 MHz, DMSO-d_6): δ: 3.86 (3H, s, OCH_3), 4.57 (2H, s), 6.31 (1H, d, J = 14.0Hz), 6.83 (1H, dd, J = 7.2, 1.3Hz), 6.89 (2H, d, J = 13.5Hz), 6.94 (1H, d, J = 7.2Hz), 7.02 (1H, d, J = 7.2Hz), 7.03 (2H, s), 7.16 (1H, dd, J = 7.2, 1.3Hz), 7.21 (1H, dd, J = 6.9, 1.3Hz), 7.23 (1H, s), 7.31 (1H, m), 7.41 (1H, dd, J = 6.9, 1.3Hz), 7.45 (1H, m), 7.48 (1H, d, J = 14.0Hz), 7.60 (2H, d, J = 13.5Hz), 9.21 (1H, s, OH), 9.45 (1H, s, OH); 13C-NMR (100 MHz, CDCl_3): δ: 198.71, 164.28, 150.92, 149.33, 147.85, 147.07, 132.87, 130.38, 127.69, 127.07, 126.63, 123.48, 122.72, 115.43, 114.75, 112.18, 111.05, 56.15, 55.73, 51.63; HR-ESI-MS m/z: 544.5562 [M+H]^+ (Caled for C_{28}H_{32}O_{6}) 544.5560.
Curcumin-Diisoferulic Acid (7g)

Yield 95%, yellow solid, mp 192–195°C; 1H-NMR (400 MHz, DMSO-d$_6$) δ: 3.75 (6H, s, OCH$_3$), 3.86 (6H, s, OCH$_3$), 4.57 (2H, s), 6.31 (2H, d, J=14.0Hz), 6.90 (2H, d, J=7.3Hz), 6.89 (2H, d, J=13.5Hz), 6.94 (2H, d, J=7.2Hz), 7.02 (2H, d, J=7.2Hz), 7.19 (2H, d, J=7.3Hz), 7.23 (2H, s), 7.48 (2H, d, J=14.0Hz), 7.60 (2H, d, J=13.5Hz), 9.21 (2H, s, OH); 13C-NMR (100 MHz, CDCl$_3$) δ: 198.71, 164.28, 150.92, 149.33, 147.85, 147.07, 142.72, 138.27, 130.38, 127.69, 127.07, 126.63, 123.48, 122.72, 115.43, 114.75, 112.18, 111.05, 56.15, 55.73, 51.63; HR-ESI-MS m/z: 720.7271 [M+H]$^+$ (Caled for C$_{43}$H$_{36}$O$_{12}$ 720.7270).

Bisdemethoxycurcumin-Diisoferulic Acid (7h)

Yield 93%, yellow solid, mp 194–197°C; 1H-NMR (400 MHz, DMSO-d$_6$) δ: 3.86 (6H, s, OCH$_3$), 4.57 (2H, s), 6.31 (2H, d, J=14.0Hz), 6.89 (2H, d, J=13.5Hz), 6.94 (2H, d, J=7.2Hz), 7.02 (2H, d, J=7.2Hz), 7.03 (2H, s), 7.21 (2H, dd, J=6.9, 1.3Hz), 7.23 (2H, s), 7.41 (2H, dd, J=6.9, 1.3Hz), 7.45 (2H, m), 7.48 (2H, d, J=14.0Hz), 7.60 (2H, d, J=13.5Hz), 9.21 (2H, s, OH); 13C-NMR (100 MHz, CDCl$_3$) δ: 198.71, 164.28, 150.92, 149.33, 147.85, 147.07, 142.72, 138.27, 130.38, 129.05, 127.69, 125.28, 122.72, 115.43, 114.75, 112.18, 111.05, 56.15, 55.73, 51.63; HR-ESI-MS m/z: 660.6746 [M+H]$^+$ (Caled for C$_{38}$H$_{28}$O$_{8}$ 660.6750).

Curcumin-Di-trans-3-hydroxycinnamic Acid (7i)

Yield 97%, yellow solid, mp 196–199°C; 1H-NMR (400 MHz, DMSO-d$_6$) δ: 3.83 (6H, s, OCH$_3$), 4.57 (2H, s), 6.31 (2H, d, J=14.0Hz), 6.70 (2H, s), 6.83 (2H, dd, J=7.5, 2.3Hz), 6.89 (2H, d, J=13.5Hz), 6.90 (2H, d, J=7.3Hz), 7.03 (2H, s), 7.16 (2H, dd, J=7.5, 2.3Hz), 7.19 (2H, d, J=7.3Hz), 7.31 (2H, m), 7.48 (2H, d, J=14.0Hz), 7.60 (2H, d, J=13.5Hz), 9.45 (1H, s, OH); 13C-NMR (100 MHz, CDCl$_3$) δ: 198.71, 164.28, 150.92, 149.33, 147.82, 147.07, 142.72, 138.27, 135.34, 130.38, 130.05, 127.07, 126.63, 123.48, 122.72, 118.18, 117.55, 114.03, 113.15, 111.05, 55.73, 51.63; HR-ESI-MS m/z: 660.6750 [M+H]$^+$ (Caled for C$_{39}$H$_{36}$O$_{10}$ 660.6750).

Bisdemethoxycurcumin-Di-trans-3-hydroxycinnamic Acid (7j)

Yield 91%, yellow solid, mp 190–193°C; 1H-NMR (400 MHz, DMSO-d$_6$) δ: 4.57 (2H, s), 6.31 (2H, d, J=14.0Hz), 6.70 (2H, s), 6.83 (2H, dd, J=7.5, 2.3Hz), 6.89 (2H, d, J=13.5Hz), 7.03 (2H, s), 7.16 (2H, dd, J=7.5, 2.3Hz), 7.21 (2H, dd, J=7.5, 2.3Hz), 7.31 (2H, m), 7.41 (2H, dd, J=6.9, 1.3Hz), 7.45 (2H, m), 7.48 (2H, d, J=14.0Hz), 7.60 (2H, d, J=13.5Hz), 9.45 (2H, s, OH); 13C-NMR (100 MHz, CDCl$_3$) δ: 198.71, 164.28, 150.92, 149.33, 147.07, 142.72, 138.27, 135.34, 130.38, 130.05, 129.05, 125.28, 122.78, 121.14, 121.14, 117.55, 114.03, 113.15, 111.05, 55.73, 51.63; HR-ESI-MS m/z: 660.6228 [M+H]$^+$ (Caled for C$_{39}$H$_{36}$O$_{10}$ 660.6230).

Acknowledgments

This study was supported by the National Natural Science Foundation of China (No. 21372054).
Conflict of Interest  The authors declare no conflict of interest.

References
1) Lee C. S., Kim Y. J., Lee M. S., Han E. S., Lee S. J., *Life Sci.*, **83**, 481–489 (2008).
2) Jutooru I., Chadalapaka G., Chintharlapalli S., Papineni S., Safe S., *Mol. Carcinog.*, **48**, 692–702 (2009).
3) Hibi S., Iwase H., Yoshioka K., Takahashi H., *Int. J. Mol. Med.*, **17**, 215–219 (2006).
4) Satomi Y., Nishino H., Shibata S., *Anticancer Res.*, **25** (6B), 4043–4047 (2005).
5) Cousins M., Adelberg J., Chen F., Rieck J., *Ind. Crops Prod.*, **25**, 129–135 (2007).
6) Chattopadhyay I., Biswas K., Bandyopadhyay U. Banerjee R. K., *Curr. Sci.*, **87**, 44–53 (2004).
7) Goel A., Kunnumakkara A. B., Aggarwal B. B., *Biochem. Pharmacol.*, **75**, 787–809 (2008).
8) Motterlini R., Foresti R., Bassi R., Green C. J., *Free Radic. Biol. Med.*, **28**, 1303–1312 (2000).
9) Menon V. P., Sudheer A. R., *Adv. Exp. Med. Biol.*, **595**, 105–125 (2007).
10) Tubas M. A. K., Ilhami G., *Chem. Biol. Interact.*, **174**, 27–37 (2008).
11) Jayaprakasha G. K., Jagannohan Rao L., Sakariash K. K., *Food Chem.*, **98**, 720–724 (2006).
12) Wang Y. F., Xu J. N., Li J., Chen C., Xi C. Y., Yan J. L., *Neurosci. Lett.*, **160**, 89–95 (2014).
13) Preetha A., Sherin G. T., Ajakumar B. K., Chitra S., Kuzhuvelil B. H., Bokkyung S., Sheela T. T., Krishna M., Indira K. P., Kallikat N. R., Bharat B. A., *Bio. Pharm.*, **76**, 1590–1611 (2008).
14) Oelkraug C., Lange C. M., Wenzel E., Fricke S., Hartke M., Simasi J., Schubert A., *Anticancer Res.*, **34**, 4781–4788 (2014).
15) Ohtsu H., Xiao Z. Y., Ishida J., Nagai M., Wang H., Itohara K., H., Su C. Y., Shih C., Chiang T. Y., Chang E., Lee Y., Tsai M. Y., Chang C., Lee K. H., *J. Med. Chem.*, **45**, 5037–5042 (2002).