Synthesis and Anticancer evaluation of (-)-Arctigenin Derivatives

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Summary: Seven (-)-arctigenin derivatives **1-7** were designed and synthesized by using Mannich and acylation methods to improve the activity and bioavailability of (-)-arctigenin. Structures of compounds **1-7** were elucidated on the basis of spectroscopic analysis and chemical evidence. Anticancer activity of these compounds on SGC7901 was assayed in *vitro*.

Keywords: (-)-Arctigenin, Synthesis, Synthetic derivatives, Anticancer activity.

Introduction

Fructus arctii, the dried ripe fruit of *Arctium lappa L.* was used as a folk medicine for thousand years in China. Chemical investigations of fructus arctii have revealed that dibenzylbutyrolactone lignans, such as arctiin and (-)-arctigenin, were the main components. Over the last decade, the research into (-)-arctigenin has been received considerable interest to the chemists as the compound have wide range of biological activities, including activities as anti-tumor [1, 2], anti-viral [3] and function as platelet-activating-factor (PAF) antagonists [4].

Based on a wide range biological activities that (-)-arctigenin was used as an excellent precursor for further structure modification. In order to get more potent, lower toxic and higher bioavailability agents, derivatives of (-)-arctigenin **1-7 (**Scheme-1, 2**)** were synthesized. As a part of our ongoing efforts to explore derivatives with increased polarity, we designed a series of novel derivatives **1-4** seeking to connect amino-methyl to C5' by Mannich reaction to evaluate the anti-tumor action of the lactone ring of (-)-arctigenin. Furthermore, to enhance the bioavailability of (-)-arctigenin, pro-drug method was applied in the structure modification to obtain O-acylation derivatives **5-7**. And biological activities of derivatives **1-7** on anticancer (SGC7901) were also assayed *in vitro*.

Results and Discussion

The Mannich reaction was carried out to yield aminomethylation derivatives and the novel Mannich derivatives **1-4** were obtained by treating (-)-arctigenin with formaldehyde and secondary amine (Scheme-1) heated at 65-80℃ for 1-5 h. On the basis of the spectra data of ^{1}H-NMR and ^{13}C-NMR, HSQC and HMBC, the structures of synthesized derivatives **1-4** was elucidated. The researching revealed that Mannich reaction was an efficient method for introducing the aminomethyl to the structure. In the reaction, hydrochlorate of secondary amine was used to instead of secondary amine in order to avoid showing cleavage at the lactone of (-)-arctigenin.

It was found that three acylating agents (acetic anhydride and succinic anhydride and benzoyl chloride) and (-)-arctigenin were added into the methylene dichloride heated at 38-40℃ and acylations were performed at the C-OH (C4') (Scheme-2). The reactions were finished within 5 h and three O-acylating derivatives **5-7** were purified by silica gel column chromatograph. The three synthetic compounds **5-7** were characterized by ^{1}H-NMR, ^{13}C-NMR spectra [5-7].

All the synthetic derivatives **1-7** were examined for anticancer activity with human cancer cell SGC7901 (human gastric carcinoma). The results indicated that the anticancer bioactivity of **5** was stronger than that of (-)-arctigenin and others were weaker. It can conclude that aminomethylation at C5' were unsuitable for the structure modification and the different groups acylation at C4' (-OH) exhibited various degree of anticancer bioactivity. And result was shown in Table-1.

Table-1: Cytotoxic activities of derivatives **1-7** on SGC7901.

Compounds	IC50(μMol/L) SGC7901
(-)-arctigenin	74
1	-
2	-
3	-
4	-
5	55
6	100
7	>100
5-fluorouracil	45

Positive control: 5-fluorouracil

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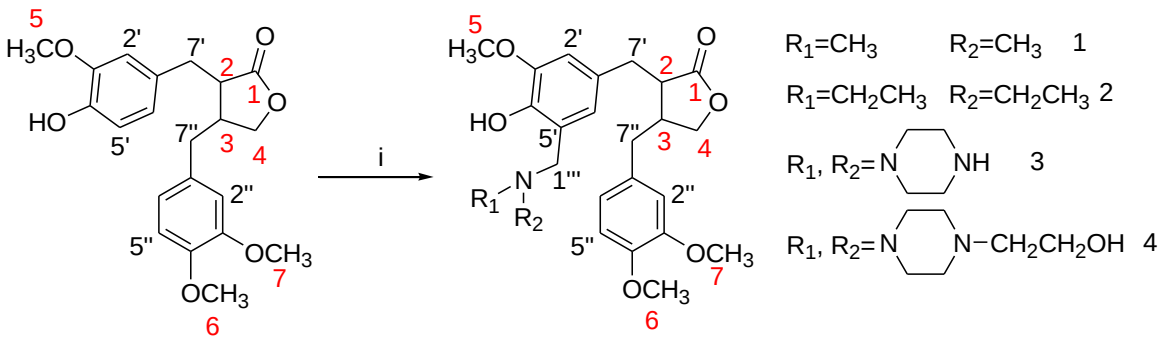
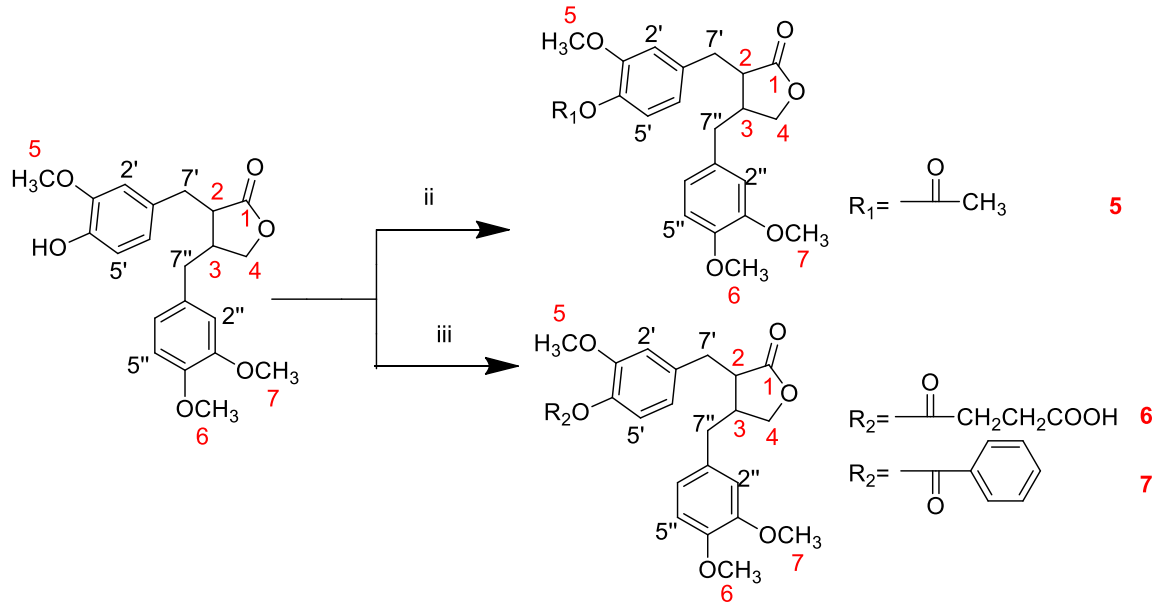


Scheme-1: The synthetic Mannich derivatives of (-)-arctigenin (Reagents and conditions: (i) HCHO, CH_3COOH, R_1R_2NH, heated 1-12 h.)



Scheme-2: The synthetic O-acetylation derivatives of (-)-arctigenin (Reagents and conditions: (ii) anhydrous acetic anhydride, CH_2Cl_2, sodium acetate, heated 5 h; (iii) succinic anhydride or benzoyl chloride, CH_2Cl_2, triethylamine, heated 7 min- 2 h.).

Experimental

Plant material: The fructus arctii was collected in Liaoning Province, P. R. China, and were identified by Prof. Kang Tingguo, College of Pharmacy, Liaoning University of Traditional Chinese Medicine. A voucher specimen (NO.arctii20071016) has been deposited at the Pharmacognosy Laboratory, College of Pharmacy, Liaoning University of TCM. Fructus arctii was defatted with petroleum ether and hydrolyzed with 5% dilute HCl. The reactive solution was filtrated and the residue was afforded. Then the residue was extracted with ethanol and concentrated in vacuum to gum. Furthermore, the gum was purified by silica gel column chromatography ($CHCl_3$-MeOH, 80:1, v/v) to yield (-)-arctigenin.

Synthesis of Mannich Derivatives **1-4**

Mannich method [8] was used to synthesize aminomethylation derivatives **1-4** of (-)-arctigenin. (-)-Arctigenin (372 mg, 1 mmol), paraformaldehyde (300 mg, 10 mmol) and the four secondary amines (dimethylamine, diethylamine, piperazine and 4-hydroxyethylpiperazine) (1 mmol) were respectively dissolved in glacial acetic acid (3 ml) and the mixture was heated at 65-80°C until

completion of the reaction as indicated by TLC. The reaction solution was distilled in vacuum and was dissolved with the same volume water. The mixtures were filtered and then the filtrates were basified with 5% Na_2CO_3 to yield precipitations. The precipitations were purified by recrystallization to afford **1**, **2**, **4** and thin layer preparation chromatograph on silica gel (25:1 methylene dichloride-methanol) to afford **3** respectively.

Synthesis of Derivative **5**

Compound **5** was prepared with the treatment of (-)-arctigenin (372 mg, 1 mmol) and anhydrous acetic anhydride (3 ml) and sodium acetate (50 mg, 3 mmol) stirring for 5 h at 40°C. Then, the reaction mixture was acid by 10% hydrochloric acid and the organic materials were extracted with methylene dichloride twice. The extracts were washed with saturated sodium bicarbonate solution, saturated brine and water. And the extracts were dried over with anhydrous sodium sulfate and concentrated in vacuum to afford **5**.

Synthesis of Derivative **6-7**

Compound **6-7** was prepared with the treatment of (-)-arctigenin (372 mg, 1 mmol) with succinic anhydride (200 mg, 2 mmol) and benzoyl chloride (2 ml) were respectively dissolved in methylene dichloride. triethylamine (0.5 ml) was slowly added at room temperature. After stirring for 7 min - 2 h at room temperature, the reaction mixture was acidified with the treatment of 10% hydrochloric acid and the organic materials were extracted with methylene dichloride twice. The extracts were washed with saturated sodium bicarbonate solution, saturated brine and water. And the extracts were dried over with anhydrous sodium sulfate and concentrated in vacuum to afford **6** and **7**.

4-(3", 4"-dimethoxybenzyl)-3-(5'-((dimethylamino) methyl)-4'-hydroxy-3'-methoxybenzyl)-dihydrofuran-2(3H)-one **1**

Yield: 57%; ^{1}H-NMR (500 MHz, $CDCl_3$) : δ (ppm): 2.55 (1H, m, H-2), 2.55 (1H, m, H-3), 3.86 (1H, dd, J = 4.9, 11.3 Hz, H-4a), 4.11 (1H, dd, J = 4.5, 11.2 Hz, H-4b), 6.59 (1H, d, J = 1.9 Hz, H-2'), 6.38 (1H, d, J = 1.8 Hz, H-6'), 2.93 (1H, dd, J = 8.4, 13.8 Hz, H-7'a), 2.85 (1H, dd, J = 6.0, 13.3 Hz, H-7'b), 6.49 (1H, d, J = 1.8 Hz, H-2"), 6.74 (1H, d, J = 8.2 Hz, H-5"), 6.52 (1H, dd, J = 1.8, 8.2 Hz, H-6"), 2.64 (1H, dd, J = 8.4, 13.8 Hz, H-7"a), 2.47 (1H, dd, J = 6.0, 13.3 Hz, H-7"b), 3.79 (9H, s, H-5, 6, 7), 3.61 (2H, q, H-1"') and 2.33 (6H, s, H-2"', 3"'); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ (ppm): 178.8(1), 149.0(4"), 147.9(3'), 147.9(3"), 146.1(4'), 130.5(1"), 128.0(1'), 121.7(5'), 121.3(6'), 120.6(6"),112.2(2'), 112.0(2"), 111.4(5"), 71.3(4), 62.0(1"'), 55.9(5), 55.9(6), 55.6(7), 44.3(2"'), 44.3(3"'), 46.7(2), 41.4(3), 38.2(7") and 34.6(7'). HR-MS: m/z (%) = 430.2167 $[M+H]^+$.

4-(3", 4"-dimethoxybenzyl)-3-(5'-((diethylamino) methyl)-4'-hydroxy-3'-methoxybenzyl)-dihydrofuran-2(3H)-one **2**

Yield: 63%; ^{1}H-NMR (500 MHz, $CDCl_3$) : δ (ppm): 2.55 (1H, m, H-2), 2.55 (1H, m, H-3), 3.86 (1H, dd, J = 2.0, 8.2 Hz, H-4a), 4.11 (1H, dd, J = 7.1, 7.6 Hz, H-4b), 6.58 (1H, d, J = 1.7 Hz, H-2'), 6.35 (1H, d, J = 1.4 Hz, H-6'), 2.94 (1H, dd, J = 5.2, 14.1 Hz, H-7'a), 2.85 (1H, dd, J = 6.8, 14.1 Hz, H-7'b), 6.50 (1H, d, J = 1.9 Hz, H-2"), 6.74 (1H, d, J = 8.2 Hz, H-5"), 6.53 (1H, dd, J = 1.9, 8.1 Hz, H-6"), 2.64 (1H, m, H-7"a), 2.47 (1H, m, H-7"b), 3.85 (3H, s, H-5), 3.82(6H, s, H-6, 7); 3.70 (2H, q, H-1"'), 2.60 (4H, m, H-2"', 3"') and 1.10(6H, t, H-4"', 5"'); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ (ppm): 178.8(1), 149.1(4"), 148.0(3"), 147.9(3'), 146.5(4'), 130.6(1"), 127.7(1'), 122.2(5'), 121.0(6'), 120.6(6"), 111.9(2"), 111.5(5"), 112.0(2'), 71.2(4), 55.9(5), 55.9(6), 56.8(7), 46.7(2), 46.4(2"'), 46.4(3"'), 41.1(3), 38.2(7"), 34.6(7'), 11.3(4"') and 11.3(5"'). HR-MS: m/z (%) = 458.2464 $[M+H]^+$.

4-(3", 4"-dimethoxybenzyl)-3-(5'-((piperazine) methyl)-4'-hydroxy-3'-methoxybenzyl-)-dihydrofuran-2(3H)-one **3**

Yield: 41%; ^{1}H-NMR (500 MHz, $CDCl_3$): δ (ppm): 2.55 (1H, m, H-2), 2.55 (1H, m, H-3), 3.85 (1H, m, H-4a), 4.13 (1H, dd, J = 6.9, 9.7 Hz, H-4b), 6.60 (1H, s, H-2'), 6.36 (1H, s, H-6'), 2.93 (1H, dd, J = 5.2, 14.4 Hz, H-7'a), 2.85 (1H, dd, J = 6.7, 14.5 Hz, H-7'b), 6.50 (1H, d, J = 1.7 Hz, H-2"), 6.76 (1H, d, J = 8.2 Hz, H-5"), 6.54 (1H, dd, J = 1.5, 8.2 Hz, H-6"), 2.65 (1H, m, H-7"a), 2.47 (1H, m, H-7"b), 3.85 (3H, s, H-5), 3.83(3H, s, H-6), 3.82(3H, s, H-7), 3.65 (2H, q, H-1"'), 2.51 (4H, m, H-2"', 3"') and 2.84.(4H, m, H-4"', 5"'); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ (ppm): 178.7(1), 149.1(4"), 147.9(3"), 147.9(3'), 145.8(4'), 130.5(1"), 128.2(1'), 121.3(5'), 121.1(6'), 120.6(6"), 112.1(2'), 112.1(2"), 111.5(5"), 71.2(4), 61.7(1"'), 55.9(5), 55.9(6), 55.9(7), 53.4(2"'), 53.4(3"'),46.7(2), 45.8(4"'), 45.8(5"'), 41.2(3), 38.2(7")and 34.6(7'). HR-MS: m/z (%) = 471.2476 $[M+H]^+$.

4-(3", 4"-dimethoxybenzyl)-3-(5'-((4-hydroylethyl-piperazine)methyl)-4'-hydroxy-3'-methoxybenzyl-)-dihydrofuran-2(3H)-one **4**

Yield: 86%; ^{1}H-NMR (500 MHz, $CDCl_3$): δ (ppm): 2.55 (1H, m, H-2), 2.55 (1H, m, H-3), 3.87 (1H, dd, J = 7.5, 9.2 Hz, H-4a), 4.13 (1H, dd, J = 7.2, 9.2 Hz, H-4b), 6.60 (1H, d, J = 1.4 Hz, H-2'), 6.36 (1H, d, J = 1.1 Hz, H-6'), 2.93 (1H, dd, J = 5.2, 14.4 Hz, H-7"a), 2.85 (1H, dd, J = 6.8, 14.2 Hz, H-7"b), 6.50 (1H, d, J = 1.8 Hz, H-2"), 6.75 (1H, d, J = 8.2 Hz, H-5"), 6.53 (1H, dd, J = 1.8, 8.2 Hz, H-6"), 2.65 (1H, m, H-7"a), 2.47 (1H, m, H-7"b), 3.86 (3H, s, H-5), 3.83(6H, s, H-6, 7), 3.65 (2H, q, H-1"'), 2.56 (8H, t, J = 5.4, 10.7 Hz, H-2"', 3"', 4"', 5"'), 2.61 (2H, m, H-6"') and 3.62(2H, t, J = 5.4, 10.7 Hz, H-7"'); ^{13}C-NMR (CDC_{l3}, 125 MHz): δ (ppm): 178.6(1), 159.0(4"), 147.9(3'), 147.9(3"), 145.7(4'), 130.5(1"), 128.1(1'), 121.2(5'), 121.1(6'), 120.5(6"), 112.1(2'), 112.0(2"), 111.4(5"), 71.2(4), 61.0(1"'), 59.1(6"'), 57.8(7"') , 55.9(5), 55.9(6), 55.9(7), 52.6(2"'), 52.6(3"'), 52.4(4"'), 52.4(5"'), 46.6(2), 41.1(3), 38.1(7") and 34.5(7'). HR-MS: m/z (%) = 515.2693 $[M+H]^+$.

4-(3", 4"-dimethoxybenzyl)-3-(4'-acetoxy-3'-methoxybenzyl)-dihydrofuran-2(3H)-one **5**

Yield: 93%; ^{1}H-NMR (500 MHz, $CDCl_3$): δ (ppm): 2.96 (1H, m, H-2), 2.97 (1H, m, H-3), 3.89 (1H, dd, J = 7.8, 9.0 Hz, H-4a), 4.17 (1H, dd, J = 7.3, 9.0 Hz, H-4b), 6.75 (1H, d, J = 1.6 Hz, H-2'), 6.92 (1H, d, J = 8.0 Hz, H-5'), 6.66 (1H, dd, J = 1.8, 8.0 Hz, H-6'), 2.59 (2H, m, H-7'), 6.49 (1H, d, J = 1.9 Hz, H-2"), 6.77 (1H, d, J = 8.0 Hz, H-5"), 6.53 (1H, dd, J = 1.9, 8.0 Hz, H-6"), 2.60 (2H, m, H-7"), 3.84 (3H, s, H-5), 3.81 (3H, s, H-6), 3.77 (3H, s, H-7) and 2.29 (3H, s, H-2"'); ^{13}C-NMR (CDC_{l3}, 125 MHz): δ (ppm): 178.6(1), 169.0(1"') , 151.2(3'), 149.1(3"), 148.0(4"), 138.7(4'), 136.7(1'), 130.3(1"), 122.7(5'), 121.5(6"), 120.6(6'), 111.5(2'), 113.4(5"), 112.0(2"), 71.3(4), 55.9(5), 55.9(6), 55.9(7), 46.4(2), 41.0(3), 38.2(7"), 34.6(7') and 20.7(2"').

4-(3", 4"-dimethoxybenzyl)-3-(4'-(4'"-carboxyl-propionate-3'-methoxybenzyl-dihydrofuran-2(3H)-one **6**

Yield: 89%; ^{13}C-NMR (CDC_{l3}, 125 MHz): δ (ppm): 178.6(1), 176.9(4"'), 170.3(1"'), 151.1(3'), 149.1(3"), 147.9(4"), 138.6(1'), 136.8(4'), 130.3(1"), 122.5(5'), 122.4(6"), 120.6(6'), 113.4(5"), 112.0(2"), 111.5(2'), 72.3(4), 55.9(5), 55.9(6), 55.8(7), 46.4(2), 41.0(3), 38.1(7"), 34.6(7'), 28.9(3"') and 28.7(2"').

4-(3", 4"-dimethoxybenzyl)-3-(4'-benzoyloxy-3'-methoxybenzyl)-dihydrofuran-2(3H) -one **7**

Yield: 53%; ^{1}H-NMR (500 MHz, $CDCl_3$): δ (ppm): 3.00 (1H, t, H-2), 3.00 (1H, t, H-3), 3.91 (1H, dd, J = 7.2, 9.1 Hz, H-4a), 4.19 (1H, dd, J = 7.7, 9.1 Hz, H-4b), 6.80 (1H, d, J = 1.9 Hz, H-2'), 7.06 (1H, d, J = 8.0 Hz, H-5'), 6.72 (1H, dd, J = 1.9, 8.0 Hz, H-6'), 2.62 (2H, m, H-7'), 6.52 (1H, d, J = 1.9 Hz, H-2"), 6.78 (1H, d, J = 8.1 Hz, H-5"), 6.55 (1H, dd, J = 2.0, 8.2 Hz, H-6"), 2.62 (2H, m, H-7"), 3.85 (3H, s, H-5), 3.83 (3H, s, H-6), 3.76 (3H, s, H-7), 8.19 (1H, dd, J = 8.5, 1.3 Hz, H-3"'), 7.62(1H, m, H-4"'), 7.50(1H, m, H-5"'), 7.50(1H, m, H-6"') and 8.19(1H, dd, J = 8.45, 1.30 Hz, H-7"'); ^{13}C-NMR (CDC_{l3}, 125 MHz): δ (ppm): 178.6(1), 164.7(1"'), 151.5(3'), 149.1(3"), 148.0(4"), 138.9(4'), 136.7(1'), 133.5(1"), 130.6(4"'), 130.4(3"'), 130.3(7"'), 129.4(2"'), 128.9(6"'), 128.6(5"'), 122.8(5'), 121.5(6"), 120.6(6'), 113.5(5"), 112.0(2"), 111.5(2'), 71.3(4), 55.9(5), 55.9(6), 53.4(7), 46.5(2), 46.5(3), 38.2(7") and 34.7(7').

Anticancer Activity of (-)-arctigenin and Synthetic Derivatives

The cytotoxicity assay was carried out on SGC-7901 which was purchased from Institute of Biochemistry and Cell Biology [9]. The cell line was cultured in RPMI-1640 containing 10% (v/v) FBS for 48 h. Each tested compound was dissolved in DMSO at the concentration of 100 mm, then diluted in a tissue culture medium and filtered before use. The cell was seeded in 96 well tissue culture plates and treated with the tested compounds or vehicle (0.1% DMSO) at various concentrations and incubated for 48 h followed by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay at 570 nm with positive control 5-fluorouracil. IC50 values of the tested compounds on cell line were calculated.

Conclusion

(-)-Arctigenin was selected as an excellent precursor for further structure modification to improve its bioavailability and anticancer activity owing to its wide-range biological activities. The synthetic derivatives of **1-7** were obtained with the treatment of secondary amines, anhydride and benzoyl chloride separately. **1-4** were mannich derivatives and **5-7** were O-acylation derivatives. The structures of **1-7** were elucidated with the aid of spectroscopic data, and their anticancer bioactivity was evaluated by human cancer cell SGC7901.

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