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Original article

Synthesis and cytotoxicity evaluation of a novel justicidin G analogue and its phosphate ester

Sheng-Peng Wang [a], Yuan-Feng Tong [a,*], Dong-Mei Wang [a], Nan Wang [b], Zheng Yan [b], Ping Huang [a], Song Wu [a,*]

[a] State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing 100050, China
[b] Beijing Key Laboratory of Active Substance Discovery and Druggability Evaluation, Institute of Materia Medica, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing 100050, China

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ABSTRACT

The novel justicidin G analogue **13** and its phosphate ester **15** were synthesized as potential anticancer agents in several steps starting from commercially available methyl gallate and veratraldehyde. The cytotoxicity of the intermediates was tested against HCT-8, BEL-7402, KETR3, HELA, BGC-823, KB and MCF-7 cell lines by the MTT test, and compound **15** exhibited significant cytotoxicity in HELA and KB cell lines.

1. Introduction

Justicidin G is a natural aryl-naphthalene lignan lactone isolated from the plant of *Justicia procumbens* L., which has shown significant antitumor activity [1–3]. Justicidin G has a similar chemical structure to that of etoposide and teniposide, but displays stronger anticancer activity and fewer side effects and is now being further investigated as a new drug development candidate [4–7]. Considering the poor solubility of justicidin G and the successful structural modification experience of etoposide phosphate ester [8], we made an attempt to synthesis the glycosylated and phosphate ester derivatives of justicidin G. However, the product yields of both derivatives were very low. Moreover, our previous study of justicidinoside B (the glycosylated product of justicidin G) indicated that derivatives with substituents at the 6′-OH position exhibited hindered rotation around the aryl-naphthalene bond, which led to difficult structural modifications and poor chemical stability [9,10]. Hence, we have further investigated the novel justicidin G analogue **13** with a 5′-OH group and its phosphate ester **15**. Compounds **10**–**15** were evaluated for their *in vitro* cytotoxicity against a panel of human tumour cell lines.

2. Experimental

The synthesis of novel justicidin G analogue **13** and its phosphate ester **15** were realized in several steps starting from commercially available methyl gallate and veratraldehyde (Scheme 1). Compound **3** was synthesized in two steps from veratraldehyde in 90% yield [11]. Compound **8** was obtained in four steps from methyl gallate in 81% yield [12]. Treatment of compound **3** with BuLi and compound **8** under nitrogen atmosphere at −78 °C afforded compound **9** in 70% yield [9]. The synthesis of compound **10** was accomplished *via* a Diels–Alder reaction from compound **9** and DEADC under acidic conditions [3]. Compound **10** was reduced with $NaBH_4$ in tetrahydrofuran under refluxing to afford compound **11** in 92% yield [10]. Methylation of compound **11** with CH_3I/K_2CO_3 in acetone under refluxing yielded compound **12** [9]. Compound **13** was prepared by treatment of the compound **12** with 10% Pd/C in methanol at ambient temperature. Treatment of compound **13** with $EtN(i\text{-}Pr)_2/CCl_4$/4-DMAP/$HPO(OBn)_2$ in acetonitrile at −30 °C afforded compound **14** in 92% yield [8]. Compound **15** was synthesized by treatment of the compound **14** with 10% Pd/C in methanol at ambient temperature.

* Corresponding authors.
E-mail addresses: tongyuanf@imm.ac.cn (Y.-F. Tong), ws@imm.ac.cn (S. Wu).

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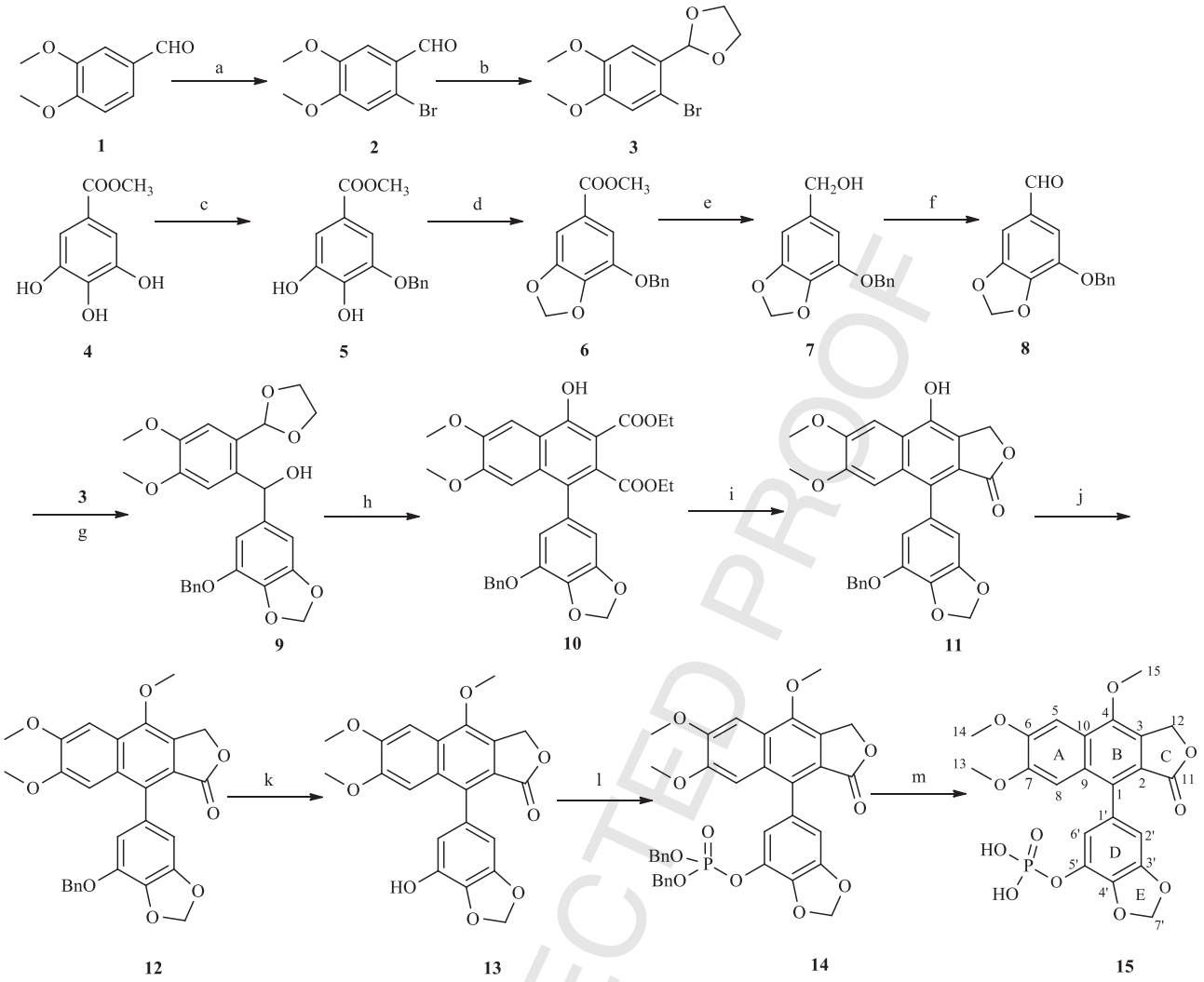

Scheme 1. Synthesis of justicidin G analogue and its phosphate ester. Conditions and reagents: (a) Br_2, CH_3OH, r.t., 12 h, 93%; (b) ethanediol, $TsOH{\cdot}H_2O$, toluene, reflux, 3 h, 96%; (c) NaH, $B(OCH_3)_3$, BnBr, DMF, 6 h, 92%; (d) CH_2Br_2, DMF, 110 °C, 1 h, 96%; (e) $LiAlH_4$, THF, 60 °C, 4 h, 93%; (f) PCC, CH_2Cl_2, 3 h, 95%; (g) *n*-BuLi, THF, −78 °C, 6 h, 70%; (h) DEADC, AcOH, CH_2Cl_2, 140 °C, 12 h, 46%; (i) $NaBH_4$, THF, reflux, 3 h, 92%; (j) CH_3I, K_2CO_3, acetone, reflux, 1 h, 96%; (k) H_2, 10% Pd/C, CH_3OH, r.t., 3 h, 97%; (l) $EtN(i\text{-}Pr)_2$, 4-DMAP, CCl_4, $HPO(OBn)_2$, CH_3CN, −30 °C, 5 h, 92%; (m) H_2, 10% Pd/C, CH_3OH, r.t., 3 h, 93%.

All these compounds were characterized by NMR and HR-ESI-MS analyses [13–15].

3. Results and discussion

Herein, we report a novel and facile synthesis of compounds **13** and **15** in good yields. Compound **10** was a key intermediate for the total synthesis of compound **15**. In a previous study, the reproducibility of the step converting **9** to **10** was less than desirable. In this work we optimized the conditions for this reaction and screened different solvents, amounts of acidic reagent, reaction temperatures and time. We found that the amount of the acidic reagent and reaction temperature were the main parameters affecting the yield of the production of compound **10**. The optimal reaction conditions were found to be 2–3 equiv. of acetic acid under 140 °C in an appropriate volume of methylene chloride, whereas excessive solvent will reduce the product yield.

The cytotoxicity of compounds **10–15** was tested against HCT-8, BEL-7402, KETR3, HELA, BGC-823, KB and MCF-7 cell lines by the MTT method. The results are summarized in Table 1. Justicidin G was used as a control compound. From the screening results, it is evident that all compounds except compound **12** exhibited some level of activity against these cell lines. HCT-8, HELA and KB were more sensitive than BEL-7402 and KETR3 to the justicidin G analogues. It is noteworthy that compound **15** as a phosphate ester not only possessed better water-solubility (3.5 g/100 mL) [16], but also raised its cytotoxicity against HELA and KB to nanomolar level.

Table 1
Cytotoxicity of compounds **10–15** against human tumour cells (IC50, μmol/L).[a]

Compound	Human tumour cells						
	HCT-8	BEL-7402	KETR3	HELA	BGC-823	KB	MCF-7
10	12.5	20.7	38.6	9.9	7.9	11.0	30.3
11	26.1	42.3	29.9	24.1	88.4	42.4	37.9
12	>100	>100	>100	>100	>100	>100	>100
13	46.0	86.1	48.1	7.5	13.1	45.9	>100
14	10.8	81.8	41.0	4.0	62.1	8.0	19.1
15	1.7	18.3	1.7	0.019	1.2	0.0036	9.7
Justicidin G	4.4	8.8	5.4	0.4	1.2	0.9	8.5

[a] Inhibitory concentration (IC50, μmol/L) as obtained by the MTT assay.

4. Conclusion

In conclusion, we have synthesized a novel justicidin G analogue **13** with a 5′-OH group and its phosphate ester **15** for

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the first time, and their structures were characterized by NMR spectroscopy and HR-ESI-MS. The cytotoxicity of compounds **10–15** was evaluated in seven cell lines by the MTT method and the results showed that five of them exhibited good antitumor activity against these cell lines. Moreover, the cytotoxicity of compound **15** against HELA and KB was improved to nanomolar level. This result is helpful in discovering new antitumor agents.

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[13] Data of compounds **3** and **8**. Compound **3**: White solid, mp 95–96 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.74 (s, 3H, –OCH_3), 3.77 (s, 3H, –OCH_3), 3.98–3.89 (m, 2H, C–CH_2–C), 4.14–4.03 (m, 2H, C–CH_2–C), 5.82 (s, 1H, Ar–CH–O), 7.03 (s, 1H, Ar–H), 7.12 (s, 1H, Ar–H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 190.8, 155.2, 149.3, 126.4, 120.0, 116.6, 111.1, 65.5, 63.5, 57.1, 56.3. HRESIMS Calcd. for $C_{11}H_{14}BrO_4$ $[M+H]^+$: 289.0070, found: 289.0064. Compound **8**: White solid, mp 66–68 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.23 (s, 2H, Ar–CH_2–O), 6.14 (s, 2H, O–CH_2–O), 7.09 (s, 1H, Ar–H), 7.61–7.19 (m, 6H, Ar–H×6), 9.75 (s, 1H, –CHO). ^{13}C NMR (100 MHz, DMSO-d_6): δ 191.5, 149.9, 143.2, 141.6, 137.0, 132.1, 129.2, 128.8, 128.6, 113.6, 103.4, 103.1, 71.3. HRESIMS Calcd. for $C_{15}H_{13}O_4$ $[M+H]^+$: 257.0808, found: 257.0799.
[14] Data of compounds **9**–**14**. Compound **9**: White solid, mp 115–116 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.66 (s, 3H, –OCH_3), 3.72 (s, 3H, –OCH_3), 3.97–3.84 (m, 2H, C–CH_2–C), 4.11–3.98 (m, 2H, C–CH_2–C), 5.10 (s, 2H, Ar–CH_2–O), 5.75 (s, 1H, Ar–CH–O), 5.90 (s, 1H, Ar–CH–Ar), 5.92 (s, 2H, O–CH_2–O), 6.47 (s, 1H, Ar–H), 6.70 (s, 1H, Ar–H), 6.92 (s, 1H, Ar–H), 6.97 (s, 1H, Ar–H), 7.48–7.21 (m, 5H, Ar–H×5). ^{13}C NMR (125 MHz, DMSO-d_6): δ 149.7, 148.8, 147.9, 142.1, 140.5, 137.4, 137.1, 134.2, 128.8, 128.4, 128.2, 126.7, 111.0, 109.9, 108.3, 101.5, 101.2, 100.5, 71.0, 69.3, 65.1, 56.1, 56.0. HRESIMS Calcd. for $C_{26}H_{26}NaO_8$ $[M+Na]^+$: 489.1520, found: 489.1512. Compound **10**: White solid, mp 162–164 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.94 (t, 3H, J = 7.1 Hz, –CH_3), 1.25 (t, 3H, J = 7.1 Hz, –CH_3), 3.60 (s, 3H, –OCH_3), 3.93 (s, 3H, –OCH_3), 3.95 (q, 2H, J = 7.1 Hz, C–CH_2–C), 4.33 (q, 3H, J = 7.1 Hz, C–CH_2–C), 5.14 (s, 2H, Ar–CH_2–O), 6.06 (s, 1H, O–CH–O), 6.09 (s, 1H, O–CH–O), 6.45 (s, 1H, Ar–H), 6.56 (s, 1H, Ar–H), 6.71 (s, 1H, Ar–H), 7.48–7.27 (m, 5H, Ar–H×5), 7.63 (s, 1H, Ar–H), 11.92 (s, 1H, Ar–OH). ^{13}C NMR (125 MHz, DMSO-d_6): δ 169.0, 168.6, 157.3, 152.2, 151.5, 149.1, 148.1, 141.6, 136.7, 134.5, 131.4, 130.7, 129.4, 128.4, 127.9, 127.6, 111.7, 105.6, 104.7, 103.2, 102.6, 101.4, 76.9, 70.4, 55.5, 55.2, 52.3, 51.6, 13.6, 13.5. HRESIMS Calcd. for $C_{32}H_{31}O_{10}$ $[M+H]^+$: 575.1912, found: 575.1902. Compound **11**: White solid, mp 206–207 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.58 (s, 3H, –OCH_3), 3.92 (s, 3H, –OCH_3), 5.13 (s, 2H, Ar–CH_2–O), 5.36 (s, 2H, Ar–CH_2–O), 6.09 (s, 2H, O–CH_2–O), 6.53 (s, 1H, Ar–H), 6.62 (s, 1H, Ar–H), 6.90 (s, 1H, Ar–H), 7.46–7.24 (m, 5H, Ar–H×5), 7.62 (s, 1H, Ar–H), 10.50 (s, 1H, Ar–OH). ^{13}C NMR (150 MHz, DMSO-d_6): δ 169.5, 150.5, 149.7, 148.1, 144.9, 141.7, 136.8, 134.7, 129.4, 129.4, 129.3, 128.4, 127.9, 127.7, 123.2, 121.7, 118.7, 111.4, 105.5, 105.0, 101.3, 100.7, 70.4, 66.6, 55.5, 55.1. HRESIMS Calcd. for $C_{28}H_{23}O_8$ $[M+H]^+$: 487.1387, found: 487.1391. Compound **12**: White solid, mp 223–224 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.58 (s, 3H, –OCH_3), 3.93 (s, 3H, –OCH_3), 4.12 (s, 3H, –OCH_3), 5.13 (s, 2H, Ar–CH_2–O), 5.70 (s, 2H, Ar–CH_2–O), 6.10 (s, 2H, O–CH_2–O), 6.54 (s, 1H, Ar–H), 6.62 (s, 1H, Ar–H), 6.89 (s, 1H, Ar–H), 7.45–7.25 (m, 5H, Ar–H×5), 7.50 (s, 1H, Ar–H). ^{13}C NMR (125 MHz, acetone-d_6): δ 168.9, 151.3, 150.1, 148.3, 147.3, 141.8, 136.9, 134.9, 132.6, 129.5, 129.1, 128.4, 128.0, 127.7, 124.9, 123.9, 119.1, 111.4, 105.7, 104.8, 101.4, 100.6, 70.6, 66.8, 59.2, 55.8, 55.3. HRESIMS Calcd. for $C_{29}H_{25}O_8$ $[M+H]^+$: 501.1544, found: 501.1535. Compound **13**: White solid, mp 142–143 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.66 (s, 3H, –OCH_3), 3.93 (s, 3H, –OCH_3), 4.12 (s, 3H, –OCH_3), 5.69 (s, 2H, Ar–CH_2–O), 6.03 (s, 2H, O–CH_2–O), 6.32 (s, 1H, Ar–H), 6.35 (s, 1H, Ar–H), 6.97 (s, 1H, Ar–H), 7.50 (s, 1H, Ar–H), 9.73 (s, 1H, Ar–OH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.6, 151.9, 150.6, 148.9, 147.8, 141.2, 134.2, 133.5, 130.1, 129.4, 125.5, 124.4, 119.6, 113.9, 106.3, 103.2, 101.5, 101.2, 67.4, 59.7, 56.3, 55.9. HRESIMS Calcd. for $C_{22}H_{19}O_8$ $[M+H]^+$: 411.1074, found: 411.1072. Compound **14**: Yellowish solid, mp 157–158 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.60 (s, 3H–OCH_3), 3.95 (s, 3H–OCH_3), 4.14 (s, 3H–OCH_3), 5.14–5.16 (m, 4H, Ar–CH_2–O×2), 5.73 (s, 2H, Ar–CH_2–O), 6.12 (s, 1H, O–CH–O), 6.13 (s, 1H, O–CH–O), 6.68 (s, 1H, Ar–H), 6.81 (s, 1H, Ar–H), 6.92 (s, 1H, Ar–H), 7.39–7.10 (m, 10H, Ar–H×10), 7.52 (s, 1H, Ar–H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 169.4, 151.8, 150.3, 150.2, 148.1, 140.1, 139.5, 139.5, 133.3, 133.3, 130.4, 129.7, 129.7, 126.8, 126.8, 119.9, 119.9, 113.2, 110.9, 106.3, 101.7, 101.4, 98.2, 67.0, 60.2, 56.1, 55.8 ^{31}P NMR (200 MHz, DMSO-d_6): δ −6.28. HRESIMS Calcd. for $C_{36}H_{31}NaO_{11}P$ $[M+Na]^+$: 693.1496, found: 693.1492.
[15] Compound **15**: White solid, mp 212–213 °C. The HRESIMS of **15** established a molecular formula of $C_{22}H_{19}O_{11}P$ [m/z 513.0642 $(M+Na)^+$, Calcd. for $C_{22}H_{19}NaO_{11}P$: 513.0640], indicating 14 degrees of unsaturation. ^{1}H NMR spectrum showed three methoxy groups [δ_H 3.70 (s, 3H, H-13), 3.94 (s, 3H, H-14), 4.13 (s, 3H, H-15)] and four aromatic protons [δ_H 7.51 (s, 1H, H-5), 7.05 (s, 1H, H-8), 6.70 (s, 1H, H-2′), 6.83 (s, 1H, H-6′)]. ^{13}C NMR and DEPT spectra exhibited 22 carbons, including three methyls, two methylenes, sixteen quaternary carbons and one carbonyl. ^{31}P NMR spectrum revealed the existence of one phosphate ester. The HMBC correlations of H-5/C-4, C-7, C-9 and H-8/C-1, C-6, C-10 revealed the present of naphthyl (ring A and ring B). The correlations from H-12 to C-2, C-3, C-4 and C-11 suggested the present of ring C. The correlations of H-13/C-7, H-14/C-6 and H-15/C-4 gave the substituted positions of three methoxy groups. The linkage of ring B and D was elucidated by the HMBC correlations from H-2′ and H-6′ to C-1 and C-1′. The correlations from H-7′ to C-4′ and C-5′ revealed the presence of ring E. The planar structure of 15 was thus established. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.51 (s, 1H, H-5), 7.05 (s, 1H, H-8), 5.71 (s, 2H, H-12), 3.70 (s, 3H, H-13), 3.94 (s, 3H, H-14), 4.13 (s, 3H, H-15), 6.70 (s, 1H, H-2′), 6.83 (s, 1H, H-6′), 6.13 (d, 2H, J = 4.1 Hz, H-7′). ^{13}C NMR (125 MHz, DMSO-d_6): δ 132.3 (C-1), 116.9 (C-2), 119.4 (C-3), 147.8 (C-4), 101.3 (C-5), 151.7 (C-6), 150.5 (C-7), 106.0 (C-8), 128.6 (C-9), 129.7 (C-10), 169.4 (C-11), 67.3 (C-12), 55.7 (C-13), 56.1 (C-14), 59.6 (C-15), 125.3 (C-1′), 107.6 (C-2′), 148.9 (C-3′), 137.2 (C-4′), 148.9 (C-5′), 116.9 (C-6′), 102.1 (C-7′). ^{31}P NMR (200 MHz, DMSO-d_6): δ −5.83.
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