Fitoterapia 106 (2015) 41–45

Contents lists available at ScienceDirect

Fitoterapia

journal homepage: www.elsevier.com/locate/fitote

Cytotoxic and antibacterial activities of the analogues of pogostone

Zheng-wei Tang [a,b,c], Cheng Peng [a,b,c,*], Min Dai [d], Bo Han [a]

[a] College of Pharmacology, Chengdu University of Traditional Chinese Medicine, Chengdu 611137, China
[b] Chengdu University of Traditional Chinese Medicine, State Key Laboratory Breeding Base of Systematic Research, Development and Utilization of Chinese Medicine Resources, Chengdu 611137, China
[c] The Ministry of Education Key Laboratory of Standardization of Chinese Herbal Medicine, Chengdu 611137, China
[d] Department of Pharmacology, Chengdu Medicine College, Chengdu 610065, China

ARTICLE INFO

Article history:
Received 20 June 2015
Received in revised form 5 August 2015
Accepted 6 August 2015
Available online 10 August 2015

Keywords:
Pogostone
Analogues
Synthesis
Antibacterial and cytotoxic activities
MRSA

ABSTRACT

Six new (A5-A6, A8-A11) and six known (A1-A4, A7, PO) α-pyrone compounds were synthesized with dehydroacetate and aldehydes in tetrahydrofuran at room temperature. And their structures were determined by ^{1}H-NMR, ^{13}C-NMR and mass spectroscopy. In the bioscreening experiments, ten compounds (A1-A5, PO, A7-A10) exhibited antibacterial activities against *Staphylococcus aureus* ATCC 25923 with minimum inhibitory concentration (MIC) values of 4–512 mg/L, and nine compounds (A1-A5, PO, A7–A8, A10) exhibited antibacterial activities against Methicillin-resistant *S. aureus* (MRSA) ATCC 43300 with MIC values of 4–256 mg/L. Moreover, compound A10 showed the highest antibacterial activity against *S. aureus* ATCC 25923 and MRSA with MIC values of 4 mg/L, while the MIC values of Amoxicillin were 8 mg/L and >256 mg/L, respectively. Two compounds (A8 and PO) exhibited antibacterial activities against *Escherichia coli* ATCC 25922 with MIC values of 32–512 mg/L. However, only one compound (A8) exhibited significant antibacterial activity against *Pseudomonas aeruginosa* CVCC 3360 with MIC value of 256 mg/L. Moreover, A10 exhibited significant inhibition of proliferation in the four cell lines MCF-10, A549, A2780 and MFC, and showed stronger inhibitive activity of these four selected cell lines than cisplatin in the cytotoxic assay. Thus, this study suggests that pogostone analogues, especially A10, represented a kind of promising antibacterial and antineoplastic agents.

1. Introduction

Since the antibiotics were introduced into clinical practice bacterial pathogens have been developing resistance, which reduces or eliminates the effectiveness of these agents [1,2]. In addition, opportunistic pathogens with innate resistance to antibiotics have become emerging problems, particularly in hospital settings. In developed countries, such as USA, bacterial strains that have acquired multiple drug resistance, include *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* species, and *S. aureus* is the most serious one [3]. For example, methicillin-resistant *S. aureus* (MRSA) was discovered in a healthcare setting and once thought to be a small problem, but now MRSA infections are becoming very common at anywhere. Meanwhile, MRSA have become resistant to the multiple classes of antibiotics (beta lactams, macrolides, quinolones, etc.), including glycopeptides vancomycin, the common drug of last resort [4,5].

Pogostone (PO) is the effective component of the antimicrobial and insecticide activities of *Pogostemonis Herba* [6,7], which has a variety of activities, including anti-inflammatory, analgesic, antipyretic, antiemetic, antimicrobial actions, anti-mutation, immunological regulation, anti-gastric ulcer, insecticide and inhibitory activity on platelet-activating factor (PAF) activation [8–16]. In the previous studies revealed that pogostone had a notable activities against *Candida albicans* and insect [17,18], oral and topical PO administration effectively reduced the bacterial load in vagina of vulvovaginal *S. aureus* mice models [19], and protected mouse from the challenge experiment with lethal dose of *S. aureus*, *Escherichia coli* and MRSA [20,21]. Moreover, the pharmacolinetics assay showed that PO was easily absorbed after oral administration in rat [22,23]. The previous studies manifested that the PO and its analogues may have considerable prospects in the antibacterial application.

As part of our ongoing search of promising new antibacterial compounds, we synthesized pogostone and its analogues. Six new (A5–A6, A8–A11) and six known (A1–A4, A7, PO) α-pyrone compounds were synthesized and their chemical structures were given in Fig. 1. Their potential antimicrobial effects on *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *P. aeruginosa* CVCC 3360 and MRSA ATCC 43300 were evaluated. Additionally, their cytotoxic activities against mammalian tumor cell lines MCF-10, A549, A2780 and MFC were also evaluated.

* Corresponding author at: College of Pharmacology, Chengdu University of Traditional Chinese Medicine, Chengdu 611137, China.

http://dx.doi.org/10.1016/j.fitote.2015.08.003
0367-326X/

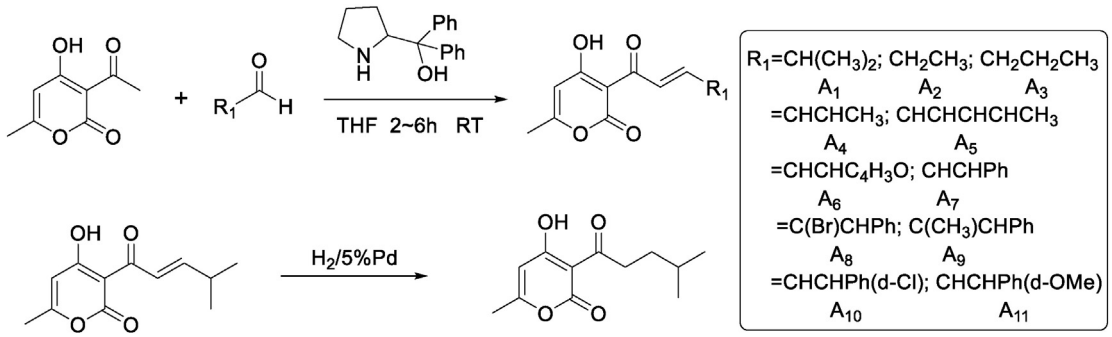

Fig. 1. Synthesis of pogostone and its analogues.

2. Materials and methods

2.1. Chemical synthesis

The synthesis route used to synthesize the pogostone and its analogues is outlined in Fig. 1. Under a nitrogen atmosphere, dehydroacetate (DHA), aldehydes and stirrer were added into the dry tetrahydrofuran (THF), which just rightly could dissolve the solid at a temperature of 5 °C. Briefly, a secondary amine catalyst (NOH) was added into the mixture and stirring for 2 ~ 6 h at the room temperature. Upon completion, the reaction mixture was plated into refrigerator at −4 °C for 30 min, then filtrated and the residue was washed with little ethyl acetate then purified by normal hexane to obtain yellow crystals (A_1–A_{11}).

The yellow crystals were dissolved in ethyl acetate, and transferred in a hydrogenator with 5% Pd–C. The hydrogen was introduced in the hydrogenator and the pressure was maintained at 0.1 Mp for 4 h with constant stirring at room temperature. Upon completion, the reaction mixture was diluted with acetone, filtered through a double-deck quantitative filter paper, followed by washing the paper with the acetone. The liquid was concentrated under reduced pressure and then separated by G_{254} Silicagel column with moving phase (petroleum ether: ethyl acetate = 10:1). The products were concentrated under reduced pressure and purified by n-hexane. There were no significant differences in the cytotoxic and antibacterial activities of these compounds comparing to pogostone, thus the data and compounds did not show.

The resulting compounds PO and A_1–A_{11} were characterized by ^{1}H-NMR, ^{13}C-NMR and mass spectroscopy.

2.2. Bacteria

S. aureus ATCC 25923 (American Type Culture Collection), *E. coli* ATCC 25922, *P. aeruginosa* CVCC 3360 (China Veterinary Culture Collection Center), and Methicillin-resistant *S. aureus* ATCC 43300. Bacteria were cultured in the Trypticase Soy broth (TSB) at 37 °C.

2.3. Carcinoma cell lines

Human breast carcinoma cells MCF-10, human lung carcinoma cells A549, human ovarian carcinoma cells A2780 and mice gastric carcinoma cells MFC. All types of cells were grown in DMEM with 5% fetal calf serum (Hohhot Cao Yuan Lv Ye Bio-engineering Materials Co., Ltd. 140101, Shanghai, China) at 37 °C with 5% CO_2.

2.4. Determination of minimal inhibitory concentrations and minimal bactericidal concentrations

The minimum inhibitory concentration (MIC) of each compound was determined using a broth microdilution assay [24]. The compounds and positive control (Amoxicillin) were diluted in 5% DMSO and added to TSB medium (0.1 mL) with bacterial inoculums (1.0×10^4 CFU per well) to achieve the wanted concentrations. Each compound was determined in triplicate for each inhibitor concentration. The microplates were incubated at 37 °C for 24 h, with shaking at 200 × g. After incubation, the plates were tested with the Multimode Reader (Thermo Scientific Skanlt Software for Varioskan Flash version 2.4.5, USA) at 600 nm. The highest dilution with no bacterial growth was detected and recognized as the MIC. Then, 5 μL solutions with no bacterial growth were added to Mueller-Hinton Agar (MHA) (OXOID LTD., BAINGSTOKE, HAMPSHIRE, ENGLAND) plates and incubated at 37 °C for 24 h for MBC test. The highest dilution with no bacterial growth on MHA plates was identified as the MBC. Blank 5% DMSO without any compounds was diluted at the same time to the highest concentration of the compounds as the blank controls.

2.5. Cell proliferation assay

Cytotoxic activity was evaluated using the CellTiter 96 Aqueous Non-Radioactive Cell Proliferation Assay (MTT) following the manufacturer's instructions using the human breast (MCF-10), lung (A549), ovarian (A2780) and mice gastric (MFC) carcinoma cell lines [25]. All types of cells were grown in DMEM with 5% fetal calf serum, and harvested using Tryspin/EDTA and diluted to 1.0×10^5 cells per well in a 96-well plate. Ten concentrations covering five orders of magnitude were tested with three replicates per concentration. Cells were incubated for 48 h at 37 °C with 5% CO_2. MTT/PBS solution was added to the wells and absorbance was measured at 490 nm four hours later. Data was analyzed using Graphpad Prism version 5.0 (GraphPad Software, La Jolla, CA) using a variable slope inhibition dose response curve. And the cisplatin was the positive control with the same conduction.

3. Results and discussion

3.1. The spectral data of compounds

To the best of our knowledge, although the study on the synthesis of pogostone had been reported [17], this route could raise the yield of pogostone from 4.48% to 58% and reduce the reaction time. Moreover, the reaction could be accomplished at the room temperature. The structures of pogostone and its analogues were shown in Fig. 2. The spectral data of each compound is given below:

4-hydroxy-6-methyl-3-(4-methylpentanoyl)-2H-pyran-2-one (PO): white crystal, yield 58%; ^{1}HNMR ($CDCl_3$, 400 MHz): δ 6.04 (s, 1H), 3.21 (t, J = 7.8 Hz, 2H), 2.51 (s, 3H), 1.61 (m, 1H), 1.48 (m, 2H), 0.89 (d, J = 6.4 Hz, 6H); ^{13}CNMR ($CDCl_3$, 100 MHz): δ 207.64, 181.02, 168.72, 158.97, 100.23, 99.41, 38.64, 31.98, 27.02, 21.56, 21.54, 20.60; ESI HRMS: calcd. For $C_{12}H_{16}O_4$ + Na 224.1049, found 224.1051;

(*E*)-4-hydroxy-6-methyl-3-(4-methylpent-2-enoyl)-2*H*-pyran-2-one (A_1): yellow crystal, yield 82%; ^{1}H-NMR (400 MHz, $CDCl_3$)

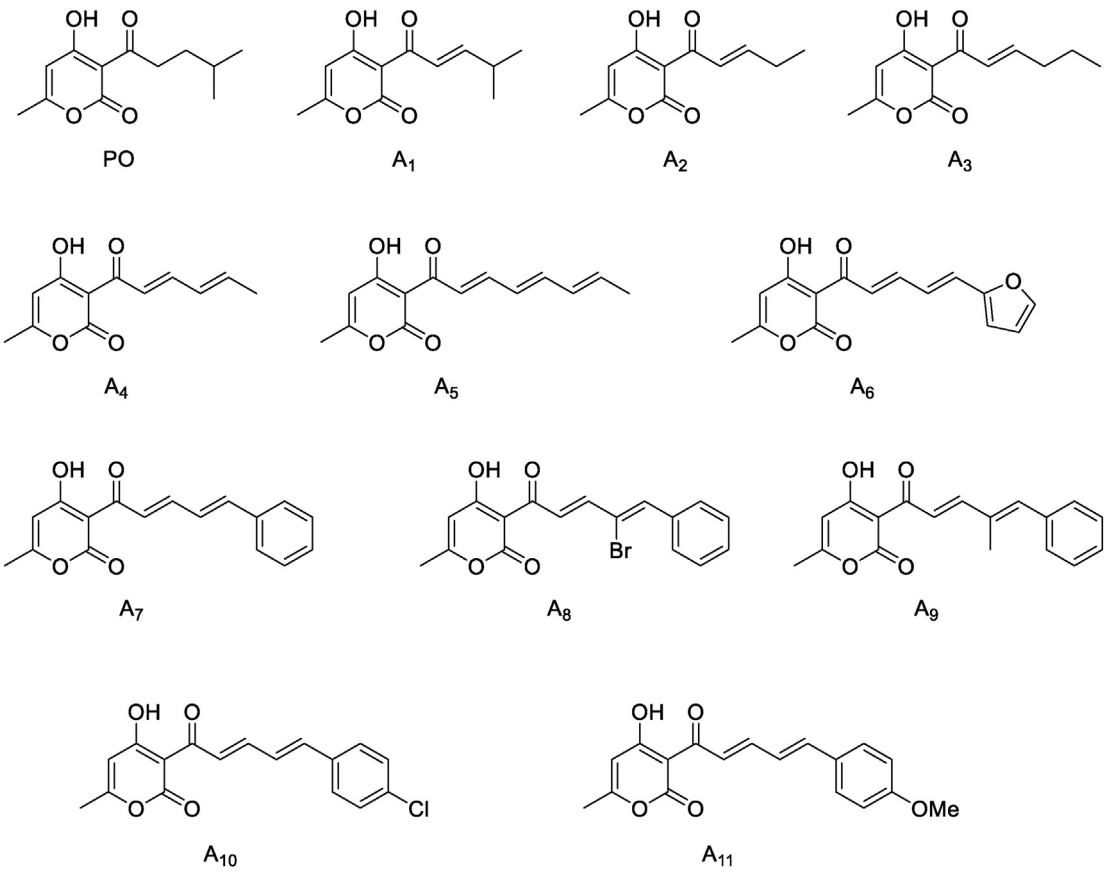

Fig. 2. Structure of pogostone and its analogues.

δ 6.86 (dd, $J_1 = 11.2$ Hz, $J_2 = 6.8$ Hz, 1H), 6.27 (d, $J = 11.2$ Hz, 1H), 6.08 (s, 1H), 2.36 − 2.24 (m, 1H), 2.07 (s, 3H), 1.91 (d, $J = 7.2$ Hz, 3H), 1.78 (d, $J = 7.2$ Hz, 3H) ppm; ^{13}C-NMR (100 MHz, $CDCl_3$) δ 181.23, 174.83, 163.93, 162.44, 152.44, 126.60, 103.41, 100.66, 36.01, 18.29, 17.55, 16.48 ppm; ESI HRMS: calcd. For $C_{12}H_{14}O_4$ + Na 245.0790, found 245.0793;

(*E*)-4-hydroxy-6-methyl-3-(pent-2-enoyl)-2*H*-pyran-2-one (A_2): yellow crystal, yield 88%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 6.81 − 6.79 (m, 1H), 6.37 (d, $J = 11.2$ Hz, 1H), 6.02 (s, 1H), 2.19 (s, 3H), 2.02 − 1.86 (m, 2H), 1.14 (t, 3H) ppm; ^{13}C-NMR (100 MHz, $CDCl_3$) δ 181.16, 175.03, 164.95, 162.80, 152.38, 134.88, 105.87, 103.13, 27.89, 21.48, 17.59 ppm; ESI HRMS: calcd. For $C_{11}H_{12}O_4$ + Na 231.0633, found 231.0629;

(*E*)-3-(hex-2-enoyl)-4-hydroxy-6-methyl-2*H*-pyran-2-one (A_3): yellow crystal, yield 87%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 6.79 − 6.72 (m, 1H), 6.45 (d, $J = 11.2$ Hz, 1H), 6.12 (s, 1H), 2.30 − 2.24 (m, 5H), 1.66 − 1.53 (m, 2H), 0.86 (t, $J = 7.2$ Hz, 3H) ppm; ^{13}C-NMR (100 MHz, $CDCl_3$) δ 184.48, 174.58, 164.08, 162.68, 150.08, 129.44, 106.35, 101.77, 36.43, 25.73, 24.72, 16.52 ppm; ESI HRMS: calcd. For $C_{12}H_{14}O_4$ + Na 245.0790, found 245.0795;

3-((2*E*, 4*E*)-hexa-2,4-dienoyl)-4-hydroxy-6-methyl-2*H*-pyran-2-one (A_4): yellow crystal, yield 81%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.81 (d, $J = 11.2$ Hz, 1H), 7.30 (d, $J = 11.2$ Hz, 1H), 6.62 (d, $J = 11.2$ Hz, 1H), 6.13 (s, 1H), 5.80 − 5.76 (m, 1H), 2.12 (s, 3H), 1.80 (d, $J = 7.2$ Hz, 3H) ppm; ^{13}C-NMR (100 MHz, $CDCl_3$) δ 180.69, 173.68, 162.25, 160.10, 142.24, 134.00, 129.94, 123.37, 101.82, 100.32, 23.16, 19.63 ppm; ESI HRMS: calcd. For $C_{12}H_{12}O_4$ + Na 243.0633, found 243.0625;

4-hydroxy-6-methyl-3-((2*E*, 4*E*, 6*E*)-octa-2, 4, 6-trienoyl)-2*H*-pyran-2-one (A_5): yellow crystal, yield 82%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.25 (dd, $J_1 = 12.4$ Hz, $J_2 = 9.6$ Hz, 1H), 6.77 (d, $J = 12.4$ Hz, 1H), 6.32-6.25 (m, 2H), 6.12 (dd, $J_1 = 12.8$ Hz, $J_2 = 9.2$ Hz, 1H), 5.94 (d, $J = 0.8$ Hz, 1H), 5.83 − 5.76 (m, 1H), 2.25 (s, 3H), 1.88 (d, $J = 7.2$ Hz, 3H) ppm; ^{13}C-NMR (100 MHz, $CDCl_3$) δ 181.27, 175.24, 165.74, 162.42, 137.58, 133.53, 133.44, 128.70, 128.52, 121.37, 95.28, 93.59, 35.26, 22.30 ppm; ESI HRMS: calcd. For $C_{14}H_{14}O_4$ + Na 269.0790, found 269.0792;

3-((2*E*, 4*E*)-5-(furan-2-yl)penta-2,4-dienoyl)-4-hydroxy-6-methyl-2*H*-pyran-2-one (A_6): yellow crystal, yield 85%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.87 (d, $J = 8.0$ Hz, 1H), 7.55 (dd, $J_1 = 12.4$ Hz, $J_2 = 9.6$ Hz, 1H), 7.16 (d, $J = 8.0$ Hz, 1H), 7.02 − 6.88 (m, 3H), 6.48 (dd, $J_1 = 10.0$ Hz, $J_2 = 6.0$ Hz, 1H), 6.13 (s, 1H), 2.21 (s, 3H) ppm; ^{13}C-NMR (100 MHz, $CDCl_3$) δ 175.47, 172.85, 164.76, 163.68, 149.30, 148.99, 137.40, 132.71, 124.57, 120.68, 116.03, 115.16, 102.75, 100.25, 23.77 ppm; ESI HRMS: calcd. For $C_{15}H_{12}O_5$ + Na 295.0582, found 295.0590;

4-hydroxy-6-methyl-3-((2*E*,4*E*)-5-phenylpenta-2,4-dienoyl)-2*H*-pyran-2-one (A_7): yellow crystal, yield 78%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.51 − 7.22 (m, 6H), 6.78 (d, $J = 11.2$ Hz, 1H), 6.28 (dd, $J_1 = 12.4$ Hz, $J_2 = 9.6$ Hz, 1H), 6.18 (d, $J = 12.4$ Hz, 1H), 6.01 (s, 1H), 2.09 (s, 3H) ppm; ^{13}C-NMR (100 MHz, $CDCl_3$) δ 181.42, 175.54, 164.32, 163.64, 150.23, 140.13, 132.84, 129.40, 129.17, 128.97, 128.93, 128.01, 125.27, 120.85, 100.39, 95.82, 20.44 ppm; ESI HRMS: calcd. For $C_{17}H_{14}O_4$ + Na 305.0790, found 305.0794;

3-((2E,4Z)-4-bromo-5-phenylpenta-2,4-dienoyl)-4-hydroxy-6-methyl-2H-pyran-2-one (A_8): yellow crystal, yield 88%. ^{1}H-NMR

(400 MHz, $CDCl_3$) δ 7.59 (d, J = 9.6 Hz, 1H), 7.26 − 7.11 (m, 5H), 6.87 (s, 1H), 6.59 (d, J = 9.6 Hz, 1H), 6.07 (s, 1H), 2.29 (s, 3H) ppm; ^{13}C-NMR (100 MHz, $CDCl_3$) δ 181.41, 174.52, 162.25, 160.10, 144.95, 134.04, 133.14, 130.51, 128.49, 126.34, 126.31, 125.28, 123.37, 115.03, 102.46, 100.86, 19.84 ppm; ESI HRMS: calcd. For $C_{17}H_{13}BrO_4$ + Na 382.9895, found 382.9891;

4-hydroxy-6-methyl-3-((2*E*,4*E*)-4-methyl-5-phenylpenta-2,4-dienoyl)-2*H*-pyran-2-one (A_9): yellow crystal, yield 85%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.49 (d, J = 12.4 Hz, 1H), 7.28 − 7.13 (m, 5H), 6.59 (d, J = 12.4 Hz, 1H), 6.25 (s, 1H), 6.06 (s, 1H), 2.26 (s, 3H), 2.19 (s, 3H) ppm; ^{13}C-NMR (100 MHz, $CDCl_3$) δ 181.32, 175.47, 165.21, 164.48, 155.61, 149.22, 132.71, 131.32, 128.85, 127.86, 127.06, 126.39, 124.57, 122.10, 99.01, 96.84, 23.77, 23.57 ppm; ESI HRMS: calcd. For $C_{18}H_{16}O_4$ + Na 319.0946, found 319.0951;

3-((2*E*,4*E*)-5-(4-chlorophenyl)penta-2,4-dienoyl)-4-hydroxy-6-methyl-2*H*-pyran-2-one (A_{10}): yellow crystal, yield 86%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.40 (d, J = 8.0 Hz, 2H), 6.99 − 6.81 (m, 3H), 6.71 (d, J = 11.2 Hz, 1H), 6.51 − 6.38 (m, 2H), 5.98 (s, 1H), 2.26 (s, 3H) ppm; ^{13}C-NMR (100 MHz, $CDCl_3$) δ 180.99, 173.71, 160.57, 159.03, 148.99, 137.40, 135.26, 134.71, 131.32, 128.06, 124.57, 124.07, 122.09, 119.97, 102.38, 93.64, 23.77 ppm; ESI HRMS: calcd. For $C_{17}H_{13}ClO_4$ + Na 339.0400, found 339.0397; and

4-hydroxy-3-((2*E*,4*E*)-5-(4-methoxyphenyl)penta-2,4-dienoyl)-6-methyl-2*H*-pyran-2-one (A_{11}): yellow crystal, yield 82%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.81 (d, J = 8.0 Hz, 2H), 7.58 (d, J = 11.2 Hz, 1H), 7.28 − 7.20 (m, 3H), 6.89 − 6.77 (m, 2H), 6.17 (s, 1H), 3.60 (s, 3H), 1.89 (s, 3H) ppm; ^{13}C-NMR (100 MHz, $CDCl_3$) δ 179.38, 173.71, 164.38, 162.05, 157.40, 151.82, 141.90, 129.91, 129.10, 127.65, 125.97, 123.58, 113.25, 112.90, 95.42, 93.64, 52.37, 17.74 ppm; ESI HRMS: calcd. For $C_{18}H_{16}ClO_5$ + Na 335.0895, found 335.0890.

3.2. In vitro antibacterial activity of the compounds

We synthesized pogostone and a series of its analogues and evaluated their antibacterial activities. Table 1 summarized the MIC results of the antibacterial assays against bacteria. In contrast to the results that all compounds had no antibacterial activities against *P. aeruginosa*, except A8 (256 mg/L); A5 (8 mg/L) and A10 (4 mg/ L) showed a strong activity against *S. Aureus* including MRSA, 32 and 64 times that of pogostone (256 mg/L), respectively. But A6 and A11 (MIC > 1024 mg/L) showed no appreciable antibacterial activities against all the tested strains. The MBC results in Table 2 showed that the best compounds (A5 and A10) also had significant antibacterial activity against *S. aureus* comparing to PO, with 8 mg/L of A5 and 4 mg/L of A10. As per CLSI standards, a MBC/MIC ratio of 1 to 2 is considered indicative of bactericidal behavior [26]. By contrast, almost of compounds exhibit identical MBC and MIC values (i.e., MBC/MIC ratios of 1 to 2) for *S. aureus*, indicative of a bactericidal mode of action against *S. aureus* as well as MRSA. Both Tables 1 and 2 indicated that the synthesis compounds showed a strong difference between *S. aureus* (Gram positive bacteria) and *E. coli*, *P. aeruginosa* (Gram negative bacteria).

MIC was determined as the lowest concentration that cause complete growth inhibition (bacteriostatic), while the MBC was the lowest concentration that killed 99.9% of the cells (bactericidal). These two kind of antibacterial effects do not exactly share the same mechanism. The mechanism of antibacterial effect can be summarized as the action on the cell (directly or indirectly) wall and plasma membrane, and the synthesis inhibition on bacterial DNA and RNA. Our results showed the antibacterial effects on Gram positive bacteria and Gram negative bacteria were very difference, and there was a huge difference in the composition of Gram positive and negative bacteria. Therefore, we guessed the action on the cell wall may be the mechanism of these compounds and could be confirmed in the further study.

Table 1
Minimal inhibitory concentrations (MICs) against bacteria of compounds (mg/L).

Compound	MIC(mg/L)			
	S. aureus	*E. coli*	*P. aeruginosa*	MRSA
	ATCC 25923	ATCC 25922	CVCC 3360	ATCC 43300
Amoxicillin	0.5	16	>256	>256
Pogostone	256	512	>1024	256
A1	64	1024	>1024	64
A2	128	1024	>1024	256
A3	128	1024	>1024	128
A4	64	>1024	>1024	128
A5	8	>1024	>1024	8
A6	>1024	>1024	>1024	>1024
A7	128	>1024	>1024	128
A8	64	32	256	64
A9	512	>1024	>1024	1024
A10	4	>1024	>1024	4
A11	>1024	>1024	>1024	>1024

Table 2
Minimal bactericidal concentrations (MBCs) against bacteria of compounds (mg/L).

compound	MBC(mg/L)			
	S. aureus	*E. coli*	*P. aeruginosa*	MRSA
	ATCC 25923	ATCC 25922	CVCC 3360	ATCC 43300
Amoxicillin	8	128	>256	>256
Pogostone	256	512	>1024	512
A1	128	>1024	>1024	128
A2	128	>1024	>1024	256
A3	256	>1024	>1024	256
A4	64	>1024	>1024	128
A5	8	>1024	>1024	16
A6	>1024	>1024	>1024	>1024
A7	256	>1024	>1024	256
A8	64	32	256	64
A9	>1024	>1024	>1024	>1024
A10	4	>1024	>1024	8
A11	>1024	>1024	>1024	>1024

3.3. In vitro antiproliferative activity of the compounds

Meanwhile, their cytotoxic activities against the mammalian tumor cell lines were evaluated. This is the first report on the antiproliferative activity evaluation of pogostone and its analogues. The cytotoxic activities of the compounds against human breast (MCF-10), lung (A549), ovarian (A2780) and mice gastric (MFC) carcinoma cell lines were

Table 3
Cytotoxic activities (IC_{50}) of the compounds.

compound	IC_{50}(mg/L)			
	MCF-10	A549	A2780	MFC
Cisplatin	10.21	14.12	21.04	18.06
Pogostone	54.65	48.24	62.16	58.21
A1	112.42	143.52	212.04	>256
A2	142.15	221.38	>256	216.24
A3	110.26	164.28	>256	184.26
A4	68.21	125.48	168.24	88.26
A5	36.52	152.12	44.26	62.10
A6	150.23	86.24	221.04	>256
A7	88.12	92.15	82.64	112.04
A8	18.12	22.41	28.10	26.15
A9	156.41	141.56	>256	210.32
A10	5.24	4.02	4.82	5.41
A11	118.21	212.04	>256	>256

shown in Table 3, noted that all compounds could inhibit the proliferation of cells. PO exhibited significant cytotoxic activity against A549, A2780, MCF-10, and MFC with IC_{50} values range from 48.24 to 62.16 mg/L. A8 (IC_{50} range from 18.12 to 28.10 mg/L) showed a strong activity against four cell lines. Moreover, A10 (IC_{50} range from 4.02 to 5.41 mg/L) exhibited the strongest cytotoxic activities against all the tested cell lines, while the IC_{50} values of the positive control (cisplatin) were range from 10.21 to 21.04 mg/L. Thus, A10 showed a stronger inhibition of proliferation in the four cell lines than cisplatin, and may have considerable prospects in the antineoplastic application. This finding was in accordance with the former research of the α-pyrone compounds (violapyrones H, I, B and C) with notable cytotoxicity [27].

4. Conclusion

The above results on antibacterial activity of PO and its analogues, from the structure-effect relationship perspective, prompted us that the antibacterial activity was related to the function group and its length, which chaining from 3′ position of the pyranoid ketone ring. When a number of function groups were 3 carbon–carbon double bonds (A5), the compound showed a strong antibacterial activity. Especially, when the function group chaining with electron withdrawing group (A8 and A10), the compound would have a stronger antibacterial activity than not. However, when the function group chaining with electron donating group (A6, A9 and A11), the activity was vanished. We found that the cytotoxic activity was very strong, when the compound contain halogen atom (A8 and A10). Taken together, the pogostone analogues, with proper structure modification, may develop as a series of novel anticancer or antibacterial agents.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgments

This work was supported by grant no. 2012JY0114 from the Technological Office of SiChuan Province. The authors deeply appreciated associate professor Bo Han for the assistance of synthesis, and Min Dai for the determination of MIC and MBC.

References

[1] L.B. Rice, Antimicrobial resistance in gram-positive bacteria, Am. J. Med. 119 (6 Suppl. 1) (2006) S11–S19.
[2] C.A. Arias, B.E. Murray, Antibiotic-resistant bugs in the 21st century—a clinical super-challenge, N. Engl. J. Med. 360 (5) (2009) 439–443.
[3] H.W. Boucher, G.H. Talbot, J.S. Bradley, J.E. Edwards, D. Gilert, et al., Bad bugs, no drugs: no ESKAPE! An update from the infections diseases society of America, Clin. Infect. Dis. 48 (1) (2009) 1–12.
[4] F.D. Lowy, Antimicrobial resistance: the example of *Staphylococcus aureus*, J. Clin. Invest. 111 (9) (2003) 1265–1273.
[5] R.L. Lock, E.J. Harry, Cell-division inhibitors: new insights for future antibiotics, Nat. Rev. Drug Discov. 7 (4) (2008) 324–338.
[6] E. Klein, W. Rojahn, Isolation, structure and synthesis of dhelwangin, Tetrahedron Lett. 27 (1969) 2279–2280.
[7] Q.M. Zhou, C. Peng, X.H. Li, L. Guo, L. Xiong, et al., Study on constituents of the aerial parts of Pogostemon Cablin, Zhong Yao Cai 36 (6) (2013) 915–918.
[8] C.Y. Lim, B.Y. Kim, S.H. Lim, S.I. Chao, Effects of Pogostemon cablin Blanco extract on hypoxia induced rabbit cardiomyocyte injury, Pharmacogn. Mag. 11 (42) (2015) 311–319.
[9] S.C. Zhao, Q. Jia, F.L. Liao, The anti-inflammatory and analgesic pharmacological study of Patchouli extract, Chin. Tradit. Plant Med. 29 (2007) 285–287.
[10] Y. Yang, K. Kinoshita, K. Koyama, K. Takahashi, T. Tai, Y. Nunoura, et al., Anti-emetic principles of *Pogostemon cablin* (Blanco) Benth, Phytomedicine 6 (2) (1999) 89–93.
[11] X.R. Liu, R. Fan, Y.Y. Zhang, M.J. Zhu, Study on antimicrobial activities of extracts from *Pogostemon cablin* (Blanco) Benth, Food Sci. Technol. 24 (2009) 220–227.
[12] M. Miyazawa, Y. Okuno, S. Nakamura, H. Kosaka, Antimutagenic activity of flavonoids from Pogostemon cablin, J. Agric. Food Chem. 48 (3) (2000) 642–647.
[13] S.S. Qi, L.P. Hu, W.N. Chen, H.B. Sun, X.D. Ma, Immunological regulation effects of essential oil in leaves of Cablin Patchouli herbal on mice, Chin. Arch. Tradit. Chin. Med. 27 (2009) 774–776.
[14] J.Y. Su, X. Luo, X.J. Zhang, X.L. Deng, Immunosuppressive activity of pogostone on T cells Blocking proliferation via S phase arrest, Int. Immunopharmacol. 26 (2) (2015) 328–337.
[15] H. Chen, H. Liao, Y. Liu, Y. Zhang, X. Wu, et al., Protective effects of pogostone from *Pogostemonis Herba* against ethanol-induced gastric ulcer in rats, Fitoterapia 100 (2015) 110–117.
[16] Y.C. Tsai, H.C. Hsu, W.C. Yang, W.J. Tsai, C.C. Chen, T. Watanabe, Alpha-Bulnesene, a PAF inhibitor isolated from the essential oil of *Pogostemon cablin*, Fitoterapia 78 (1) (2007) 7–11.
[17] Yu-Yang Yi, Jing-Jin He, Jun-Quan Su, Song-Zhi Kong, Ji-Yan Su, et al., Synthesis and antimicrobial evaluation of pogostone and its analogues, Fitoterapia 84 (2013) 135–139.
[18] T.Y. Cao, J.D. Xian, H.F. Wang, Antifeedant activity of pogostone against Sposoptera Litura, J. Environ. Entomol. 35 (5) (2013) 623–628.
[19] Y.X. Zhou, C. Peng, F. Wan, X.Y. Cao, Effect of Patchouli oil on acute bacterial upper respiratory infections model mice, Pharmacol. Clin. Chin. Mater. Med. 30 (4) (2014) 60–61.
[20] F. Peng, F. Wan, L. Xiong, C. Peng, M. Dai, In vitro and in vivo antibacterial activity of pogostone, Chin Med J (Engl) 127 (23) (2014) 40001–40005.
[21] X. Yang, X. Zhang, S.P. Yang, W.Q. Liu, Evaluation of antibacterial activity of Pathouli oil, Iran. J. Pharm. Res. 12 (3) (2013) 307–316.
[22] Y.C. Li, H.C. Liang, H.M. Chen, L.R. Tan, Y.Y. Yi, Z. Qin, et al., Anti-*Candida albicans* activity and pharmacokinetics of pogostone isolated from *Pogostemonis Herba*, Phytomedicine 20 (1) (2012) 77–83.
[23] R.Q. Zhang, Y.X. Li, C. Peng, Pharmacokinetic study of pogostone in rats after oral administration by GC/MS, J. Chromatogr. Sci. 5 (2) (2014) 76–78.
[24] Yun Fei Li, Hong Wu, Rong Gao Sun, Kai Yun Liu, Hua Qi Zhang, et al., Inhibited biofilm formation and improved antibacterial activity of a novel nanoemulsion against cariogenic Streptococcus mutans in vitro and in vivo, Int. J. Nanomedicine 10 (2015) 447–462.
[25] Alan J. Weaver Jr., Joyce B. Shepard, Royce A. Wilkinson, Robert L. Watkins, Sarah K. Walton, et al., Antibacterial activity of THAM Trisphenylguanide against methicillin-resistant *Staphylococcus aureus*, PLoS One 9 (5) (2014) e97742.
[26] CLSI, Methods for Dilution Antimicrobial Sucseptibility Tests for Bacteria That Grow Aerobically, 8th ed. Clinical and Laboratory Standards Institute, Wayne, PA, 2009.
[27] Hee Jae Shin, Hwa-Sun Lee, Jong Seok Lee, Junho Shin, Min Ah Lee, et al., Violapyrones H and I, new cytotoxic compounds isolated from *Streptomyces* sp. Associated with the Marine Starfish Acanthaster planci, Mar. Drugs 12 (2014) 3283–3291.