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Cytotoxic and antibacterial activities of the analogues of pogostone

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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.

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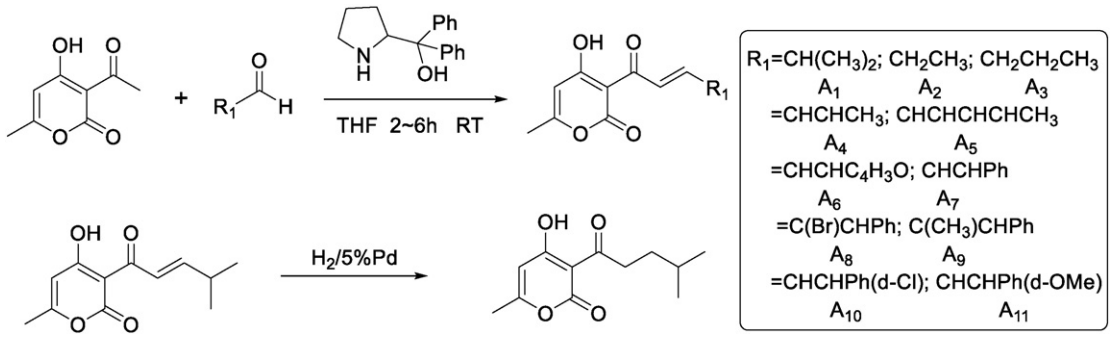

Fig. 1. Synthesis of pogostone and its analogues.

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, $CDCl_3$)