Antitumor and Antibacterial Derivatives of Oridonin: A Main Composition of Dong-Ling-Cao

Dahong Li, Tong Han, Shengtao Xu, Tingting Zhou, Kangtao Tian, Xu Hu, Keguang Cheng, Zhanlin Li, Huiming Hua and Jinyi Xu

Key Laboratory of Structure-Based Drug Design & Discovery, Ministry of Education, School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang 110016, China; lidahong0203@163.com (D.L.; hantong1221@163.com (T.H); tingtingzhou1118@hotmail.com (T.Z.); kangtaotian@126.com (K.T); huxu105@163.com (X.H); lzl1030@hotmail.com (Z.L.)

State Key Laboratory of Natural Medicines and Department of Medicinal Chemistry, China Pharmaceutical University, Nanjing 210009, China; cpuxst@163.com

State Key Laboratory of New-Tech for Chinese Medicine Pharmaceutical Processes, National Post-Doctoral Research Workstation, Jiangsu Kanion Pharmaceutical Co. Ltd., Lianyungang 222001, China

State Key Laboratory for the Chemistry and Molecular Engineering of Medicinal Resources, School of Chemistry and Pharmacy, Guangxi Normal University, Guilin 541004, China

*Correspondence: kgcheng2008@gmail.com (K.C.); huimhua@163.com (H.H.); jinyixu@china.com (J.X.); Tel.: +86-24-2398-6465 (H.H.); +86-25-8327-1445 (J.X.)

Academic Editor: Derek J. McPhee

Received: 5 March 2016; Accepted: 27 April 2016; Published: 30 April 2016

Abstract: Isodon rubescens has been used as a traditional green tea for more than 1000 years and many medicinal functions of I. rubescens are also very useful, such as its well-known antitumor and antibacterial activities. Oridonin, a bioactive ent-kaurane diterpenoid, is the major ingredient of this medicinal tea. Herein, 22 novel oridonin derivatives were designed and synthesized. The antibacterial activity was evaluated for the first time. Compound 12 was the most promising one with MIC of 2.0 µg/mL against B. subtilis, which was nearly 3-fold stronger than positive control chloromycetin. The antiproliferative property was also assayed and compound 19 showed stronger activity than taxol. The apoptosis-inducing ability, cell cycle arrest effect at S phase and influence of mitochondrial membrane potential by 19 in CaEs-17 cancer cells were first disclosed. Based on the above results, the cell apoptosis induced by compound 19 in CaEs-17 cells was most probably involved in the intrinsic apoptotic pathway.

Keywords: Isodon rubescens; medicinal tea; diterpenoid; oridonin; medicinal chemistry

1. Introduction

Isodon rubescens (Chinese name Dong-ling-cao), belongs to the Isodon genus of the Labiatae family. It has been used as a tea drink for more than 1100 years from the Tang Dynasty (AD 618–907) in Taihang Mountain Area, and was recorded in a traditional Chinese book “Qi-xian-zhi” around the year 1660. The leaves are still used as a kind of tea (Figure 1) nowadays, especially in Henan Province of the People’s Republic of China. In the meantime, many medicinal functions of I. rubescens are very useful, such as antitumor and antibacterial activities. It is also a kind of antibiotic and antiphlogistic folk medicine and was first listed in Pharmacopoeia of the People’s Republic of China in the year 1977. The major chemical composition of I. rubescens is diterpenoids, such as oridonin, ponidin, pedalitin, taibairubescensins A and B, xindongnins A and B, and so on [1–8]. Of these, oridonin is the major and most important ingredient of this medicinal tea. Numerous reports have shown that oridonin possesses remarkable antitumor activity both in vitro and in vivo [9–11]. Nevertheless, only a few studies have concerned the antibacterial (only antimycobacterial) activity of oridonin.
Nevertheless, only a few studies have concerned the antibacterial (only antimycobacterial) activity of oridonin or its derivatives, especially the antitumor activity of spirolactone-type 6,7-secokaurane diterpenoids, enmein-type 6,7-secokaurane diterpenoids and their derivatives [14–17]. Only a small number of studies could be found about the antibacterial and antitumor use of the major component oridonin or its derivatives in the field of medicinal chemistry.

For the above reasons, 22 oridonin derivatives were designed and synthesized. The antibacterial activity of oridonin and its derivatives against *Escherichia coli* (ATCC 25922), *Bacillus subtilis* (CMCC 63501), *Staphylococcus aureus* (ATCC 29213), and *Monilia albicans* (ATCC 10231) was evaluated for the first time. The antiproliferative properties were also assayed. The apoptosis-inducing ability, cell cycle arrest effect and influence of mitochondrial membrane potential by the typical derivative 19 in CaEs-17 cancer cells were further disclosed.

2. Results and Discussions

2.1. Synthesis of Compounds 2–12 and 14–24

Compound 13 was synthesized from 1 by selected oxidation with Jones Reagent and no further purification was needed for the next step. Target compounds 2–12 and 14–24 were obtained by treatment of compound 1 or 13 with corresponding acid in the presence of EDCI and DMAP in dichloromethane (DCM) at room temperature for 8–12 h. 14-OH was first regio-selectively esterified in this reaction condition [14,18]. Flash chromatography could only be taken at the last step of the synthetic route of each target compound (Scheme 1).

![Scheme 1. Synthetic routine of compounds 2–12 and 14–24.](image-url)
2.2. Antimicrobial Activity

The antibacterial activity of 1–24 against M. albicans, B. subtilis, S. aureus, and E. coli were evaluated and listed in Table 1. Most of them were active against Gram-negative bacterium S. aureus and B. subtilis. No obvious inhibitory activity (MIC > 100 µg/mL) was observed in any of the synthetic derivatives against fungus M. albicans and Gram-negative bacterium E. coli. Oridonin (1) exhibited moderate potency against S. aureus and B. subtilis with MIC of 31.2 µg/mL. Among the derivatives 2–12, 2 and 10 were weaker than parent compound 1, and the others showed similar or better activity. As for 2–5 with R of aliphatic substituents, 4 was the strongest with R of n-heptyl and the MIC values were 15.6 and 3.9 µg/mL against S. aureus and B. subtilis, correspondingly. The fragments contained fluorine atoms were also introduced into diterpenoids scaffold (6–10 and 18–22), since fluorinated compounds always had unique biological and physicochemical properties, and they were widely spread in modern drugs. The derivatives with 4-trifluoromethylphenyl (6) and mono-fluorine substituted benzyl (7–9) exhibited similar potency and were stronger than that with multiple-fluorine substituted benzyl (10). 12 with R of 2-(1H-indolyl) was the most promising one with MICs of 3.9 and 2.0 µg/mL, respectively, which was similar as positive control chloromycetin against S. aureus and 3-fold stronger against B. subtilis. This confirmed that introducing hetero-N atom contained moistures into the natural lead molecules (usually only obtained C, H and O atoms) could always enhance the bioactivity and/or drug-like properties. 1-Position oxidized oridonin derivative (13) was weaker than oridonin, and the antibacterial activity of its derivatives (14–24) was also not as potent as the corresponding ones (2–12) of oridonin, while similar SARs could be concluded. According to the so called ‘rule of five’ proposed by Lipinski in 1997, Clog p values of less than 5 suggested appropriate membrane permeability for a potential drug candidate. All the designed derivatives showed Clog p values below 5 which could penetrate into cells to some extent and no further relationships between antimicrobial activity and Clog p values could be concluded.

| Compound | E. coli | S. aureus | B. subtilis | M. albicans | Clog p |
|----------|---------|-----------|-------------|-------------|-------|
| 1        | >100    | 31.2      | 31.2        | >100        | 1.70  |
| 2        | >100    | 62.5      | 31.2        | >100        | 0.65  |
| 3        | >100    | 31.2      | 15.6        | >100        | 1.21  |
| 4        | >100    | 15.6      | 3.9         | >100        | 2.35  |
| 5        | >100    | 15.6      | 7.8         | >100        | 1.84  |
| 6        | >100    | 31.2      | 15.6        | >100        | 0.91  |
| 7        | >100    | 31.2      | 15.6        | >100        | 0.91  |
| 8        | >100    | 15.6      | 15.6        | >100        | 0.47  |
| 9        | >100    | 62.5      | 31.2        | >100        | 0.76  |
| 10       | >100    | 15.6      | 7.8         | >100        | 1.96  |
| 11       | >100    | 3.9       | 2.0         | >100        | 1.17  |
| 12       | >100    | 62.5      | 31.2        | >100        | 0.06  |
| 13       | >100    | 15.6      | 15.6        | >100        | 1.96  |
| 14       | >100    | >100      | >100        | >100        | 1.70  |
| 15       | >100    | 62.5      | 31.2        | >100        | 2.26  |
| 16       | >100    | 15.6      | 7.8         | >100        | 3.40  |
| 17       | >100    | 15.6      | 15.6        | >100        | 2.89  |
| 18       | >100    | 62.5      | 15.6        | >100        | 2.70  |
| 19       | >100    | 31.2      | 15.6        | >100        | 1.96  |
| 20       | >100    | 31.2      | 15.6        | >100        | 1.96  |
| 21       | >100    | 31.2      | 15.6        | >100        | 1.52  |
| 22       | >100    | >100      | 62.5        | >100        | 1.81  |
| 23       | >100    | 31.2      | 7.8         | >100        | 3.01  |
| 24       | >100    | 3.9       | 3.9         | >100        | 2.22  |
| chloromycetin | 3.9  | 3.9       | 7.8         | NT          |       |
| fluconazole | NT     | NT        | 3.91        | NT          |       |

1 NT, not test; 2 Clog p values were calculated by ChemBioDraw Ultra 12.0 (ChemBridge Corp., San Diego, CA, USA).
2.3. Antiproliferative Activity

The antiproliferative activity of the derivatives together with their corresponding parent compounds 1 and 13 was evaluated against Bel-7402, K562, MGC-803 and CaEs-17 cells, and the results were listed in Table 2. All the synthetic derivatives showed better antiproliferative activity than their parent compounds 1 and 13. 1-Position oxidized oridonin analogue (13) showed stronger antiproliferative activity than oridonin (1) against four selected cell lines with IC\textsubscript{50} values of 2.98, 4.34, 3.98 and 7.23 µM. Most of the derivatives (14–24) of 13 with bigger Clog \( p \) values were also stronger than corresponding derivatives of 1. Similar SARs could be concluded from the derivatives of 1 and 13. Among 14–17 with R of aliphatic substituents, 17 with 1-adamantyl group showed bigger IC\textsubscript{50} values against four tested cell lines than the other three with R of cyclopentyl, cyclohexyl or n-heptyl. Other pharmacophores like indolyl were used as well and 24 with R of 2-(1H-indolyl) showed the most potent cytotoxicity of all tested compounds against Bel-7402 cells with IC\textsubscript{50} value of 0.81 µM. Therefore, in our further research, more derivatives with hetero-N atom will be designed and synthesized. Among the mono-fluorine substituted benzyl derivatives (19–21), 19 with 4-fluorophenyl substituent exhibited the strongest activity with IC\textsubscript{50} values of 0.98, 0.29, 0.60, and 0.22 µM, correspondingly. The value against CaEs-17 cell line was the smallest one among all the derivatives. So, compound 19 was selected for further mechanism study against CaEs-17 cells.

| Compound | Bel-7402 | K562 | MGC-803 | CaEs-17 |
|----------|----------|------|---------|---------|
| 1        | 7.48 ± 0.53 | 4.76 ± 0.32 | 5.69 ± 0.39 | 11.03 ± 1.02 |
| 2        | 1.18 ± 0.03 | 2.03 ± 0.17 | 1.18 ± 0.21 | 3.36 ± 0.22 |
| 3        | 1.01 ± 0.04 | 1.97 ± 0.18 | 1.12 ± 0.10 | 3.25 ± 0.31 |
| 4        | 0.96 ± 0.06 | 1.83 ± 0.23 | 1.08 ± 0.06 | 3.20 ± 0.29 |
| 5        | 0.97 ± 0.11 | 1.84 ± 0.36 | 1.14 ± 0.23 | 3.16 ± 0.37 |
| 6        | 1.63 ± 0.81 | 0.25 ± 0.02 | 0.81 ± 0.10 | 0.61 ± 0.13 |
| 7        | 1.07 ± 0.52 | 0.31 ± 0.04 | 0.37 ± 0.04 | 0.43 ± 0.10 |
| 8        | 1.13 ± 0.14 | 0.37 ± 0.07 | 0.61 ± 0.01 | 0.28 ± 0.22 |
| 9        | 1.39 ± 0.72 | 0.59 ± 0.19 | 1.03 ± 0.55 | 0.29 ± 0.04 |
| 10       | 3.80 ± 0.92 | 2.66 ± 0.13 | 4.02 ± 0.75 | 7.23 ± 1.03 |
| 11       | 0.90 ± 0.02 | 1.87 ± 0.07 | 1.37 ± 0.09 | 3.92 ± 0.36 |
| 12       | 0.82 ± 0.22 | 1.74 ± 0.23 | 1.12 ± 0.17 | 3.63 ± 0.29 |
| 13       | 2.98 ± 0.14 | 4.34 ± 0.04 | 3.98 ± 0.66 | 7.23 ± 0.73 |
| 14       | 0.98 ± 0.06 | 1.77 ± 0.13 | 1.31 ± 0.14 | 2.72 ± 0.30 |
| 15       | 0.93 ± 0.11 | 1.76 ± 0.24 | 1.08 ± 0.09 | 2.56 ± 0.25 |
| 16       | 0.95 ± 0.07 | 1.81 ± 0.24 | 1.09 ± 0.13 | 3.13 ± 0.22 |
| 17       | 0.99 ± 0.10 | 1.91 ± 0.45 | 1.17 ± 0.15 | 3.46 ± 0.44 |
| 18       | 2.81 ± 0.41 | 0.86 ± 0.03 | 1.02 ± 0.38 | 0.89 ± 0.30 |
| 19       | 0.98 ± 0.05 | 0.29 ± 0.05 | 0.60 ± 0.40 | 0.22 ± 0.09 |
| 20       | 1.66 ± 0.39 | 0.35 ± 0.06 | 0.87 ± 0.05 | 0.64 ± 0.11 |
| 21       | 1.45 ± 0.42 | 0.47 ± 0.01 | 0.90 ± 0.22 | 0.51 ± 0.03 |
| 22       | 3.17 ± 0.65 | 2.16 ± 0.37 | 3.94 ± 0.71 | 8.55 ± 0.80 |
| 23       | 1.07 ± 0.13 | 1.72 ± 0.24 | 1.25 ± 0.16 | 3.62 ± 0.18 |
| 24       | 0.81 ± 0.08 | 1.66 ± 0.26 | 1.09 ± 0.24 | 3.57 ± 0.16 |
| Taxol \(^1\) | 1.89 ± 0.09 | 0.41 ± 0.02 | 0.85 ± 0.06 | 0.43 ± 0.03 |

\(^1\) Taxol was used as a positive control. Data were means ± SD of three experiments.

2.4. Apoptosis-Inducing Ability of 19 in CaEs-17 Cell Line

Apoptosis is a process of programmed cell death, and cancer cells usually have an abnormal ability of proliferation, mainly due to the defective apoptosis. Thus, activation of apoptosis could reduce the accumulation of cancer cells. In order to examine the involvement, at least in part, of apoptosis in the loss of cancer cell viability of compound 19 in CaEs-17 cells, an annexin V-FITC/PI binding
assay was carried out. CaEs-17 cells were treated with different concentrations of 19 and percentages of apoptotic cells were determined by flow cytometry. As shown in (Figure 2), 19 exhibited potent dose-dependent activity in the induction of apoptosis. Treatment of CaEs-17 cells with 19 at 0.125, 0.25, and 0.5 µM, apoptotic cell rates (early and late) were 20.83, 30.94, and 53.15%, as compared with 6.11% in an untreated vehicle control, indicating that 19 was able to induce apoptotic cell death in CaEs-17 cells.

Figure 2. Apoptosis-inducing ability of compound 19 in CaEs-17 cell line.

2.5. Cell Cycle Effect on CaEs-17 Cells by 19

The cell cycle is the series of events that take place in a cell, leading to its division and duplication (replication). The apoptosis inducing activity of compound 19 was also characterized by flow cytometric analysis of the DNA profile. CaEs-17 cells were treated with compound 19 at concentrations of 0.125, 0.25, and 0.5 µM, which resulted in accumulation of 29.95%, 43.43%, and 57.52% of cells at the S phase, respectively, compared with the untreated cells of 27.31% (Figure 3). There were also a decline of G1 phase cells of 59.89%, 48.23%, and 36.56%, and G2 phase cells of 10.15%, 8.34% and 5.92%, respectively. So, compound 19 influenced cell-cycle progression of CaEs-17 cells at low sub-micromolar concentrations and arrested at S phase.

2.6. Effect on Mitochondrial Membrane Potential

In order to determine the involvement of mitochondrial in compound 19 mediated apoptosis in CaEs-17 cells, the changes on mitochondrial membrane potential were monitored by flow cytometry. CaEs-17 cells were incubated with different concentrations (0, 0.125, 0.25, and 0.5 µM) of compound 19 and the percentage of apoptotic cells increased in a concentration-dependent fashion (2.85%, 13.70%, 22.70% and 34.16%, respectively, Figure 4). These results indicated that the treatment of compound 19 in CaEs-17 cells triggered the collapse of mitochondrial membrane potentials and induced apoptosis.
Figure 3. Cell cycle effect on CaEs-17 cells of compound 19.

Figure 4. Effect on mitochondrial membrane potential of CaEs-17 cells by compound 19.
3. Materials and Methods

3.1. Chemistry

3.1.1. General

All the solvents and chemicals were purchased from Yuwang Chemical Industries, Ltd. (Shenyang, China) and not further purified. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and fetal bovine serum (FBS) were bought from Sigma Aldrich (Sigma Chemical Co., Shanghai, China). Column chromatography was performed with silica gel (200–300 mesh, Qingdao Ocean Chemical Co. Ltd., Qingdao, China). TLC plates were precoated with silica gel GF 254 (Qingdao Ocean Chemical Co. Ltd.). Melting points (m.p.) were measured with XT-4 micro melting point apparatus and uncorrected. NMR was recorded with a Bruker AV-300 or AV-500 spectrometer (Bruker Corp., Karlsruhe, Germany) using TMS as an internal standard. Mass spectra were obtained using FTMS-2000 (Thermo Fisher Scientific, Waltham, MA, USA).

3.1.2. General Procedure to Synthesize 2–12 and 14–24

Compound 13 (72 mg, 0.2 mmol) was synthesized from 1 by oxidation with Jones Reagent and no further purification was needed for the next step [14]. Compound 1 or 13 (72 mg, 0.2 mmol) was dissolved in 10 mL of dichloromethane and mixed with corresponding acid (0.24 mmol), EDCI and DMAP. The reaction was stirred at room temperature and monitored by TLC. After 6–8 h, the mixture was poured into 10 mL of 10% HCl, and then extracted with DCM three times (each 10 mL). The organic layer was combined, washed with water and saturated brine, sequentially, then dried over anhydrous Na2SO4, and concentrated in vacuo. The crude product was purified by column chromatography (MeOH/DCM 1:150 v/v) to give the target compounds 2–12 and 14–24.

Compound 19: white solid, 24% yield, m.p. 114–116 °C; IR (KBr) υmax 3404, 2957, 2361, 1707, 1643, 1604, 1508, 1458, 1280, 1242, 1155, 1051, 965, 909 cm⁻¹; 13C-NMR (DMSO-d6, 125 MHz), δ (ppm) 211.47, 205.02, 166.92, 164.25, 149.59, 132.39, 132.31, 126.06, 122.07, 115.67, 115.50, 96.91, 74.91, 73.46, 64.93, 61.50, 59.87, 50.78, 48.59, 41.63, 38.41, 35.73, 32.84, 30.49, 30.06, 23.14, 19.20; 1H-NMR (CDCl3, 500 MHz), δ (ppm) 7.95 (2H, m, Ar-H), 7.06 (2H, d, J = 8.7 Hz, Ar-H), 6.30 (1H, s, 17-CH2), 6.10 (1H, s, 14-CH), 5.64 (1H, s, 17-CH2), 5.48 (1H, d, J = 11.5 Hz, 6-OH), 4.34, 4.03 (each 1H, d, J = 9.9 Hz, 13-CH); MS (ESI) m/z: 485.2 [M + H]+, 507.2 [M + Na]+, 523.2 [M + K]+.

3.2. Biology

3.2.1. Antibacterial Assay

The antibacterial activity against *M. albicans*, *S. aureus*, *B. subtilis*, and *E. coli* were evaluated. Generally, the minimal inhibitory concentrations (MICs) were measured by micro-broth dilution method. The stock solution of target compounds was diluted and added into microtitration plates. The specified concentration of fungus or bacterium was added and incubated. After that, MTT solution was added, and the absorbance at 570 nm was measured. Taxol was used as a positive control.

3.2.2. MTT Assay

Bel-7402 (human hepatoma), K562 (human leukemia), MGC-803 (human esophageal cancer), and CaEs-17 (human gastric cancer) cell lines were obtained from the American Type Culture Collection (Manassas, VA, USA). The cell lines were cultured in RPMI 1640 or DMEM with high glucose or low glucose supplemented with 10% FBS at 37 °C in 5% CO2. The antiproliferative activity was performed by the MTT method [21,22]. In brief, Bel-7402 cells were placed in 96-well plates and incubated for 24 h. Concentrations of the compounds (purity > 98%) were added and incubated for another 96 h. After that, MTT solution was added, and the absorbance at 570 nm was measured. Taxol was used as a positive control.
as the positive control. The results were obtained from three independent experiments carried out in duplicate. All measurements of antiproliferative activities were repeated in triplicate and data were expressed as mean ± SD (standard deviations). An ANOVA test using SPSS 16.0 (Statistical Program for Social Sciences, SPSS Inc., Chicago, IL, USA) was used to analyze the experimental data.

3.2.3. Cell Apoptosis

The CaEs-17 cells were incubated with compound 19 at certain concentrations for 3 days and apoptosis was analyzed using Annexin-V and propidium iodide (PI) double staining method by flow cytometry similar as previous reports [23] according to operation manual.

3.2.4. Effect of Cell Cycle

CaEs-17 cells were plated in 6-well plates and incubated for 24 h, and then certain concentrations of compound 19 were added. After 48 h treatment, cells were fixed with 70% ethanol, treated with RNase, and stained with PI. DNA content was measured using a flow cytometer [24].

3.2.5. Mitochondrial Membrane Potential

CaEs-17 cells were cultured overnight and incubated with the test compound or vehicle in triplicate for 48 h. The cells were stained with the lipophilic cationic dye (JC-1), according to the manufacturer’s instruction (Keygen, KGA601, Nanjing, China). The percentage of cells with healthy or collapsed mitochondrial membrane potentials was monitored by flow cytometry [25].

4. Conclusions

In this study, 22 derivatives (2–12 and 14–24) of oridonin (1) and 1-position oxidized oridonin (13) were designed and synthesized. All the title compounds were first evaluated for their antimicrobial activity against M. albicans, B. subtilis, S. aureus and E. coli, and antiproliferative activity against Bel-7402, K562, MGC-803 and CaEs-17 cells. Preliminary SARs were also determined. Compound 12 with R of 2-(1H-indolyl) was the most promising antimicrobial candidate with MICs of 3.9 and 2.0 µg/mL against S. aureus and B. subtilis, respectively. Derivative 19 with R of 4-fluorophenyl showed the smallest IC_{50} value of 0.22 µM among all the derivatives against CaEs-17 cell line, which was almost 50-fold stronger than lead oridonin (1) and nearly 1-fold more potent than taxol (0.43 µM). The apoptosis-inducing effect of 19 was further investigated using CaEs-17 cells. It was found that 19 arrested CaEs-17 cell cycle at S phase and incubation with 19 induced apoptosis and mitochondrial depolarization at low sub-micromolar concentrations in dose-dependent manner. Apoptosis was divided into intrinsic and extrinsic pathways. The intrinsic pathway mainly depended on the loss of mitochondria membrane potential. Therefore, the cell apoptosis induced by 19 in CaEs-17 cells was most probably involved in the intrinsic apoptotic pathway. It was expected that the remarkable biological profile of oridonin analogs made them possible as promising candidates for the development of novel agents from functional foods.

Supplementary Materials: Supplementary materials of NMR spectra of 1, 13 and 19, and spectra data of other target compounds could be found online at: http://www.mdpi.com/1420-3049/21/5/575/s1.

Acknowledgments: This work was financially supported by the National Natural Science Foundation of China (81373280, 21502121), Project Funded by China Post-Doctoral Science Foundation (2015M570258), General Scientific Research Projects of Department of Education in Liaoning Province (L2014382), Key Laboratory for the Chemistry and Molecular Engineering of Medicinal Resources (Guangxi Normal University), Ministry of Education of China (CMEMR2013-B07) and Career Development Support Plan for Young and Middle-aged Teachers in Shenyang Pharmaceutical University.

Author Contributions: D.L., H.H. and J.X. conceived and designed the experiments; D.L., T.H., T.Z., K.T., and X.H. performed the experiments, D.L., K.C. and Z.L. analyzed the data; D.L., S.X., H.H., and J.X. wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest.
Abbreviations

The following abbreviations are used in this manuscript:

- SAR: Structure Activity Relationship
- $^{1}$H-NMR: Proton Nuclear Magnetic Resonance
- TMS: Tetramethyilsilane
- MS: Mass Spectrometry
- ESI: Electrospray Ionization
- DCM: Dichloromethane
- MTT: 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
- FBS: Fetal Bovine Serum
- MIC: Minimal Inhibitory Concentration
- NT: Not Test

References

1. Liu, X.; Yang, J.; Wang, W.G.; Li, Y.; Wu, J.Z.; Pu, J.X.; Sun, H.D. Diterpene alkaloids with an aza-$\alpha$-kaurene skeleton from Isodon rubescens. *J. Nat. Prod.* 2015, 78, 196–201. [CrossRef] [PubMed]
2. Liu, X.; Zhan, R.; Wang, W.G.; Du, X.; Li, X.N.; Yang, J.H.; Zhang, P.; Li, Y.; Pu, J.X.; Wu, J.Z.; et al. A new diterpene glycoside from Rabdosia rubescens. *Chem. Pharm. Bull.* 2013, 61, 90–95. [CrossRef] [PubMed]
3. Liu, X.; Wang, W.G.; Du, X.; Li, X.N.; Kong, L.M.; Li, Y.; Pu, J.X.; Wu, J.Z.; Sun, H.D. Enmein-type diterpenoids from the aerial parts of Isodon rubescens and their cytotoxicity. *Fitoterapia* 2012, 83, 1451–1455. [CrossRef] [PubMed]
4. Zou, J.; Pan, L.; Li, Q.; Pu, J.; Yao, P.; Zhu, M.; Banas, J.A.; Zhang, H.; Sun, H. Rubesanolides C-E: abietane diterpenoids isolated from Isodon rubescens and evaluation of their anti-biofilm activity. *Org. Biomol. Chem.* 2012, 10, 5039–5044. [CrossRef] [PubMed]
5. Zou, J.; Pan, L.; Li, Q.; Zhao, J.; Pu, J.; Yao, P.; Gong, N.; Lu, Y.; Kondratyuk, T.P.; Pezzuto, J.M.; et al. Rubesanolides A and B: Diterpenoids from Isodon rubescens. *Org. Lett.* 2011, 13, 1406–1409. [CrossRef] [PubMed]
6. Huang, S.X.; Xiao, W.L.; Li, L.M.; Li, S.H.; Zhou, Y.; Ding, L.S.; Lou, L.G.; Sun, H.D. Bisrubescensins A–C: Three new dimeric ent-kauranoids isolated from Isodon rubescens. *Org. Lett.* 2006, 8, 1157–1160. [CrossRef] [PubMed]
7. Liu, H.M.; Yan, X.; Kiuchi, F.; Liu, Z. A new diterpene glycoside from Rabdosia rubescens. *Chem. Pharm. Bull.* 2000, 48, 148–149. [CrossRef] [PubMed]
8. Li, B.L.; Chen, S.N.; Shi, Z.X.; Chen, Y.Z. ent-Kaurene diterpenoids from Isodon rubescens. *Phytochemistry* 2000, 53, 855–859. [CrossRef]
9. Zhao, Z.; Chen, Y. Oridonin, a promising antitumor natural product in the chemotherapy of hematological malignancies. *Curr. Pharm. Biotechnol.* 2014, 15, 1083–1092. [CrossRef] [PubMed]
10. Liu, Z.; Ouyang, L.; Peng, H.; Zhang, W.Z. Oridonin: Targeting programmed cell death pathways as an anti-tumour agent. *Cell Prolif.* 2012, 45, 499–507. [CrossRef] [PubMed]
11. Li, C.Y.; Wang, E.Q.; Cheng, Y.; Bao, J.K. Oridonin: An active diterpenoid targeting cell cycle arrest, apoptotic and autophagic pathways for cancer therapeutics. *Int. J. Biochem. Cell B.* 2011, 43, 701–704. [CrossRef] [PubMed]
12. Xu, S.; Pei, L.; Li, D.; Yao, H.; Cai, H.; Yao, H.; Wu, X.; Xu, J. Synthesis and antimycobacterial evaluation of natural oridonin and its enmein-type derivatives. *Fitoterapia* 2014, 99, 300–306. [CrossRef] [PubMed]
13. Xu, S.; Li, D.; Pei, L.; Yao, H.; Wang, C.; Cai, H.; Yao, H.; Wu, X.; Xu, J. Design, synthesis and antimycobacterial activity evaluation of natural oridonin derivatives. *Bioorg. Med. Chem. Lett.* 2014, 24, 2811–2814. [CrossRef] [PubMed]
14. Wang, L.; Li, D.; Xu, S.; Cai, H.; Yao, H.; Zhang, Y.; Jiang, J.; Xu, J. The conversion of oridonin to spirolactone-type or enmein-type diterpenoid: synthesis and biological evaluation of ent-6,7-seco- oridonin derivatives as novel potential anticancer agents. *Eur. J. Med. Chem.* 2012, 52, 242–250. [CrossRef] [PubMed]
15. Li, D.; Cai, H.; Jiang, B.; Liu, G.; Wang, Y.; Wang, L.; Yao, H.; Wu, X.; Sun, Y.; Xu, J. Synthesis of spirolactone-type diterpenoid derivatives from kaurene-type oridonin with improved antiproliferative effects and their apoptosis-inducing activity in human hepatoma Bel-7402 cells. Eur. J. Med. Chem. 2013, 59, 322–328. [CrossRef] [PubMed]

16. Li, D.; Xu, S.; Cai, H.; Pei, L.; Wang, L.; Wu, X.; Yao, H.; Jiang, J.; Sun, Y.; Xu, J. Library construction and biological evaluation of enmein-type diterpenoid analogues as potential anticancer agents. Chem. Med. Chem. 2013, 8, 812–818. [CrossRef] [PubMed]

17. Li, D.; Xu, S.; Cai, H.; Pei, L.; Zhang, H.; Wang, L.; Yao, H.; Wu, X.; Jiang, J.; Sun, Y.; et al. Enmein-type diterpenoid analogs from natural kaurene-type oridonin: Synthesis and their antitumor biological evaluation. Eur. J. Med. Chem. 2013, 64, 215–221. [CrossRef] [PubMed]

18. Fujita, E.; Nagao, Y.; Kohno, T.; Matsuda, M.; Ozaki, M. Antitumor activity of acylated oridonin. Chem. Pharm. Bull. 1981, 29, 3208–3213. [CrossRef] [PubMed]

19. Bahadori, M.B.; Valizadeh, H.; Asghari, B.; Dinparast, L.; Farimani, M.M.; Bahadori, S. Chemical composition and antimicrobial, cytotoxicity, antioxidant and enzyme inhibitory activities of Salvia spinosa L. J. Funct. Foods 2015, 18, 727–736. [CrossRef]

20. Liao, J.; Yang, F.; Zhang, L.; Chai, X.; Zhao, Q.; Yu, S.; Zou, Y.; Meng, Q.; Wu, Q. Synthesis and biological evaluation of novel fluconazole analogues bearing 1,3,4-oxadiazole moiety as potent antifungal agents. Arch. Pharm. Res. 2015, 38, 470–479. [CrossRef] [PubMed]

21. Qiao, A.; Wang, Y.; Xiang, L.; Zhang, Z.; He, X. Novel triterpenoids isolated from hawthorn berries functioned as antioxidant and antiproliferative activities. J. Funct. Foods 2015, 13, 308–313. [CrossRef] [PubMed]

22. Gurkan-Alp, A.S.; Gök, H.; Alp, M.; Ozkan, T.; Sunguroglu, A. Synthesis and anticancer effects of some novel 2-(4-phenoxyphenyl)-1H-benzimidazole derivatives on K562 cell line. Arch. Pharm. Res. 2015, 38, 650–658. [CrossRef] [PubMed]

23. Yan, X.; Yu, Y.; Ji, P.; He, H.; Qiao, C. Antitumor activity of endoperoxide-iron chelator conjugates-design, synthesis and biological evaluation. Eur. J. Med. Chem. 2015, 102, 180–187. [CrossRef] [PubMed]

24. Kao, Y.L.; Kuo, Y.M.; Lee, Y.R.; Yang, S.F.; Chen, W.R.; Lee, H.J. Apple polyphenol induces cell apoptosis, cell cycle arrest at G2/M phase, and mitotic catastrophe in human bladder transitional carcinoma cells. J. Funct. Foods 2015, 14, 384–394. [CrossRef]

25. Lepiarczyk, M.; Kuźa, Z.; Bielawska, A.; Czarnomysy, R.; Gornowicz, A.; Bielawski, K. Cytotoxic activity of octahydropyrazin[2,1-a:5,4-a']diquinoline derivatives in human breast cancer cells. Arch. Pharm. Res. 2015, 38, 628–641. [CrossRef] [PubMed]

Sample Availability: Samples of the target compounds are available from the authors.