One-pot synthesis and biological evaluation of (2E,4E)-4-arylidene-2-styryl-5-oxopyrrolidine derivatives

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Abstract
Many marine alkaloids possess interesting structures and antitumor activities. Thus, we have synthesized (2E,4E)-4-arylidene-2-styryl-5-oxopyrrolidine derivatives of the marine alkaloids, rhopaladins A–D. The cytotoxicities of these derivatives against C-33A, CaSki, SiHa, HeLa, HepG2, and LO2 cells are evaluated by MTT assays. The results show that (2E,4E)-2-(4-chlorostyryl)-4-benzylidene-N-cyclohexyl-1-(4-fluorophenyl)-5-oxopyrrolidine-2-carboxamide significantly inhibits cancer cell proliferation, with IC50 values against C-33A, CaSki, SiHa, HeLa, and HepG2 cells of 5.56, 9.15, 12.5, 21.4, and 14.5 μM, respectively, and an IC50 value of 86.77 μM against the normal LO2 cell line.

Keywords
(2E,4E)-4-arylidene-2-styryl-5-oxopyrrolidine, cancer, cytotoxicity evaluation, one-pot, synthesis

Introduction
Cancer is one of the most serious diseases, and is considered to be the leading cause of death and threat to public health in all countries of the World.1,2 Cervical cancer is one of the ten most common cancers in women, and it ranks fourth for both incidence and mortality; 85% of cervical cancers occur in less-developed regions.3,4 Cancer treatment methods include, among others, surgery, radiotherapy, and chemotherapy. Chemotherapy, as a classic treatment, has anticancer significance in the treatment of different stages of tumor cell growth.3,4 Hence, the synthesis of new anticancer compounds is necessary for the treatment of cancer.

Compounds containing the pyrrolidone moiety can be used as the basic nuclear framework in the synthesis of anticancer and antiviral drugs, and are widely used in the medicine and in the chemical industry. For the treatment of diseases, natural marine products are considered superior to terrestrial natural products because of their chemical novelty and their ability to exhibit different biological activities.6 Alkaloids exist widely in marine organisms and have diverse physiological activities, such as antifungal activity, antimicrobial activity, anti-inflammatory and cytotoxicity.6–9 Pyrrolidone-related marine natural compounds have unique chemical structures and often display strong antifungal and antibacterial properties. Inasmuch as the rhopaladins A–D alkaloids exhibit inhibitory activity against cyclin-dependent kinase 4 and C-erbβ-2 kinase, they demonstrate significant cytotoxicity against human tumor cells.10

We have previously synthesized (E)-2-aryloyl-4-arylidene-5-oxopyrrolidinerhopaladin analogues from a Baylis–Hillman acid (2-bromomethyl-3-p-fluorobenzyl-2-propenoic acid), primary amines, arylglyoxals and isocyanides via a one-pot approach based on Ugi condensation and intramolecular SN

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These rhopaladin analogues inhibit the proliferation of the CaSki cervical cancer cell line, induce cell apoptosis, and down-regulate E6/E7 mRNA expression. 12,13 In addition, they have high selectivity toward cervical cancer cells. Therefore, novel (2E,4E)-4-arylidene-2-styryl-5-oxopyrrolidines has been designed and synthesized using the same one-pot approach by replacing the arylglyoxal with (E)-3-arylacrolein (Scheme 1).

Results and discussion

One-pot synthesis of (2E,4E)-4-arylidene-2-styryl-5-oxopyrrolidines 6

The Ugi condensation reaction was complete in 24 h at room temperature, and the Ugi intermediates 5 were converted to 5-oxopyrrolidines in the presence of Cs2CO3. Seven (2E,4E)-4-arylidene-2-styryl-5-oxopyrrolidine derivatives 6a–g were produced in good to excellent yields of 83%–91%, with that of compound 6a (yield 91%) being the highest (Scheme 2). The yields of the products were related to the R group of isocyanide 4. The structures of compounds 6a–g were confirmed from their spectroscopic data.

In vitro cytotoxicity assay

MTT assays were carried out to determine the growth inhibitory effects of compounds 6 toward: five cancer cell lines (C-33A, CaSki, SiHa, HeLa, and HepG2) and one normal hepatocyte cell lines (LO2). We treated cells with different concentrations of compounds 6 and determined the IC50 values of each compound, with cisplatin and RPDPB (prior synthetic compound11 (2E,N-(tert-butyl)-2-(4-chlorobenzoyl)-4-(4-fluorobenzylidene)-1-isopropyl-5-oxopyrrolidine-2-carbox-amide) as the reference control. The results in Table 1 show that compounds 6a–f have low cytotoxicity toward the cancer cells. However, the IC50 values of compound 6g against the C-33A, CaSki, SiHa, HeLa, HepG2, and LO2 cells were 5.56, 9.15, 12.5, 21.4, 14.5, and 86.77 μM, respectively. For the positive control, the IC50 values of cisplatin were 13.16, 12.5, 37.06, 21.3, 19.27, and 17.3 μM, and the IC50 values of RPDPB were...
over 100, 23.7, over 100, 24.8, over 100, and over 100 μM, respectively. The results show that compound 6g has obvious cytotoxicity, and has low cytotoxicity against normal hepatocyte LO2 cells. The cytotoxicity of compound 6a–g was related to the Ar3 group. With halogen atom in Ar3 group, its cytotoxicity will increase, especially with fluorine atom.

**Table 1.** The in vitro cytotoxic effects of compounds 6a–g.

| Entry | Structure | Anti-proliferative effects (IC50, μM) | C-33A | CaSki | SiHa | HeLa | HepG2 | LO2 |
|-------|-----------|----------------------------------|------|------|------|------|-------|-----|
| 6a    | ![Structure](image) | >100 | 74.7 | 91.3 | 69.3 | 86.7 | >100 |
| 6b    | ![Structure](image) | >100 | >100 | >100 | >100 | >100 | >100 |
| 6c    | ![Structure](image) | >100 | >100 | >100 | >100 | >100 | >100 |
| 6d    | ![Structure](image) | 58.4 | >100 | 69.3 | >100 | >100 | >100 |
| 6e    | ![Structure](image) | >100 | >100 | >100 | >100 | >100 | >100 |
| 6f    | ![Structure](image) | >100 | >100 | >100 | >100 | >100 | >100 |
| 6g    | ![Structure](image) | 5.56 | 9.15 | 12.5 | 21.4 | 14.5 | 86.77 |
| RPDPB | ![Structure](image) | >100 | 23.7 | >100 | 24.8 | >100 | >100 |
| Cisplatin | ![Structure](image) | 13.16 | 12.5 | 37.06 | 21.3 | 19.27 | 17.3 |

*The compound RPDPB as a represent of RPDP in Scheme 1 for the previous analogues.*
Conclusion

We have synthesized 4-arylidene-2-styryl-5-oxopyrrolidine rhopaladin analogues 6 via a one-pot procedure and have evaluated their cytotoxicity against C-33A, CaSki, SiHa, HeLa, HepG2, and LO2 cells. The results of these cytotoxicity assays showed that compound 6g could significantly inhibit cancer cell proliferation.

Experimental

General information

Melting points were measured with an X-4 melting point instrument (uncorrected thermometer) produced by Beijing Ruili Analytical Instrument Co., Ltd. Mass spectrometry was performed with a Finnigan trace MS analyzer (direct injection method). Elemental analysis was performed using an elemental analyzer (uncorrected thermometer) produced by Beijing Ruili Analytical Instrument Co., Ltd. Melting points were measured with an X-4 melting point instrument (uncorrected thermometer). Mass spectrometry was performed with a Finnigan trace MS analyzer (direct injection method). Elemental analysis was performed using an elemental analyzer (uncorrected thermometer)

One-pot synthesis of (2E,4E)-4-arylidene-2-styryl-5-oxopyrrolidines 6

A mixture of aromatic amine 2 (1 mmol) and substituted (E)-3-arylacrolein 3 (1 mmol) was stirred in methanol (5 mL) at room temperature for 30 min. After precipitation had occurred, Baylis–Hillman acid (2-chloromethyl-3-aryl-3-propenoic acid) 1 (1 mmol) and isocyanide 4 (1 mmol) were added successively, and the mixture was stirred at room temperature for 24 h. After the reaction was complete by TLC monitoring, the mixture was cooled at -18°C overnight and the resulting precipitate was filtered, and recrystallized from ether to give compounds 6 white solids.

(2E,4E)-4-(4-Chlorobenzylidene)-2-(4-chlorostyryl)-N-tert-butyl-5-oxo-1-p-tolylpyrrolidine-2-carboxamide (6a): White crystals (0.47 g, yield 89%); m.p. 214–215°C; 1H NMR (400 MHz, CDCl3): δ 7.57-7.01 (m, 14H, 12*ArH and 2*=CH), 7.02 (d, J = 16.2 Hz, 1H, =CH), 6.38 (d, J = 16.2 Hz, 1H, =CH), 5.64 (s, 1H, NH), 3.43 (d, J = 16.8 Hz, 1H, CH2), 1.21 (s, 3H, 3*CH3); 13C NMR (100 MHz, CDCl3): δ 169.9, 169.0, 135.6, 135.3, 135.2, 132.6, 131.2, 130.9, 129.1, 128.9, 128.6, 128.3, 127.7, 126.8, 126.7, 124.6, 69.7, 51.8, 41.0, 28.3; EI-MS: m/z (%) = 518 (M+, 5), 418 (M+-CONHBut-t, 100), 315 (21), 307 (63), 204 (33), 103 (50), 77 (23), 57 (40). Anal. calcd for C30H27Cl2N2O2: C, 69.79; H, 5.67; N, 5.25; found: C, 69.87; H, 5.72; N, 5.31.

(2E,4E)-4-(4-Chlorobenzylidene)-2-(4-chlorostyryl)-N-tert-butyl-5-oxo-1-p-tolylpyrrolidine-2-carboxamide (6d): White crystals (0.48 g, yield 89%); m.p. 230–232°C; 1H NMR (400 MHz, CDCl3): δ 7.57-7.01 (m, 14H, 12*ArH and 2*=CH), 6.38 (d, J = 16.4 Hz, 1H, =CH), 5.64 (s, 1H, NH), 3.43 (d, J = 16.4 Hz, 1H, CH2), 1.21 (s, 3H, 3*CH3); 13C NMR (100 MHz, CDCl3): δ 169.8, 168.9, 135.8, 135.1, 134.3, 132.2, 133.8, 133.4, 133.2, 130.9, 129.5, 129.3, 129.0, 128.7, 128.3, 128.1, 127.9, 123.1, 69.7, 51.7, 41.2, 28.2; EI-MS: m/z (%) = 518 (M+, 6), 418 (M+-CONHBut-t, 100), 341 (68), 204 (73), 77 (34), 59 (73). Anal. calcd for C30H27Cl2N2O2: C, 69.36; H, 5.43; N, 5.39; found: C, 69.28; H, 5.47; N, 5.43.

(2E,4E)-4-(4-Chlorobenzylidene)-2-(4-chlorostyryl)-N-cyclohexyl-5-oxopyrrolidine-2-carboxamide (6f): White crystals (0.47 g, yield 90%); m.p. 230–232°C; 1H NMR (400 MHz, CDCl3): δ 7.57-7.01 (m, 14H, 12*ArH and 2*=CH), 6.38 (d, J = 16.4 Hz, 1H, =CH), 5.61 (s, 1H, NH), 3.41 (d, J = 16.8 Hz, 1H, CH2), 3.32 (d, J = 17.2 Hz, 1H, CH2), 1.15 (s, 9H, 3*CH3); 13C NMR (100 MHz, CDCl3): δ 170.0, 168.9, 137.0, 135.3, 134.2, 133.8, 133.3, 132.8, 130.9, 129.2, 129.1, 129.0, 128.7, 128.2, 127.9, 127.4, 125.9, 123.1, 69.7, 51.7, 41.2, 28.2; EI-MS: m/z (%) = 518 (M+, 6), 418 (M+-CONHBut-t, 100), 341 (68), 204 (73), 77 (34), 57 (93). Anal. calcd for C30H28Cl2N2O2: C, 69.87; H, 5.72; N, 5.31.

(2E,4E)-4-(4-Chlorobenzylidene)-2-(4-chlorostyryl)-N-tert-butyl-5-oxo-1-phenylpyrrolidine-2-carboxamide (6g): White crystals (0.48 g, yield 89%); m.p. 230–232°C; 1H NMR (400 MHz, CDCl3): δ 7.57-7.01 (m, 14H, 12*ArH and 2*=CH), 6.38 (d, J = 16.4 Hz, 1H, =CH), 5.64 (s, 1H, NH), 3.43 (d, J = 16.4 Hz, 1H, CH2), 1.21 (s, 3H, 3*CH3); 13C NMR (100 MHz, CDCl3): δ 169.8, 168.9, 135.8, 135.1, 134.3, 132.2, 133.8, 133.4, 133.2, 130.9, 129.5, 129.3, 129.0, 128.7, 128.3, 128.1, 127.9, 123.1, 69.7, 51.7, 41.2, 28.2; EI-MS: m/z (%) = 518 (M+, 6), 418 (M+-CONHBut-t, 100), 341 (68), 204 (73), 91 (21), 76 (13), 57 (100). Anal. calcd for C30H28Cl2N2O2: C, 69.87; H, 5.72; N, 5.31.
(100 MHz, CDCl3): δ 169.9, 169.0, 136.0, 135.5, 135.4, 134.0, 133.1, 131.9, 131.0, 129.8, 129.6, 129.2, 128.8, 127.9, 127.6, 127.5, 124.4, 118.9, 69.2, 48.8, 41.4, 32.3, 25.2, 24.5; EI-MS: m/z (%): 622 (M+, 2), 496 (M+−CONHCy, 25), 467 (100), 341 (34), 204 (44), 155 (19), 111 (33), 76 (41). Anal. calcd for C_{32}H_{29}BrClN_{2}O_{2}: C, 61.56; H, 4.74; N, 4.45.

(2E,4E)-2-(4-Chlorostyryl)-4-benzylidene-N-cyclohexyl-1-(4-fluorophenyl)-5-oxopyrrolidine-2-carboxamide (6g): White crystals (0.47 g, yield 89%); m.p. 142–144 °C; 1H NMR (600 MHz, CDCl3): δ 7.54-7.26 (m, 14H, 13*ArH and =CH), 3.75-3.72 (m, 1H, CH), 3.44 (d, J= 16.8 Hz, 1H, CH2), 3.34 (d, J= 16.8 Hz, 1H, CH2), 1.80-0.85 (m, 10H, 5*CH2); 13C NMR (100 MHz, CDCl3): δ 170.0, 169.2, 163.9, 135.5, 133.9, 130.3, 131.7, 131.0, 128.9, 128.6, 128.3, 126.9, 126.7, 126.6, 124.4, 116.1, 115.9, 101.0, 69.4, 48.7, 41.2, 32.6, 24.6, 24.5; EI-MS: m/z (%): 528 (M+, 2), 402 (M+−CONHCy, 100), 307 (84), 170 (67), 111 (17), 95 (23), 77 (24). Anal. calcd for C_{42}H_{30}ClF_{2}N_{2}O_2: C, 72.65; H, 5.72; N, 5.30; found: C, 72.48; H, 4.74; N, 4.45.

**Cell culture and treatment**

The human cervical C-33A, CaSki, HeLa cancer cell lines, and normal hepatocyte LO2 cells were obtained from the Experiment Center of Medicine, Dongfeng Hospital, Hubei University of Medicine. The human cervical SiHa cancer cell line was purchased from the China Center for Type Culture Collection, CCTCC. The C-33A and HeLa cells were cultured in Dulbecco’s modified Eagle medium (DMEM, Gibco), and the SiHa cells were cultured in Eagle’s Minimum Essential Medium (MEM, Corning), and the CaSki and LO2 cells were cultured in RPMI Medium 1640 basic (Gibco), meanwhile, all mediums contain 10% fetal bovine serum (FBS, Corning). All cells were fostered in an incubator with a humidified atmosphere of 5% CO2 at 37°C.

**Cell viability in vitro assays**

Cell viability was estimated via the MTT assay. The specific experimental steps are as follows: cells were trypsinized with 0.25% trypsin and incubated in 96-well plates for 24h before drug administration. The cells were then treated with different concentrations of compounds 6 (3.125, 6.25, 12.5, 25, 50, and 100 μM) for 48h. Untreated cells served as the control group; cisplatin was used as a positive control. After incubation, MTT (0.5 g/mL) was added in the same medium containing for 4h. Next, dimethyl sulfoxide (DMSO, 150 μL) was added to dissolve the generated formazan crystals. Subsequently, the absorbance at 490nm was determined using an enzyme-labeled meter (BioTek, Inc., BioTek MQX200).

**Declaration of conflicting interests**

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**Supplemental material**

Supplemental material for this article is available online.

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