Design, Synthesis, and Preliminary Antitumor Activity Evaluation of Novel Alkaloid Derivatives

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Abstract
A novel alkaloid scaffold was designed through scaffold-hopping strategy based on the active pyrazines alkaloid isolated previously. A total of 25 derivatives were synthesized based on this scaffold and evaluated for their antitumor activities. Among all these tested compounds, 9f exhibited most excellent antitumor activities toward H460 cells, TMD-8 cells, and MV4-11 cells in vitro by 3-(4, 5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide assay with IC50 values of 29.8, 14.9, and 18.8 μM, respectively.

Keywords
Fu zi, scaffold-hopping, antitumor activity, structure-activity relationship, alkaloid

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Cancer is one of most serious public health problems representing the leading cause of morbidity and mortality worldwide, which causes great social and economic burdens.1 Natural products have been considered as important sources for drug discovery, which could be developed for the treatment of various diseases. “Fu zi,” also named Radix Aconiti Lateralis Praeparata, is the lateral root of Aconitum carmichaelii, which is widely distributed in Sichuan Province of China and is often used as agents toward various diseases.2-4 Our previous study on “Fu zi” resulted in the isolation of 7 nonditerpene alkaloids, among which 2 new pyrazines (Figure 1, compounds 1 and 2) showed moderate antitumor activity.5 Given this result, 2 lead compounds are considered as potentially novel scaffold or pharmacophore with antitumor activity. In the present study, the scaffold-hopping strategy was utilized to design new scaffold based on the lead compounds.6 A water-soluble group, secondary amine moiety, was introduced as a linkage between hydrophobic head and hydrophilic tail (Figure 1). Then, we modified the hydrophobic head with various aromatic rings to identify new compounds with better antitumor activity. Also, compounds with contrary configuration of lead compounds were synthesized and evaluated for their antitumor activities to ascertain the structure-activity relationships that determine the antitumor activities of the synthesized compounds.

The starting materials were (S)-3-aminopropane-1,2-diol (4) and various aromatic aldehydes (3a-3p). Scheme 1 shows that (S)-3-aminopropane-1,2-diol (4) was treated with different aromatic aldehydes (3a-3p) in dry Dichloromethane (DCM) to achieve the aromatic imines (5). Once the completion of the transformation monitored by thin-layer chromatography (TLC), reducing agents, including NaBH4, STAB, and NaBH3CN, were added to the reaction mixture.7-9 The reactions were allowed to stir at room temperature to give the final products. However, only reaction treated with NaBH4 performed good conversion. The reduced target compounds 6a to 6p were isolated using column chromatography over silica gel with elution by DCM and DCM-MeOH with a gradually increasing volume of MeOH.

Derivatives 9a to 9i were synthesized similar to derivatives 6a to 6p, which were depicted in scheme 2.

All these target compounds were evaluated for their anti-proliferative activities against tumor cell lines, including...
H460 cells (human nonsmall-cell lung cancer cells), TMD-8 cells (human diffuse large B-cell lymphoma cells), and MV4-11 cells (biphenotypic B myelomonocytic leukemia cells), in vitro by 3-(4, 5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. Each experiment was repeated 3 times. The results of these studies are summarized in Table 1.

As illustrated in Table 1, the S-configuration compound 6g, in which the 3,5-di-tert-butyl-2-hydroxyl phenyl was introduced as the hydrophobic head, exhibited good antitumor activities against 2 tested tumor cell lines, including H460 and MV4-11 cells, with IC50 values of 40.8 and 34.3 µM, respectively. The S-configuration compound 6i with a 5-(4-nitrophenyl)furan-2-yl moiety as hydrophobic head showed good activities against all tested cell lines, with IC50 values of 53.8, 62.6, and 43.5 µM, respectively. In addition, compound 6g showed weak activity against TMD-8 (IC50 = 65.6 µM), whereas compound 6i moderately inhibited its growth (IC50 = 62.6 µM). The R-configuration compound 9h, featuring same hydrophobic head of compound 6g, presented same level of antitumor activities of 6g against all 3 tumor cell lines, with IC50 values of 40.8, 65.6, and 34.3 µM, respectively. Notably, among all compounds, 9f with the same hydrophobic head of compound 6i exhibited most excellent activities against tested tumor cell lines, with IC50 values of 29.8, 14.9, and 18.8 µM, respectively. The aforementioned results demonstrated that R-configuration analogs were more potent than the S-configuration analogs from the lead compounds.

Figure 1. Design of the target compound based on the lead compounds through scaffold hopping.

Scheme 1. Synthetic route of compounds 6a-6p. Reagents and conditions: (a) DCM, r.t. 3 hours. (b) NaBH4, DCM, r.t. 3 hours.
Scheme 2. Synthetic route of compounds 9a-9i. Reagents and conditions: (a) DCM, r.t. 3 hours. (b) NaBH₄, DCM, r.t. 3 hours.

Table 1. Antitumor Activity Evaluation of Synthesized Compounds.

| Compounds | In vitro inhibition of human cancer cells proliferation (IC₅₀, μM) |
|-----------|---------------------------------------------------------------|
|           | H460      | TMD-8     | MV4-11       |
| 6a        | >100      | >100      | >100         |
| 6b        | >100      | >100      | >100         |
| 6c        | >100      | >100      | >100         |
| 6d        | >100      | >100      | >100         |
| 6e        | >100      | >100      | >100         |
| 6f        | >100      | >100      | >100         |
| 6g        | 40.8 ± 13.9 | 65.6 ± 27.8 | 34.3 ± 6.8 |
| 6h        | >100      | >100      | >100         |
| 6i        | 53.8 ± 23.3 | 62.6 ± 20.2 | 43.5 ± 13.0 |
| 6j        | >100      | >100      | >100         |
| 6k        | >100      | >100      | >100         |
| 6l        | >100      | >100      | >100         |
| 6m        | >100      | >100      | >100         |
| 6n        | >100      | >100      | >100         |
| 6o        | >100      | >100      | >100         |
| 6p        | >100      | >100      | >100         |
| 9a        | >100      | >100      | >100         |
| 9b        | >100      | >100      | >100         |
| 9c        | >100      | >100      | >100         |
| 9d        | >100      | >100      | >100         |
| 9e        | >100      | >100      | >100         |
| 9f        | 29.8 ± 11.3 | 14.9 ± 5.8 | 18.8 ± 7.1  |
| 9g        | >100      | >100      | >100         |
| 9h        | 57.5 ± 19.5 | 69.5 ± 24.6 | 44.8 ± 14.7 |
| 9i        | >100      | >100      | >100         |
| Paclitaxel | 0.044 ± 0.013 | 0.049 ± 0.020 | 0.033 ± 0.009 |

*The data are expressed as the mean ± SD. All experiments were independently performed at least 3 times.

*Used as a positive control.
In conclusion, 25 new pyrazines alkaloid derivatives were successfully synthesized and evaluated cytotoxic activity against 3 cancer cell lines. The results indicate that some of these derivatives possess at least moderate cytotoxic activity. Among all these tested compounds, 9f exhibited most excellent antitumor activities, which implied that continued searching for new antitumor agents based on the aforementioned scaffold is promising.

### Experimental

#### General

The reactions were monitored by TLC with silica gel GF254 plates, which were visualized by ultraviolet (UV) light (254 nm). ¹H Nuclear Magnetic Resonance (NMR) spectra were measured on a Bruker 600 and 400 MHz spectrometer at 25°C, and referenced to Me4Si. Chemical shifts are reported in ppm (δ) using the residual solvent line as an internal standard. Splitting patterns are designed as s, singlet; d, doublet; t, triplet; m, multiplet. NMR spectra were taken in Dimethylsulfoxide (DMSO) with Tetramethylsilane (TMS) internal standard on a Bruker SF-400 spectrometer (operating frequency 400 MHz). Starting materials and solvents were purchased from common commercial suppliers and were used without further purification.

#### General Procedure for Preparation of Conjugates Between Triterpenoid and 1,2-Diaminobenzene 6a-6p and 9a-9i

(5)-3-((3,4-Dimethylbenzyl)amino)propane-1,2-diol (6a). Light yellow oil. Yield = 27.8%. C₁₂H₁₆NO₂. ¹H NMR (400 MHz, DMSO-d₆) δ 7.27-7.22 (m, 1H), 6.89 (m, 2H), 6.81 (dd, J = 8.2, 2.8 Hz, 1H), 3.80 (m, 5H), 3.69 (dd, J = 11.4, 3.6 Hz, 1H), 3.57 (dd, J = 11.4, 4.8 Hz, 1H), 2.83 (dd, J = 12.2, 3.8 Hz, 1H), 2.71 (dd, J = 12.2, 7.2 Hz, 1H). ¹C NMR (100 MHz, CDCl₃) δ 159.75, 140.72, 129.56, 120.57, 113.93, 112.63, 70.15, 65.46, 55.21, 53.64, 51.54. HR-ESI-MS (positive ion mode): m/z 212.1268 [M+H]+ (calcd for C₁₀H₁₄CINO₂, 211.1208).

(5)-3-((2-Chloro-4-fluorobenzyl)amino)propane-1,2-diol (6c). White solid. Yield = 37.1%. C₁₁H₁₃ClFNO₂ mp 66.0 to 66.5°C. ¹H NMR (400 MHz, DMSO-d₆) δ 7.55 (dd, J = 8.5, 6.6 Hz, 1H), 7.38 (dd, J = 8.9, 2.6 Hz, 1H), 7.20 (dd, J = 8.5, 2.6 Hz, 1H), 4.57 (d, J = 4.7 Hz, 1H), 4.49 (s, 1H), 3.76 (s, 2H), 3.55 (m, 1H), 3.32 (m, 2H), 2.59 (dd, J = 11.7, 4.3 Hz, 1H), 2.44 (dd, J = 11.7, 7.2 Hz, 1H), 2.10 (s, 1H). ¹C NMR (100 MHz, DMSO-d₆) δ 162.40, 134.93, 133.54, 131.61, 116.80, 114.60, 71.01, 64.94, 52.60, 50.15. HR-ESI-MS (positive ion mode): m/z 234.0686 [M+H]+ (calcd for C₁₄H₁₃ClFNO₂, 233.0619).

(5)-3-((3,4-Diethylbenzyl)amino)propane-1,2-diol (6d). White solid. Yield = 26.2%. C₁₄H₁₅NO₂ mp 83.1 to 83.3°C. ¹H NMR (400 MHz, DMSO-d₆) δ 7.59 (d, J = 1.8 Hz, 1H), 7.55 (d, J = 8.2 Hz, 1H), 7.31 (dd, J = 8.2, 2.0 Hz, 1H), 4.54 (d, J = 4.4 Hz, 1H), 4.47 (s, 1H), 3.70 (s, 2H), 3.54 (m, 1H), 3.32 (m, 2H), 2.55 (dd, J = 11.8, 4.6 Hz, 1H), 2.40 (dd, J = 11.8, 7.2 Hz, 1H), 2.19 (s, 1H). ¹C NMR (100 MHz, DMSO-d₆) δ 142.95, 131.23, 130.66, 130.17, 129.31, 128.64, 71.01, 64.96, 52.45, 52.17. HR-ESI-MS (positive ion mode): m/z 250.0389 [M+H]+ (calcd for C₁₄H₁₃ClNO₂, 249.0323).

(5)-3-((4-Chlorobenzyl)amino)propane-1,2-diol (6e). White solid. Yield = 15.4%. C₁₃H₁₂ClNO₂ mp 77.4 to 77.9°C. ¹H NMR (400 MHz, DMSO-d₆) δ 7.39 (m, 4H), 4.53 (brs, 1H), 3.70 (s, 2H), 3.55 (m, 1H), 3.31 (m, 2H), 2.57 (dd, J = 11.7, 4.5 Hz, 1H), 2.42 (dd, J = 11.7, 7.2 Hz, 1H). ¹C NMR (100 MHz, CDCl₃) δ 137.36, 133.00, 129.62, 128.67, 70.20, 65.43, 58.16, 53.06, 51.54, 18.35. HR-ESI-MS (positive ion mode): m/z 216.0777 [M+H]+ (calcd for C₁₀H₁₃ClNO₂, 215.0713).

(5)-3-((3-Chloro-4-fluorobenzyl)amino)propane-1,2-diol (6f). White solid. Yield = 17.3%. C₁₄H₁₃ClNO₂ mp 76.1 to 76.6°C. ¹H NMR (400 MHz, DMSO-d₆) δ 7.53 (d, J = 7.8 Hz, 1H), 7.36-7.30 (m, 2H), 4.55 (brs, 1H), 3.69 (s, 2H), 3.54 (m, 1H), 3.32 (m, 2H), 2.55 (dd, J = 11.8, 4.4 Hz, 1H), 2.40 (dd, J = 11.8, 7.2 Hz, 1H). ¹C NMR (100 MHz, DMSO-d₆) δ 157.65, 139.48, 130.22, 128.85, 119.57, 116.97, 70.97, 64.97, 52.42, 52.16. HR-ESI-MS (positive ion mode): m/z 234.0680 [M+H]+ (calcd for C₁₄H₁₃ClNO₂, 233.0619).

(5)-3-((3-Methylbenzyl)amino)propane-1,2-diol (6g). Light yellow oil. Yield = 19.5%. C₁₈H₂₃NO₃S. ¹H NMR (400 MHz, DMSO-d₆) δ 7.07 (d, J = 2.3 Hz, 1H), 6.86 (d, J = 2.2 Hz, 1H), 3.87 (m, 2H), 3.58 (m, 1H), 3.52 (m, 2H), 2.64 (dd, J = 11.8, 4.4 Hz, 1H), 2.45 (dd, J = 11.8, 7.2 Hz, 1H), 1.35 (s, 9H), 1.23 (s, 9H). ¹C NMR (100 MHz, CDCl₃) δ 153.01, 136.01, 130.11, 129.19, 125.41, 124.96, 69.67, 67.78, 65.82, 65.07, 58.92, 58.32, 55.87, 54.29, 52.06, 34.34, 30.31, 18.40. HR-ESI-MS (positive ion mode): m/z 310.2384 [M+H]+ (calcd for C₁₈H₂₃NO₃S, 309.2304).
(S)-3-((Pyridin-4-ylmethyl)amino)propane-1,2-diol (6h). Light yellow oil. Yield = 22.9%. C17H21NO2. 1H NMR (400 MHz, DMSO-d6) δ 8.49 (d, J = 6.0 Hz, 1H), 7.35 (d, J = 5.8 Hz, 2H), 4.54 (d, J = 4.4 Hz, 1H), 3.68 (s, 2H), 3.53 (m, 1H), 3.57-3.45 (m, 2H), 2.56 (dd, J = 11.2, 4.8 Hz, 1H), 2.44 (dd, J = 11.2, 7.2 Hz, 1H). 1C NMR (100 MHz, CDCl3) δ 194.89, 187.82, 129.99, 69.96, 65.39, 53.27, 51.35, 18.84. HR-ESI-MS (positive ion mode): m/z 296.1510 [M+H]+ (calcd for C17H21NO2, 296.1532).

(S)-3-((4-Nitrophenyl)furan-2-yl)methylene)propane-1,2-diol (6i). Brown solid. Yield = 34.6%. C13H16N2O2. 1H NMR (400 MHz, DMSO-d6) δ 8.27 (d, J = 9.0 Hz, 2H), 7.91 (d, J = 9.0 Hz, 2H), 7.24 (d, J = 3.4 Hz, 1H), 6.49 (d, J = 3.4 Hz, 1H), 4.60 (s, 1H), 3.81 (s, 2H), 3.61-3.51 (m, 1H), 2.67 (dd, J = 11.8, 4.4 Hz, 1H), 2.51 (m, 1H). 1C NMR (100 MHz, CDCl3) δ 157.58, 149.84(2C), 148.87, 122.99(2C), 69.87, 65.56, 52.63, 51.84. HR-ESI-MS (positive ion mode): m/z 283.1122 [M+H]+ (calcd for C9H17NO4, 282.1059).

(S)-3-((1-Methyl-5-nitro-1H-imidazol-2-yl)methyl)amino)propane-1,2-diol (6j). Yellow oil. Yield = 30.6%. C10H13F2NO2. 1H NMR (400 MHz, DMSO-d6) δ 8.51 (d, J = 1.8 Hz, 1H), 7.02 (d, J = 8.0 Hz, 1H), 6.88 (t, J = 16 Hz, 1H), 3.68 (s, 2H), 3.78 (m, 1H), 3.32 (m, 2H), 2.58 (m, J = 4.0 Hz, 1H). 1C NMR (100 MHz, CDCl3) δ 194.89, 187.82, 129.99, 69.96, 65.39, 53.27, 51.35, 18.84. HR-ESI-MS (positive ion mode): m/z 296.1510 [M+H]+ (calcd for C17H21NO2, 296.1532).

(S)-3-((3-Nitro-4-(pyrrolidin-1-yl)benzyl)amino)propane-1,2-diol (6k). White solid. Yield = 33.0%. C14H15NO4. 1H NMR (400 MHz, DMSO-d6) δ 8.01 (d, J = 8.0 Hz, 1H), 7.02 (s, 1H), 6.97 (d, J = 8.0 Hz, 1H), 4.24 (brs, 3H), 3.77 (m, 1H), 3.62 (m, 2H), 2.58 (m, J = 4.0 Hz, 1H). 1C NMR (100 MHz, CDCl3) δ 163.73, 136.04, 135.67, 129.78(2C), 125.81, 69.96, 65.39, 53.27, 51.35, 19.75,19.43. HR-ESI-MS (positive ion mode): m/z 210.1473 [M+H]+ (calcd for C10H11ClNO2, 209.1416).

(S)-3-((3,4-Difluorobenzyl)lamino)propane-1,2-diol (6l). White oil. Yield = 38.7%. C19H15NO2. 1H NMR (400 MHz, DMSO-d6) δ 8.51 (d, J = 1.8 Hz, 1H), 7.01 (s, 1H), 6.97 (d, J = 8.0 Hz, 1H), 4.24 (brs, 3H), 3.77 (m, 1H), 3.62 (m, 2H), 2.58 (m, J = 4.0 Hz, 1H). 1C NMR (100 MHz, CDCl3) δ 163.73, 136.04, 135.67, 129.78(2C), 125.81, 69.96, 65.39, 53.27, 51.35, 18.84. HR-ESI-MS (positive ion mode): m/z 210.1473 [M+H]+ (calcd for C10H11ClNO2, 209.1416).

(B)-3-((2-Chloro-4-fluorobenzyl)amino)propane-1,2-diol (9b). White solid. Yield = 33.7%. C15H15NO2. 1H NMR (400 MHz, DMSO-d6) δ 7.26 (d, J = 12 Hz, 1H), 7.04 (d, J = 8 Hz, 1H), 6.88 (t, J = 16 Hz, 1H), 3.78 (m, 2H), 3.72 (m, 1H), 3.55 (2H), 3.02 (brs, 3H), 2.65 (m, 2H). 1C NMR (100 MHz, DMSO-d6) δ 162.41, 134.89, 133.55, 131.63, 116.80, 114.60, 70.99, 64.94, 52.59, 50.13. HR-ESI-MS (positive ion mode): m/z 232.1212.
(R)-3-((3,4-Dichlorobenzyl)amino)propane-1,2-diol (9e). White solid. Yield = 29.5%. C_{10}H_{14}ClNO_2, mp 81.7 to 81.9°C. 1H NMR (400 MHz, DMSO-d_6) δ 7.59 (d, J = 2.0 Hz, 1H), 7.56 (d, J = 8.2 Hz, 1H), 7.32 (dd, J = 8.2, 2.0 Hz, 1H), 4.55 (s, 1H), 3.70 (s, 2H), 3.53 (m, 1H), 3.40-3.24 (m, 3H), 2.55 (dd, J = 11.8, 4.6 Hz, 1H), 2.40 (dd, J = 11.8, 7.2 Hz, 1H). 1C NMR (100 MHz, CDCl_3) δ 139.68, 132.47, 131.17, 130.47, 130.10, 127.58, 70.24, 65.39, 52.65, 51.51. HR-ESI-MS (positive ion mode): m/z 250.0383 [M+H]^+ (calcd for C_{10}H_{14}ClNO_2, 249.0323).

(R)-3-((3-Chloro-4-fluorobenzyl)amino)propane-1,2-diol (9f). Light yellow oil. Yield = 31.4%. C_{10}H_{13}ClFNO_2, mp 81.7 to 81.9°C. 1H NMR (400 MHz, DMSO-d_6) δ 7.35 (dd, J = 8.0, 6.4 Hz, 1H), 7.20-7.14 (m, 1H), 7.04 (dd, J = 8.4, 2.2 Hz, 1H), 4.58 (s, 1H), 3.74 (s, 2H), 3.56 (s, 1H), 3.33 (s, 2H), 2.58 (dd, J = 11.8, 4.4 Hz, 1H), 2.43 (dd, J = 11.8, 7.2 Hz, 1H). 1C NMR (100 MHz, CDCl_3) δ 164.17, 142.02, 130.05, 123.75, 115.09, 114.21, 70.23, 65.42, 53.24, 51.55. HR-ESI-MS (positive ion mode): m/z 200.1060 [M+H]^+ (calcd for C_{10}H_{14}FNO_2, 199.1009).

Antitumor Activity

Antitumor activity assays were carried out by MTT method on H460 cells (human nonsmall-cell lung cancer), TMD-8 cells, and MV4-11 cells (biphenotypic B myelomonocytic leukemia cells). Cells were plated into 96-well plates at a density of 1 × 10^4 cells per well in 100 mL of medium and grown for 48 hours. The cells were then exposed to the tested compounds at different concentrations (0.025-20 μM) for 48 hours. A 0.5% MTT solution was added to each well. After further incubation for another 4 hours, formazan formed from MTT was extracted in 150 µL of DMSO for 15 minutes standby. Absorbance at 570 nm was then determined on a microplate reader. In brief, the mean percentage of cell survival rates relative to that of untreated cells was estimated from the data of 6 individual experiments.

Declaration of Conflicting Interests

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