Synthesis and Antitumor Activity of Evodiamine Derivatives With Nitro, Amino, and Methoxy Groups

Ruolan Yang, Jingjing Ma, Hui Guo, Qinghua Meng, Yuwei Wang, Hao Yan, Ruyi Jin, Zhi Li and Lingjie Meng

Abstract
MS, and IR, $^{1}$H NMR and $^{13}$C NMR spectroscopy were employed to elucidate 4 novel evodiamine (EVO) derivatives with nitro, amino, and methoxy groups, namely 2-NO$_2$-EVO ($7a$), 10-OCH$_3$-2-NO$_2$-EVO ($7b$), 2-NH$_2$-EVO ($8a$), and 10-OCH$_3$-2-NH$_2$-EVO ($8b$). The amino compounds ($8a$, $8b$) were obtained by the reduction of nitro derivatives ($7a$, $7b$) with SnCl$_2$/HCl. The anti-proliferative activities of these compounds were tested by Cell Counting Kit-8 assay for 48 h against the MDA-MB-231 and sw620 cancer cell lines, as well as the normal LO2 cells. The in vitro experiment showed that $8a$ possesses the most potent inhibitory activities against MDA-MB-231 and SW620 cells, with IC$_{50}$ values of 0.79 and 1.28 $\mu$M, respectively. The cytotoxicity of $8a$ against the 2 cancer cell lines was higher than that of EVO.

Keywords
synthesis, evodiamine derivatives, antitumor, cell viability, apoptosis

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Introduction
Cancer is the biggest threat to public health due to its increasing incidence and high mortality rate. Chemotherapeutic drugs are the most popular treatment options, but they are also accompanied by many serious side effects, high toxicity, and drug resistance. In the past few decades, many plant products have demonstrated effective therapeutic effects against cancer. Evodiamine (Evo, structure see Figure 1) is an alkaloid isolated from the fruit of Evodia fructus (a traditional Chinese herb), which has been reported to possess good inhibitory effects against lung cancer A549, cervical cancer HeLa, liver cancer HepG2, colon cancer HCT-116, and other cancer cell lines by regulating the cell cycle, apoptosis, and autophagy. So far, EVO remains inappropriate for clinical application due to its poor physical and chemical traits, while its antitumor potential is promising. In order to improve these defects, pharmacologists have tried to add active groups (-F, -Cl, -OH, and -NH$_2$) through structural modification. For example, some derivatives of EVO with -F, -OH, and -NH$_2$ groups, such as 3-fluoro-EVO, 10-hydroxyl-EVO, and 3-amino-10-hydroxyl-EVO, were designed and synthesized. Nitro and amino groups, 2 common functional groups in pharmaceutical chemistry, can be found in many antitumor drugs, antibiotics, and insecticides. For example, 9-nitro-camptothecin exhibited a good therapeutic effect against advanced pancreatic cancer. The insecticidal activity could be enhanced by nitro group substitution at the periphery of 1,10-phenanthroline. So, the introduction of nitro and amino groups are important chemical modifications that can alter the hydrophobic and electronic properties of a molecule. Generally, structural modification of EVO is achieved at the 3 and 10 positions, while there have been scarce reports concerning 2-position derivatives. To this end, we attempted to modify EVO at the 2 and 10 positions and explore the relevant biological activities with a view to comprehensively understanding the chemical space surrounding the alkaloid.

In this work, MS, and IR, $^{1}$H NMR and $^{13}$C NMR spectroscopy were employed to elucidate 4 EVO derivatives with nitro...
and amino groups, namely 2-NO₂-EVO (7a), 10-OCH₃-2-NO₂-EVO (7b), 2-NH₂-EVO (8a), and 10-OCH₃-2-NH₂-EVO (8b) (Scheme 1). The nitro derivatives of EVO (7a, 7b) were reduced to amino compounds (8a, 8b) with stannous chloride in hydrochloric acid (HCl). Through Cell Counting Kit-8 (CCK-8) assay, the proliferation inhibition of compounds (7a, 7b, 8a, and 8b) was examined against human breast carcinoma MDA-MB-231, human colon carcinoma SW620, and LO2 cell lines, as well as the healthy human liver LO2 cell line.

**Results and Discussion**

**Synthesis and Characterization of Compounds**

**Synthesis of Compounds 7a, 7b, 8a, and 8b**

The synthetic route is shown in Scheme 1. Compounds 7a and 7b were synthesized based on the coupling of substituted 3,4-dihydro-β-carboline (3a and 3b) with substituted N-methyl isatoic anhydride (6), following the method described previously. The nitro group can be reduced over palladium carbon with hydrogen, but in this method, the catalyst is difficult to prepare, and using hydrogen in the laboratory can be dangerous. So, the nitro derivatives of EVO were reduced to amino compounds (8a and 8b) with stannous chloride in HCl, but in very low yield, with lots of byproducts produced.

**NMR Spectra and HRMS of Compounds 7a, 7b, 8a, and 8b**

The chemical structures of 7a, 7b, 8a, and 8b were characterized by ¹H NMR spectroscopy, as displayed in Figure S15. Compared to the ¹H NMR spectrum of 7a (Figure S15A), the chemical shift at 10.99 ppm belonging to the imino proton (⁻NH₂, indole ring) and the singlet at 3.23 ppm, attributed to the methyl (N-CH₃) of 7a, shifted to 10.99 ppm and 2.92 ppm in the ¹H NMR spectrum of 8a (Figure S15B). The new singlet peak at 5.80 ppm represents the amino (⁻NH₂) proton signal. Similarly, compared to the ¹H NMR spectrum of 7b (Figure S15C), the singlet at 10.81 ppm belonging to the imino proton (⁻NH₂, indole ring), the chemical shift at 3.74 ppm ascribed to the methyl (O-CH₃) and at 3.23 ppm for the methyl (N-CH₃) of 7b shifted to 10.63, 3.74, and 2.98 ppm, respectively, in the ¹H NMR spectrum of 8b (Figure S15D). The new singlet peak at 5.66 ppm represents the amino (⁻NH₂) proton signal. The chemical structures of 7a, 7b, 8a, and 8b were also characterized by ¹³C NMR spectroscopy, as shown in Figure S7, S9, S11, and S13. Compared to the ¹³C NMR spectrum of 7a (Figure S7), a new singlet peak at 55.78 ppm represents the methyl (O-CH₃) signal in the ¹³C NMR spectrum of 7b (Figure S9). Similarly, compared to the ¹³C NMR spectrum of 8a (Figure S11), there is a new singlet peak at 55.80 ppm, representing the methyl (O-CH₃) signal in the ¹³C NMR spectrum of 8b (Figure S13). The molecular weights of 7a, 7b, 8a, and 8b were characterized by high-resolution mass-spectrometry (HRMS), as shown in Figures S8, S10, S12, and S14.

In the infrared spectrum, peaks at about 3353 cm⁻¹ in 7a and 3356 cm⁻¹ in 8a (Figure S16A) were assigned to the imino bond (⁻NH₂, indole ring), and a new dual peak (absent in 7a) at around 3260 and 3216 cm⁻¹ was attributed primarily to the amino bond (⁻NH₂) of 8a, which is similar to that reported. Similarly, the peaks at about 3403 cm⁻¹ in 7b and 3446 cm⁻¹ in 8b (Figure S16B) were assigned to the imino bond (⁻NH₂, indole ring), while a new dual peak (absent in 7b) at around 3402 and 3340 cm⁻¹ were assigned mostly to the amino bond (⁻NH₂) of 8b. These IR signals explain the chemical differences between 7a/8a and 7b/8b. Thus, the reduction step was clearly supported by the amino bond (⁻NH₂) of 8a/8b compared with 7a/7b.

**In Vitro Cytotoxicity Assay**

The antiproliferative activity of compounds (EVO, 7a, 7b, and 8a) was evaluated against MDA-MB-231, SW620, and LO2.
cells for 48 h, in which the cells without any treatment were used as the control. The cell survival rates denote mean ± SD, which are listed in Tables S1 to S2 Supporting Information. For IC₅₀ value (µM) calculation of compounds, the cell viability results from CCK-8 assays were fitted for different systems using non-linear regression (inhibitor vs normalized response with variable slope). Figure 2A to C summarize the results. From Figure 2A and B, it can been seen that the cytotoxicity of the compounds (EVO, 7a, 7b, and 8a) against the 2 cancer cells increased as the concentration increased, while compound 8b showed almost no cytotoxicity. IC₅₀ values of EVO, 7a, 7b, and 8a against MDA-MB-231 cancer cells were 1.03, 3.87, 44.95, and 0.79 µM, respectively, suggesting that compound 8a exhibited the most potent activities against MDA-MB-231 cells. Figure 2B shows that 7a, 7b, and 8b were inactive (IC₅₀>100 µM) in comparison with EVO (IC₅₀ = 3.47 µM), while compound 8a exhibited the most potent activity against SW620 cells, with an IC₅₀ value of 1.28 µM. The 2-nitro EVO derivative 7a was inactive, but its reduced product 8a showed potent activity against the MDA-MB-231 and SW620 cells. Figure 2C shows that all the compounds had little toxicity to LO2 cells, while most of the compounds were active against MDA-MB-231 and SW620 cells, implying that these compounds kill cancer cells selectively over normal cells.

Cell Apoptosis Assay

To ascertain further that 8a induced apoptosis in MDA-MB-231 cells, a propidium iodide (PI)—annexin V-FITC binding assay was conducted, followed by flow cytometry (FCM) measurement of apoptotic ratios. In Figure 3A, the total apoptotic ratio of the control group without any treatment was 4.41% by a sum of the early apoptotic ratio (2.76%) and the late apoptotic ratio (1.65%). In Figure 3B, the total apoptotic ratio of 8a was 56.9% by a sum of the early apoptotic ratio (8.39%) and the late apoptotic ratio (48.54%) of MDA-MB-231 cells. The results demonstrated that 8a exerted its antiproliferative effects possibly via its proapoptotic activity on the MDA-MB-231 cell lines.24

Materials and Methods

Materials

Tryptamine, 5-methoxytryptamine, 4-nitroantranilic acid, and triphosgene were purchased from Aladdin Industrial Corporation. Sodium hydroxide (NaOH), phosphorus oxychloride (POCl₃), iodomethane (CH₃I), and stannous chloride dihydrate (SnCl₂·H₂O₂) were procured from Energy Chemical; fetal bovine serum (FBS) and Dulbecco’s modified Eagle’s medium (DMEM) from Gibco BRL; and CCK-8 kit from Beyotime, China. Ultrapure water was prepared with a Milli-Q purification system from Millipore at 18.2 MΩ cm⁻¹. The chemical reagents used were all analytically pure. ESI mass spectra were gathered on Aglient 7250 and JEOL-JMS-T100LP AccuTOF spectrometers.

Synthesis of the Compounds

3,4-Dihydro-β-carboline (3a). Tryptamine (2.048 g, 12.8 mmol) was dissolved in ethyl formate (100 mL) and stirred under gentle reflux overnight at 60 °C. After solvent removal, a light yellow oily liquid was obtained. Using DCM (30 mL) for dissolution, POCl₃ (5 mL) was added dropwise, while the temperature was maintained at 0 °C for 2 h. After the addition, the mixture was allowed to warm to room temperature and stirred for an additional 3 h. Solvent was removed and the residue was completely dissolved in aqueous acetic acid (10%). DCM (100 mL) was added and separated. The aqueous layer was neutralized with ammonia solution, and a large amount of precipitate was generated. After filtration, the residue was washed with cooled EtOAc to give 1.46 g (yield 67%) of a yellow solid.24 1H NMR (400 MHz, DMSO-d₆) δ 11.30 ppm (s, 1H), 8.36 ppm (t, J = 2.2 Hz, 1H), 7.56 ppm (d, J = 7.9 Hz, 1H), 7.4 ppm (d, J = 8.2 Hz, 1H), 7.16 ppm (m, 1H), 7.027.06 ppm (m, 1H), 3.76 ppm (m, 2H), and 2.77 ppm (m, 2H).

6-Methoxy-3,4-dihydro-β-carboline (3b). 5-Methoxy-tryptamine (4.05 g, 20 mmol) was dissolved in ethyl formate (100 mL) and stirred under gentle reflux overnight at 60 °C. After solvent removal, a light yellow oily liquid was obtained. This was dissolved in DCM (80 mL) before POCl₃ (5 mL) was added dropwise, while the temperature was maintained at 0 °C for 2 h. After the addition, the mixture was allowed to warm to room temperature and stirred for an additional 3 h. Solvent was removed and 10% acetic acid aqueous solution was added until completely dissolved. After the addition of ammonia solution to adjust the alkalinity, a large amount of precipitate was generated. After filtration, the residue was washed with ethyl acetate, obtaining 3.5 g of white solid, with a yield of 87%.24 1H NMR (DMSO-d₆, 400 MHz) (ppm) δ 11.14 (1H, s), 8.31 (1H, s, J = 2.2 Hz), 7.30 (1H, d, J = 8.9 Hz), 7.01 (1H, d, J = 2.4 Hz), 6.83 (1H, d, J = 8.9, 2.5 Hz), 3.74 to 3.78 (5H, m), and 2.74 to 2.79 (2H, m).

7-Nitro-isatoic anhydride (5). 2-Amino-4-nitrobenzoic acid (4.57 g, 25.1 mmol) was dissolved in anhydrous THF (100 mL). Then, triphosgene (2.96 g, 10 mmol) was added and the reaction refluxed at 70 °C for 5 h. On pouring the solution into ice water (500 mL), a large amount of precipitate was generated. A yellow solid (4.85 g) was obtained by filtration with a yield of 93%.1H NMR (DMSO-d₆, 400 MHz) (ppm) δ 12.08 ppm (s, 1H), 8.16 ppm (d, J = 8.6 Hz, 1H), 7.97 ppm (dd, J = 8.6, 2.2 Hz, 1H), and 7.87 ppm (d, J = 2.1 Hz, 1H).13C NMR (101 MHz, DMSO-d₆) δ 159.2, 152.4, 147.1, 142.6, 131.4, 117.8, 115.9, 110.7 ppm.
Figure 2. In vitro cytotoxicity of evodiamine (EVO), 7a, 7b, 8a, and 8b against MDA-MB-231 cancer cells (A), SW620 cancer cells (B) and LO2 cells (C).

Figure 3. In vitro cell apoptosis. Flow cytometry analysis of MDA-MB-231 cells incubated with culture medium as control (A) and 20.00 µM of 8a (B) for 48 h. (Q1-UL, necrotic cells; Q1-UR, late apoptotic; Q1-LR, early apoptotic cells; and Q1-LL, living cells).
N-methyl-7-nitro-isatoic anhydride (6). 7-Nitroastraphalic anhydride (1.015 g, 4.88 mmol) was dissolved in 50 mL DMF, and cooled to 0 °C in an ice-water bath. NaH (0.3 g, 12.5 mmol) was added and the mixture was stirred at 0 °C for an additional 1 h. After that, MeI (3.142 g, 22.14 mmol) was added, dropwise. The reaction was allowed to warm up to room temperature and was stirred for an additional 4 h. The reaction solution was poured into ice water (300 mL), followed by filtration, and washing the precipitate with water to obtain a yellow solid product, 0.67 g (yield 62%). 1H NMR (400 MHz, DMSO-d6) δ 8.25 ppm (d, J = 8.6 Hz, 1H), 8.13 ppm (d, J = 1.3 Hz, 1H), 8.07 ppm (dd, J = 8.5, 2.0 Hz, 1H), and 3.56 ppm (s, 3H). 13C NMR (101 MHz, DMSO-d6) δ 158.4, 152.9, 147.9, 143.6, 131.6, 118.0, 117.1, 110.4, 32.6 ppm.

10-Methoxy-2-nitro evodiamine (7a). N-methyl-7-nitro-isatoic anhydride (1.11 g, 5 mmol) and 3,4-dihydro-β-carboline (0.85 g, 5 mmol) were dissolved in 100 mL anhydrous DCM and heating under reflux overnight. The solvent was removed and the residue washed with water. The precipitate was obtained as an orange solid product (1.16 g), with a yield of 66.5%, m.p. 260.52-261.9 °C. 1H NMR (DMSO-d6) δ 10.99 ppm (s, 1H), 7.96 ppm (d, J = 8.4 Hz, 1H), 7.68 ppm (d, J = 2.0 Hz, 1H), 7.62 ppm (dd, J = 8.5, 2.1 Hz, 1H), 7.46 ppm (d, J = 7.8 Hz, 1H), 7.36 ppm (j, J = 8.1 Hz, 1H), 7.12 ppm (t, J = 8.0 Hz, 1H), 7.01 ppm (t, J = 7.2 Hz, 1H), 6.35 ppm (s, 1H), 4.65 ppm (dd, J = 13.0, 5.4 Hz, 1H), 3.44 ppm (p, 1H), 3.24 ppm (s, 3H), 2.95 to 3.04 ppm (m, 1H), and 2.74 to 2.79 ppm (m, 1H). 13C NMR (101 MHz, DMSO-d6) δ 163.6, 151.4, 148.6, 136.8, 131.4, 130.1, 126.5, 122.5, 122.1, 119.5, 118.7, 113.3, 112.2, 112.0, 109.8, 71.1, 42.3, 36.9, 19.7 ppm. MS(ESI): 349.16529 (C19H16N4O3, [M + H]). Anal. Caled for C19H16N4O3: C 65.51, H 4.63, N 16.81.

2-Amino evodiamine (8a). 2-Nitro evodiamine (1.164 g, 3.34 mmol) was suspended in DMF (50 mL), and HCl (5 mL) was added. SnCl2·(H2O)2 (4 g, 17.7 mmol) was dissolved in 30 mL DMF. The previously made Vilsmeier reagent was added dropwise and stirred overnight at 50 °C. Then, saturated sodium hydroxide solution was added until the pH reached 9. After filtering, water (20 mL) was added and the mixture extracted with EtOAc (3 × 100 mL). The combined organic layers were washed with brine (50 mL) and dried with Na2SO4. After solvent removal, the residue was purified on silica gel, eluting with EtOAc/hexanes (1:1→1:2, Rf = 0.17) to obtain a solid (100 mg) compound (8a), light yellow in color, yield 9.4%, m.p. 261.7-262.0 °C. 1H NMR (DMSO-d6) δ 10.90 ppm (s, 1H), 7.43 ppm (j, J = 7.8 Hz, 2H), 7.36 ppm (d, J = 8.1 Hz, 1H), 7.09 ppm (m, 1H), 6.967.00 ppm (m, 1H), 6.086.10 ppm (m, 1H), 6.006.02 ppm (m, 1H), 6.00 ppm (s, 1H), 5.80 ppm (s, 2H), 4.56 ppm (dd, J = 13.0, 4.9 Hz, 1H), 2.92 ppm (s, 3H), 2.90 ppm (s, 1H), 2.74 ppm (s, 1H), and 2.67 ppm (m, 1H). 13C NMR (101 MHz, CDCl3) δ 165.5, 154.3, 150.5, 136.8, 132.4, 130.1, 129.9, 126.6, 118.6, 118.4, 112.2, 111.7, 107.5, 107.1, 99.3, 70.9, 41.5, 36.9, 19.9 ppm. MS(ESI): 319.15484 (C19H16N4O, [M + H]). Anal. Caled for C19H16N4O: 71.68, H 5.70, N 17.60; found C71.92, H 5.57, N 17.12.

10-Methoxy-2-amino evodiamine (8b). To 10-methoxy-2-nitro evodiamine (1.2 g, 3.17 mmol) dissolved in 50 mL DMF, 15 mL HCl was added. SnCl2·(H2O)2 (4 g, 17.7 mmol) was dissolved in 30 mL DMF. The previously made Vilsmeier reagent was added dropwise and stirred overnight at 50 °C. Then, saturated sodium hydroxide solution was added until the pH reached 9. After filtering, water (20 mL) was added and extracted with EtOAc (3 × 100 mL). The combined organic layers were washed with brine (50 mL) and dried with Na2SO4. After solvent removal, the residue was purified on silica gel, eluting with (DCM/Ethanol = 100:1, Rf = 0.23) to give compound 8b, which was a yellow solid (170 mg), yield 15.4%, m.p. 264.5264.9 °C. 1H NMR (DMSO-d6, 400 MHz) δ 10.63 ppm (s, 1H), 7.117.21 ppm (m, 2H), 7.12 ppm (d, J = 2.2 Hz, 1H), 7.03 ppm (d, J = 2.3 Hz, 1H), 6.71 ppm (dd, J = 8.7, 2.4 Hz, 1H), 6.48 ppm (dd, J = 8.4, 2.2 Hz, 1H), 6.38 ppm (d, J = 2.2 Hz, 1H), 5.66 ppm (s, 2H), 3.74 ppm (s, 3H), 3.39 ppm (m, 2H), 2.98 ppm (s, 3H), and 2.81 ppm (t, J = 7.4 Hz, 2H). 13C NMR (101 MHz, DMSO-d6) δ 163.2, 151.4, 148.6, 136.8, 131.4, 130.1, 126.5, 122.5, 119.5, 118.7, 113.3, 112.2, 112.0, 109.8, 71.1, 42.5, 36.9, 19.7 ppm. MS(ESI): 349.16529 (C19H16N4O2, [M + H]). Anal. Caled for C19H16N4O2: C 68.95, H 5.70, N 19.69; found C68.95, H 5.70, N 19.68.

Characterization of Compounds 7a, 7b, 8a, and 8b

For elucidation of the chemical structures of 7a, 7b, 8a, and 8b, an ADVANCEIII spectrometer (400 MHz) was utilized to perform 1H NMR analysis. The molecular weights of 8a and 8b were further characterized by HRMS.
Culture of SW620 and MDA-MB-231 Cells
Human breast carcinoma MDA-MB-231 and human colon carcinoma SW620 cells, purchased from the Type Culture Collection of Chinese Academy of Sciences in Shanghai, China, were cultured in 75 cm² flasks using DMEM medium containing 1% Pen-Strep (vol/vol) and 10% FBS under 5% CO₂ at 37 °C. Medium replacement was implemented at 2-d intervals, and, following cellular confluence, the cells were carried on the passage with a ratio of 1:3. The fourth generation cells were used in this experiment.

In Vitro Cytotoxicity
CCK-8 assay was performed for evaluating the antiproliferative activity of the compounds against MDA-MB-231 and SW620 carcinoma cells.27 For cell culturing, 5000 cells were seeded into each well of a 96-well plate containing 200 µL medium. After culturing for 24 h, the medium in each well was replaced with fresh medium (20 µL) containing samples in varying concentrations, in order to attain final sample concentrations of 0.015, 0.045, 0.137, 0.411, 1.234, 3.703, 11.11, 33.33, and 100 µM. After 48 h of incubation, the medium was removed and replaced with fresh culture medium containing CCK-8 reagent, which was incubated for another 4 h. The absorbance at 450 nm was read utilizing a microplate reader. The formula for cell viability was calculated as follows28:

\[
\text{Cell viability(%) = } \frac{A_s - A_{pc}}{A_{nc} - A_{pc}} \times 100\%.
\]  

where \(A_s\), \(A_{nc}\), and \(A_{pc}\) are the absorbances of sample, negative control, and positive control at 450 nm, respectively. The cells incubated without drugs were regarded as the negative control, while the wells containing medium only without cells were regarded as the positive control. All the experiments were conducted in triplicates.

Cell Apoptosis Assay
Dual-staining with PI and Annexin V-FITC was employed to determine whether the death of cancer cells incubated with 2-NH₂-EVO (8a) was induced by apoptosis. After seeding 5 × 10⁵ MDA-MB-231 cells into each well of a 24-well plate overnight, 20.00 µM of 8a was added and incubated for 48 h. The untreated cells were used as control. After 48 h, the cells were dual-stained using PI and annexin V-FITC. With the aid of FCM, the PI and/or annexin V-FITC-positive cell percentages were recorded inside the quadrants.

Statistical Analysis
Data, all of which were acquired from at least 3 separate trials (n = 3), were processed and expressed as means ± SDs. Statistical analysis was carried out through one-way analysis of variance using GraphPad Prism 8, and \(P\) values of <.05 were considered to indicate significant inter-mean differences. For calculation of IC₅₀ values, GraphPad Prism 8 was used as well.

Conclusion
In this work, 4 novel EVO derivatives with nitro and amino groups were designed and synthesized. The antiproliferative activities of all compounds were tested against MDA-MB-231 and SW620 cancer cells for 48 h and compared to EVO. Among them, 8a exhibited the most effective cytotoxicity against MDA-MB-231 and SW620 cells with IC₅₀ values of 0.79 and 1.28 µM, respectively. Flow cytometric analysis revealed that the MDA-MB-231 cells, which were subjected to 48-h culturing with 20.00 µM 8a, had a remarkable apoptotic effect.

Authors’ Note
Ruolan Yang and Jingjing Ma contributed equally to this work. H.G. conceived and designed the experiments; R.L.Y. performed the experiments; J.J.M., Z.L., Q.H.M., and H.Y analyzed the data; R.Y.J. finished calculation; L.J.M contributed reagents/materials/analysis tools; R.L.Y. and H.G. wrote the paper.

Declaration of Conflicting Interests
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ORCID iD
Hui Guo https://orcid.org/0000-0003-3211-4806

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References
1. Siegel RL, Miller KD, Jemal A. Cancer statistics 2020. CA Cancer J Clin. 2020(70):7-30. doi:10.3322/caac.21590
2. Shah U, Shah R, Acharya S, Acharya N. Novel anticancer agents from plant sources. Chin J Nat Med. 2013;11(1):16-23. doi:10.1016/S1875-5364(13)60002-3
3. Khazir J, Mir BA, Pilcher L, Riley DL. Role of plants in anticancer drug discovery. Phytochem Lett. 2014;7:173-181. doi:10.1016/j.phytot.2013.11.010
4. Jiang J, Hu C. Evodiamine: a novel anti-cancer alkaloid from Evodia rutaecarpa. *Molecules.* 2009;14(5):1852-1859. doi:10.3390/molecules14051852
5. Zhao Z, He X, Han W. Genus Tetradium L. A comprehensive review on traditional uses, phytochemistry, and pharmacological activities. *J Ethnopharmacol.* 2019;231:337-354. doi:10.1016/j.jep.2018.11.035
6. Lin L, Ren L, Wen I, Wang Y, Qi J. Effect of evodiamine on the proliferation and apoptosis of A549 human lung cancer cells. *Med Mol Rep.* 2016;14(3):2832-2838. doi:10.3892/mmr.2016.5575
7. Yang J, Wu LJ, Tashino S, Onodera S, Ikejima T. Reactive oxygen species and nitric oxide regulate mitochondria-dependent apoptosis and autophagy in evodiamine-treated human cervix carcinoma HeLa cells. *Free Radiuc Res.* 2008;42(5):492-504. doi:10.1080/10715760802112791
8. Qiu C, Gao LN, Yan K, Cai YL, Zhang YA. Promising antitumor activity of evodiamine incorporated in hydroxypropyl-beta-cyclodextrin: pro-apoptotic activity in human hepatoma HepG2 cells. *Chem J Cent.* 2016;10:406. doi:10.1186/s13065-016-0191-y
9. Zhao LC, Li J, Liao K, et al. Evodiamine induces apoptosis and inhibits migration of HCT-116 human colorectal cancer cells. *Int J Mol Sci.* 2015;16(11):27411-27421. doi:10.3390/ijms161126031
10. Hong JY, Hyun PS, Min HY, Joo PH, Kook IS. Anti-proliferative effects of evodiamine in human lung cancer cells. *J Cancer Prev.* 2014;19(1):7-13. doi:10.15440/jcpr.2014.19.1.7
11. Guo XX, Li XP, Zhou P. Evodiamine induces apoptosis in SMMC-7721 and HepG2 cells by suppressing NOD1 signal pathway. *Int J Mol Sci.* 2018;19(1):3419-3434. doi:10.3390/ijms19113419
12. Tu YJ, Fan X, Yang X, Zhang C, Liang H. Evodiamine activates autophagy as a cytoprotective response in murine Lewis lung carcinoma cells. *Oncol Rep.* 2013;29(2):481-489. doi:10.3892/or.2012.2125
13. Wu J, Wang J, Zhao H. Commentary regarding “solubilities of evodiamine in twelve organic solvents from T = (283.2 to 323.2) K”. *J Chem Thermodyn.* 2019;129:145-147. doi:10.1016/j.jct.2018.09.015
14. Fan JP, Yang XM, Xu XK, Xie YL, Zhang XH. Solubility of rutaecarpine and evodiamine in (ethanol + water) mixed solvents at temperatures from (288.2 to 328.2) K. *J. Chem. Thermodyn.* 2015;83:85-89. doi:10.1016/j.jct.2014.12.004
15. Hu X, Wang Y, Xue JI, et al. Design and synthesis of novel nitrogen mustard-evodiamine hybrids with selective antiproliferative activity. *Bioorganic Med Chem Lett.* 2017;27(22):4989-4993. doi:10.1016/j.bmcl.2017.10.014
16. Deng JD, Lei S, Jiang Y, Wen HX, Tan W, Wan Z. A concise synthesis and biological study of evodiamine and its analogues. *Chem Commun.* 2019;55(21):3089-3092. doi:10.1039/C9CC00434C
17. Wang SZ, Dong GQ, Chen SQ, et al. Scaffold diversity inspired by the natural product evodiamine: discovery of highly potent and multitargeting antitumor agents. *J Med Chem.* 2015;58(16):6678-6696. doi:10.1021/acs.jmedchem.5b00910
18. Fang K, Dong GQ, Gong H, et al. Design synthesis and biological evaluation of E-ring modified evodiamine derivatives as novel antitumor agents. *Chin Chem Lett.* 2014;25(7):978-982. doi:10.1016/j.ccl.2014.03.043
19. Song S, Chen Z, Li S, Huang Y, Wan Y, Song H. Design synthesis and evaluation of N13-substituted evodiamine derivatives against human cancer cell lines. *Molecules.* 2013;18(12):15750-15768. doi:10.3390/molecules181215750
20. Dong G, Wang S, Miao Z, et al. New tricks for an old natural product: discovery of highly potent evodiamine derivatives as novel antitumor agents by systemic structure-activity relationship analysis and biological evaluations. *J Med Chem.* 2012;55(17):7593-7613. doi:10.1021/jm300605m
21. Stehlin JS, Giovanella BC, Natelson EA, et al. Study of 9-nitrocamptothecin (RFS-2000) in patients with advanced pancreatic cancer. *Int J Oncol.* 1999;14(5):821-831. doi:10.3892/ijo.14.5.821
22. Gu JI, Lee K, Juvik JA, Rebeiz CC, Bouton CE, Rebeiz CA. Porphyrinic insecticides. IV: structure-activity study of substituted phenanthrenones. * Pest Manag Sci.* 2006;69(1):19-30. doi:10.1002/ps.2780390104
23. Chin Chung M, Longhin Bosquesi P, Leandro dos Santos JA. Prodrug approach to improve the physico-chemical properties and decrease the genotoxicity of nitro compounds. *Curr Pharm Des.* 2011;17(32):3515-3526. doi:10.2174/138161211798194512
24. Nie LF, Wang SS, Cao JG, et al. Straightforward synthesis, characterization, and cytotoxicity evaluation of hybrids of natural alkaloid evodiamine/rutaecarpine and thiieno[2,3-d]pyrimidinones. *J Asian Nat Prod Res.* 2018;20(1):69-82. doi:10.1080/10286020.2018.1540599
25. Moreno-Fuquen R, Mercedesincapic-Ótero M, Becerra D, Millán JC, Macias MA. Synthesis of 1-arylsulfonyl-3-methylsulfanyl-5-amino-1,2,4-triazoles and their analysis by spectroscopy, x-ray crystallography and theoretical calculations. *J Mol Struct.* 2021;1226(part A):129-317. doi:10.1016/j.molstruc.2020.129317
26. Li Y, Lin J, Ma J, et al. Methotrexate-Camptothecin prodrug nanoassemblies as a versatile nanoplatform for biomodal imaging-guided self-active targeted and synergistic chemotherapy. *ACS Appl Mater Interfaces.* 2017;9(40):34650-34665. doi:10.1021/acsamat.7b02780
27. Sui H, Zhou LH, Zhang YL, et al. Evodiamine/rutaecarpine and thieno[2,3-d]pyrimidinones. *Nat Prod Res.* 2018;22(1):69-82. doi:10.1080/10286020.2018.1540599
28. Rao K, Zhong Q, Bielski ER, Da Rocha SRP. Nanoparticles of 5-amino-1,2,4-triazoles and their analysis by spectroscopy, x-ray crystallography and theoretical calculations. *J Mol Struct.* 2021;1226(part A):129-317. doi:10.1016/j.molstruc.2020.129317
29. Sui H, Zhou LH, Zhang YL, et al. Evodiamine/rutaecarpine and thieno[2,3-d]pyrimidinones. *Nat Prod Res.* 2018;22(1):69-82. doi:10.1080/10286020.2018.1540599