RESEARCH ARTICLE

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2018, 9, 1131Design, synthesis and bioactivity investigation of
tetrandrine derivatives as potential anti-cancer
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Twenty-four 14-sulfonamide-tetrandrine derivatives as potential anti-cancer agents were synthesized. The synthetic derivatives were investigated for their cytotoxic activity against human cancer cell lines MDA-MB-231, PC3, WM9, HEL and K562. Initially, the IC₅₀ values (50% inhibitory concentrations) of all of the compounds were determined. These derivatives exhibited potent, but distinct, inhibitory effects on the above-mentioned cell lines. Among them, compound **23**, which was modified with a 2-naphthalenesulfonyl group at the 14-amino position, showed impressive inhibition of all five cancer cell lines, and especially of MDA-MB-231 cells with an IC₅₀ value of 1.18 ± 0.14 μM. Further mechanism exploration showed that **23** induced potent apoptotic cell death on MDA-MB-231 cancer cells in a concentration-dependent manner. The results revealed that **23** might be a potential anti-cancer drug candidate.

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1. Introduction

Since the last decade, cancer has been ranked as the most serious disease due to its uncontrolled cell growth, and it is a leading cause of death worldwide.^{1,2} It is estimated that by 2030, the number of cancer deaths worldwide will reach more than 11.4 million.^{3–6} Therefore, the global demand for anti-cancer drugs is on the rise. Since 2010, sales of anti-cancer drugs have reached 36.4 billion US dollars, ranking them as first among all kinds of drugs.⁷ As a result, the prevalence of cancer and its resistance to existing therapeutic agents necessitate the development of new drugs that may overcome the limitations of existing drugs.⁸

Natural medicines play a crucial role in anti-cancer drug discovery.⁹ About two-thirds of anti-neoplastic drugs are either original natural products or molecules derived from these.^{10–12} Tetrandrine (Tet) is a typical dibenzylisoquinoline alkaloid,^{13,14} which displays a variety of biological activities,

including anti-inflammation,¹⁵ suppression of tumor proliferation,¹⁶ immunomodulation and so on.^{17,18} Recently, it has been reported that tetrandrine improves CIA in mice by restoring the balance between Th17 and Treg cells *via* the aryl hydrocarbon receptor, and it restricts the migration and invasion of RA fibroblast-like synoviocytes by activating the PI3K/Akt and JNK signaling pathways and down-regulating the expression of Rac1, Cdc42, and RhoA GTPases.^{19–21} In addition, Karnika Singh *et al.* reported for the first time that tetrandrine inhibited the proliferation of PaCa cells and PaCa tumor growth by indirectly impairing the activities of CDK4/6.²² Interestingly, Gauri Shishodia *et al.* tested the effectiveness of tetrandrine in the sensitization of PCa cells to TRAIL and the results showed that the treatment of PCa cells with tetrandrine could induce expression of DR4, DR5 and TNFR1, and sensitize these cells to TRAIL-mediated cell death.²³ Lee *et al.* reported that tetrandrine could cause apoptosis of human lung cancer cells.²⁴

In addition, structural modification of tetrandrine is the strategy of choice to enhance its pharmacological activity, as well as to decrease its toxicity. Wang *et al.* introduced halogen groups at the 5-position of tetrandrine to obtain derivatives that could reduce the expression of Pg-P,²⁵ and thereby reverse the effect of MDR (multidrug resistance) on a variety of drug-resistant cancer cell lines.^{26–28} In addition, Wu *et al.* synthesized a series of tetrandrine derivatives *via* Sonogashira and Suzuki reactions, which showed good inhibitory activity on HepG2 (human hepatocellular carcinoma), A549 (human lung cancer) and BGC-823 (human gastric cancer) cell lines.²⁹

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Previously, Ochoa Lara's research group reported a series of bis-alkylated tetrandrine derivatives that were found to be effective dicationic hosts in water for carboxylates, nucleotides, and DNA.^{30–32} More recently, Calvillo-Páez *et al.* synthesized two mono-alkylated tetrandrine derivatives and found that these two derivatives exhibit high affinity ($K \sim 10^5 \text{ M}^{-1}$) and sequence selectivity for dsDNA in an intercalation binding mode.³³ Our previous study also showed that substitution at the 14-position of tetrandrine could enhance its inhibitory activity on HCC (human hepatocellular carcinoma).³⁴ Indeed, a series of novel 14-urea tetrandrine derivatives recently synthesized by us showed moderate to strong anti-proliferative activities against the human cancer cell lines HEL (leukemia), K562 (leukemia), PC3 (prostate), MDA-MB-231 (breast) and WM9 (melanoma).³⁵

On the other hand, the sulfonamide functional group is a basic commonplace unit, typically found in several drugs. Furthermore, sulfonamides are known to exhibit several types of bioactivity, such as diuretic, antiglaucoma, anticonvulsant, and antiobesity effects.^{36–38} For example, *N*-acylsulfonamides can inhibit enzymatic hydrolysis and are demonstrated as potential drug candidates for the treatment of cancer.^{39–41} Indeed, some *N*-acylsulfonamides have recently progressed into clinical trials, including ABT-199 (venetoclax), a chemical analogue of navitoclax with highly selective Bcl-2 inhibitor properties^{42–44} (Fig. 1).

A wide range of biologically active compounds with sulfonamide units are expected to be used as agents for the treatment of cancers, viral infections and some cardiovascular diseases. For that reason and also inspired by our previous studies, twenty-four new sulfonamide-tetrandrine derivatives were designed and synthesized. Meanwhile, all of the compounds were investigated for their anti-cancer activities, and some of those compounds were highly active toward cancer cell lines, outperforming their parent compounds.

2. Results and discussion

2.1 Chemistry

The synthetic procedure for compounds 1–24 is described in Scheme 1. According to previously reported procedures,³⁵ tetrandrine was selectively nitrified at the C-14 position to obtain the compound Tet-NO₂. The nitro group was then efficiently transformed into an amino group. All of the compounds were then efficiently sulfonamidated in the presence of DMAP with satisfactory yields from Tet-NH₂ (Scheme 1). All of the structures of the sulfonamide-tetrandrine derivatives were verified using IR, ¹H NMR, ¹³C NMR and HRMS.

Fig. 1 Chemical structure of ABT-199.

2.2 Biological evaluation

2.2.1 *In vitro* cytotoxicity assay. The inhibitory effects of these novel tetrandrine derivatives on cancer cells were tested on five human cancer cell lines: HEL, PC3, MDA-MB-231, WM9, and K562. The anti-proliferative effects of the compounds were examined using MTT assays after 48 h of drug treatment. Vincristine, Tet-NO₂, Tet-NH₂ and tetrandrine were used as positive controls. The IC₅₀ values for each derivative are shown in Table 1 and the values are the average of at least three independent experiments.

As shown in Table 1, the anti-cancer activity of these sulfonamide-tetrandrine derivatives was significantly enhanced compared to that of their parent compound. Derivatives in which the benzene ring was substituted with electron-withdrawing groups at the 14-amino position showed increased anti-proliferative activities against all five cancer cell lines. In HEL cells, all of the derivatives except compounds 20 and 22 exhibited better activity than that of Tet-NO₂, Tet-NH₂ and tetrandrine. Among them, compound 11 displayed the highest anti-cancer potential with an IC₅₀ value of $1.57 \pm 0.05 \mu\text{M}$. In PC3 cells, the inhibitory effect of all of the derivatives was obviously stronger than that of the positive controls. Notably, compound 10 with an inter-nitro substituted benzenesulfonyl group at the 14-amino position showed better activity with an IC₅₀ value of $1.94 \pm 0.11 \mu\text{M}$. In WM9 cells, the IC₅₀ values of derivatives 5 and 8 were $1.98 \pm 0.42 \mu\text{M}$ and $1.68 \pm 0.22 \mu\text{M}$, respectively. Among these, the IC₅₀ value of compound 8 was lower than that of the positive controls. For K562 cells, most of the derivatives showed potent anti-proliferative activity. Remarkably, the IC₅₀ values of derivatives 8, 10, 17 and 23 were lower than those of the positive controls. Finally, in MDA-MB-231 cells, the IC₅₀ values of all of the derivatives were significantly lower than those of the positive controls. In particular, compound 23 showed potent inhibitory activity against MDA-MB-231 cells with an IC₅₀ value of $1.18 \pm 0.14 \mu\text{M}$.

Independently, the cytotoxic activity on the normal human liver cell line HL7702 of all of the compounds was evaluated using MTT assays. Furthermore, the natural product Tet was included for comparison. Amongst the compounds screened for cytotoxic activity on the normal human liver cell line HL7702, compounds Tet, 19 and 16 showed weak activities with IC₅₀ values of $44.25 \pm 0.21 \mu\text{M}$, $32.11 \pm 0.10 \mu\text{M}$ and $28.23 \pm 0.43 \mu\text{M}$, respectively. On the other hand, compound 8 showed moderate capabilities for cytotoxic activity with an IC₅₀ value of $8.73 \pm 0.11 \mu\text{M}$.

2.3 Annexin V-FITC/PI flow cytometry assay

The cytotoxicity mechanism of 23 on MDA-MB-231 cancer cells was further investigated using an Annexin V-FITC/propidium iodide staining assay. MDA-MB-231 cells were treated with 1.25 and 2.5 μM 23 for 24 h and stained with Annexin V-FITC and propidium iodide for 15 min, and samples were analyzed using flow cytometry. As illustrated in Fig. 2, compound 23 induced apoptosis of MDA-MB-231 cells

Scheme 1 Synthesis of tetrandrine derivatives. (a) Mixed acid (12.0 eq, HNO₃:(CH₃CO)₂O = 10:7, v/v), CH₂Cl₂, 0 °C to r.t. (94%) (b) Pd/C (20%), N₂H₄·H₂O (30.0 eq), MeOH, 80 °C, 3.5 h (83%). (c) RSOOCl (1.0 eq), DMAP (0.3 eq), CH₂Cl₂, 0 °C to r.t., 4–5 h (89–77%).

in a concentration-dependent manner. Specifically, while the percentage of apoptotic cells in the control was about 4.0%, a higher percentage of apoptosis (55%) was observed with compound 23 (concentration 2.5 μM).

2.4 The tetrandrine derivative 23 affects the protein levels of apoptosis indicators in MB-MDA-231 cells

It is generally accepted that Caspase-3 is the most important terminal cleavage enzyme during apoptosis. PARP is involved in DNA repair and transcriptional regulation. During apoptosis, PARP is cleaved by Caspase-3 into two fragments of 31 kD and 85 kD. This cleavage makes PARP unable to function normally, causing apoptosis.⁴⁵ To understand the mechanism of apoptosis induced by compound 23, a western blot was performed to detect the expression levels of apoptosis-related proteins. Proteins were extracted from MDA-MB-231 cells treated with compound 23 at 2.5 μM for 24 h. As shown in Fig. 3, the treatment of MDA-MB-231 cells with compound 23 resulted in increased expression of pro-apoptotic protein Bax, and the appearance of cleaved PARP. Meanwhile, the expression of anti-apoptotic-related proteins (Bcl-xl and Bcl-2) decreased accordingly. These results revealed that compound 23 is a potent apoptosis inducer.

3. Conclusions

In this study, 24 novel sulfonamide–tetrandrine derivatives were synthesized in good yields and tested for their cytotoxic activities. The inhibitory activity of most of the derivatives against the tested cancer cell lines was significantly higher than that of the original compound, tetrandrine. The cytotoxic activity of some of the derivatives was considerably higher than that of the positive controls. In particular, halogen substitution on the benzene ring at the C-14 sulfonamide moiety could significantly enhance the cytotoxic activity. Mechanistic studies indicated that compound 23, and likely other derivatives, downregulated the expression of Bcl-2 and Bcl-xl, upregulated the pro-apoptotic Bax level and activated PARP cleavage to induce apoptosis. Our present results suggest that compound 23 might act as a potent cytotoxic drug candidate for the treatment of breast cancer.

4. Experimental section

4.1 Chemistry

Tetrandrine was isolated and purified from the roots of *Stephania tetrandra* by our group with a purity of ≥98%. Reagents and solvents were purchased from commercial sources. Solvents were purified according to the guidelines in the Purification of Laboratory Chemicals. Column chromatography was performed on silica gel (Qingdao, 200–300 mesh) using the indicated eluents. Thin-layer (0.25 mm) chromatography was carried out on silica gel plates (Qingdao). NMR spectra were recorded on 400 MHz (Varian) or 500 MHz (Wipm) spectrometers in appropriate solvents using TMS as an internal standard or the solvent signals as secondary standards and the chemical shifts are shown on the δ scale. Multiplicities of ¹H NMR signals are designated as s (singlet), d (doublet), t (triplet), and m (multiplet). ¹³C NMR spectra were recorded on 100 MHz or 125 MHz spectrometers. High-resolution mass spectra were obtained using Bruker ESI-QTOF mass spectrometry. IR spectra were recorded using a FTIR spectrometer (IR 200) and the KBr disk method was adopted. Melting points (uncorrected) were determined on WRX-4 Micro Melting Point Apparatus. The chemical purity was determined using HPLC (HP1100). The HPLC conditions used CAPCELL PAK C₁₈(4.6 mm × 250 mm), and the mobile phase consisted of acetonitrile and 0.1% triethylamine (80:20) with a gradient elution at a flow rate of 1 mL min^{−1}. The detection wavelength was 282 nm and the sample quantity was 10 μL.

Tetrandrine. White amorphous solid; mp: 173–175 °C; purity: 98.9%; IR (KBr, cm^{−1}): 2934, 2836, 1606, 1414, 1110, 861; ¹H NMR (CDCl₃, 400 MHz) δ (ppm): 7.34 (dd, *J* = 8.0, 2.4 Hz, 1H), 7.14 (dd, *J* = 8.0, 2.0 Hz, 1H), 6.88 (t, *J* = 4.4 Hz, 1H), 6.81 (dd, *J* = 8.4, 2.8 Hz, 1H), 6.54 (d, *J* = 1.6 Hz, 1H), 6.50 (s, 1H), 6.32–6.28 (m, 2H), 5.99 (s, 1H), 3.92 (s, 3H), 3.87 (dd, *J* = 11.2, 5.6 Hz, 1H), 3.74 (s, 3H), 3.72 (s, 1H), 3.56–3.38 (m, 2H), 3.37 (s, 3H), 3.24 (dd, *J* = 12.8, 5.6 Hz, 1H), 3.18 (s, 3H), 3.02–2.66 (m, 8H), 2.61 (s, 3H), 2.51 (d, *J* = 10.4 Hz, 1H), 2.41 (dd, *J* = 16.0, 5.2 Hz, 1H), 2.33 (s, 3H). ¹³C NMR (CDCl₃, 100 MHz) δ (ppm): 153.6, 152.7, 149.2, 148.6, 146.8, 145.7, 143.4, 141.8, 135.1, 134.9, 134.5, 132.5, 130.1, 128.6, 128.0, 123.4, 123.2, 122.6, 121.8, 120.6, 116.1, 112.9, 111.3, 104.7, 77.3, 63.6, 61.3, 56.1, 55.9, 45.2, 44.1, 42.5, 42.2, 41.9, 37.9, 25.3, 21.6. MS (ESI): *m/z* 623.3 [M + H]⁺.

Table 1 The IC₅₀ values of tetrandrine derivatives against the growth of five cancer cell lines and the normal human liver cell line HL7702

Compound	R	IC ₅₀ (μM), 48 h					
		HEL	K562	PC3	WM9	MDA-MB-231	HL7702
1		2.60 ± 0.32	3.57 ± 0.22	3.37 ± 0.41	2.58 ± 0.10	5.67 ± 0.37	14.67 ± 0.17
2		6.00 ± 0.05	4.14 ± 0.33	6.64 ± 0.17	4.03 ± 0.53	5.12 ± 0.19	12.48 ± 0.54
3		6.77 ± 0.29	7.26 ± 0.19	5.19 ± 0.36	6.35 ± 0.45	5.33 ± 0.27	22.54 ± 0.25
4		5.83 ± 0.39	7.12 ± 0.17	5.57 ± 0.29	5.80 ± 0.46	6.69 ± 0.31	18.60 ± 0.27
5		3.87 ± 0.54	3.07 ± 0.19	4.44 ± 0.32	1.98 ± 0.42	7.48 ± 0.61	13.48 ± 0.09
6		3.04 ± 0.21	5.84 ± 0.19	5.89 ± 0.06	4.19 ± 0.11	7.89 ± 0.08	16.32 ± 0.14
7		3.48 ± 0.29	3.57 ± 0.30	4.63 ± 0.18	4.09 ± 0.40	7.42 ± 0.51	7.91 ± 0.27
8		2.90 ± 0.09	1.46 ± 0.33	3.47 ± 0.39	1.68 ± 0.22	3.81 ± 0.31	8.73 ± 0.11
9		5.72 ± 0.46	5.05 ± 0.37	7.303 ± 0.19	3.257 ± 0.35	2.51 ± 0.27	16.18 ± 0.44
10		2.17 ± 0.20	1.78 ± 0.12	1.94 ± 0.11	3.36 ± 0.29	6.37 ± 0.39	10.07 ± 0.81
11		1.57 ± 0.05	3.28 ± 0.29	3.24 ± 0.41	4.33 ± 0.33	7.79 ± 0.42	12.13 ± 0.39
12		2.59 ± 0.10	2.67 ± 0.11	2.97 ± 0.39	3.73 ± 0.53	4.81 ± 0.09	17.06 ± 0.20
13		3.83 ± 0.37	3.72 ± 0.37	3.98 ± 0.41	8.51 ± 0.51	5.60 ± 0.50	9.38 ± 0.11
14		6.01 ± 0.09	5.95 ± 0.11	5.76 ± 0.12	7.93 ± 0.21	6.84 ± 0.25	19.49 ± 0.34
15		3.68 ± 0.13	4.03 ± 0.20	4.44 ± 0.10	4.24 ± 0.23	7.12 ± 0.44	14.10 ± 0.15
16		8.59 ± 0.45	3.22 ± 0.29	3.99 ± 0.06	4.11 ± 0.18	4.56 ± 0.21	28.23 ± 0.43
17		5.68 ± 0.03	1.47 ± 0.04	3.41 ± 0.15	3.70 ± 0.34	3.88 ± 0.17	13.74 ± 0.31
18		4.81 ± 0.15	3.24 ± 0.36	3.29 ± 0.09	4.16 ± 0.17	5.31 ± 0.41	21.21 ± 0.42
19		5.31 ± 0.47	6.76 ± 0.22	5.46 ± 0.36	2.13 ± 0.19	5.43 ± 0.32	32.11 ± 0.10
20		9.58 ± 0.87	3.19 ± 0.05	4.86 ± 0.43	4.07 ± 0.33	5.25 ± 0.29	26.17 ± 0.19
21		2.87 ± 0.19	3.61 ± 0.28	3.81 ± 0.18	4.43 ± 0.32	6.55 ± 0.41	8.11 ± 0.47
22		10.75 ± 0.53	3.47 ± 0.19	4.75 ± 0.09	3.31 ± 0.18	6.61 ± 0.35	17.47 ± 0.29
23		2.33 ± 0.41	1.68 ± 0.02	3.96 ± 0.29	4.74 ± 0.58	1.18 ± 0.14	14.42 ± 0.87
24		2.85 ± 0.23	1.39 ± 0.19	4.30 ± 0.33	4.53 ± 0.13	6.26 ± 0.30	12.71 ± 0.07
Tet-NO ₂		7.36 ± 0.29	9.05 ± 0.51	6.81 ± 0.35	12.12 ± 0.58	11.29 ± 0.52	—

Table 1 (continued)

Compound	R	IC ₅₀ (μM), 48 h					
		HEL	K562	PC3	WM9	MDA-MB-231	HL7702
Tet-NH ₂		6.66 ± 0.11	3.00 ± 0.33	2.67 ± 0.51	6.36 ± 0.20	3.55 ± 0.35	—
Tet		7.45 ± 0.34	11.76 ± 0.27	5.86 ± 0.29	6.10 ± 0.31	8.94 ± 0.24	44.25 ± 0.21
Vincristine		5.97 ± 0.27	5.36 ± 0.39	5.31 ± 0.18	4.28 ± 0.22	9.86 ± 0.17	—

4.1.1 General procedure for the synthesis of Tet-NO₂ and Tet-NH₂. Under the protection of an argon atmosphere, concentrated HNO₃ (69%, 0.6 mL, 9.6 mmol) was added dropwise into (CH₃CO)₂O (1.5 mL, 16.0 mmol) at -10 °C and stirred for 10 min. The reaction mixture was then allowed to warm up to room temperature and stirred for 20 min. Then, a solution of tetrandrine (500 mg, 0.8 mmol) in CH₂Cl₂ (5 mL) was added dropwise at 0 °C and the reaction was monitored using TLC. Upon completion, the reaction mixture was quenched with a saturated aqueous solution of NaHCO₃, extracted with CH₂Cl₂ (3 × 20 mL), dried over anhydrous MgSO₄ and concentrated under reduced pressure. The residue was purified using chromatography on silica gel (CH₂Cl₂:MeOH = 50:1 v/v, 0.1% TEA) to afford Tet-NO₂.

14-Nitro-tetrandrine (Tet-NO₂). Light yellow amorphous solid; yield 94%; mp: 176–177 °C; IR (KBr, cm⁻¹): 3053, 2935, 2840, 1603, 1578, 1316; ¹H NMR (CDCl₃, 400 MHz) δ (ppm): 7.41–7.34 (m, 2H), 7.11 (dd, *J* = 8.2, 2.6 Hz, 1H), 6.75 (dd, *J* = 8.2, 2.6 Hz, 1H), 6.52 (d, *J* = 7.2 Hz, 2H), 6.28 (d, *J* = 7.8 Hz, 2H), 5.97 (s, 1H), 3.97 (s, 3H), 3.89 (dd, *J* = 10.8, 6.0 Hz, 1H), 3.74 (s, 3H), 3.69–3.60 (m, 1H), 3.51–3.39 (m, 2H), 3.36 (s, 3H), 3.30–3.22 (m, 2H), 3.17 (s, 3H), 3.00–2.66 (m, 6H), 2.62 (s, 3H), 2.52 (d, *J* = 13.0 Hz, 1H), 2.34–2.29 (m, 1H), 2.19 (s, 3H); ¹³C NMR (CDCl₃, 100 MHz) δ (ppm): 152.3, 152.1, 151.5, 148.7, 148.2, 146.5, 144.2, 143.5, 137.5, 136.4, 133.1, 130.5, 130.4, 128.9, 128.1, 127.6, 121.6, 121.4, 121.3, 119.9, 117.2, 112.5, 108.2, 105.8, 63.6, 61.7, 60.3, 56.3, 55.8, 55.7, 45.3, 43.2, 42.8, 41.5, 37.9, 36.8, 25.3, 21.6. HRMS (ESI-QTOF): *m/z* 668.2967 [M + H]⁺, calcd for C₃₈H₄₂N₃O₈: 668.2972.

The mixture of 14-nitro-tetrandrine Tet-NO₂ (200 mg, 0.30 mmol), palladium on carbon (10%, 40 mg) and hydrazine hydrate (85%, 0.33 mL, 9.0 mmol) in absolute methanol (30

mL) was allowed to stir at 65 °C for 2–3 h before the reaction was completed. It was then cooled to room temperature and filtered. The filtrate was evaporated under reduced pressure. The residue was diluted with CH₂Cl₂, washed with water, and dried over anhydrous MgSO₄. After removing the solvent under reduced pressure, the crude product was recrystallized from petroleum ether and acetone (1/3, v/v) to yield Tet-NH₂.

14-Amino-tetrandrine (Tet-NH₂). White amorphous solid; yield 83%; mp: 163–165 °C; purity: 99.0%; IR (KBr, cm⁻¹): 3402, 2933, 2836, 1610, 1506, 1348, 1211, 1109, 874, 628; ¹H NMR (CDCl₃, 400 MHz) δ (ppm): 7.28 (d, *J* = 9.6 Hz, 1H), 7.18 (dd, *J* = 2.0, 8.0 Hz, 1H), 6.60 (dd, *J* = 2.0, 8.4 Hz, 1H), 6.50 (s, 1H), 6.46 (s, 1H), 6.31 (s, 1H), 6.29 (s, 1H), 6.12 (dd, *J* = 1.6, 8.0 Hz, 1H), 5.87 (s, 1H), 3.94 (d, *J* = 9.2 Hz, 1H), 3.87 (s, 3H), 3.80 (dd, *J* = 5.2, 11.2 Hz, 1H), 3.73 (s, 3H), 3.69–3.60 (m, 1H), 3.49–3.40 (m, 1H), 3.35 (s, 3H), 3.26 (dd, *J* = 5.2, 12.4 Hz, 1H), 3.11 (s, 3H), 3.05 (dd, *J* = 14.4, 9.0 Hz, 1H), 2.99–2.80 (m, 4H), 2.79–2.63 (m, 2H), 2.61 (s, 3H), 2.42 (s, 3H), 2.39–2.29 (m, 2H); ¹³C NMR (CDCl₃, 100 MHz) δ (ppm): 156.6, 151.6, 149.4, 148.7, 148.5, 144.2, 142.0, 140.8, 138.0, 133.2, 132.6, 129.3, 128.0, 127.6, 127.4, 122.6, 122.1, 121.3, 120.9, 120.5, 120.2, 112.3, 105.8, 100.6, 64.2, 61.5, 59.9, 56.1, 55.6, 55.5, 44.9, 43.2, 42.3, 40.8, 40.0, 38.7, 24.6, 20.6. HRMS (ESI-QTOF): *m/z* 638.3221 [M + H]⁺, calcd for C₃₈H₄₃N₃O₆: 638.3230.

4.1.2 General procedure for the synthesis of 1 to 24. To a solution of 14-amino-tetrandrine Tet-NH₂ (100 mg, 0.16 mmol) in CH₂Cl₂ (3 mL) under the protection of an argon atmosphere, sulfonyl chloride (0.16 mmol, 1.0 eq) and DMAP (0.03 mmol, 0.2 eq) were added and the mixture was stirred at room temperature for 3–4 h. After the reaction was completed, the mixture was extracted using CH₂Cl₂ (repeated

Fig. 2 Compound 23 induced apoptosis in MDA-MB-231 cells. MDA-MB-231 cells were treated with the tested compound at 1.25 μM and 2.5 μM for 24 h, stained with Annexin V-FITC and PI, incubated at room temperature for 15 min and analyzed using flow cytometry.

Fig. 3 Compound 23 affects the protein levels of the apoptotic genes in MDA-MB-231 cells. Western blots using the indicated antibodies revealed that treatment with compound 23 activated the apoptosis pathway in MDA-MB-231 cells. The concentration of the compound was 2.5 μM.

three times). The combined organic phase was washed using brine and dried over anhydrous MgSO_4 before vacuum suction filtration. The crude product was purified using chromatography on silica gel ($\text{CH}_2\text{Cl}_2/\text{MeOH}$, 50/1 v/v, 0.1% TEA) to afford the pure product.

14-(Methylsulfonamide)tetradrine (1). White amorphous solid; yield 76%; mp: 165–167 °C; purity: 98.1%; IR (KBr, cm^{-1}): 3413, 2938, 2848, 1611, 1508, 1359, 1162, 1117, 871, 586; ^1H NMR (CDCl_3 , 500 MHz) δ (ppm): 7.27 (dd, $J = 8.2$, 2.1 Hz, 1H), 7.18 (dd, $J = 8.1$, 2.6 Hz, 1H), 6.60 (dd, $J = 8.4$, 2.6 Hz, 1H), 6.50 (s, 1H), 6.46 (s, 1H), 6.31 (s, 1H), 6.29 (s, 1H), 6.12 (dd, $J = 8.5$, 2.1 Hz, 1H), 5.87 (s, 1H), 5.29 (s, 1H), 3.95 (d, $J = 9.0$ Hz, 1H), 3.87 (s, 3H), 3.80–3.75 (m, 1H), 3.73 (s, 3H), 3.69–3.60 (m, 1H), 3.49–3.40 (m, 1H), 3.34 (s, 3H), 3.25–3.20 (m, 1H), 3.10 (s, 3H), 3.05 (dd, $J = 12.0$, 9.6 Hz, 1H), 2.95–2.82 (m, 4H), 2.79–2.63 (m, 2H), 2.59 (s, 3H), 2.42 (s, 3H), 2.39–2.29 (m, 2H); ^{13}C NMR (CDCl_3 , 125 MHz) δ (ppm): 156.1, 155.1, 152.0, 149.2, 148.7, 148.3, 144.2, 138.1, 132.8, 129.6, 127.4, 121.3, 121.2, 121.0, 120.8, 112.2, 105.8, 70.5, 64.3, 61.0, 60.1, 56.1, 55.7, 55.5, 44.9, 42.6, 42.2, 40.4, 40.2, 39.3, 38.9, 29.7, 28.1, 24.5, 22.8, 20.6, 19.2. HRMS (ESI-QTOF): m/z 738.3733 $[\text{M} + \text{H}]^+$, calcd for $\text{C}_{39}\text{H}_{45}\text{N}_3\text{O}_8\text{S}$: 738.2825.

14-(Propylsulfonamide)tetradrine (2). White amorphous solid; yield 85%; mp: 156–158 °C; purity: 98.0%; IR (KBr, cm^{-1}): 3421, 2938, 2845, 1610, 1508, 1455, 1232, 1141, 1118, 873, 846; ^1H NMR (CDCl_3 , 500 MHz) δ (ppm): 7.32 (dd, $J = 8.5$, 2.5 Hz, 1H), 7.25 (s, 1H), 7.21 (dd, $J = 8.0$, 2.5 Hz, 1H), 6.59 (dd, $J = 10.5$, 2.5 Hz, 1H), 6.51 (s, 1H), 6.50 (s, 1H), 6.32 (s, 1H), 6.15 (dd, $J = 8.5$, 3.5 Hz, 1H), 5.91 (s, 1H), 3.93 (s, 3H), 3.92–3.85 (m, 2H), 3.75 (s, 3H), 3.58–3.49 (m, 2H), 3.38 (s, 3H), 3.37–3.34 (m, 1H), 3.11 (s, 3H), 3.10–2.91 (m, 7H), 2.82–2.74 (m, 2H), 2.65 (s, 3H), 2.51 (d, $J = 4.5$ Hz, 1H), 2.48 (s, 3H), 2.46 (s, 1H), 1.92–1.81 (m, 2H), 1.25 (s, 1H), 1.01 (t, $J = 7.5$ Hz, 3H); ^{13}C NMR (CDCl_3 , 125 MHz) δ (ppm): 153.6, 153.4, 150.01, 149.7, 148.8, 146.9, 143.8, 138.3, 132.8, 131.6, 130.7, 130.1, 128.2, 124.9, 123.9, 122.6, 122.3, 120.3, 120.2, 115.9, 114.9, 113.2, 112.0, 106.1, 67.0, 64.7, 63.4, 60.4, 56.2, 55.9, 55.7, 53.8, 53.4, 40.8, 40.6, 39.9, 21.6, 21.2, 17.3, 13.1, 13.0. HRMS (ESI-QTOF): m/z 744.3293 $[\text{M} + \text{H}]^+$, calcd for $\text{C}_{41}\text{H}_{49}\text{N}_3\text{O}_8\text{S}$: 744.3319.

14-(Butylsulfonamide)tetradrine (3). White amorphous solid; yield 86%; mp: 158–160 °C; purity: 98.1%; IR (KBr, cm^{-1}): 3418, 2940, 2873, 1610, 1508, 1455, 1232, 1137, 1117, 872, 847; ^1H NMR (CDCl_3 , 400 MHz) δ (ppm): 9.21 (s, 1H), 7.50 (d, $J = 7.2$ Hz, 1H), 7.24–7.16 (m, 1H), 6.91–6.87 (m, 1H), 6.82 (d, $J = 8.2$ Hz, 1H), 6.66 (s, 1H), 6.45 (s, 1H), 6.33 (d, $J = 8.0$ Hz, 1H), 6.06 (s, 1H), 4.42–4.07 (m, 4H), 3.94 (s, 3H), 3.81 (s, 3H), 3.71 (s, 3H), 3.47 (s, 3H), 3.39 (s, 2H), 3.24 (s, 3H), 3.21–3.10 (m, 4H), 2.97–2.76 (m, 6H), 2.04–1.93 (m, 2H), 1.76 (s, 3H), 1.57–1.48 (m, 2H), 1.00 (t, $J = 7.3$ Hz, 3H); ^{13}C NMR (CDCl_3 , 100 MHz) δ (ppm): 153.6, 153.4, 150.1, 149.7, 148.8, 146.9, 143.8, 132.8, 131.6, 130.7, 130.1, 128.2, 124.9, 123.9, 122.6, 122.4, 120.3, 120.2, 115.9, 114.9, 113.25, 112.05, 106.1, 67.0, 64.7, 63.4, 60.4, 56.2, 55.9, 55.7, 51.8, 44.4, 43.9, 40.8, 40.6, 39.9, 36.2, 25.4, 21.6, 21.6, 21.2, 13.5. HRMS (ESI-

QTOF): m/z 758.3438 $[\text{M} + \text{H}]^+$, calcd for $\text{C}_{42}\text{H}_{51}\text{N}_3\text{O}_8\text{S}$: 758.3475.

14-(Trifluoromethylsulfonamide)tetradrine (4). Yellow amorphous solid; yield 71%; mp: 148–150 °C; IR (KBr, cm^{-1}): 3434, 2934, 2851, 1609, 1505, 1448, 1314, 1198, 1104, 869, 849; ^1H NMR (CDCl_3 , 400 MHz) δ (ppm): 7.31–7.25 (m, 3H), 7.19 (dd, $J = 8.0$, 3.2 Hz, 1H), 6.55 (dd, $J = 8.8$, 3.2 Hz, 1H), 6.48 (s, 1H), 6.40 (s, 1H), 6.36 (s, 1H), 6.12 (dd, $J = 8.4$, 2.0 Hz, 1H), 5.91 (s, 1H), 4.29 (d, $J = 9.6$ Hz, 1H), 3.90 (s, 3H), 3.80 (dd, $J = 11.2$, 5.2 Hz, 2H), 3.76 (s, 3H), 3.46–3.41 (m, 1H), 3.38 (s, 3H), 3.37–3.20 (m, 4H), 3.12 (s, 3H), 3.10–2.83 (m, 5H), 2.73 (s, 3H), 2.59 (s, 3H), 2.54 (d, $J = 15.2$ Hz, 1H); ^{13}C NMR (CDCl_3 , 100 MHz) δ (ppm): 153.6, 149.9, 149.0, 147.2, 143.9, 138.5, 132.7, 132.4, 130.8, 125.5, 125.1, 123.9, 122.8, 122.5, 122.2, 120.4, 120.4, 114.8, 112.1, 106.2, 77.2, 77.0, 76.7, 67.0, 64.6, 63.1, 60.4, 56.1, 56.1, 55.9, 55.7, 55.5, 55.2, 44.5, 40.9, 40.7, 39.9, 21.6, 21.2. HRMS (ESI-QTOF): m/z 770.2686 $[\text{M} + \text{H}]^+$, calcd for $\text{C}_{39}\text{H}_{42}\text{F}_3\text{N}_3\text{O}_8\text{S}$: 770.2723.

14-(Benzenesulfonamide)tetradrine (5). Dark yellow amorphous solid; yield 85%; mp: 170–171 °C; IR (KBr, cm^{-1}): 3413, 2938, 2848, 1611, 1508, 1448, 1359, 1232, 1162, 1117, 1080, 871; ^1H NMR (CDCl_3 , 400 MHz) δ (ppm): 7.77–7.73 (m, 2H), 7.48–7.44 (m, 1H), 7.39–7.36 (m, 2H), 7.29 (dd, $J = 6.6$, 2.4 Hz, 1H), 7.23 (s, 1H), 7.18 (dd, $J = 8.0$, 2.6 Hz, 1H), 6.55 (dd, $J = 6.4$, 2.4 Hz, 1H), 6.47 (s, 1H), 6.39 (s, 1H), 6.29 (s, 1H), 6.12 (dd, $J = 6.8$, 2.0 Hz, 1H), 5.84 (s, 1H), 3.92 (s, 3H), 3.87–3.83 (m, 2H), 3.73 (s, 3H), 3.55–3.47 (m, 1H), 3.37 (s, 1H), 3.35 (s, 3H), 3.31 (d, $J = 4.4$ Hz, 1H), 3.14 (dd, $J = 11.2$, 5.2 Hz, 1H), 3.07 (s, 3H), 2.89–2.99 (m, 3H), 2.80–2.73 (m, 2H), 2.63 (s, 3H), 2.50 (s, 3H), 2.48 (d, $J = 3.6$ Hz, 1H), 2.44 (d, $J = 3.6$ Hz, 1H), 2.37 (dd, $J = 12.4$, 7.6 Hz, 1H), 2.14 (d, $J = 12.0$ Hz, 1H); ^{13}C NMR (CDCl_3 , 100 MHz) δ (ppm): 153.6, 153.4, 149.9, 149.5, 148.1, 146.9, 143.7, 139.4, 138.4, 132.8, 132.8, 131.7, 130.7129.6, 128.9, 127.9, 127.8, 125.0, 123.8, 122.6, 122.3120.3, 120.1, 115.7, 115.0, 114.8, 112.9, 112.0, 106.2, 67.0, 64.7, 63.6, 60.4, 55.9, 55.7, 55.5, 44.4, 43.8, 40.8, 40.6, 39.9, 36.0, 21.5, 21.2. HRMS (ESI-QTOF): m/z 778.3182 $[\text{M} + \text{H}]^+$, calcd for $\text{C}_{44}\text{H}_{47}\text{N}_3\text{O}_8\text{S}$: 778.3162.

14-(2-Fluorobenzenesulfonamide)tetradrine (6). White amorphous solid; yield 80%; mp: 156–158 °C; purity: 98.5%; IR (KBr, cm^{-1}): 3415, 2939, 2842, 1612, 1508, 1359, 1232, 1168, 1117, 1077, 826; ^1H NMR (CDCl_3 , 500 MHz) δ (ppm): 8.02 (dd, $J = 7.5$, 2.0 Hz, 1H), 7.51–7.46 (m, 1H), 7.28 (dd, $J = 7.0$, 2.5 Hz, 2H), 7.23 (td, $J = 7.5$, 1.0 Hz, 1H), 7.16 (dd, $J = 8.0$, 2.5 Hz, 1H), 7.10 (dd, $J = 8.0$, 2.5 Hz, 1H), 7.02 (s, 1H), 6.53 (dd, $J = 8.0$, 2.5 Hz, 1H), 6.48 (s, 1H), 6.41 (s, 1H), 6.33 (s, 1H), 6.12 (dd, $J = 8.5$, 2.0 Hz, 1H), 5.87 (s, 1H), 3.96 (d, $J = 9.5$ Hz, 1H), 3.82 (s, 3H), 3.79 (d, $J = 5.0$ Hz, 1H), 3.75 (s, 3H), 3.65 (td, $J = 10.5$, 5.0 Hz, 1H), 3.48 (s, 2H), 3.38 (s, 3H), 3.26 (dd, $J = 12.0$, 5.0 Hz, 1H), 3.18 (dd, $J = 15.0$, 5.5 Hz, 1H), 3.10 (s, 3H), 3.05–2.86 (m, 4H), 2.79–2.69 (m, 2H), 2.60 (s, 3H), 2.56 (s, 3H), 2.36 (d, $J = 15.0$ Hz, 1H); ^{13}C NMR (CDCl_3 , 125 MHz) δ (ppm): 165.8, 163.3, 155.8, 152.2, 149.1, 148.7, 148.4, 146.0, 143.8, 138.0, 137.3, 137.2, 134.1, 132.9, 131.5, 129.5, 129.0, 128.9, 128.4, 127.5, 126.9, 126.8, 120.9, 120.9, 120.8, 120.0, 116.0, 115.7, 112.1, 107.4, 105.6, 64.0, 61.3, 60.0, 56.2,

55.6, 55.4, 44.8, 42.3, 42.3, 39.9, 39.1, 38.5, 24.6, 20.5. HRMS (ESI-QTOF): m/z 796.3036 $[M + H]^+$, calcd for $C_{44}H_{46}FN_3O_8S$: 796.3068.

14-(2-Chlorobenzenesulfonamide)tetrandrine (7). White amorphous solid; yield 82%; mp: 157–158 °C; IR (KBr, cm^{-1}): 3419, 2938, 2839, 1611, 1507, 1453, 1329, 1232, 1164, 1113, 864, 589; 1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.81 (d, J = 8.0 Hz, 2H), 7.77 (s, 1H), 7.53 (d, J = 8.0 Hz, 1H), 7.46 (t, J = 8.2 Hz, 1H), 7.32 (dd, J = 8.2, 2.2 Hz, 1H), 7.24 (dd, J = 8.0, 2.6 Hz, 1H), 6.63–6.60 (m, 2H), 6.48 (s, 1H), 6.26 (s, 1H), 6.15 (dd, J = 8.4, 2.6 Hz, 1H), 5.89 (s, 1H), 4.05 (d, J = 8.0 Hz, 1H), 3.97 (s, 3H), 3.82 (dd, J = 8.2, 2.6 Hz, 1H), 3.73 (s, 3H), 3.37 (s, 3H), 3.26–3.21 (m, 2H), 3.09 (s, 3H), 3.01–2.66 (m, 7H), 2.61 (s, 3H), 2.52 (s, 3H), 2.45–2.40 (m, 2H), 2.33 (dd, J = 13.6, 5.0 Hz, 1H); ^{13}C -NMR ($CDCl_3$, 100 MHz) δ (ppm): 165.6, 155.9, 152.1, 149.3, 148.5, 148.3, 145.5, 144.1, 138.4, 138.2, 134.2, 134.0, 132.8, 132.1, 131.2, 129.9, 129.6, 128.5, 127.6, 127.3, 127.0, 126.1, 125.8, 121.5, 121.3, 120.9, 120.5, 112.2, 107.1, 105.6, 64.1, 61.2, 60.0, 56.2, 55.7, 55.5, 45.0, 43.0, 42.4, 40.8, 40.0, 38.8, 24.7, 20.7. HRMS (ESI-QTOF): m/z 812.2736 $[M + H]^+$, calcd for $C_{44}H_{46}ClN_3O_8S$: 812.2772.

14-(3-Bromobenzenesulfonamide)tetrandrine (8). White amorphous solid; yield 86%; mp: 157–158 °C; IR (KBr, cm^{-1}): 3413, 2936, 2842, 1611, 1508, 1459, 1331, 1232, 1163, 1116, 880, 589; 1H NMR ($CDCl_3$, 500 MHz) δ (ppm): 7.75 (d, J = 2.5 Hz, 1H), 7.73 (s, 1H), 7.65 (s, 1H), 7.63 (s, 1H), 7.32 (dd, J = 8.0, 2.5 Hz, 1H), 7.23 (dd, J = 8.0, 2.5 Hz, 1H), 6.63–6.59 (m, 2H), 6.48 (s, 1H), 6.26 (s, 1H), 6.15 (dd, J = 8.5, 2.0 Hz, 1H), 5.89 (s, 1H), 4.04 (d, J = 9.0 Hz, 1H), 3.96 (s, 3H), 3.82 (dd, J = 11.0, 5.5 Hz, 1H), 3.73 (s, 3H), 3.48–3.42 (m, 1H), 3.37 (s, 3H), 3.25–3.19 (m, 2H), 3.09 (s, 3H), 3.02–2.65 (m, 6H), 2.61 (s, 3H), 2.47 (s, 3H), 2.44–2.29 (m, 3H), 2.03 (s, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm): 166.2, 155.9, 152.1, 149.3, 148.6, 148.3, 145.4, 144.1, 138.2, 135.6, 133.9, 132.8, 132.2, 131.5, 129.6, 129.1, 128.4, 127.5, 127.0, 125.8, 125.7, 121.5, 121.2, 121.0, 120.6, 112.2, 107.1, 105.7, 64.1, 61.2, 60.0, 56.2, 55.6, 55.5, 44.9, 43.0, 42.3, 40.8, 40.0, 38.7, 24.7, 20.6. HRMS (ESI-QTOF): m/z 856.2224 $[M + H]^+$, calcd for $C_{44}H_{46}BrN_3O_8S$: 856.2267.

14-(2-Nitrobenzenesulfonamide)tetrandrine (9). Light yellow amorphous solid; yield 87%; mp: 150–152 °C; IR (KBr, cm^{-1}): 3401, 2938, 2839, 1642, 1543, 1506, 1451, 1359, 1116, 587; 1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.87 (d, J = 8.0 Hz, 2H), 7.66 (d, J = 8.0 Hz, 2H), 7.36 (d, J = 8.4 Hz, 1H), 7.27 (d, J = 2.4 Hz, 1H), 7.22 (dd, J = 8.0, 2.4 Hz, 1H), 6.60–6.52 (m, 2H), 6.38 (s, 1H), 6.33 (s, 1H), 6.12 (d, J = 8.4 Hz, 1H), 5.89 (s, 1H), 4.12–4.08 (m, 2H), 3.94 (s, 3H), 3.84 (t, J = 12.4 Hz, 2H), 3.75 (s, 3H), 3.39 (s, 3H), 3.35 (d, J = 13.6 Hz, 1H), 3.23–3.16 (m, 2H), 3.12 (s, 3H), 3.07–2.92 (m, 4H), 2.89 (s, 1H), 2.81 (s, 3H), 2.53 (s, 4H), 2.47–2.38 (m, 1H), 2.18 (d, J = 14.8 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 153.5, 153.4, 150.0, 149.8, 148.3, 148.2, 146.9, 143.8, 138.3, 133.3, 132.9, 132.0, 131.9, 131.8, 130.8, 129.8, 127.8, 125.0, 124.9, 124.3, 122.5, 122.4, 120.3, 120.2, 115.8, 115.0, 112.6, 112.2, 106.4, 64.7, 63.3, 63.2, 60.8, 56.0, 55.7, 55.5, 44.6, 43.7, 41.1, 40.7, 40.3, 36.0, 21.6, 21.3.

HRMS (ESI-QTOF): m/z 823.2977 $[M + H]^+$, calcd for $C_{44}H_{46}N_4O_{10}S$: 823.3013.

14-(3-Nitrobenzenesulfonamide)tetrandrine (10). Light yellow amorphous solid; yield 90%; mp: 151–152 °C; IR (KBr, cm^{-1}): 3411, 2937, 2844, 1609, 1532, 1507, 1452, 1353, 1232, 1118, 875, 589; 1H NMR ($CDCl_3$, 500 MHz) δ (ppm): 8.72 (dd, J = 4.0, 2.0 Hz, 1H), 8.32–8.29 (m, 1H), 8.03–8.00 (m, 1H), 7.57 (t, J = 8.0 Hz, 1H), 7.29–7.26 (m, 2H), 7.22 (s, 1H), 7.16 (dd, J = 8.4, 2.6 Hz, 1H), 6.53 (dd, J = 8.0, 2.4 Hz, 1H), 6.46 (s, 1H), 6.38 (s, 1H), 6.30 (s, 1H), 6.12 (dd, J = 8.4, 2.4 Hz, 1H), 5.84 (s, 1H), 3.93 (s, 3H), 3.91 (s, 1H), 3.80 (dd, J = 12.4, 5.4 Hz, 1H), 3.49–3.38 (m, 2H), 3.36 (s, 3H), 3.28–3.21 (m, 2H), 3.07 (s, 3H), 3.04–2.85 (m, 4H), 2.80–2.67 (m, 3H), 2.59 (s, 3H), 2.56 (s, 3H), 2.54–2.44 (m, 2H), 2.23 (d, J = 14.6 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 164.6, 155.8, 152.1, 149.3, 148.5, 148.3, 145.6, 144.1, 138.2, 136.4, 135.5, 134.1, 132.9, 132.5, 131.9, 130.6, 129.6, 129.1, 128.6, 127.7, 127.2, 126.9, 125.9, 121.5, 121.1, 120.9, 120.5, 112.2, 107.1, 105.7, 77.2, 76.7, 64.1, 61.3, 60.0, 56.2, 55.7, 55.5, 43.1, 42.4, 40.9, 40.0, 38.7, 24.8, 20.7. HRMS (ESI-QTOF): m/z 823.2978 $[M + H]^+$, calcd for $C_{44}H_{46}N_4O_{10}S$: 823.3013.

14-(4-Fluorobenzenesulfonamide)tetrandrine (11). White amorphous solid; yield 80%; mp: 158–161 °C; IR (KBr, cm^{-1}): 3425, 2935, 2841, 1609, 1506, 1336, 1216, 1163, 1107, 1070, 835, 545; 1H NMR ($CDCl_3$, 500 MHz) δ (ppm): 7.73 (dd, J = 8.5, 5.0 Hz, 2H), 7.27 (d, J = 8.5 Hz, 2H), 7.22 (s, 1H), 7.17 (dd, J = 8.0, 2.5 Hz, 1H), 7.03 (t, J = 8.5 Hz, 2H), 6.54 (dd, J = 8.5, 2.5 Hz, 1H), 6.45 (s, 1H), 6.39 (s, 1H), 6.28 (s, 1H), 6.11 (dd, J = 8.5, 2.5 Hz, 1H), 5.83 (s, 1H), 3.92 (s, 3H), 3.84 (d, J = 9.5 Hz, 1H), 3.77 (dd, J = 11.0, 5.5 Hz, 1H), 3.72 (s, 3H), 3.46–3.39 (m, 1H), 3.33 (s, 4H), 3.21 (dd, J = 12.0, 5.5 Hz, 1H), 3.12 (dd, J = 14.0, 6.0 Hz, 1H), 3.06 (s, 3H), 2.99–2.66 (m, 6H), 2.57 (s, 3H), 2.48 (s, 4H), 2.38–2.32 (m, 1H), 2.16 (d, J = 15.0 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 155.7, 153.2, 152.1, 149.3, 148.6, 148.50, 144.0, 140.2, 134.6, 134.2, 132.9, 132.3, 130.0, 129.7, 128.5, 127.8, 126.9, 122.8, 121.3, 120.9, 120.7, 119.1, 117.1, 112.2, 106.4, 105.7, 64.1, 61.3, 60.1, 56.3, 55.7, 55.5, 45.0, 43.2, 42.4, 40.8, 39.6, 38.7, 29.7, 24.8, 20.5, 14.2. HRMS (ESI-QTOF): m/z 796.3035 $[M + H]^+$, calcd for $C_{44}H_{46}FN_3O_8S$: 796.3068.

14-(4-Chlorobenzenesulfonamide)tetrandrine (12). Yellow amorphous solid; yield 85%; mp: 157–158 °C; IR (KBr, cm^{-1}): 3412, 2938, 2838, 1611, 1507, 1453, 1359, 1232, 1164, 1114, 766, 589; 1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.65 (d, J = 8.4 Hz, 2H), 7.33 (d, J = 8.2 Hz, 2H), 7.30 (m, 2H), 7.23 (s, 1H), 7.18 (dd, J = 8.0, 2.6 Hz, 1H), 6.55 (dd, J = 8.0, 2.2 Hz, 1H), 6.46 (s, 1H), 6.40 (s, 1H), 6.29 (s, 1H), 6.12 (dd, J = 8.4, 2.0 Hz, 1H), 5.84 (s, 1H), 3.93 (s, 3H), 3.86–3.77 (m, 2H), 3.73 (s, 3H), 3.51–3.43 (m, 1H), 3.35 (s, 3H), 3.32 (m, 2H), 3.12 (dd, J = 14.0, 5.8 Hz, 1H), 3.07 (s, 3H), 3.01 (m, 3H), 2.82–2.77 (m, 2H), 2.59 (s, 3H), 2.49 (s, 3H), 2.44 (d, J = 4.8 Hz, 1H), 2.34 (dd, J = 16.0, 11.2 Hz, 1H), 2.16 (d, J = 14.6 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 155.8, 152.2, 149.1, 148.8, 148.5, 146.1, 143.8, 139.7, 138.4, 138.0, 134.0, 132.9, 131.5, 129.6, 128.9, 128.4, 127.8, 127.0, 126.8, 121.0, 121.0, 120.9, 120.8, 119.9, 112.1, 107.4, 105.6, 77.2, 64.1, 61.3, 60.1, 56.3, 55.6,

55.4, 44.9, 42.3, 40.0, 39.1, 38.7, 29.7, 24.6, 20.5, 14.1. HRMS (ESI-QTOF): m/z 812.2742 $[M + H]^+$, calcd for $C_{44}H_{46}ClN_3O_8S$: 812.2772.

14-(4-Bromobenzenesulfonamide)tetrandrine (13). White amorphous solid; yield 85%; mp: 155–157 °C; IR (KBr, cm^{-1}): 3422, 2937, 2842, 1611, 1508, 1458, 1329, 1281, 1219, 1162, 1116, 742, 606; 1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.96 (s, 1H), 7.64–7.58 (m, 2H), 7.32 (dd, $J = 8.4, 2.2$ Hz, 1H), 7.27–7.18 (m, 4H), 6.55 (dd, $J = 8.4, 2.6$ Hz, 1H), 6.49 (s, 1H), 6.40 (s, 1H), 6.31 (s, 1H), 6.11 (dd, $J = 8.6, 2.2$ Hz, 1H), 5.86 (s, 1H), 3.97 (dd, $J = 11.4, 5.2$ Hz, 1H), 3.91 (s, 3H), 3.86 (d, $J = 9.4$ Hz, 1H), 3.74 (s, 3H), 3.67–3.59 (m, 1H), 3.47–3.39 (m, 2H), 3.36 (s, 3H), 3.15 (dd, $J = 14.2, 5.8$ Hz, 1H), 3.09 (s, 3H), 3.02–2.89 (m, 3H), 2.88–2.75 (m, 3H), 2.72 (s, 3H), 2.51 (s, 3H), 2.50–2.44 (m, 1H), 2.20 (d, $J = 14.6$ Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 153.6, 153.5, 149.9, 149.7, 148.3, 146.9, 143.7, 138.6, 138.4, 132.8, 132.1, 131.7, 130.7, 129.6, 129.4, 127.7, 127.6, 124.9, 123.8, 122.9, 122.6, 122.3, 120.3, 120.1, 115.7, 114.8, 112.8, 112.0, 106.1, 67.0, 64.7, 63.4, 60.4, 55.9, 55.7, 55.6, 55.4, 44.4, 43.8, 40.9, 40.6, 39.9, 21.5, 21.2. HRMS (ESI-QTOF): m/z 856.2230 $[M + H]^+$, calcd for $C_{44}H_{46}BrN_3O_8S$: 856.2267.

14-(6-chloropyridine-3-sulfonamide)tetrandrine (14). White amorphous solid; yield 87%; mp: 160–162 °C; IR (KBr, cm^{-1}): 3407, 2938, 2841, 1611, 1508, 1448, 1421, 1356, 1232, 1219, 1116, 841, 777; 1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 9.99 (s, 1H), 9.76 (s, 1H), 8.71 (d, $J = 2.2$ Hz, 1H), 8.14 (dd, $J = 8.4, 2.6$ Hz, 1H), 7.53 (s, 1H), 7.531 (s, 1H), 7.19 (dd, $J = 8.2, 2.4$ Hz, 1H), 6.84 (dd, $J = 8.2, 2.6$ Hz, 1H), 6.66 (s, 1H), 6.48 (s, 1H), 6.47 (s, 1H), 6.32 (d, $J = 8.0$ Hz, 1H), 6.24 (d, $J = 10.2$ Hz, 1H), 6.06 (s, 3H), 4.43 (d, $J = 9.4$ Hz, 1H), 4.30 (d, $J = 10.6$ Hz, 1H), 4.17–4.12 (m, 1H), 3.81 (s, 3H), 3.76 (d, $J = 6.0$ Hz, 2H), 3.57 (s, 3H), 3.46 (s, 3H), 3.38 (s, 1H), 3.23 (s, 3H), 3.18 (d, $J = 6.4$ Hz, 2H), 2.97 (d, $J = 4.4$ Hz, 3H), 2.87 (d, $J = 4.6$ Hz, 3H), 2.80 (d, $J = 4.8$ Hz, 1H), 2.73 (d, $J = 16.0$ Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 155.5, 153.5, 149.9, 148.9, 148.5, 147.0, 143.7, 138.4, 137.9, 135.4, 132.8, 131.8, 130.8, 129.6, 127.1, 125.0, 124.6, 123.8, 122.6, 122.3, 120.3, 120.1, 115.9, 114.7, 112.6, 112.0, 106.1, 64.7, 63.4, 60.4, 55.9, 55.8, 55.8, 55.7, 55.1, 44.4, 43.9, 40.8, 40.6, 39.9, 36.1, 21.6, 21.2. HRMS (ESI-QTOF): m/z 813.2688 $[M + H]^+$, calcd for $C_{43}H_{45}ClN_4O_8S$: 813.2725.

14-(4-Nitrobenzenesulfonamide)tetrandrine (15). Dark yellow amorphous solid; yield 85%; mp: 150–152 °C; IR (KBr, cm^{-1}): 3419, 2935, 2840, 1734, 1607, 1508, 1448, 1216, 1161, 1118, 853, 735; 1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.23–8.19 (m, 2H), 7.92–7.88 (m, 2H), 7.31 (dd, $J = 8.2, 2.2$ Hz, 1H), 7.19 (dd, $J = 8.2, 2.6$ Hz, 1H), 6.55 (dd, $J = 8.4, 2.6$ Hz, 1H), 6.48 (s, 1H), 6.37 (s, 1H), 6.30 (s, 1H), 6.11 (dd, $J = 8.4, 2.4$ Hz, 1H), 5.86 (s, 1H), 4.14–4.09 (m, 1H), 3.94 (s, 3H), 3.89 (dd, $J = 14.8, 8.0$ Hz, 2H), 3.73 (s, 3H), 3.67–3.53 (m, 1H), 3.45 (dd, $J = 12.0, 5.2$ Hz, 1H), 3.36 (s, 3H), 3.34–3.27 (m, 1H), 3.17 (dd, $J = 14.0, 5.8$ Hz, 1H), 3.08 (s, 3H), 3.05–2.74 (m, 6H), 2.67 (s, 3H), 2.53 (s, 3H), 2.50 (d, $J = 4.6$ Hz, 1H), 2.33 (dd, $J = 14.8, 9.4$ Hz, 1H), 2.17 (d, $J = 14.8$ Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 156.1, 152.3, 149.4, 149.0, 149.0, 148.8, 147.3, 146.0,

144.0, 138.0, 132.9, 131.7, 129.7, 127.5, 126.6, 126.3, 123.9, 121.1, 121.0, 120.9, 120.9, 119.0, 112.0, 107.0, 105.7, 64.2, 61.4, 60.3, 60.1, 56.3, 55.6, 55.47, 53.41, 44.7, 42.2, 41.8, 39.7, 39.1, 38.8, 29.6, 23.7, 21.0, 20.5, 14.1. HRMS (ESI-QTOF): m/z 823.2977 $[M + H]^+$, calcd for $C_{44}H_{46}N_4O_{10}S$: 823.3013.

14-(3-Methoxybenzenesulfonamide)tetrandrine (16). Yellow amorphous solid; yield 85%; mp: 176–178 °C; IR (KBr, cm^{-1}): 3430, 2936, 2835, 1596, 1506, 1448, 1260, 1217, 1156, 1110, 1021, 909, 828; 1H NMR ($CDCl_3$, 500 MHz) δ (ppm): 7.65 (d, $J = 8.5$ Hz, 2H), 7.28–7.25 (m, 2H), 7.16 (dd, $J = 8.0, 2.5$ Hz, 1H), 6.82 (d, $J = 8.5$ Hz, 2H), 6.54 (dd, $J = 8.5, 2.0$ Hz, 1H), 6.45 (s, 1H), 6.39 (s, 1H), 6.28 (s, 1H), 6.11 (dd, $J = 8.5, 2.0$ Hz, 1H), 5.83 (s, 1H), 3.91 (s, 3H), 3.83 (d, $J = 9.5$ Hz, 1H), 3.78 (s, 3H), 3.72 (s, 3H), 3.46–3.35 (m, 2H), 3.33 (s, 3H), 3.21 (dd, $J = 12.0, 5.5$ Hz, 1H), 3.11 (dd, $J = 14.0, 5.5$ Hz, 1H), 3.06 (s, 3H), 2.99–2.65 (m, 5H), 2.57 (s, 3H), 2.48 (s, 3H), 2.40 (dd, $J = 8.5, 2.0$ Hz, 2H), 2.14 (d, $J = 14.5$ Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm): 162.3, 155.9, 152.1, 149.2, 148.7, 148.5, 145.9, 143.9, 138.0, 134.1, 133.0, 132.9, 131.7, 129.5, 128.5, 128.9, 127.4, 127.2, 126.9, 121.0, 120.9, 120.9, 120.8, 120.4, 113.8, 112.1, 107.5, 105.6, 64.1, 61.2, 60.0, 56.2, 55.7, 55.5, 55.4, 45.0, 42.4, 40.1, 39.2, 38.8, 24.6, 20.5. HRMS (ESI-QTOF): m/z 808.3251 $[M + H]^+$, calcd for $C_{45}H_{49}N_3O_9S$: 808.3268.

14-(3,5-Dichlorobenzenesulfonamide)tetrandrine (17). Pink amorphous solid; yield 85%; mp: 155–156 °C; IR (KBr, cm^{-1}): 3423, 2935, 2840, 1609, 1568, 1506, 1448, 1351, 1216, 1165, 1114, 864, 800; 1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.67 (d, $J = 2.0$ Hz, 2H), 7.45 (t, $J = 2.0$ Hz, 1H), 7.35 (dd, $J = 8.2, 2.0$ Hz, 1H), 7.22 (dd, $J = 8.2, 2.6$ Hz, 1H), 7.15 (s, 1H), 6.56 (dd, $J = 8.4, 2.6$ Hz, 2H), 6.52 (s, 1H), 6.42 (s, 1H), 6.34 (s, 1H), 6.12 (dd, $J = 8.4, 2.2$ Hz, 1H), 5.89 (s, 1H), 4.12–4.08 (m, 1H), 4.02 (dd, $J = 11.0, 5.2$ Hz, 1H), 3.92 (s, 3H), 3.75 (s, 3H), 3.73–3.67 (m, 1H), 3.60 (dd, $J = 13.0, 5.2$ Hz, 1H), 3.45 (dd, $J = 13.6, 5.0$ Hz, 1H), 3.39 (s, 3H), 3.19 (dd, $J = 14.0, 5.8$ Hz, 1H), 3.13 (s, 3H), 3.06–2.89 (m, 3H), 2.81 (t, $J = 12.0$ Hz, 1H), 2.74 (s, 3H), 2.65–2.58 (m, 2H), 2.55 (s, 3H), 2.29 (d, $J = 14.8$ Hz, 1H), 2.05 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 156.6, 152.3, 149.3, 149.0, 148.7, 145.4, 144.5, 144.2, 137.9, 135.5, 132.8, 132.2, 131.9, 131.8, 129.7, 126.7, 126.6, 125.7, 125.0, 121.3, 121.1, 120.9, 120.8, 119.0, 111.9, 106.0, 105.8, 64.4, 61.4, 60.3, 60.1, 56.2, 55.6, 55.7, 44.6, 42.2, 41.4, 39.6, 39.2, 38.8, 29.6, 23.3, 21.0, 20.5, 14.1. HRMS (ESI-QTOF): m/z 846.2330 $[M + H]^+$, calcd for $C_{44}H_{45}Cl_2N_3O_8S$: 846.2383.

14-(2,4,6-Trimethylbenzenesulfonamide)tetrandrine (18). Yellow amorphous solid; yield 91%; mp: 154–156 °C; IR (KBr, cm^{-1}): 3429, 2936, 2853, 1671, 1610, 1507, 1449, 11261, 1217, 1159, 1107, 808; 1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.56–7.48 (m, 1H), 7.21 (dd, $J = 8.4, 2.6$ Hz, 1H), 6.98 (s, 2H), 6.85 (d, $J = 8.0$ Hz, 1H), 6.66 (s, 1H), 6.47 (s, 1H), 6.41 (s, 1H), 6.33 (d, $J = 8.4$ Hz, 1H), 6.06 (s, 1H), 5.81 (s, 1H), 4.42 (d, $J = 12.6$ Hz, 1H), 4.29 (d, $J = 10.6$ Hz, 1H), 4.16 (d, $J = 12.6$ Hz, 1H), 3.96 (dd, $J = 15.8, 11.0$ Hz, 1H), 3.82 (s, 3H), 3.71 (s, 9H), 3.47 (s, 2H), 3.38 (s, 3H), 3.24 (s, 3H), 3.20–3.06 (m, 3H), 3.01–2.83 (m, 6H), 2.72 (d, $J = 15.8$ Hz, 1H), 2.32 (s, 3H), 1.94 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 168.1, 156.1, 152.0,

149.2, 148.5, 148.4, 145.1, 144.1, 138.1, 136.2, 133.9, 133.5, 132.9, 130.2, 130.0, 129.6, 128.4, 128.1, 127.5, 126.9, 126.3, 125.7, 125.3, 124.8, 124.7, 121.4, 121.2, 121.1, 120.9, 120.6, 112.2, 106.3, 105.6, 64.10, 60.8, 59.9, 56.3, 55.6, 55.4, 44.9, 42.3, 41.8, 40.1, 39.9, 38.8, 24.7, 20.2. HRMS (ESI-QTOF): m/z 820.3572 $[M + H]^+$, calcd for $C_{47}H_{53}N_3O_8S$: 820.3607.

14-(2,4,6-Triisopropylbenzenesulfonamide)tetrandrine (19). Yellow amorphous solid; yield 90%; mp: 170–172 °C; IR (KBr, cm^{-1}): 3411, 2958, 1610, 1507, 1462, 1424, 1360, 1282, 1232, 1220, 1133, 864, 669; 1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.53–7.48 (m, 1H), 7.19 (dd, J = 8.0, 2.0 Hz, 1H), 6.96 (s, 2H), 6.86–6.79 (m, 1H), 6.64 (s, 1H), 6.45 (s, 1H), 6.39 (s, 1H), 6.34–6.27 (m, 1H), 6.04 (s, 1H), 5.79 (s, 1H), 4.39 (d, J = 11.4 Hz, 2H), 4.27 (d, J = 10.6 Hz, 1H), 4.14 (d, J = 12.0 Hz, 1H), 3.97–3.91 (m, 1H), 3.80 (s, 3H), 3.69 (s, 15H), 3.60 (s, 1H), 3.45 (s, 3H), 3.35 (s, 3H), 3.22 (s, 3H), 2.96–2.85 (m, 7H), 2.69 (d, J = 12.0 Hz, 1H), 2.53 (s, 1H), 2.30 (s, 3H), 1.92 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 153.7, 153.3, 149.9, 149.3, 148.1, 146.9, 143.7, 142.3, 139.6, 138.3, 133.4, 132.8, 131.7, 131.5, 130.6, 129.8, 127.9, 124.8, 123.8, 122.5, 122.3, 120.2, 120.0, 115.3, 115.0, 112.6, 112.0, 106.1, 67.0, 64.7, 63.3, 60.3, 55.9, 55.6, 55.2, 44.3, 43.7, 40.9, 40.5, 39.9, 36.1, 23.2, 21.5, 21.2, 20.8. HRMS (ESI-QTOF): m/z 904.4529 $[M + H]^+$, calcd for $C_{53}H_{65}N_3O_8S$: 904.4571.

14-(4-Tertbutylbenzenesulfonamide)tetrandrine (20). Yellow amorphous solid; yield 85%; mp: 167–169 °C; IR (KBr, cm^{-1}): 3429, 2937, 1610, 1507, 1449, 1331, 1218, 1159, 1112, 834, 576; 1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.71–7.67 (m, 2H), 7.41–7.37 (m, 2H), 7.31 (dd, J = 8.4, 2.6 Hz, 1H), 7.20 (dd, J = 8.2, 2.6 Hz, 2H), 6.55 (dd, J = 8.4, 2.6 Hz, 1H), 6.50 (s, 1H), 6.39 (s, 1H), 6.37 (s, 1H), 6.11 (dd, J = 8.4, 2.2 Hz, 1H), 5.88 (s, 1H), 5.30 (s, 1H), 4.14–4.00 (m, 1H), 3.89 (s, 3H), 3.75 (s, 3H), 3.63 (d, J = 12.4 Hz, 1H), 3.48 (s, 1H), 3.38 (s, 3H), 3.17 (dd, J = 14.2, 6.0 Hz, 1H), 3.11 (s, 3H), 3.04–2.89 (m, 3H), 2.82 (d, J = 12.0 Hz, 1H), 2.75 (s, 3H), 2.61 (t, J = 12.2 Hz, 1H), 2.51 (s, 3H), 2.48 (d, J = 4.6 Hz, 1H), 2.22–2.15 (m, 1H), 2.05 (s, 1H), 1.28 (s, 9H), 1.25 (t, J = 4.0 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 156.7, 155.8, 152.0, 149.5, 148.8, 145.3, 144.5, 138.2, 137.8, 132.8, 132.2, 131.6, 129.7, 127.4, 126.5, 126.3, 125.7, 121.2, 121.1, 121.0, 120.9, 120.2, 112.0, 106.6, 105.8, 77.0, 76.7, 64.5, 61.2, 60.3, 60.2, 60.1, 56.2, 55.6, 55.5, 53.4, 46.7, 42.3, 41.4, 40.0, 39.36, 39.0, 35.0, 31.0, 29.6, 23.2, 20.5. HRMS (ESI-QTOF): m/z 834.3813 $[M + H]^+$, calcd for $C_{48}H_{55}N_3O_8S$: 834.3788.

14-(4-Trifluoromethylbenzenesulfonamide)tetrandrine (21). White amorphous solid; yield 80%; mp: 160–162 °C; IR (KBr, cm^{-1}): 3433, 2935, 1672, 1611, 1609, 1507, 1449, 1323, 1218, 1164, 1106, 807, 708; 1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.04–7.96 (m, 1H), 7.83–7.78 (m, 2H), 7.36 (dd, J = 8.2, 2.2 Hz, 1H), 7.22 (dd, J = 8.2, 2.2 Hz, 4H), 6.57 (dd, J = 8.4, 2.6 Hz, 1H), 6.53 (s, 1H), 6.39 (s, 1H), 6.33 (s, 1H), 6.12 (dd, J = 8.6, 2.2 Hz, 1H), 5.89 (s, 1H), 4.14–4.07 (m, 1H), 3.91 (s, 3H), 3.86–3.79 (m, 2H), 3.75 (s, 3H), 3.71 (d, J = 10.0 Hz, 1H), 3.39 (s, 3H), 3.22–3.14 (m, 2H), 3.12 (s, 3H), 3.05–2.93 (m, 4H), 2.89 (s, 1H), 2.84 (d, J = 12.0 Hz, 1H), 2.80 (s, 3H), 2.52 (s, 3H), 2.50–2.43 (m, 1H), 2.19 (d, J = 14.6 Hz, 1H); ^{13}C NMR

($CDCl_3$, 100 MHz) δ (ppm): 162.5, 156.7, 152.1, 151.5, 149.7, 149.0, 148.6, 145.7, 144.6, 139.6, 137.8, 132.8, 131.8, 131.3, 129.8, 128.5, 127.9, 127.1, 126.4, 125.8, 121.2, 120.9, 120.9, 120.5, 119.6, 119.0, 112.0, 107.0, 105.9, 77.0, 76.7, 64.5, 61.3, 60.1, 56.2, 55.6, 55.4, 42.2, 41.1, 39.9, 39.1, 36.4, 29.6, 22.8, 20.5. HRMS (ESI-QTOF): m/z 846.2330 $[M + H]^+$, calcd for $C_{45}H_{46}F_3N_3O_8S$: 846.2311.

14-(4-Acetaminobenzenesulfonamide)tetrandrine (22). Light yellow amorphous solid; yield 85%; mp: 166–168 °C; IR (KBr, cm^{-1}): 3425, 2939, 2845, 1609, 1507, 1453, 1232, 1158, 1133, 871, 663; 1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.77 (s, 1H), 7.64 (d, J = 8.6 Hz, 2H), 7.55 (d, J = 8.6 Hz, 2H), 7.27 (dd, J = 8.2, 2.4 Hz, 2H), 7.22 (s, 1H), 7.16 (dd, J = 8.0, 2.6 Hz, 1H), 6.55 (dd, J = 8.4, 2.6 Hz, 1H), 6.45 (s, 1H), 6.40 (s, 1H), 6.29 (s, 1H), 6.12 (dd, J = 8.4, 2.2 Hz, 1H), 5.84 (s, 1H), 3.91 (s, 3H), 3.86–3.74 (m, 2H), 3.73 (s, 3H), 3.43 (m, 2H), 3.34 (s, 3H), 3.24–3.18 (m, 1H), 3.11 (dd, J = 13.8, 5.6 Hz, 1H), 3.06 (s, 3H), 3.00–2.82 (m, 3H), 2.81–2.64 (m, 2H), 2.58 (s, 3H), 2.49 (s, 3H), 2.44 (d, J = 4.0 Hz, 1H), 2.36 (dd, J = 14.6, 9.4 Hz, 1H), 2.15 (s, 3H), 2.12 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 165.5, 156.7, 152.1, 151.6, 149.7, 149.0, 148.6, 145.7, 144.6, 139.6, 137.8, 132.8, 131.8, 131.3, 129.8, 128.5, 127.9, 127.2, 126.4, 125.8, 121.3, 120.9, 120.9, 120.5, 119.6, 112.0, 107.0, 105.9, 77.0, 76.7, 64.5, 61.3, 60.1, 56.2, 55.6, 55.5, 42.2, 41.1, 39.9, 39.2, 36.4, 31.4, 29.6, 22.8, 20.5. HRMS (ESI-QTOF): m/z 834.3760 $[M + H]^+$, calcd for $C_{46}H_{50}N_4O_9S$: 834.3788.

14-(2-Naphthalenesulfonamide)tetrandrine (23). Light yellow amorphous solid; yield 85%; mp: 157–158 °C; purity: 98.1%; IR (KBr, cm^{-1}): 3405, 2936, 1608, 1506, 1448, 1330, 1262, 1215, 1122, 875, 817; 1H NMR ($CDCl_3$, 500 MHz) δ (ppm): 7.86 (d, J = 8.5 Hz, 1H), 7.82 (d, J = 8.5 Hz, 2H), 7.72 (dd, J = 8.0, 2.5 Hz, 1H), 7.58–7.51 (m, 2H), 7.28 (s, 1H), 7.26–7.23 (m, 1H), 7.14 (dd, J = 8.5, 2.5 Hz, 1H), 6.53 (dd, J = 8.5, 2.0 Hz, 1H), 6.44 (s, 1H), 6.36 (s, 1H), 6.26 (s, 1H), 6.10 (dd, J = 8.5, 2.0 Hz, 1H), 5.80 (s, 1H), 3.91 (s, 3H), 3.85 (d, J = 9.5 Hz, 1H), 3.76 (dd, J = 8.5, 2.0 Hz, 1H), 3.70 (s, 3H), 3.46–3.36 (m, 2H), 3.33 (s, 3H), 3.23–3.16 (m, 2H), 3.04 (s, 3H), 2.99–2.82 (m, 3H), 2.78–2.64 (m, 2H), 2.56 (s, 3H), 2.51 (s, 3H), 2.47–2.41 (m, 2H), 2.11 (d, J = 14.0 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 157.3, 155.9, 152.1, 152.0, 149.1, 148.7, 148.6, 145.8, 145.5, 143.9, 138.3, 138.0, 134.5, 133.8, 132.9, 132.1, 132.0, 131.8, 129.5, 129.1, 129.0, 128.4, 128.2, 127.8, 127.5, 127.3, 127.1, 126.6, 122.3, 121.0, 120.8, 120.1, 112.1, 107.1, 105.6, 64.1, 61.3, 60.0, 56.2, 55.6, 55.4, 44.9, 42.2, 40.0, 39.2, 24.5, 20.5. HRMS (ESI-QTOF): m/z 828.3292 $[M + H]^+$, calcd for $C_{48}H_{49}N_3O_8S$: 828.3319.

14-(Quinoline-8-sulfonamide)tetrandrine (24). Pink amorphous solid; yield 85%; mp: 171–173 °C; purity: 96.2%; IR (KBr, cm^{-1}): 3413, 2936, 1611, 1562, 1457, 1232, 1217, 1116, 837, 987; 1H NMR ($CDCl_3$, 500 MHz) δ (ppm): 9.01–8.98 (m, 1H), 8.63 (d, J = 7.5 Hz, 1H), 8.18 (d, J = 8.5 Hz, 1H), 8.00 (d, J = 9.0 Hz, 1H), 7.61 (t, J = 8.0 Hz, 1H), 7.45 (dd, J = 8.0, 4.0 Hz, 1H), 7.25 (d, J = 10.0 Hz, 2H), 7.12–7.07 (m, 2H), 6.51–6.47 (m, 2H), 6.35 (s, 2H), 6.10 (dd, J = 8.5, 2.0 Hz, 1H), 5.84 (s, 1H), 3.97–3.88 (m, 2H), 3.80 (d, J = 8.0 Hz, 1H), 3.78 (s, 3H), 3.76 (s, 3H), 3.52–3.43 (m, 1H), 3.38 (s, 3H), 3.35–3.21

(m, 2H), 3.12 (d, $J = 10.0$ Hz, 1H), 3.09 (s, 3H), 3.08–2.85 (m, 3H), 2.77 (m, 2H), 2.62 (s, 3H), 2.60 (s, 3H), 2.55 (d, $J = 2.5$ Hz, 1H), 2.24–2.68 (d, $J = 14.5$ Hz, 1H), 2.62 (s, 3H), 2.60 (s, 3H), 2.55 (d, $J = 2.5$ Hz, 1H), 2.24 (d, $J = 14.5$ Hz, 1H); ^{13}C NMR (CDCl_3 , 100 MHz) δ (ppm): 153.7, 153.4, 152.7, 150.0, 149.3, 149.1, 148.0, 146.9, 143.8, 143.3, 138.3, 136.3, 135.7, 133.3, 132.7, 131.6, 131.5, 130.6, 129.2, 129.2, 128.6, 125.4, 124.8, 124.0, 122.7, 120.3, 120.0, 115.5, 115.2, 112.0, 111.5, 106.2, 64.8, 63.3, 60.4, 56.0, 55.7, 55.1, 44.4, 43.7, 41.2, 40.7, 40.0, 36.0, 29.6, 21.6, 21.3. HRMS (ESI-QTOF): m/z 829.3232 $[\text{M} + \text{H}]^+$, calcd for $\text{C}_{47}\text{H}_{48}\text{N}_4\text{O}_8\text{S}$: 829.3250.

4.2 Biological tests

4.2.1 Cell lines and cell culture. Human erythroleukemic cell lines HEL and K562, human breast cell line MDA-MB-231, human prostate cell line PC3 and human melanoma cell line WM9 were obtained from Molecular and Cell Biology Research, Sunnybrook Health Sciences Centre, Toronto, Ontario, Canada. Human hepatocyte HL7702 was obtained from the Key Laboratory of Chemistry for Natural Products of Guizhou Province and Chinese Academy of Sciences. All cell lines were cultured in RPMI (HEL, K562 and HL7702) or DMEM (MDA-MB-231, PC3, and WM9) medium (high glucose) supplemented with 5% FBS (HyClone, GE Healthcare, Parramatta, Australia) and 1% ampicillin/streptomycin.

4.2.2 MTT test method. The anti-proliferative effects of the novel tetrandrine derivatives were examined using MTT assays. Briefly, human cancer cell lines MDA-MB-231, PC3, WM9, HEL and K562 were cultured in 96-well plates maintained at 37 °C in 95% humidity and 5% CO_2 for 48 h. Different concentrations (20.0–0.5 μM) of compounds were used in the treatments. After 48 h of treatment, the cells were washed twice with PBS, 20 μL of MTT solution (5 mg mL^{-1} in PBS) was added to each well, and the plate was incubated at 37 °C for 4 h. After centrifugation, 100 μL of DMSO was added to each well to dissolve the formazan crystals. The absorbance was measured at 490 nm using an ELISA microplate reader. IC_{50} values were determined for the replicates of the 96-well plates from three independent experiments.

4.2.3 Flow cytometry. MDA-MB-231 cells were incubated with compounds or DMSO as a vehicle control for 24 hours. The cells were then washed with cold PBS, and stained with Annexin V-FITC and propidium iodide (PI) using an apoptosis detection kit (BD Biosciences, USA).

4.2.4 Western blotting. Total protein was collected from MDA-MB-231 cells using RIPA lysis buffer and separated on 10% SDS-PAGE, as previously described.⁴⁴ Protein was then transferred to a polyvinylidene fluoride (PVDF, 0.2 μm) membrane, and blocked using 5% BSA solution for 2 h at room temperature. The membrane was then incubated with specific primary antibodies overnight at 4 °C, followed by incubation with the corresponding HRP-conjugated secondary antibodies for 2 h at room temperature. The expression of a particular protein was measured by applying ECL Select substrate (Li-Cor, USA). Polyclonal rabbit antibody for Bcl-2 and

β -actin was obtained from Abcam (Abcam, Cambridge, UK); Bcl-xl, Caspase-3, Bax and PARP antibodies were obtained from Cell Signaling Technology (CST, Danvers, USA).

Conflicts of interest

The authors declare no conflict of interest.

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