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Synthesis and Bioactivity Evaluation of Nepetaefolin F and Its Analogues

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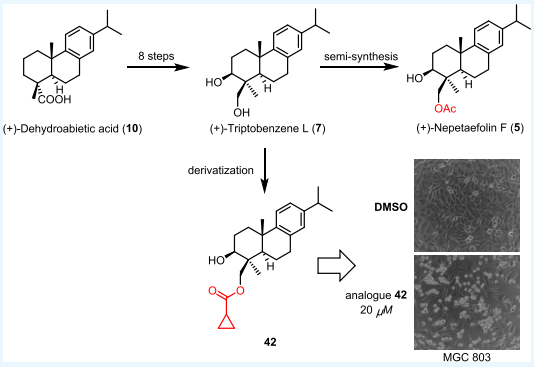
ABSTRACT: Nepetaefolin F (**5**), an abietane diterpenoid, showed significant inhibitory activity against human cancer cells *in vitro* with an IC_{50} value of 6.3 μM. The syntheses of nepetaefolin F and its analogues are presented herein. The cytotoxicity against various cancer cell lines was evaluated; notably, the cyclopropanecarboxylate ester **42** displayed significant antitumor activity against MGC 803 cells with an IC_{50} value of 20.9 μM. Further studies revealed that **42** could upregulate the expression of p62, microtubule-associated protein 1 light-chain 3 β (LC3 B-I), cleaved caspase-3, and cleaved caspase-9 and downregulate the expression of Beclin-1 and LC3B-II. Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis showed that **42** could modulate multiple signaling pathways, especially for peroxisome proliferator-activated receptor (PPAR) and AMP-activated protein kinase (AMPK), which are closely related to autophagy. These results suggested that compound **42** is a promising lead by inhibiting cell proliferation and autophagy, as inducing cell apoptosis in MGC 803 cells.



Gastric cancer is the third most common cause of cancer death.[1,2] Surgical resection is the main treatment for early gastric cancer; however, the addition of chemotherapy preoperatively (neoadjuvant), postoperatively (adjuvant), or perioperatively increases the survival benefit,[3] and systemic chemotherapy is the main treatment for advanced gastric cancer.[4]

Natural products have been recognized as fruitful medicinal resources for new drug discovery due to their unique chemical scaffolds and various biological activities.[5] Between 1981 and 2019, approximately 25% of new anticancer agents approved by the Food and Drug Administration (FDA) were based on natural products.[6] Several promising cytotoxic compounds and clinical first-line antineoplastic drugs are originated from diterpenoids (Figure 1), such as tanshinones (**1**, tanshinone I),[7,8] oridonin (**2**),[9,10] paclitaxel,[11,12] and ginkgolides (**3**, ginkgolide B).[13,14] To date, only one commercial abietane-type drug, Ecabet (**4**), was approved in 1993 for the treatment of gastric ulcers.[15] Recently, we have isolated a number of abietane diterpenoids in *Caryopteris nepetaefolia* (Benth.) Maxim., a folk herb endemic in China with anti-inflammatory activity.[16] Among them, nepetaefolins F (**5**) and G (**6**) showed significant inhibitory activity against human cancer cells *in vitro* with IC_{50} values of 6.3 μM and 9.0 μM, respectively. In 2018, Carter and co-workers reported the first asymmetric synthesis of nepetaefolin F in 10 steps with an overall yield of 14.8%, featuring a strategy based on a proline sulfonamide-catalyzed Yamada−Otani reaction and Pummerer reaction.[17] In the present study, we established an efficient and robust synthetic route to (+)-nepetaefolin F (**5**) and 27 derivatives from dehydroabietic acid. Furthermore, the cytotoxic effect of nepetaefolin F (**5**) and its analogues was evaluated on human cancer cells, including gastric cancer, ovarian cancer, breast cancer, lung cancer, and liver cancer cell lines. The underlying molecular mechanism of compound **42** on cell viability of gastric cancer cells was also studied.

■ RESULTS AND DISCUSSION

Chemistry. Commercially available dehydroabietic acid (**10**) has been widely used as the precursor for the synthesis of natural abietane analogues by medicinal chemists.[18−21] As outlined in Scheme 1, nepetaefolin F (**5**) could be assembled from aldehyde **8** through α-methylation of aldehyde. Hydroboration−oxidation of allylic alcohol **9** would be able to deliver aldehyde **8**. A decarboxylation of dehydroabietic acid **10** and subsequent allylic oxidation are convenient to provide the carbon framework and proper functional groups.

The decarboxylation of dehydroabietic acid **10** with lead tetraacetate gave a mixture of three olefins, including 19-norabieta-4(18),8,11,13-tetraene (**11**), 19-norabieta-3,8,11,13-tetraene (**12**), and 19-norabieta-4,8,11,13-tetraene (**13**) with an overall yield of 96% (Scheme 2).[22] Without further

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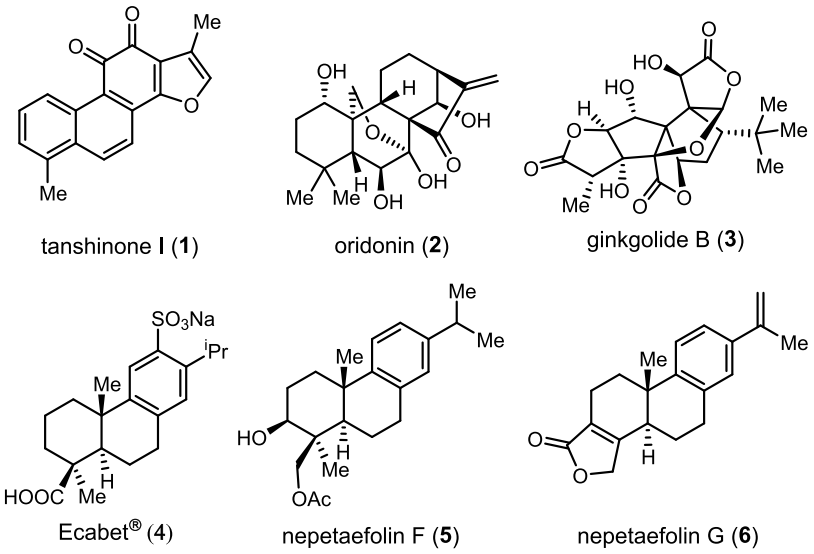
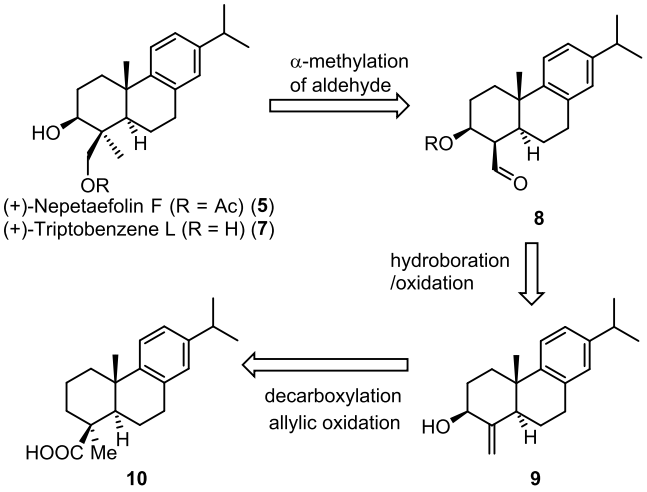


Figure 1. Structures of representative bioactive diterpenoids (**1**−**6**).

Scheme 1. Retrosynthetic Analysis of Nepetaefolin F (5)

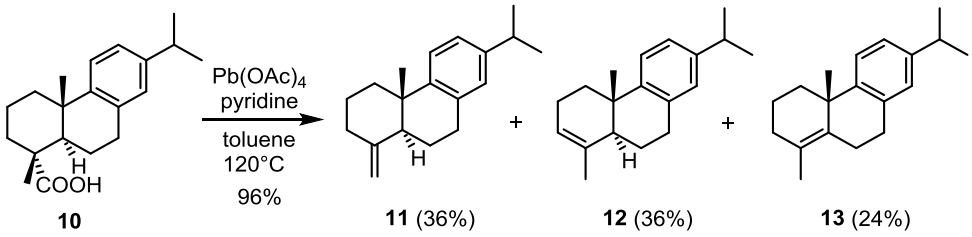


purification, treatment of this mixture with selenium dioxide (SeO_2) and *tert*-butyl hydroperoxide (^{t}BuOOH) afforded allylic alcohol **9a** as a single stereoisomer in 31% yield over two steps from **10** (Scheme 3). Interestingly, the stereochemistry was verified to be *R*-OH by extensive NMR experiments, implicating that the facial selectivity in the allylic oxidation is likely controlled by the axial methyl group at C10. Therefore, protection by the benzyl group was performed in the presence of benzyl bromide (BnBr) and sodium hydride (NaH) to generate compound **14** in 90% yield. Compound **14** was subjected to a hydroboration−oxidation sequence to give a primary alcohol **15** in 61% yield as a single isomer. Subsequent oxidation with Dess−Martin periodinane (DMP) afforded aldehyde **8a** in good yield.

With compound **8a** in hand, we then conducted the key α-methylation to furnish the quaternary carbon center at C4. To our delight, treatment of compound **8a** with potassium *tert*-butoxide (^{t}BuOK) and methyl iodide (MeI) smoothly provided the methylated product **16** with a satisfactory yield and diastereoselectivity. The unexpected elimination of the benzyloxy group might be driven by the conjugation of aldehyde, although the enal form was not detected under harsh conditions. The observed facial selectivity in methylation also took advantage of a 1,3-diaxial repulsion from the methyl group at C10. Subsequent reduction by $NaBH_4$ in MeOH furnished the primary alcohol **17**.

The next phase of synthesis is regio- and stereoselective hydroboration to complete the *cis*-1,3-diol motif in triptobenzene L (**7**). Direct exposure of **17** to borane methyl sulfide complex (BH_3·DMS) only resulted in **7** in 10% yield, together with a great amount of regio- and stereoisomers. Based on work by Vedejs and co-workers,[23] we envisioned that a hydroxyl-directed intramolecular hydroboration would be feasible to realize the desired selectivity. To our delight, application of trifluoromethanesulfonic acid (TfOH) as an additive gave compound **7** in 40% isolated yield with 2:1 dr. In this case, increasing the reaction temperature to −20 °C ensured a full conversion of **17** but led to poor regioselectivity (2:1 ratio for **7** and C2 hydroxylated product) and diastereoselectivity. Furthermore, triptobenzene L (**7**) was

Scheme 2. Decarboxylation of Dehydroabietic Acid (10)



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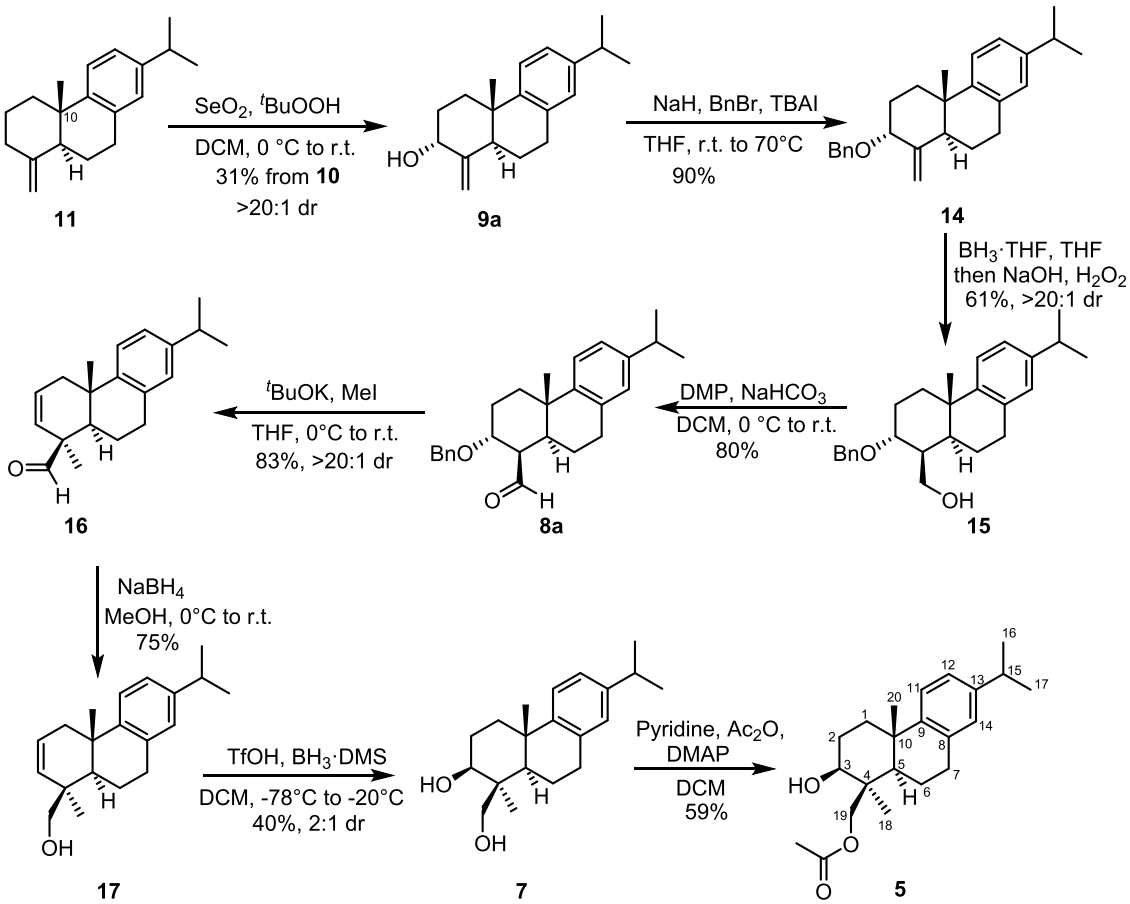
Scheme 3. Synthesis of Nepetaefolin F (5)



selectively acetylated with acetic anhydride (Ac_2O) to provide nepetaefolin F (**5**) in 59% yield.

Esterification has been considered as a useful method to expand the diversity of candidate compounds and enhance the biological activity.[24,25] With sufficient amount of triptobenzene L (**7**) in hand, we next set out to streamline the synthesis of derivatives **18**−**42** at the diol at C3 and C19 positions in a nonselective manner with different carboxylic acids (Scheme 4). In addition, derivatives **43** and **44** were prepared by the treatment of triptobenzene L (**7**) with triethylamine (TEA) and methanesulfonyl chloride (MsCl) (Scheme 5). Accordingly, 27 analogues have been synthesized with a yield range from 11 to 42%.

Biological Results. In the present study, the cytotoxicity of nepetaefolin F (**5**) and its derivatives on cell viability of various cancer cell lines was evaluated by cell counting kit-8 (CCK-8). As shown in Figure 2A−E, cyclopropanecarboxylate ester **42** showed better antitumor activity than nepetaefolin F in gastric cancer, ovarian cancer, breast cancer, lung cancer, and liver cancer cell lines, especially in MGC 803 cells. In our previous research, nepetaefolin F showed good antitumor activity (IC_{50} was 6.3 μM) in a patient-derived xenograft (PDX) model of non-small-cell lung cancer (LUPF045);[18] however, it showed low cytotoxicity to A549 cells in our study. Non-small-cell lung carcinoma includes squamous cell carcinoma, adenocarcinoma, and large-cell carcinoma. The different characteristics and heterogeneity between squamous non-small-cell lung cancer PDX cells and non-small-cell lung adenocarcinoma cell line A549 cells might result in the different inhibitory effect.[26] Therefore, in the present study, we further investigated the effect and the underlying mechanism of derivative **42** on cell viability of MGC 803 cells.

As shown in Figure 3A,B, compound **42** significantly reduced the cell viability of MGC 803 cells in a dose-dependent manner. The IC_{50} value of **42** on MGC 803 cells for 24 h was 20.9 μM. 5-Ethynyl-2′-deoxyuridine (EdU) staining was used to detect the effect of **42** on the cell proliferation of MGC 803 cells. As displayed in Figure 3C,D, after treatment for 24 h, compound **42** (20 μM) significantly reduced the number of EdU-positive cells (green fluorescence) compared with the control cells.

Ribonucleic acid sequencing (RNA-Seq) results demonstrated that there were 2755 significantly different genes ($p < 0.05$) between the cyclopropanecarboxylate ester **42**-treated cells and the control-treated cells. Among these genes, the expression of 275 genes was upregulated, and the expression of 2480 genes was downregulated after treatment with derivative **42** for 24 h (Figure 4A). The top 10 downregulated genes (MT1G, EPHA2, MT2A, RRP9, CCDC85B, CDC20, TNFRSF12A, PTGES, UBE2S, CCN1) and upregulated genes (TCEAL9, KPNA2, CASC19, CENPF, UBE2C, RBM5, TRIR, MCAM, SLC38A2, SNHG19) are presented in Figure 4B. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed for these differential genes to predict the function of differentially expressed genes. As demonstrated in Figure 4C, the enriched top 20 pathways, including PPAR signaling pathway, AMPK signaling pathway, cell cycle, fatty

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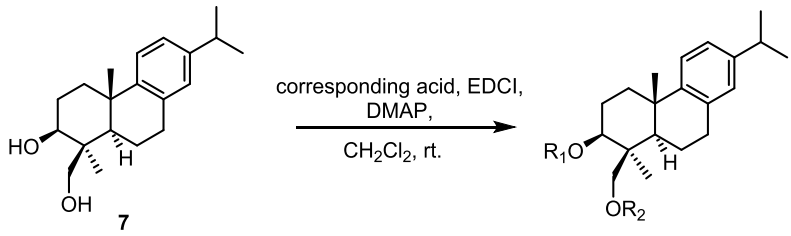
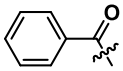
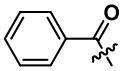
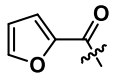
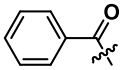
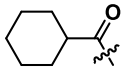
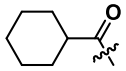
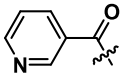
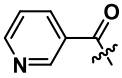
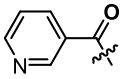
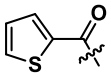
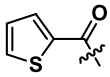
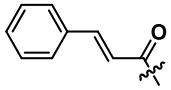
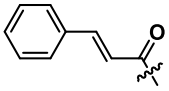
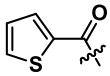
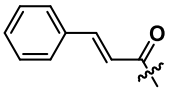
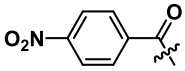
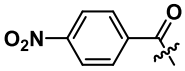
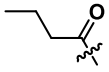
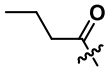
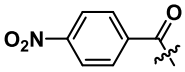
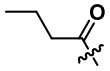
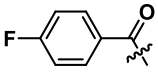
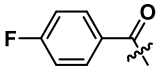
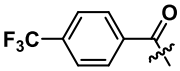
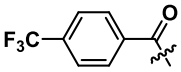
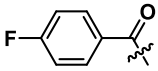
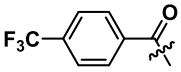
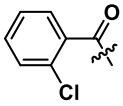
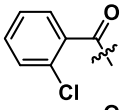
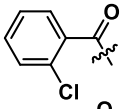
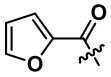
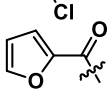
Scheme 4. Synthesis of Nepetaefolin F Analogues 18−42



Compound	R_1	R_2	yield	global yield	Compound	R_1	R_2	yield	global yield
18			22%	0.7%	31	H		37%	1.2%
19	H		26%	0.9%	32			24%	0.8%
20			24%	0.8%	33			27%	0.9%
21	H		28%	0.9%	34	H		35%	1.2%
22			26%	0.9%	35			21%	0.7%
23	H		35%	1.2%	36	H		30%	1.0%
24			25%	0.8%	37			25%	0.8%
25	H		36%	1.2%	38	H		40%	1.3%
26			26%	0.9%	39			33%	1.1%
27	H		37%	1.2%	40	H		41%	1.4%
28			27%	0.9%	41			27%	0.9%
29	H		42%	1.4%	42	H		37%	1.2%
30			25%	0.8%					

acid biosynthesis, and fatty acid degradation, were predicted to possibly mediate the inhibitory effect of compound **42** on the cell viability of MGC 803 cells. GO analysis demonstrated that cyclopropanecarboxylate ester **42** mainly modulated the regulation of mitotic nuclear division, cell cycle, cell division, organelle localization, and cell cycle process (Figure 4D). Both PPAR signaling pathway and AMPK signaling pathway were closely related to autophagy,[27−29] indicating that compound **42** possibly inhibits gastric cancer through modulating autophagy in MGC 803 cells.

After RNA-seq, we further investigated the effect of agent **42** on the protein expression of autophagy-related and apoptosis-related proteins in MGC 803 cells. Microtubule-associated protein light-chain 3 (LC3) is a marker of autophagy. When

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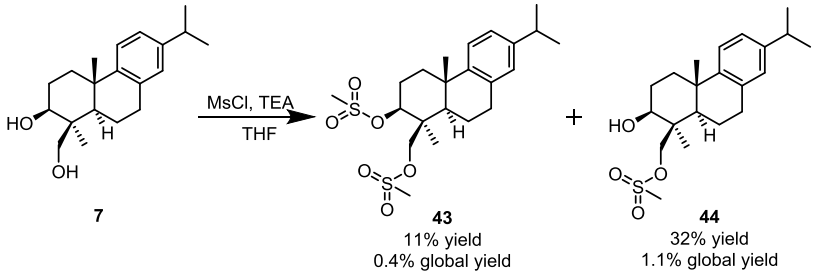
Scheme 5. Synthesis of Nepetaefolin F Analogues 43 and 44



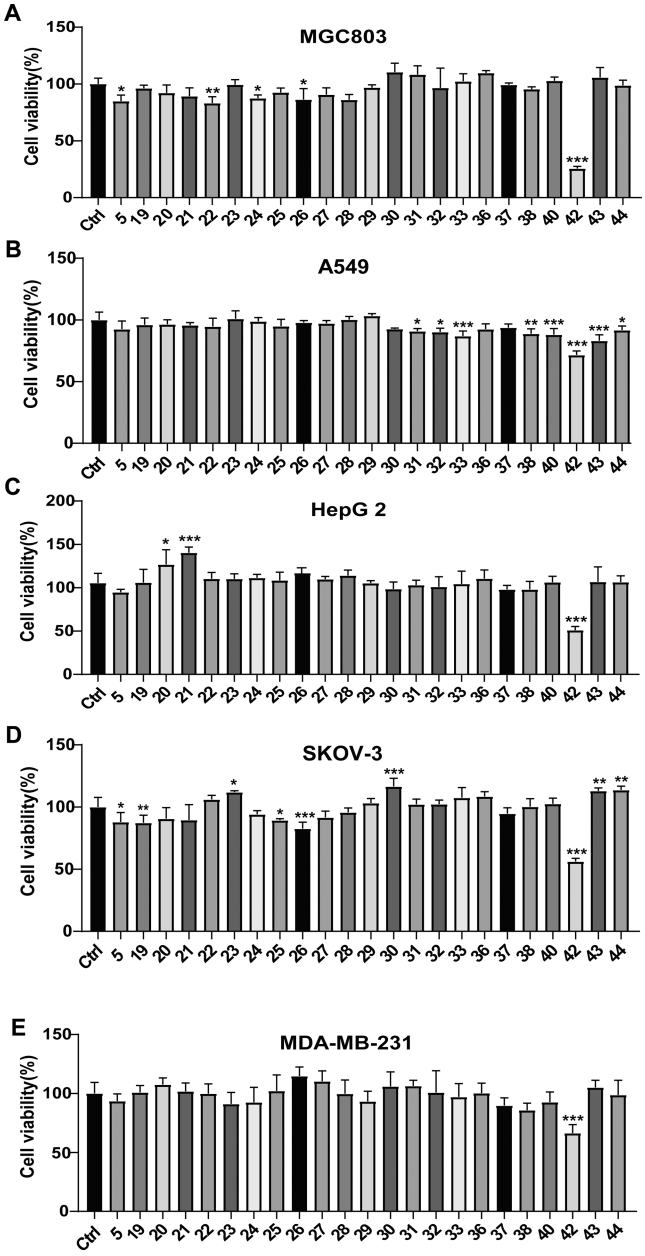


Figure 2. Effect of nepetaefolin F and its analogues at 20 μM on cell viability of various human cancer cell lines after treatment for 48 h. Data are shown as mean ± standard deviation (SD); $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$, compared with control group. $n \geq 3$.

autophagy occurs, LC3 is proteolytically cleaved by the autophagy-related gene 4 (Atg4) to produce LC3B-I. In the process of autophagy, LC3-I is combined with phosphatidylthanolamine to form LC3-phosphatidylthanolamine conjugate (LC3-II).[30] The ratio of LC3-II to LC3-I can reflect the level of autophagy.[31] P62/SQSTM1, which binds to polyubiquitin proteins and LC3-II, is another commonly used marker for autophagy, and p62 accumulates when autophagy is inhibited.[30,32,33] In this study, compared with the control cells, derivative **42** could significantly upregulate the protein expression of LC3B-II and p62 and decrease the protein expression of LC3B-I after treatment for 24 h (Figure 5A). Consistently, the expression of Beclin-1 was downregulated, which plays a central role in the activation of autophagy-related signaling pathway.[34] These above results indicated that compound **42** inhibited cell viability of MGC 803 cells through suppressing autophagy in MGC 803 cells.

Moreover, we also investigated the effect of **42** on the protein expression of apoptosis-related proteins in MGC 803 cells. Caspases are at the core of cell apoptosis. The extrinsic apoptotic pathway is dependent on the initiator caspase-8, while the intrinsic mitochondrial apoptotic pathway activates caspase-9 through cytochrome c release and then cleaves and activates downstream caspase-3.[35] As shown in Figure 5B, agent **42** enhanced the protein expression of cleaved caspase-9 and cleaved caspase-3, and decreased the protein expression of B-cell lymphoma-2 (Bcl-2); however, agent **42** showed no significant effect on the protein expression of Bcl-2-associated X protein (Bax). These results indicated that compound **42** induced apoptosis of MGC 803 cells in a mitochondrial-dependent pathway. The process of apoptosis is often accompanied by the increase of reactive oxygen species (ROS). In our present study, derivative **42** indeed enhanced ROS accumulation in MGC 803 cells after treatment for 18 and 24 h (Figure 5C).

■ CONCLUSIONS

(+)-Nepetaefolin F (**5**) was synthesized in nine steps with 2% overall yield from dehydroabietic acid, and 27 analogues were obtained by esterification. Among them, cyclopropanecarboxylate ester **42** showed better antitumor effect than nepetaefolin F and the other derivatives on cell viability of gastric cancer, ovarian cancer, breast cancer, lung cancer, and liver cancer cell lines. Derivative **42** significantly decreased the cell viability of MGC 803 cells in a dose-dependent manner, and the IC_{50} value for 24 h was 20.9 μM. The research on mechanism showed that analogue **42** suppressed cell viability, possibly through suppressing cell autophagy and enhancing mitochondrial-dependent cell apoptosis. Our findings indicate that compound **42** is a potential drug for gastric cancer therapy.

■ EXPERIMENTAL SECTION

General Experimental Procedures. All reactions were performed under an N_2 atmosphere unless otherwise stated.

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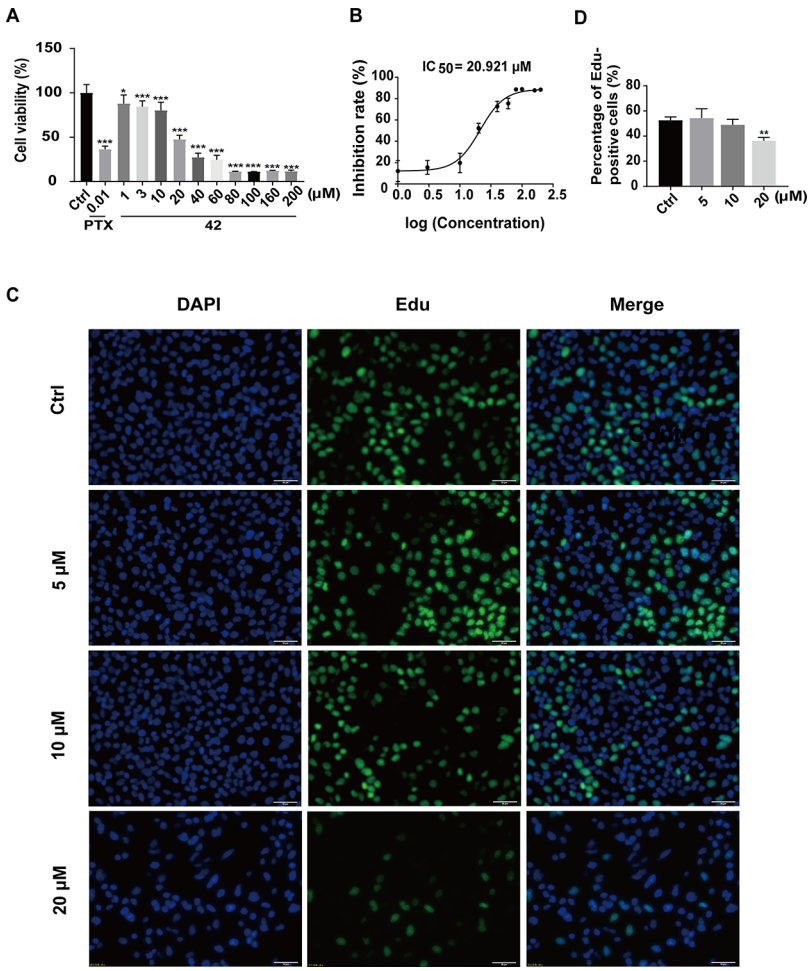

Figure 3. Compound **42** inhibited cell viability and proliferation of MGC 803 cells after treatment for 24 h. (A) Compound **42** reduced cell viability of MGC 803 cells in a dose-dependent manner after treatment for 24 h. (B) IC_{50} of compound **42** for 24 h in MGC 803 cells. (C, D) Compound **42** (20 μM) decreased cell proliferation of MGC 803 cells after treatment for 24 h detected by EdU staining. Scale bar, 50 μm. Data are shown as the means ± SD, $n \geq 3$. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared with the control group.

Commercially available reagents were used as purchased without further purification unless otherwise noted. Optical rotations were measured with an Anton Paar MCP5500 polarimeter. The NMR spectra were recorded on Bruker AVANCE-III instruments (400 MHz). HRESIMS data were obtained on FT mass spectrometers in electrospray ionization (ESI) mode. Thin layer chromatography was performed on TLC silica gel GF254 plates supplied by Yantai Jiangyou Silicon Material Company (China) and visualized by UV light or phosphomolybdic acid.

*(2R,4aS,10aR)-7-Isopropyl-4a-methyl-1-methylene-1,2,3,4,4a,9,10,10a-octahydrophenanthren-2-ol (**9a**).* To a solution of dehydroabietic acid **10** (25.0 g, 83.2 mmol) in toluene (125 mL), pyridine (15 mL) and lead tetraacetate (92.0 g, 207.5 mmol) were added at room temperature, and the mixture was refluxed for 2 h. After cooling to room temperature, the mixture was filtered through celite and concentrated under reduced pressure. The crude product was subjected to purification on silica gel chromatography, eluting with petroleum ether (PE) to afford a mixture of three olefins **11**−**13** as a colorless oil (20.3 g, 96% for three olefins).

The olefins were dissolved in dichloromethane (DCM) (500 mL) and cooled to 0 °C. Then, SeO_2 (5.7 g, 51 mmol) and ^{t}BuOOH (37.1 mL, 204 mmol, ca. 5.5 M in decane) were added, and the solution was allowed to warm to room temperature. After 2 h, the reaction mixture was quenched with saturated sodium hyposulfite ($Na_2S_2O_3$) solution (200 mL) and filtered through celite. The organic layer was separated, and the aqueous phase was extracted with DCM (100 mL × 4). The combined organic layers were washed with brine, dried over sodium sulfate (Na_2SO_4), and concentrated. The residue was purified by flash chromatography on silica gel (PE/EtOAc = 20:1) to afford **9a** as pale yellow oil (6.9 g, 31% for 2 steps, >20:1 dr): $[\alpha]_D^{25}$ + 83 (*c* 1.0, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23 (d, *J* = 8.1 Hz, 1H, H-11), 7.02 (d, *J* = 8.1 Hz, 1H, H-12), 6.95 (s, 1H, H-14), 5.08 (s, 1H, H-19), 4.75 (s, 1H, H-19), 4.37 (t, *J* = 2.5 Hz, 1H, H-3), 3.03−2.89 (m, 2H, H-7), 2.85 (m, 1H, H-5 or H-15), 2.78 (m, 1H, H-5 or H-15), 2.14−1.88 (m, 4H, H-1 or H-2 or H-6), 1.81−1.70 (m, 2H, H-1 or H-2 or H-6), 1.24 (d, *J* = 6.9 Hz, 6H, H-16 and H-17), 0.99 (s, 3H, H-20); ^{13}C NMR (100 MHz, $CDCl_3$) δ 151.9 (C-4), 146.0 (C-9 or C-13), 144.3 (C-9 or C-13), 134.8 (C-8), 127.2 (C-14), 125.4 (C-11), 124.1 (C-12), 110.0 (C-19), 73.1 (C-3), 41.7, 39.1, 33.6, 32.5, 30.3, 30.0, 24.1 (C-16), 24.1 (C-17), 22.1, 21.0; HRMS (EI): calcd for $C_{19}H_{26}O$ $(M)^+$: 270.1984, found: 270.1990.

*(2R,4aS,10aR)-2-(Benzyloxy)-7-isopropyl-4a-methyl-1-methylene-1,2,3,4,4a,9,10,10a-octahydrophenanthrene (**14**).* The solution of allylic alcohol **9a** (13.8 g, 51 mmol) in

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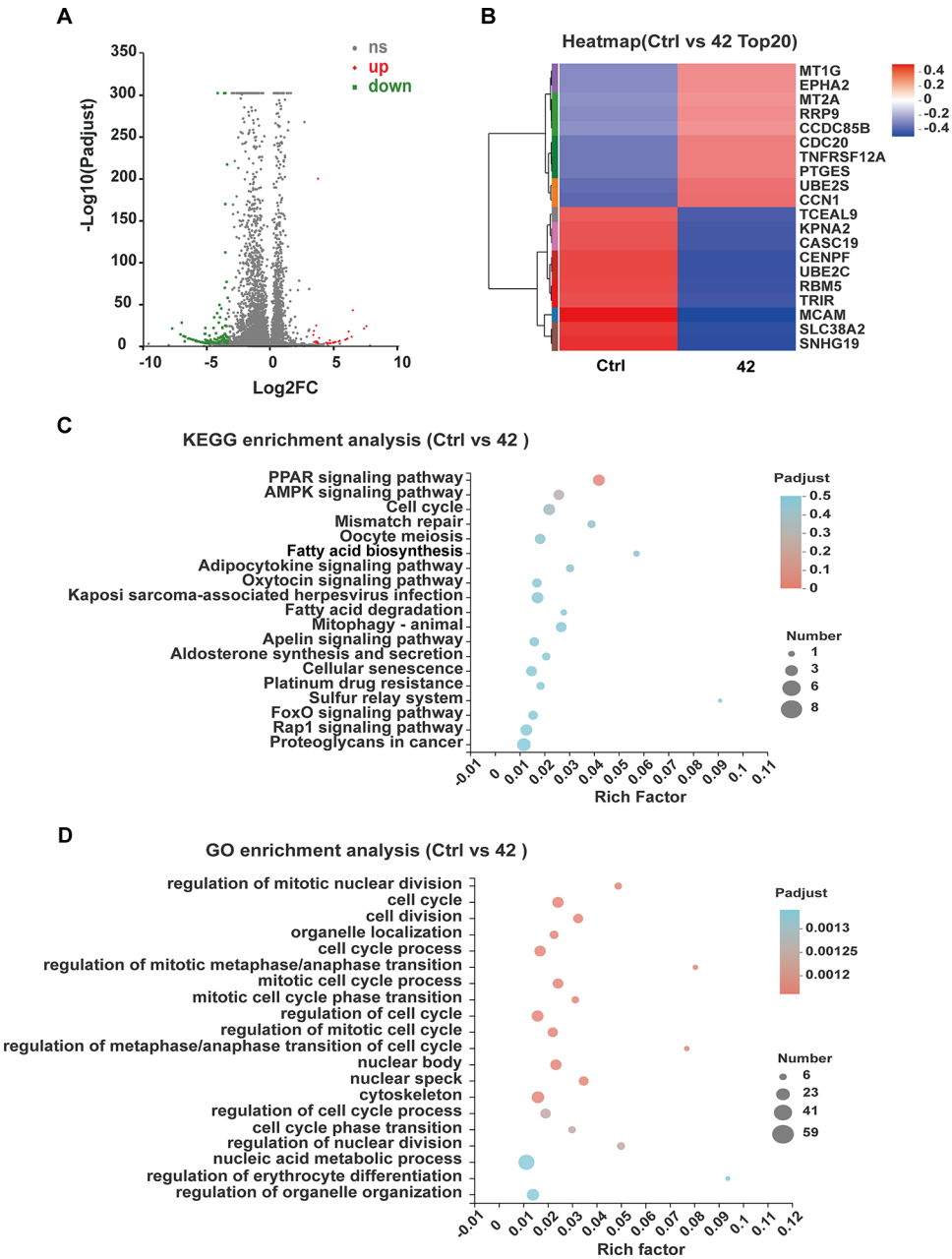

Figure 4. Transcriptional profiles and bioinformatic analysis. (A) Log2 fold changes in gene expression in MGC 803 treated with derivative **42** at 20 μM for 24 h; genes are represented as dots. (B) Heat map showing the top 10 upregulated genes and the top 10 downregulated genes in MGC 803 cells treated with derivative **42**. (C, D) KEGG pathway enrichment analysis and GO analysis for differential genes.

tetrahydrofuran (THF) (300 mL) was cooled to 0 °C; NaH (6.1 g, 152.5 mmol, 60% in mineral oil) was added into the solution above in one portion and stirred for 0.5 h, whereupon BnBr (17.4 g, 102 mmol) and tetrabutylammonium iodide (TBAI, 18.8 g, 51 mmol) were introduced. The resulting mixture was allowed to warm to 70 °C. After the reaction was completed, saturated NH_4Cl solution (200 mL) was added, the organic layer was separated, and the aqueous phase was extracted with ethyl acetate (200 mL × 3). The combined organic layers were washed with brine, dried over Na_2SO_4, and evaporated. The residue was purified by chromatography on silica gel (PE/EtOAc = 150:1) to give **14** (16.5 g, 90%) as a colorless oil: $[\alpha]_D^{25}$ + 2 (*c* 5.0, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33−7.19 (m, 6H, benzyl group and H-11), 7.01 (dd, *J* = 8.1, 2.0 Hz, 1H, H-12), 6.93 (d, *J* = 2.0 Hz, 1H, H-14), 5.05 (t, *J* = 1.5 Hz, 1H, H-19), 4.88 (t, *J* = 1.7 Hz, 1H, H-19), 4.45 (d, *J* = 12.0 Hz, 1H, CH_2-Ph), 4.27 (d, *J* = 12.0 Hz, 1H, CH_2-Ph), 3.94 (brs, 1H, H-3), 2.94−2.87 (m, 2H, H-7), 2.87−2.77 (m, 1H, H-5 or H-15), 2.66 (m, 1H, H-5 or H-15), 2.09−1.96 (m, 3H), 1.94−1.85 (m, 1H), 1.83−1.74 (m, 2H), 1.23 (d, *J* = 6.9 Hz, 6H, H-16 and H-17), 1.00 (s, 3H, H-20); ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.4 (C-4), 145.9 (C-9 or C-13), 144.5 (C-9 or C-13), 139.2, 134.8, 128.3, 127.6, 127.3, 127.2, 125.5 (C-11), 124.0 (C-12), 111.5 (C-19), 79.4 (C-3), 69.1 (CH_2-Ph), 42.1, 39.1, 33.6, 33.1, 30.1, 29.4, 24.1 (C-16 and 17), 22.5, 21.1; HRMS (EI): calcd for $C_{26}H_{32}O$ $(M)^+$: 360.2453, found: 360.2449.

*((1S,2R,4aS,10aS)-2-(Benzyloxy)-7-isopropyl-4a-methyl-1,2,3,4,4a,9,10,10a-octahydrophenanthren-1-yl)methanol (**15**).* To a solution of **14** (16.5 g, 45.8 mmol) in THF (300

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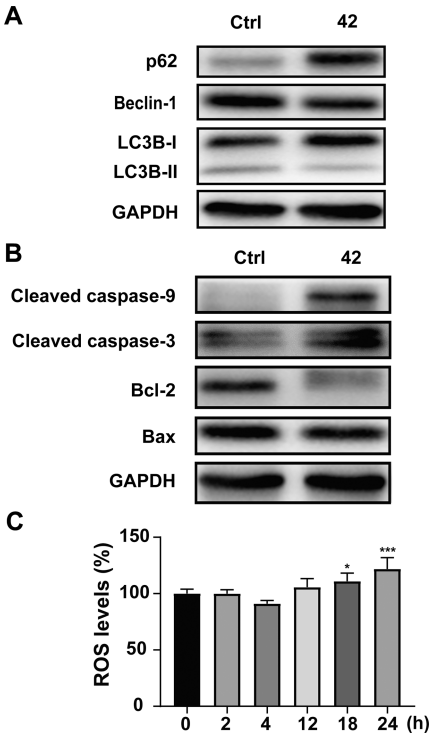

Figure 5. Compound **42** modulated autophagy and apoptosis in MGC 803 cells. (A) Derivative **42** (20 μM) treatment regulated the protein expression of autophagy-related proteins (LC3B, p62, Beclin-1) for 24 h detected by Western blot assay. (B) Derivative **42** (20 μM) modulated the protein expression of apoptosis-related proteins in MGC 803 cells. (C) Derivative **42** (20 μM) increased reactive oxygen species (ROS) levels in MGC 803 cells after treatment for 18 and 24 h. Data are shown as the means ± SD, $n \geq 3$. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared with the control group.

mL) was added BH_3·THF (137 mL, 137 mmol, ca. 1 M in THF) at 0 °C. The reaction was allowed to warm to room temperature and stirred for an additional 2 h. Next, the oxidation step was carried out at 0 °C; 2 N NaOH (91 mL, 182 mmol) and 30% H_2O_2 (26 mL, 229 mmol) were carefully added and stirred for an additional 2 h. The mixture was extracted with ethyl acetate (300 mL × 3), washed with brine, dried over anhydrous Na_2SO_4, and evaporated. The residue was purified by chromatography on silica gel (DCM/MeOH = 50:1) to give **15** (10.6 g, 61%, >20:1 dr) as pale yellow oil: $[\alpha]_D^{25}$ + 58 (*c* 1.0, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33−7.20 (m, 5H, benzyl group), 7.16 (d, *J* = 8.1 Hz, 1H, H-11), 6.99 (dd, *J* = 8.1, 1.9 Hz, 1H, H-12), 6.88 (d, *J* = 1.9 Hz, 1H, H-14), 4.55 (d, *J* = 11.9 Hz, 1H, CH_2-Ph), 4.49 (d, *J* = 11.9 Hz, 1H, CH_2-Ph), 3.83 (m, 1H, H-3), 3.79 (dd, *J* = 10.8, 2.7 Hz, 1H, H-19), 3.60 (t, *J* = 10.8 Hz, 1H, H-19), 2.98−2.75 (m, 3H, H-7 or H-5 or H-15), 2.28 (dd, *J* = 13.0, 6.7 Hz, 1H), 2.10−1.80 (m, 6H), 1.56 (ddd, *J* = 13.1, 5.3, 2.1 Hz, 1H), 1.22 (d, *J* = 6.9 Hz, 6H), 1.02 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 145.9 (C-9 or C-13), 145.8 (C-9 or C-13), 139.2, 134.9, 128.4, 127.5, 127.4, 127.1, 124.7 (C-11), 124.0 (C-12), 74.6 (C-3), 70.2 (CH_2-Ph), 61.6 (C-19), 49.2, 38.3, 36.7, 33.6, 32.4, 30.6, 24.9, 24.1 (C-16 and 17), 23.8, 22.5; HRMS (EI): calcd for $C_{26}H_{34}O_2$ $(M)^+$:378.2559, found: 378.2553.

*(1R,2R,4aS,10aS)-2-(Benzyloxy)-7-isopropyl-4a-methyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carbaldehyde (**8a**).* To a solution of **15** (10.6 g,28 mmol) in DCM (300 mL) was added Dess−Martin periodinane (33.8 g, 79.7 mmol) and $NaHCO_3$ (4.7 g, 56 mmol) at 0 °C. The reaction was allowed to warm to room temperature and stirred overnight prior to quenching with saturated $Na_2S_2O_3$ solution (200 mL). The mixture was extracted with DCM (200 mL × 3), washed with brine, dried over anhydrous Na_2SO_4, and filtered through a short pad of celite. The combined solution was concentrated under reduced pressure, and the residue was subjected to purification on silica gel chromatography and evaporated. The residue was purified by chromatography on silica gel (PE/EtOAc = 100:1) to afford **8a** (8.4 g, 80%) as pale yellow oil: $[\alpha]_D^{25}$ + 27 (*c* 0.5, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.98 (s, 1H, H-19), 7.33−7.22 (m, 5H, benzyl group), 7.18 (d, *J* = 8.0 Hz, 1H, H-11), 7.02 (dd, *J* = 8.0, 2.0 Hz, 1H, H-12), 6.92 (d, *J* = 2.0 Hz, 1H, H-14), 4.56 (d, *J* = 11.7 Hz, 1H, CH_2-Ph), 4.43 (d, *J* = 11.7 Hz, 1H, CH_2-Ph), 4.23 (t, *J* = 1.7 Hz, 1H, H-3), 3.05−2.89 (m, 2H, H-7), 2.83 (m, 1H, H-4 or H-15), 2.68 (m, 1H, H-4 or H-15), 2.55 (ddd, *J* = 13.4, 5.8, 2.1 Hz, 1H), 2.27 (m, 1H), 2.08−1.97 (m, 2H), 1.92−1.84 (m, 1H), 1.84−1.78 (m, 2H), 1.23 (d, *J* = 6.9 Hz, 6H, H-16 and H-17), 1.03 (s, 3H, H-20); ^{13}C NMR (100 MHz, $CDCl_3$) δ 203.8 (C-19), 146.2 (C-9 or C-13), 144.6 (C-9 or C-13), 138.8, 134.6, 128.5, 127.6, 127.6, 127.3, 125.0 (C-11), 124.3 (C-12), 71.7 (C-3), 70.5 (CH_2-Ph), 57.5, 39.2, 37.3, 33.6, 32.2, 30.9, 24.1 (C-16 and 17), 23.7, 23.2, 23.0; HRMS (ESI) calcd for $C_{26}H_{32}O_2Na$ $[M + Na]^+$: 399.2300, found: 399.2294.

*(1S,4aS,10aR)-7-Isopropyl-1,4a-dimethyl-1,4,4a,9,10,10a-hexahydrophenanthrene-1-carbaldehyde (**16**).* To a solution of aldehyde **8a** (4.5 g, 12 mmol) in THF (100 mL) was added ^{t}BuOK (13.2 mL, 13.2 mmol, 1 M in THF) at 0 °C. The mixture was stirred for 5 min, and MeI (0.74 mL, 12.0 mmol) was added. The reaction was allowed to warm to room temperature and stirred for 30 min prior to quenching with saturated NH_4Cl solution (100 mL). The mixture was evaporated and extracted with ethyl acetate (20 mL × 4), washed with brine, dried over anhydrous Na_2SO_4, and evaporated. The residue was purified by chromatography on silica gel (PE/EtOAc = 100:1) to give **16** (2.8 g, 83%, >20:1 dr) as pale yellow oil: $[\alpha]_D^{25}$ + 25 (*c* 1.0, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.85 (s, 1H, H-19), 7.18 (d, *J* = 8.2 Hz, 1H, H-11), 7.04 (dd, *J* = 8.2, 2.1 Hz, 1H, H-12), 6.91 (d, *J* = 2.1 Hz, 1H, H-14), 5.94 (ddd, *J* = 10.1, 5.8, 2.0 Hz, 1H, H-2), 5.67 (ddd, *J* = 10.1, 2.9, 1.2 Hz, 1H, H-3), 2.97−2.89 (m, 1H, H-7 or H-15), 2.89−2.78 (m, 2H, H-7 or H-15), 2.63 (dd, *J* = 17.4, 5.8 Hz, 1H, H-1), 2.24−2.12 (m, 2H), 1.93−1.79 (m, 2H), 1.25 (d, *J* = 3.5 Hz, 6H, H-16 and H-17), 1.22 (s, 3H, H-18 or H-20), 1.17 (s, 3H, H-18 or H-20); ^{13}C NMR (100 MHz, $CDCl_3$) δ 205.5 (C-19), 146.2 (C-9 or C-13), 143.7 (C-9 or C-13), 134.7 (C-8), 129.8 (C-3), 127.8 (C-14), 126.9 (C-2 or C-11), 126.1 (C-2 or C-11), 124.6 (C-12), 50.7, 50.0, 40.1, 36.6, 33.6, 31.5, 25.5, 24.1 (C-16 and 17), 23.3, 20.1; HRMS (ESI) calcd for $C_{20}H_{27}O$ $[M + H]^+$: 283.2062, found: 283.2063.

*((1S,4aS,10aR)-7-Isopropyl-1,4a-dimethyl-1,4,4a,9,10,10a-hexahydrophenanthrene-1-yl)methanol (**17**).* To a solution of **16** (2.8 g, 9.9 mmol) in MeOH (70 mL) was added $NaBH_4$ (382.4 mg, 9.9 mmol) at 0 °C. The reaction was allowed to warm to room temperature and stirred for 2 h prior to quenching with saturated NH_4Cl solution (50 mL). The mixture was evaporated and extracted with DCM (20 mL × 4), washed with brine, dried over anhydrous Na_2SO_4, and evaporated. The residue was purified by chromatography on silica gel (PE/EtOAc = 20:1) to give **17** (2.1 g, 75%) as pale

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yellow oil: $[\alpha]_D^{25}$ + 24 (*c* 10.0, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.22 (d, *J* = 8.2 Hz, 1H, H-11), 7.06 (dd, *J* = 8.2, 2.0 Hz, 1H, H-12), 6.93 (d, *J* = 2.0 Hz, 1H, H-14), 5.80 (ddd, *J* = 10.2, 5.8, 1.7 Hz, 1H, H-2), 5.73 (dd, *J* = 10.2, 2.6 Hz, 1H, H-3), 3.84 (d, *J* = 10.8 Hz, 1H, H-19), 3.61 (d, *J* = 10.8 Hz, 1H, H-19), 2.96−2.80 (m, 3H, H-7 and H-15), 2.62 (dd, *J* = 17.0, 5.8 Hz, 1H, H-1), 2.19 (d, *J* = 17.0 Hz, 1H, H-1), 2.01 (m, 1H), 1.81 (dd, *J* = 12.9, 1.6 Hz, 1H), 1.74−1.61 (m, 2H), 1.30 (s, 3H, H-18 or H-20), 1.27 (d, *J* = 6.9 Hz, 6H, H-16 and H-17), 1.20 (s, 3H, H-19 or H-20); ^{13}C NMR (100 MHz, $CDCl_3$) δ 145.8 (C-9 or C-13), 145.0 (C-9 or C-13), 134.8 (C-8), 132.8 (C-3), 126.7 (C-14), 126.0 (C-2 or C-11), 124.9 (C-2 or C-11), 124.4 (C-12), 66.7 (C-19), 48.6 (C-5), 40.2, 39.9, 36.6, 33.6, 31.5, 26.1, 25.8, 24.1 (C-16 and C-17), 19.6; HRMS (ESI) calcd for $C_{20}H_{29}O$ [M + H]$^+$: 285.2218, found: 283.2216.

*(+)-Triptobenzene L (**7**).* To a solution of neat $Me_2S{\cdot}BH_3$ (5.7 mL, 56.3 mmol) in DCM (60 mL) was slowly added TfOH (5.1 mL, 56.3 mmol) at −78 °C. The reaction was stirred for 35 min, and a solution of **17** (1.6 g, 5.6 mmol) in DCM (50 mL) was added. The reaction mixture was stirred for 24 h at −20 °C. The oxidation step was carried out at 0 °C, 2 N NaOH (8.4 mL, 16.8 mmol), and 30% H_2O_2 (2 mL, 17.6 mmol) was carefully added and stirred for an additional 2 h. The mixture was extracted with DCM (50 mL × 4), washed with brine, dried over anhydrous Na_2SO_4, and evaporated. The residue was purified by chromatography on silica gel (PE/EtOAc = 3:1) to give **7** (0.68 g, 40%, 2:1 dr) as a white solid: mp = 159.6−160.8 °C. $[\alpha]_D^{25}$ + 25 (*c* 1.0, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.15 (d, *J* = 8.2 Hz, 1H, H-11), 6.99 (dd, *J* = 8.2, 2.0 Hz, 1H, H-12), 6.89 (d, *J* = 2.0 Hz, 1H, H-14), 4.33 (d, *J* = 11.2 Hz, 1H, H-19), 3.51 (dd, *J* = 11.8, 4.6 Hz, 1H, H-3), 3.43 (d, *J* = 11.2 Hz, 1H, H-19), 2.94 (dd, *J* = 16.1, 6.0 Hz, 1H, H-7), 2.89−2.78 (m, 2H, H-7 and H-15), 2.34 (dt, *J* = 13.5, 3.5 Hz, 1H, H-1), 2.07−1.86 (m, 3H, H-2 and H-6), 1.72−1.59 (m, 1H, H-6), 1.54 (td, *J* = 13.5, 3.5 Hz, 1H, H-1), 1.45 (dd, *J* = 12.5, 1.9 Hz, 1H, H-5), 1.31 (s, 3H, H-18), 1.22 (d, *J* = 6.9 Hz, 6H, H-16 and H-17), 1.16 (s, 3H, H-20); ^{13}C NMR (100 MHz, $CDCl_3$) δ 146.3 (C-9), 146.0 (C-13), 134.5 (C-8), 126.9 (C-14), 124.6 (C-11), 124.2 (C-12), 80.7 (C-3), 64.3 (C-19), 50.8 (C-5), 42.9 (C-4), 37.2 (C-10), 36.9 (C-1), 33.6 (C-15), 31.2 (C-7), 28.6 (C-2), 26.1 (C-20), 24.1(C-16), 24.1 (C-17), 22.7 (C-18), 19.1 (C-6); HRMS (ESI) calcd for $C_{20}H_{30}O_2Na$ [M + Na]$^+$: 325.2143, found: 325.2144.

*(+)-Nepetaefolin F (**5**).* To a solution of **7** (40 mg, 0.13 mmol) in DCM (2 mL) was added 4-dimethylaminopyridine (DMAP) (1.7 mg, 0.013 mmol) and pyridine (21 μL, 0.27 mmol) at room temperature. The resulting mixture was stirred at room temperature for 2 min, and Ac_2O (14 μL, 0.146 mmol) was added. The reaction mixture was stirred for 2 h at room temperature. Then, the reaction was quenched by addition of saturated $NaHCO_3$ solution (2 mL). The resulting mixture was extracted with ethyl acetate (2 mL × 4), washed with brine, dried over anhydrous Na_2SO_4, and evaporated. The residue was purified by chromatography on silica gel (PE/EtOAc = 20:1) to recover starting material **7** (10 mg) and give **5** (20 mg, 59% brsm) as colorless oil: $[\alpha]_D^{25}$ + 40 (*c* 1.0, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.16 (d, *J* = 8.2 Hz, 1H, H-11), 7.00 (d, *J* = 8.2 Hz, 1H, H-12), 6.89 (s, 1H, H-14), 4.43 (d, *J* = 11.7 Hz, 1H, H-19), 4.24 (d, *J* = 11.7 Hz, 1H, H-19), 3.36 (dd, *J* = 11.3, 5.0 Hz, 1H, H-3), 2.94 (dd, *J* = 17.1, 6.2 Hz, 1H, H-7), 2.83 (m, 2H, H-7 and H-15), 2.34 (dt, *J* = 13.3, 3.6 Hz, 1H, H-1), 2.08 (s, 3H, OAc), 2.00 (dd, *J* = 13.3, 6.9 Hz, 1H, H-6), 1.91 (m, 1H, H-2), 1.86 (m, 1H, H-2), 1.78 (m, 1H, H-6), 1.56 (td, *J* = 13.0, 4.7 Hz, 1H, H-1), 1.48 (dd, *J* = 12.5, 1.9 Hz, 1H, H-5), 1.22 (s, 3H, H-18), 1.21 (d, *J* = 6.0 Hz, 6H, H-16 and H-17), 1.19 (s, 3H, H-20); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.3 (OAc), 146.1 (C-9), 146.1 (C-13), 134.5 (C-8), 126.9 (C-14), 124.9 (C-11), 124.3 (C-12), 79.1 (C-3), 65.4 (C-9), 51.2 (C-5), 42.5 (C-4), 37.3 (C-1), 37.3 (C-10), 33.6 (C-15), 31.5 (C-7), 28.2 (C-2), 25.6 (C-20), 24.1(C-16), 24.1 (C-17), 22.6 (C-18), 21.3 (OAc), 19.7 (C-6); HRMS (ESI) calcd for $C_{22}H_{32}O_3Na$ [M + Na]$^+$: 367.2249, found: 367.2247.

Cell Culture. The human gastric cancer cell line MGC 803, breast cancer cell line MDA-MB-231, and ovarian carcinoma cell line SKOV-3 were obtained from Cell Bank, Type Culture Collection of Chinese Academy of Sciences (Shanghai, People's Republic of China). The human lung cancer cell A549 and hepatocellular cancer cell HepG 2 were gifts from Professor Tao Wu (Shanghai University of Traditional Chinese Medicine). The MDA-MB-231 and HepG 2 cells were cultured in DMEM medium (Meilunbio, People's Republic of China). MGC 803 and A549 cells were cultured in RPMI 1640 medium (Meilunbio). SKOV-3 cells were cultured in McCoy's 5A medium (Gibco). The medium contained 10% heat-inactivated fetal bovine serum (Gibco), 100 units/mL penicillin, and 100 μg/mL streptomycin (Gibco). Cells were incubated at 37 °C in a humidified atmosphere of 5% CO_2.

Cell Viability Assay. Cell viability was detected by using cell counting kit-8 (Yeasen, People's Republic of China). Cells were seeded in 96-well plates at a density of 5 × 10^3 cells/mL (MGC 803, A549, HepG 2) or 8 × 10^3 cells/mL (MDA-MB-231, SKOV-3) and incubated overnight. Then, cells were treated with 20 μM nepetaefolin F and its analogues for 48 h. Paclitaxel (PTX) (Yuanye, People's Republic of China) is used as a positive drug. After treatment, 10 μL CCK-8 solution was added in each well and incubated for another 0.5−1 h. The absorbance at 450 nm was measured with a Thermo Scientific Varioskan Flash microplate reader (Thermo). Finally, the cell viability rate (%) was calculated as follows: (absorbance of treated sample − blank/absorbance of control sample − blank) × 100.

EdU Staining. Cell proliferation of MGC 803 cells was measured by EdU staining (RiBoBio, People's Republic of China). 100 μL of MGC 803 cell suspension was seeded in 96-well plates at a density of 7000 cells/well and cultured for 24 h. Then, cells were incubated with analogue **42** for another 24 h at different concentrations (5, 10, and 20 μM). Next, 100 μL of EdU medium (50 μM) was added to each well and incubated for another 2 h. After being labeled by EdU, the cells were fixed by 4% paraformaldehyde for 30 min. After that, Apollo staining and DNA staining were performed. After staining, samples were observed and photographed with a fluorescence microscope (IX81, Olympus, Japan), and then analyzed by using ImageJ software. The EdU incorporation rate was calculated as the ratio of the number of green fluorescent cells (Apollo-staining) to the number of blue fluorescent cells (Hoechst 33342 staining).

Analysis of Intracellular ROS. MGC 803 cells were seeded in a 96-well plate and treated with **42** (20 μM) for 2, 4, 12, 18, and 24 h. The intracellular levels of ROS produced by MGC 803 cells were detected by 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) (Yeasen). Fluorescence was measured at 488 nm excitation wavelength and 525 nm

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emission wavelength with a Thermo Scientific Varioskan Flash microplate reader (Thermo).

Western Blotting Assay. After treatment with **42** for 24 h, MGC 803 cells were collected and lysed in RIPA buffer (Meilunbio) containing a protease and phosphatase inhibitor cocktail (Yeasen) at 4 °C. After sonication, the supernatant of cell lysate was collected by centrifugation at 12,000 rpm at 4 °C for 15 min and then quantified by the bicinchoninic acid (BCA) protein assay kit (Yeasen). 25 μg of protein from each sample was separated by using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (10 or 12%) and transferred to poly(vinylidene difluoride) (PVDF) membranes by wet transfer. After being blocked with 5% skimmed milk at room temperature for 2 h, the PVDF membranes were incubated with primary antibodies against Bcl-2 (1:1000, D55G8, #4223, CST), Bax (1:1000, D2E11, #5023, CST), cleaved caspase-3 (1:1000, 5A1E, #9664, CST), cleaved caspase-9 (1:1000, D2D4 #7237, CST), p62 (1:1000, D5E2, #8025, CST), Beclin-1 (1:1000, D40C5, #3495, CST), LC3B (1:1000, E7X4S, #43566, CST), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (1:200000, D16H11, #5174, CST) overnight at 4 °C. After washing three times with 1 × phosphate buffer saline with 0.1% Tween-20 (PBST), the membranes were further incubated with the secondary antibody conjugated with horseradish peroxidase (1:3000, #7074, CST) at room temperature for 60 min. Finally, the membranes were incubated in an enhanced chemiluminiscent (ECL) working solution (Yeasen) for several minutes and imaged by using the visualization instrument Tanon-5200 (Tanon, People's Republic of China).

RNA-seq. MGC 803 cells were inoculated in a medium dish (4 mL/well) at a density of 1×10^5 cells /mL for 24 h. Afterward, MGC 803 cells were replaced with fresh medium with or without compound **42** (20 μM) for another 24 h. After treatment, the cells were washed twice with cold 1 × PBS, and then total RNA was isolated from MGC 803 cells by using TRIzol Reagent. RNA samples were sequenced by Shanghai Majorbio Biotechnology Co., Ltd. In brief, messenger RNA was enriched by oligo (dT) beads from the total RNA. Second, the enriched mRNA was fragmented and reversed into complementary DNA (cDNA). The synthesized cDNA was then subjected to end-repair and 'A' base addition. Finally, paired-end RNA-seq was sequenced using Illumina HiSeq xten/NovaSeq. 6000 sequencing platform. Sequencing data were analyzed by using Meggie biocloud platform.

Statistical Analysis. All of the results were presented as the mean ± standard deviation (SD), and differences among more than two groups were analyzed by one-way analysis of variance (ANOVA) with Dunnett or Tukey test using GraphPad 8.0 software (La Jolla, CA). $P < 0.05$ was considered to indicate a statistically significant difference.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c01319.

Experimental procedures for the preparation of **18**−**42**, ^{1}H NMR and ^{13}C NMR spectra for all compounds, and list of abbreviations (PDF)

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Notes

The authors declare no competing financial interest.

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