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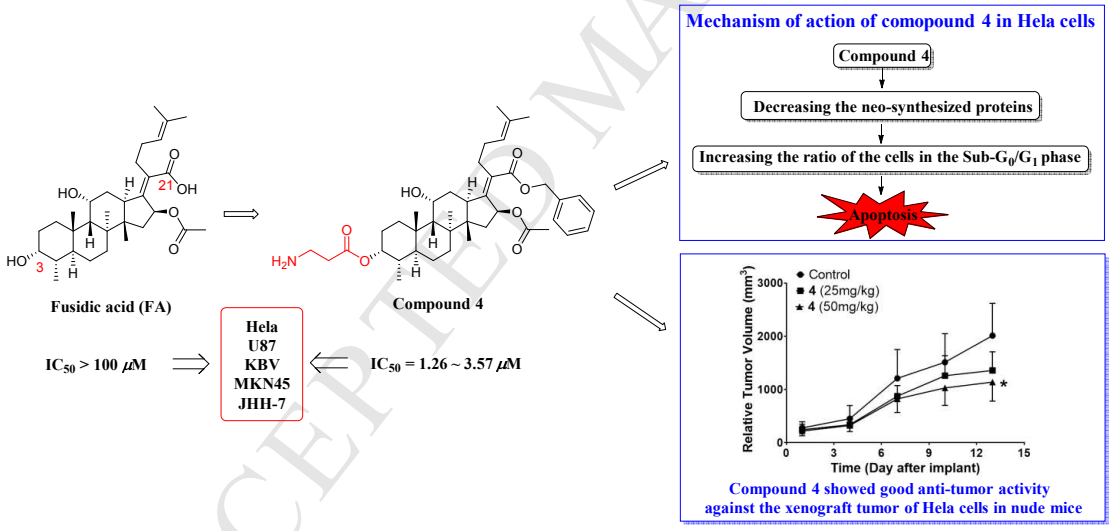
#Jingxuan Ni and Mengqi Guo have equally contributed to the work.

Graphical abstract



Discovery, synthesis of novel fusidic acid derivatives possessed amino-terminal groups at the 3-hydroxyl position with anticancer activity

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ABSTRACT: A series of novel fusidic acid (FA) derivatives were synthesized and screened for their *in vitro* cytotoxicity against the Hela, U87, KBV and MKN45 cancer cell lines. Selected FA derivatives with anti-tumor activity were firstly identified including compound **4**, which exhibited good anti-proliferative activity with IC_{50} values in the range of 1.26-3.57 μM. Further research revealed that compound **4** induced Hela cells to undergo apoptosis by increasing the ratio of the cells in the Sub-G_0/G_1 phase via decreasing the neo-synthesized proteins in a dose-dependent manner from 1-10 μM. Compound **4** also showed good *in vivo* anti-tumor activity against the xenograft tumor of Hela cells and had no apparent toxicity. This study highlights the advantage of introducing the medium-length amino-terminal groups at the 3-OH position of FA to enhance its anti-tumor activity and suggests that compound **4** provides a starting point for designing more potent derivatives in the future.

Key words: *Fusidic acid, derivatives, synthesis, anti-tumor activity, apoptosis*

INTRODUCTION

Cancer is a particularly complex, widespread and fatal disease. There were 14.1 million new cancer cases, 8.2 million cancer deaths and 32.6 million cancer patients (within 5 years after diagnosis) worldwide in 2012, and cancer deaths are expected to continue rising with an estimated 13.1 million deaths in 2030 [1,2]. Thus, cancer has become a

major cause of mortality around the world. Several lines of evidence support the view that chemotherapy has become one of the most important treatments for cancer [3-5]. Unfortunately, most anticancer drugs have serious side effects making them less than ideal for the long term treatment of cancer [6]. Therefore, the identification of new agents that are cytotoxic to cancer cells without inducing serious side effects is critical for the research and development of new anti-tumor drugs.

Fusidic acid (FA) is a steroid-based, narrow spectrum bacteriostatic antibiotic with a tetracyclic ring system [7]. It was first isolated from the fungus *Fusidium coccineum* in 1960 and has been in clinical use since 1962 for the treatment of skin, bone and joint infections caused by *Staphylococcus aureus* as well as against several other Gram-positive species [8-11]. Additionally, as FA is a marketed drug, it has been extensively studied and is known not to be cytotoxic. Despite the extensive research on FA, no FA derivatives have appeared on the market, and there have not been any reports in the literature that FA derivatives are cytotoxic.

In our group, we are committed to finding noncytotoxic FA derivatives with better antibacterial activity compared with FA. Antibacterial screening for synthesized derivatives in the Community for Open Antimicrobial Drug Discovery (http://www.co-add.org/), including antibacterial and cytotoxicity assay, was carried out to obtain derivatives with better antibacterial activity and lower cytotoxicity compared with FA. However, the results of the antibacterial screening showed that FA derivatives **1** and **10** did not possess the expected antibacterial activity but instead were cytotoxic. Compared with FA that had inhibitory activity against *S. aureus* with a MIC $\leq$ 0.25 μg/mL and no cytotoxicity against the human embryonic kidney cell line HEK293 with a CC_{50} > 32 μg/mL, compound **1** had no inhibitory activity against *S. aureus* with a MIC > 32 μg/mL and cytotoxicity against HEK293 with a CC_{50} = 10.8 μg/mL. Similarly, compound **10** had weak inhibitory activity against *S. aureus* with a MIC = 32 μg/mL and cytotoxicity against HEK293 with a CC_{50} = 20.6 μg/mL. According to the results of the antibacterial screening, we speculated that introduction of a benzyl group at the 21-COOH site and amino-terminal groups or carboxyl-terminal groups at the 3-OH site led to the cytotoxicities of compound **1** and **10**. Therefore, FA derivatives with similar structures to compound **1** or **10** were designed and synthesized to verify whether the introduction of similar structures led to the cytotoxicity of the FA derivatives, and to further explore the mechanism of the cytotoxicity of the FA derivatives.

In this work, a series of novel FA derivatives were synthesized, their cytotoxicities *in vitro* and anti-tumor activities

in vivo were evaluated and the mechanism of their anti-tumor activity was explored. If novel FA derivatives with anti-tumor activity and a defined mechanism of action can be obtained, it will further support the design and synthesis of novel FA derivatives with better anti-tumor activity.

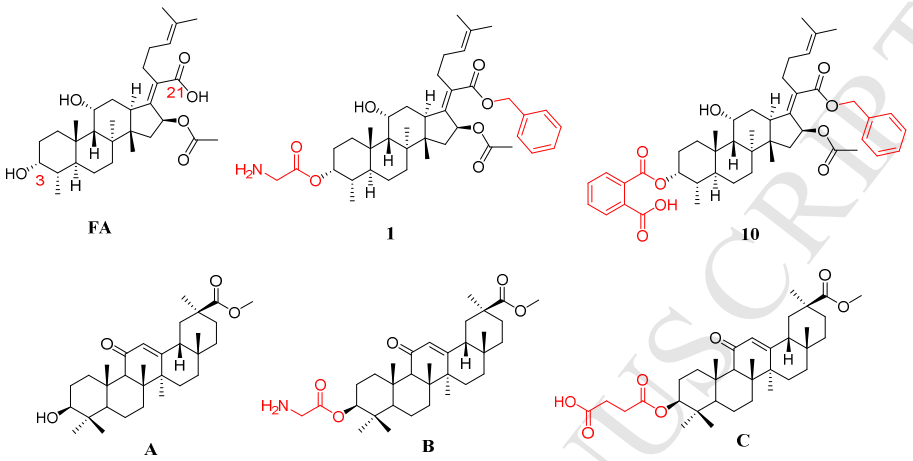


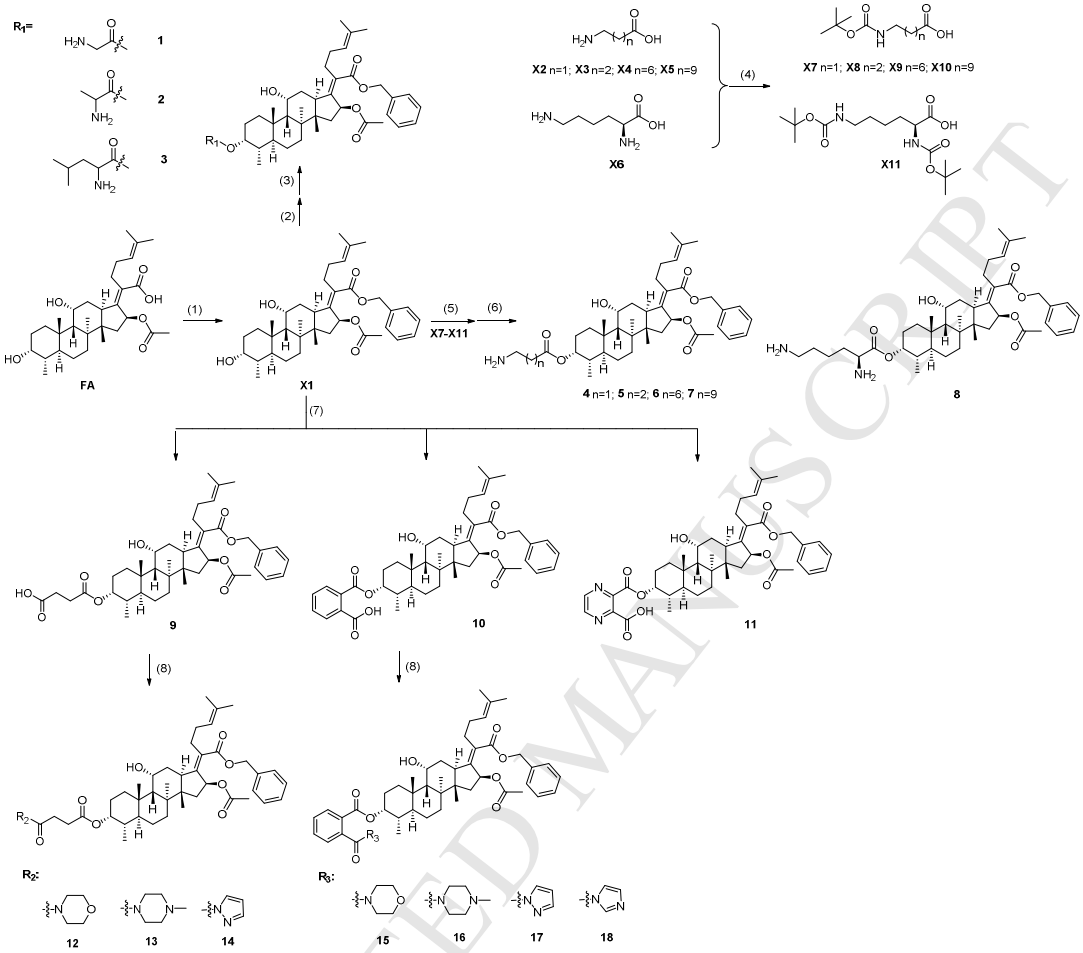
Figure 1. Structures of **FA**, **1**, **10**, glycyrrhetinic acid (GA) derivatives **A**, **B** and **C**.

RESULTS AND DISCUSSION

Synthetic chemistry

FA derivatives **1-18** were synthesized as shown in Scheme 1. First, commercially available FA was coupled with benzyl bromide in acetone in the presence of an appropriate amount of K_2CO_3 to obtain the intermediate **X1**. Three different types of derivatives can be synthesized using **X1** as a raw material according to the following methods: (1) Treatment of **X1** with different *t*-butyloxy carbonyl (boc)-protected amino acids in anhydrous CH_2Cl_2 in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) and a catalytic amount of dimethylaminopyridine (DMAP) produced intermediates. Next, intermediates were deprotected under acidic conditions to produce compounds **1-3**; (2) The amino-terminal acids **X2-X6** with different chain lengths were reacted with *di-tert*-butyl dicarbonate (boc anhydride) in *tert*-butyl alcohol (TBA) in the presence of sodium hydroxide solution to obtain the amino-protected acids **X7-X11**, respectively. Then, treatment of **X1** with **X7-X11** in anhydrous CH_2Cl_2 in the presence of EDCI and DMAP produced the corresponding intermediates. Next, the intermediates were deprotected with trifluoroacetic acid (TFA) to produce compounds **4-8**; (3) Treatment of **X1** with different anhydrides in anhydrous CH_2Cl_2 in the presence of DMAP produced compounds **9-11**. Next, **9** or **10** was reacted with different nitrogen-containing heterocyclic groups in anhydrous CH_2Cl_2 in the presence of EDCI produced compounds **12-18**.

Scheme 1. Synthesis of FA derivatives 1-18[a]



[a]Reagent and conditions: (1) BrBn, K_2CO_3, acetone, 30 °C; (2) Boc-amino acids, DMAP, EDCI, CH_2Cl_2, r.t.; (3) Hydrochloric acid, ethyl acetate, r.t.; (4) Boc anhydride, NaOH/H_2O, TBA, 0 °C-r.t.; (5) DMAP, EDCI, CH_2Cl_2, r.t.; (6) TFA, CH_2Cl_2, 0 °C-r.t.; (7) Anhydrides, DMAP, CH_2Cl_2, r.t.; (8) Nitrogen-containing heterocyclic groups, EDCI, CH_2Cl_2, r.t.

Cell growth inhibition

The anti-proliferative activity of all the synthesized compounds at 5 μM and 1.5 μM was determined by an MTT assay in four cancer cell lines: Hela (cervical cancer), KBV (multidrug-resistant oral epidermoid carcinoma), U87 (glioma) and MKN45 (gastric cancer). The results are summarized in Table 1. FA showed no significant growth inhibition effects against the Hela, KBV, U87 and MKN45 cell lines. Some of its derivatives displayed good growth inhibition effects at 5 μM and even several derivatives showed good effects at 1.5 μM. In the assay of cell growth inhibition at 5 μM, compounds **4**-**8** showed good anti-proliferative activity against four cancer cell lines that yielded survival rates of not more than 23%. Compound **2** showed good growth inhibition effects against Hela, KBV, U87 cell lines (cell viability $\leq$ 33%) and it had no effect against MKN45 cell lines. Compound **1** showed good growth

inhibition effect against KBV (cell viability was 28%) and moderate effect against U87 (cell viability was 41%). Compound **3** showed good growth inhibition effect only against Hela (cell viability was 22%). Compound **13** showed moderate effect against Hela (cell viability was 47%). Other compounds showed no significant growth inhibition effects against Hela, KBV, U87 and MKN45 cell lines. In the assay of cell growth inhibition at 1.5 μM, compound **4** still exhibited good growth inhibition effects against Hela, KBV, U87 and MKN45 cell lines (cell viability were 32%, 14%, 10% and 23%, respectively). However, at a concentration of 1.5 μM, compound **5** exhibited good growth inhibition effect only against U87 (cell viability was 10%), compound **6** exhibited good effect only against MKN45 (cell viability was 16%) and compound **7** exhibited moderate growth inhibition effects only against U87 (cell viability was 49%). Other compounds showed no significant growth inhibition effects against the Hela, KBV, U87 and MKN45 cell lines. In previous antibacterial screening, compounds **1** and **10** had cytotoxicity against HEK293 with a CC_{50} = 10.8 μg/mL and 20.6 μg/mL, respectively. However, in the MTT assay at a concentration of 5 μM, compound **1** showed a growth inhibition effect against KBV and U87 cells but had no effect against Hela and MKN45 cells, while compound **10** showed no significant growth inhibition effects against the Hela, KBV, U87 and MKN45 cell lines. Thus, we infered that differences in the compound concentrations and cell lines led to the different cytotoxicity results.

The results of the MTT assay showed that derivatives with amino-terminal groups at the 3-OH site had better anti-tumor activity compared with derivatives with carboxyl-terminal groups. And S. Schwarz et al reported that the glycine substituted glycyrrhetinic acid (GA) derivative **B** had better anti-tumor activity and the hydrogen succinate **C** had less anti-tumor activity compared with GA derivative **A** (Figure 1) [12,13]. The results of the above studies were consistent, then a preliminary structure-activity relationship was developed that derivatives with amino-terminal groups introduced at the 3-OH site had better anti-proliferative activity than derivatives with carboxyl-terminal groups at the 3-OH site. In addition, carbon chains inserted between the amino and carboxyl groups that were too short induced a decrease of anti-proliferative activity against the various cancer cell lines. 3-Glycine derivatives without a branched-chain or with a short branched-chain showed better anti-proliferative activity than 3-glycine derivatives with a long branched-chain. Derivatives with carboxyl-terminal groups or nitrogen-containing heterocyclic groups introduced at the 3-OH site had no significant anti-proliferative activity, but introduction of fat chains and saturated nitrogen-containing heterocyclic groups might favor anti-proliferative activity.

Compound **4** showed the best anti-proliferative activity against all four cancer cell lines among the eighteen FA

derivatives. Therefore, we further extended our characterization of the anti-proliferative activity of **4** in five cancer cell lines: Hela, U87, JHH-7 (liver cancer), KBV and MKN45. The IC_{50} (50% growth inhibition) values for **4** are shown in Table 2. Compound **4** displayed good anti-proliferative activity in all the tested cell lines with IC_{50} values of 1.26 μM-3.57 μM. Thus compound **4** has an effective growth inhibitory effect on a variety of tumor cells, and we further explored its possible mechanism of action and anti-tumor activity *in vivo*.

Table 1. ***In vitro*** **cytotoxicities of FA and its derivatives against Hela, KBV, U87 and MKN45 at 5 μM and 1.5 μM[a]**

Cpd.	Cell viability							
	Hela		KBV		U87		MKN45	
	5 μM	1.5 μM	5 μM	1.5 μM	5 μM	1.5 μM	5 μM	1.5 μM
FA	73%±2%	70%±2%	67%±2%	71%±4%	87%±2%	92%±2%	83%±2%	80%±3%
1	96% ± 5%	100% ± 1%	**28**% ± 9%	97% ± 1%	41% ± 7%	94% ± 3%	93% ± 1%	95% ± 1%
2	**23**% ± 10%	102% ± 5%	**15**% ± 2%	95% ± 3%	**33**% ± 8%	95% ± 2%	91% ± 1%	96% ± 2%
3	**22**% ± 1%	99% ± 1%	83% ± 2%	96% ± 1%	111% ± 3%	106% ± 4%	92% ± 2%	95% ± 1%
4	**13**% ± 9%	**32**% ± 14%	**12**% ± 8%	**14**% ± 6%	**9**% ± 1%	**10**% ± 0%	**5**% ± 0%	**23**% ± 2%
5	**23**% ± 16%	101% ± 6%	**13**% ± 9%	98% ± 4%	**9**% ± 1%	**10**% ± 0%	**6**% ± 1%	90% ± 1%
6	**19**% ± 4%	99% ± 2%	**11**% ± 6%	83% ± 8%	**10**% ± 0%	116% ± 4%	**4**% ± 0%	**16**% ± 2%
7	**19**% ± 17%	100% ± 4%	**9**% ± 3%	93% ± 5%	**10**% ± 0%	49% ± 9%	**5**% ± 0%	96% ± 1%
8	**8**% ± 1%	101% ± 8%	**18**% ± 16%	99% ± 7%	**9**% ± 1%	105% ± 1%	**8**% ± 2%	91% ± 1%.
9	69%±2%	83%±18%	66%±5%	72%±5%	84%±2%	86%±2%	76%±4%	74%±2%
10	68%±2%	89%±18%	81%±11%	78%±9%	85%±13%	81%±2%	76%±1%	78%±3%
11	69%±1%	76%±7%	108%±26%	86%±5%	90%±8%	88%±3%	87%±4%	95%±13%
12	64%±6%	76%±4%	66%±8%	78%±6%	93%±11%	91%±5%	78%±3%	82%±3%
13	47%±2%	70%±2%	71%±12%	82%±5%	88%±7%	87%±6%	75%±6%	81%±5%
14	71%±2%	75%±0%	80%±6%	77%±1%	83%±2%	87%±3%	83%±12%	88%±6%	
15	60%±5%	76%±7%	66%±12%	81%±2%	77%±2%	86%±3%	92%±9%	104%±14%
16	73%±2%	83%±5%	75%±3%	89%±9%	77%±3%	86%±5%	99%±13%	104%±9%
17	82%±3%	90%±2%	79%±2%	85%±3%	87%±3%	90%±0%	96%±4%	101%±3%

18	63%±2%	84%±4%	69%±6%	81%±4%	77%±1%	85%±6%	92%±5%	103%±8%

[a]The cell viabilities are presented as the mean ± SD of three independent experiments.

Table 2. The IC_{50} values of compound 4 against a panel of cancer cell lines

Cancer cell lines	IC_{50} (μM)	
	4	**FA**
Hela	3.57 ± 1.27	> 100
U87	2.40 ± 0.87	> 100
JHH-7	3.18 ± 1.16	> 100
KBV	1.26 ± 0.23	> 100
MKN45	1.28 ± 0.25	> 100

*Compound **4** induced the Hela cells to undergo cell apoptosis*

To explore the effect of compound **4** on the cell apoptosis, a TUNEL staining assay and flow cytometry were conducted. As shown in Figure 2, compound **4** at 2 μM, but not the parental FA, could induced the Hela cells to undergo apoptosis after 24 hours incubation as indicated by the green fluorescence cells. Similar results could be observed in the flow cytometry assay, in which compound **4** at 5 μM, but not the parental FA, could significantly increase the ratio of the cells in the Sub-G_0/G_1 phase after 48 h or 72 h incubation (Figure 3).

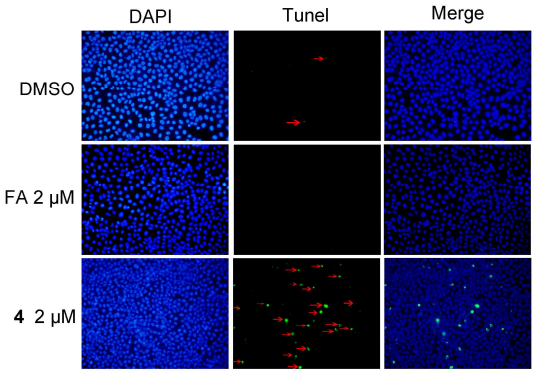


Figure 2. Effects of compound **4** on the early stage of cell apoptosis in Hela cells. Cells were seeded into 12-well plates with cover slips and treated with compound **4** at 2 μM for 24 h. Then cells were subjected to the TUNEL staining assay to determine the early stage of cell apoptosis. The red arrows indicate the TUNEL-positive cells.

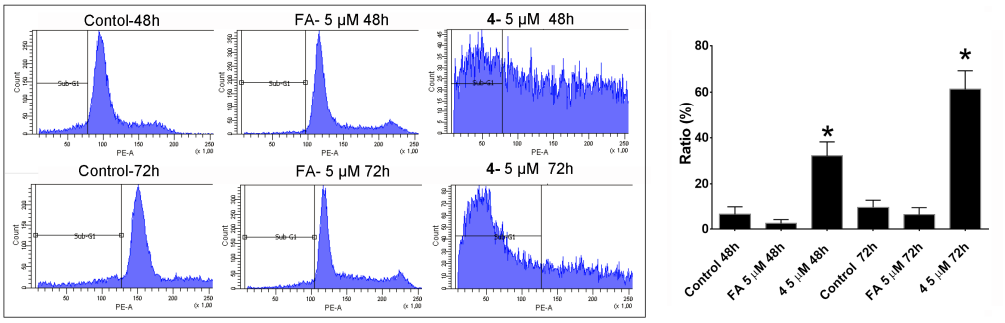


Figure 3. Effects of compound **4** on the late stage of cell apoptosis in Hela cells. Cells were seeded into 6-well plates and treated with compound **4** at 5 μM for 48 h and 72 h. Cells were then subjected to flow cytometry to determine the distribution ratio of sub-G_0/G_1. *: $p<0.05$, compared with the control group.

*Compound **4** inhibited protein synthesis*

As puromycin can be incorporated into neo-synthesized proteins, the anti-puromycin antibody was used to detect the effect of compound **4** on the neo-synthesized proteins. As shown in Figure 4, compound **4** at 5 μM, but not the parental FA, decreased the amount of puromycin-incorporated neo-synthesized proteins, which indicated that compound **4** could inhibit protein synthesis. Consistently, compound **4** decreased the neo-synthesized proteins in a dose-dependent manner in Hela cells from 1-10 μM.

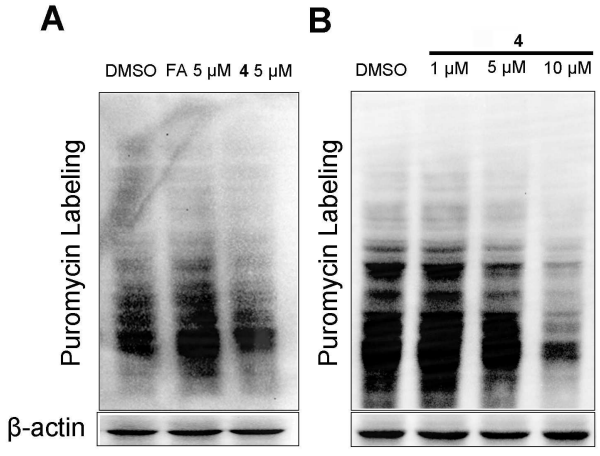


Figure 4. Effects of compound **4** on protein synthesis in Hela cells. Cells were seeded into plates and treated with compound **4** at the concentration indicated for 2 h, and then the cells were incubated with puromycin for 10 min. After washing, cells were lysed, and the puromycin-incorporated neo-synthesized proteins were detected.

*Compound **4** showed good anti-tumor activity against the xenograft tumor of Hela cells in vivo*

A Hela cell xenograft model was established using female nude mice to explore the effect of compound **4** on the anti-tumor activity *in vivo*. Considering that the half-life of FA is short, we adopted a twice daily dosing regimen. The results showed that compound **4** had satisfactory anti-tumor activity in this xenograft tumor model (Figure 5). Compared with the control, the anti-tumor activity of compound **4** was obvious and the compound had no apparent toxicity. We determined anti-tumor activity of compound **4** was dose-dependent and its toxicity was not increased.

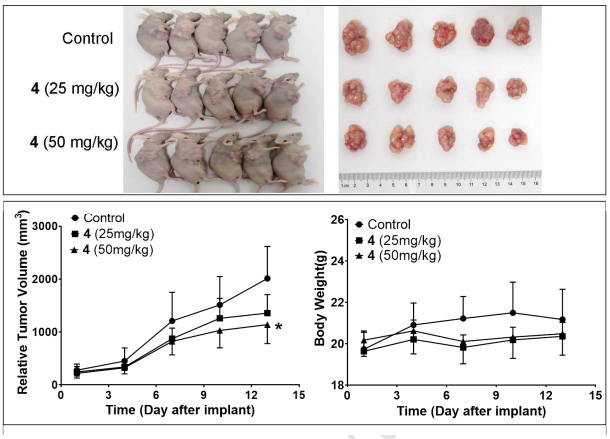


Figure 5. Effects of compound **4** on the anti-tumor activity of Hela xenograft tumors in nude mice. Mice were inoculated with Hela cells and treated with at the indicated concentrations at a dose of 25 mg/kg and 50 mg/kg. At the end of treatment, the mice were euthanized and the tumors were removed and weighed. All data are expressed as the mean ± SD (n = 5). *$p < 0.05$, compared with the control group.

Table 3. Inhibitory effects of 4 on the xenograft tumor growth of Hela in nude mice[a]

Groups	Dosage (mg/kg)	Number Initial/end	Body weight (g) Initial	Body weight (g) End	Tumor weight (g)	IR (%)
Control	0	5/5	19.7 ± 0.8	21.2 ± 1.5	1.85 ± 0.36	
4	25	5/5	19.6 ± 1.0	20.4 ± 0.7	1.19 ± 0.17*	35.4
4	50	5/5	20.2 ± 0.8	20.5 ± 1.0	1.01 ± 0.32*	45.5

[a]Data are expressed as the mean ± SD (n = 5). *$P < 0.05$, compared with control.

CONCLUSION

A series of novel FA derivatives were synthesis. All the synthesized derivatives were screened for anti-proliferative activity in Hela, KBV, U87 and MKN45 cell lines using the MTT assay. This study demonstrates that introducing amino acids and amino-terminal groups at the 3-OH position of FA generated compounds that exhibited good anti-proliferative activity, but introducing carboxyl-terminal groups or nitrogen-containing heterocyclic groups had no

obvious effect on the anti-proliferative activity. We found that compound **4** had the best anti-proliferative activity among those tested. Additionally, compound **4** induced the Hela cells to undergo apoptosis by increasing the ratio of cells in the Sub-G_0/G_1 phase as shown by the flow cytometry and TUNEL assay experiments. Compound **4** was also shown to decrease the neo-synthesized proteins in a dose-dependent manner in Hela cells from 1-10 μM. Moreover, compound **4** shows good anti-tumor activity against a xenograft tumor of Hela cells *in vivo*. Based on these results, work is ongoing to design and synthesize novel FA derivatives with the introduction of medium-length amino-terminal groups to obtain derivatives with better anti-tumor activity.

EXPERIMENTAL SECTION

Chemistry

The chemicals and reagents were analytically pure or dried with standard methods when necessary. The progress of all reactions were monitored by TLC on silica gel HSGF254 using fluorescence with a wavelength of 254 nm on a ZF7-C three-purpose UV analyzer and 10% ethanol sulfate solution for detection of the spots. All the synthesized compounds were purified by column chromatography on silica gel. All NMR spectra (^{1}H and ^{13}C NMR) were routinely measured using Bruker av400 or JNM-ECZS 400 instruments using $CDCl_3$ as solvent with TMS as the internal standard. Chemical shifts are expressed in δ values (ppm) and the coupling constants (*J*) in Hertz. High resolution mass spectra (HRMS) were recorded on Agilent QTOF 6520 or 6530 spectrometer. Melting points of compounds were recorded on Micro melting point apparatus XT3A or SGW®X-4.

General procedure for the synthesis of 1-3

Synthetic method of X1

FA (10.0 g, 19.0 mmol) was dissolved in acetone (200.0 mL), potassium carbonate (5.4 g, 39.0 mmol) and benzyl bromide (2.8 mL, 23.0 mmol) were added with stirring, and reacted at 30 °C for 6 hours. It was filtered, concentrated, diluted with ethyl acetate (50.0 mL), washed successively with 10% hydrochloric acid, water and saturated brine, dried over anhydrous sodium sulfate, filtered, and the solvent was distilled off under reduced pressure. The residue was purified by silica gel column chromatography to provide white solid **X1**.

Synthetic method of intermediates a-c

X1 (0.1 mmol) was dissolved in anhydrous dichloromethane (10.0 mL) and *tert*-butyloxy carbonyl protected amino acids (0.2 mmol), DMAP (0.3 mmol) and EDCI (0.3 mmol) were added with stirring, reacted at room temperature for

4 hours. It was washed successively with 10% hydrochloric acid, water and saturated brine, dried over anhydrous sodium sulfate, filtered, and the solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography ($V_{chloroform}$: $V_{methanol}$ = 300:1-200:1) to give intermediates **a**-**c**, respectively.

3β-[(2-amino)acetoxy]-21-fusidic acid (benzyl) ester (**1**)

a (45.0 mg, 59.0 μmol) was dissolved in ethyl acetate (10.0 mL) and 37% hydrochloric acid was added dropwise, reacted at room temperature for 5 hours. It was washed successively with saturated sodium bicarbonate solution, water and saturated brine, dried over anhydrous sodium sulfate, filtered, and the solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography ($V_{chloroform}$: $V_{methanol}$ = 60:1) to give white solid **1** (31.0 mg, 80.3% yield). m.p. 237-239 °C. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.31-7.36 (m, 5H, 5×Ar-H), 5.89 (d, J = 8.20 Hz, 1H, 16-H), 5.22 (dd, J = 5.17, 12.14 Hz, 1H, CHAr), 5.06 (t, J = 6.91 Hz, 1H, 24-H), 4.99 (d, J = 2.11 Hz, 1H, 11-OH), 4.92 (dd, J = 4.38, 12.18 Hz, 1H, CHAr), 4.65 (d, J = 23.65 Hz, 2H, -NH_2), 4.31 (s, 1H, 11-H), 3.47 (s, 1H, 3-H), 3.02 (d, J = 11.43 Hz, 1H, 13-H), 2.38-2.48 (m, 2H, 2×22-H), 2.23-2.30 (m, 1H, 12-H), 2.01-2.20 (m, 5H, 1-H, 5-H, 15-H and 2×23-H), 1.93 (s, 3H, $OCOCH_3$), 1.68-1.88 (m, 4H, 2×2-H, 7-H and 12-H), 1.63 (s, 3H, 27-CH_3), 1.54-1.60 (m, 3H, 1-H, 6-H and 9-H), 1.52 (s, 3H, 26-CH_3), 1.40-1.48 (m, 1H, 4-H), 1.35 (s, 3H, 30-CH_3), 1.25-1.32 (m, 1H, 15-H), 1.04-1.18 (m, 2H, 6-H and 7-H), 0.98 (s, 3H, 19-CH_3), 0.91 (s, 3H, 18-CH_3), 0.82 (d, J = 6.68 Hz, 3H, 28-CH_3). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 175.20, 171.97, 171.39, 149.93, 137.19, 134.03, 131.88, 130.03, 129.97, 129.74, 124.49, 111.68, 76.60, 75.86, 69.55, 67.88, 50.48, 50.16, 45.39, 40.87, 40.47, 39.11, 38.81, 38.30, 37.34, 36.36, 33.98, 32.34, 31.17, 30.47, 29.82, 28.80, 27.20, 25.75, 24.13, 22.43, 22.07, 19.43, 19.20, 17.10. HR-MS (ESI) *m/z*: calcd. for $C_{40}H_{57}NO_7$ $[M+H]^+$: 664.4208, found: 664.4221.

3β-[(2-amino)propionyloxy]-21-fusidic acid (benzyl) ester (**2**)

According to the synthesis method of **1**, **b** was reacted with 37% hydrochloric acid to give white solid 2 (46.0 mg, 86.7% yield). m.p. 232-235 °C. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.29-7.35 (m, 5H, 5×Ar-H), 5.88 (d, J = 8.27 Hz, 1H, 16-H), 5.20 (d, J = 12.18 Hz, 1H, CHAr), 5.05 (t, J = 7.16 Hz, 1H, 24-H), 4.91 (d, J = 2.46 Hz, 1H, 11-OH), 4.92 (d, J = 12.14 Hz, 1H, CHAr), 4.64 (d, J = 24.20 Hz, 2H, -NH_2), 4.31 (s, 1H, 11-H), 3.59 (q, J = 6.38 Hz, 1H, -CH-), 3.01 (d, J = 11.13 Hz, 1H, 13-H), 2.38-2.49 (m, 2H, 2×22-H), 2.24-2.28 (m, 1H, 12-H), 2.05-2.19 (m, 5H, 1-H, 5-H, 15-H and 2×23-H), 1.92 (s, 3H, $OCOCH_3$), 1.67-1.89 (m, 4H, 2×2-H, 7-H and 12-H), 1.62 (s, 3H, 27-CH_3), 1.54-1.60 (m, 3H, 1-H, 6-H and 9-H), 1.51 (s, 3H, 26-CH_3), 1.47 (s, 1H, 4-H), 1.36 (s, 3H, -CH_3), 1.35 (s, 3H, 30-CH_3), 1.24-1.31 (m, 1H,

15-H), 1.09-1.17 (m, 2H, 6-H and 7-H), 0.97 (s, 3H, 19-CH_3), 0.91 (s, 3H, 18-CH_3), 0.83 (d, J = 6.69 Hz, 3H, 28-CH_3). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 177.50, 171.97, 171.38, 149.90, 137.20, 134.06, 132.07, 131.92, 130.04, 129.98, 129.75, 124.47, 111.69, 76.58, 75.85, 69.62, 67.90, 51.65, 50.44, 50.21, 45.39, 40.92, 40.48, 39.18, 38.33, 37.38, 36.29, 34.07, 32.31, 31.18, 30.47, 29.81, 28.67, 27.20, 25.74, 24.12, 22.44, 22.08, 19.42, 19.21, 17.19. HR-MS (ESI) *m/z*: calcd. for $C_{41}H_{59}NO_7$ $[M+H]^+$: 678.4364, found: 678.4376.

3β-[(2-amino-4-methyl)pentanoyloxy]-21-fusidic acid (benzyl) ester (**3**)

According to the synthesis method of **1**, **c** was reacted with 37% hydrochloric acid to give white solid **3** (28.0 mg, 79.4% yield). m.p. 248-250 °C. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.30-7.36 (m, 5H, 5×Ar-H), 5.88 (d, J = 8.26 Hz, 1H, 16-H), 5.20 (d, J = 12.21 Hz, 1H, CHAr), 5.06 (t, J = 6.94 Hz, 1H, 24-H), 4.91-4.94 (m, 2H, CHAr and 11-OH), 4.64 (d, J = 23.61 Hz, 2H, -NH_2), 4.31 (s, 1H, 11-H), 3.49 (t, J = 7.43 Hz, 1H, -CH-), 3.02 (d, J = 11.58 Hz, 1H, 13-H), 2.38-2.52 (m, 2H, 2×22-H), 2.26-2.29 (m, 1H, 12-H), 2.07-2.20 (m, 3H, 15-H and 2×23-H), 1.96-2.04 (m, 2H, 1-H and 5-H), 1.92 (s, 3H, $OCOCH_3$), 1.68-1.88 (m, 4H, 2×2-H, 7-H and 12-H), 1.63 (s, 3H, 27-CH_3), 1.54-1.61 (m, 3H, 1-H, 6-H and 9-H), 1.52 (s, 3H, 26-CH_3), 1.38-1.46 (m, 1H, 4-H), 1.36 (s, 3H, 30-CH_3), 1.25-1.33 (m, 1H, 15-H), 1.03-1.17 (m, 2H, 6-H and 7-H), 0.98 (s, 3H, 19-CH_3), 0.94 (s, 3H, -CH_3), 0.93 (d, J = 1.78 Hz, 3H, 18-CH_3), 0.91 (s, 3H, -CH_3), 0.83 (d, J = 6.66 Hz, 3H, 28-CH_3). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 177.58, 171.94, 171.36, 149.95, 137.21, 134.01, 131.88, 130.02, 129.94, 129.72, 124.49, 111.68, 76.60, 75.85, 69.55, 67.86, 54.45, 50.49, 50.19, 46.06, 45.39, 40.92, 40.50, 39.07, 38.30, 37.38, 36.30, 34.02, 32.29, 31.17, 30.46, 29.82, 28.61, 27.19, 26.47, 25.62, 24.44, 24.17, 23.54, 22.43, 22.06, 19.43, 19.20, 17.23. HR-MS (ESI) *m/z*: calcd. for $C_{44}H_{65}NO_7$ $[M+H]^+$: 720.4834, found: 720.4849.

General procedure for the synthesis of 4-8

*Synthetic method of **X7-X11***

Aqueous sodium hydroxide (sodium hydroxide: 170.0 mg, 4.3 mmol; water: 2.0 mL) and *di-tert*-butyl dicarbonate were dissolved in *tert*-butyl alcohol and **X2-X6** (3.8 mmol) were added with stirring respectively, reacted at room temperature for 18-24 hours. It was diluted with water and 1.0 mol/L hydrochloric acid, extracted with ethyl acetate rapidly, washed successively with water and saturated brine, dried over anhydrous sodium sulfate, filtered, and the solvent was evaporated under reduced pressure. Then it gave intermediates **X7**-**X11**, respectively.

*Synthetic method of intermediates **d-h***

X1 (0.1 mmol) was dissolved in anhydrous dichloromethane (8.0 mL) and compounds **X10**-**X14** (0.2 mmol), DMAP

(0.3 mmol) and EDCI (0.3 mmol) were added with stirring respectively, reacted at room temperature for 20-24 hours. It was filtered, concentrated, diluted with ethyl acetate (10.0 mL), washed successively with water and saturated brine, dried over anhydrous sodium sulfate, filtered, and the solvent was distilled off under reduced pressure. The residue was purified by silica gel column chromatography ($V_{petroleum\ ether}$: $V_{ethyl\ acetate}$ = 20:1-8:1) to give intermediates **d**-**h**, respectively.

3β-(4-aminopropionyloxy)-21-fusidic acid (benzyl) ester (**4**)

d (35.0 mg, 45.0 μmol) was dissolved in anhydrous dichloromethane (10.0 mL) and trifluoroacetic acid (0.9 ml) was added at 0 °C, reacted at room temperature for 3 hours. The solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography ($V_{dichloromethane}$: $V_{methanol}$ = 100:1-50:1) to give white solid **4** (26.0 mg, 85.0% yield). m.p. 207-210 °C. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.31-7.34 (m, 5H, 5×Ar-H), 5.89 (d, J = 8.24 Hz, 1H, 16-H), 5.20 (d, J = 12.11 Hz, 1H, CHAr), 4.94 (d, J = 12.08 Hz, 1H, CHAr), 4.33 (s, 1H, 11-H), 3.30 (s, 1H, 3-H), 3.01 (d, J = 11.51 Hz, 1H, 13-H), 4.47 (m, 2H, -CH_2-), 4.33 (s, 1H, 11-H), 4.21-4.24 (m, 4H, 2×-CH_2-), 2.80 (t, J = 7.12 Hz, 2H, -CH_2-), 2.31-2.42 (m, 3H, 12-H and 2×22-H), 2.13-2.23 (m, 3H, 15-H and 2×23-H), 2.00-2.09 (m, 2H, 1-H and 5-H), 1.93 (s, 3H, $OCOCH_3$), 1.66-1.87 (m, 3H, 2×2-H, 7-H and 12-H), 1.51-1.62 (m, 4H, 1-H, 4-H, 6-H and 9-H), 1.47 (s, 3H, 27-CH_3), 1.43 (s, 3H, 26-CH_3), 1.32 (s, 3H, 30-CH_3), 1.26-1.27 (m, 1H, 15-H), 1.07-1.16 (m, 2H, 6-H and 7-H), 0.97 (s, 3H, 19-CH_3), 0.90 (s, 3H, 18-CH_3), 0.80 (d, J = 6.39 Hz, 3H, 28-CH_3). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 170.88, 170.09, 169.27, 148.87, 135.13, 129.31, 128.09, 128.01, 127.93, 127.86, 88.59, 75.88, 73.82, 67.44, 65.90, 48.40, 48.21, 45.55, 43.43, 39.37, 38.90, 38.40, 36.90, 36.08, 35.35, 34.42, 31.51, 30.41, 30.04, 28.37, 26.43, 25.14, 24.68, 23.90, 23.34, 22.26, 20.44, 20.17, 17.26, 14.97, 7.95. HR-MS (ESI) *m/z*: calcd. for $C_{41}H_{59}NO_7$ $[M+H]^+$: 678.4364, found: 678.4348.

3β-(4-aminobutyryloxy)-21-fusidic acid (benzyl) ester (**5**)

According to the synthesis method of **4**, **e** was reacted with trifluoroacetic acid to give white solid 5 (29.0 mg, 73.6% yield). m.p. 210-212 °C. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.31-7.35 (m, 5H, 5×Ar-H), 5.88 (d, J = 8.32 Hz, 1H, 16-H), 5.20 (d, J = 12.13 Hz, 1H, CHAr), 4.95 (d, J = 12.21 Hz, 1H, CHAr), 4.93 (s, 1H, 11-OH), 4.36 (s, 1H, 11-H), 3.53 (t, J = 7.13 Hz, 2H, -CH_2-), 3.13 (s, 1H, 3-H), 3.00 (d, J = 11.69 Hz, 1H, 13-H), 2.45-2.54 (m, 2H, 2×22-H), 2.31-2.44 (m, 1H, 12-H), 2.12-2.24 (m, 3H, 15-H and 2×23-H), 2.02-2.10 (m, 2H, 1-H and 5-H), 1.93 (s, 3H, $OCOCH_3$), 1.62-1.88 (m, 6H, 2×2-H, 6-H, 7-H, 9-H and 12-H), 1.50-1.58 (m, 2H, 1-H and 4-H), 1.47 (s, 3H, 27-CH_3),

1.43 (s, 3H, 26-CH_3), 1.33 (s, 3H, 30-CH_3), 1.27-1.31 (m, 1H, 15-H), 1.09-1.17 (m, 2H, 6-H and 7-H), 0.98 (s, 3H, 19-CH_3), 0.90 (s, 3H, 18-CH_3), 0.80 (d, J = 6.56 Hz, 3H, 28-CH_3). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 172.68, 170.70, 169.83, 149.25, 135.63, 129.86, 128.60, 128.38, 89.12, 75.63, 74.31, 68.07, 66.43, 49.03, 48.68, 46.14, 43.90, 43.42, 39.89, 39.62, 39.46, 38.90, 37.09, 36.49, 35.91, 35.25, 31.62, 30.43, 30.21, 28.84, 26.97, 25.61, 25.12, 24.40, 23.49, 23.18, 22.74, 20.94, 20.21, 17.61, 15.54, 8.45. HR-MS (ESI) *m/z*: calcd. for $C_{42}H_{61}NO_7$ $[M+H]^+$: 692.4521, found: 692.4505.

3β-(8-aminooctanoyloxy)-21-fusidic acid (benzyl) ester (**6**)

According to the synthesis method of **4**, **f** was reacted with trifluoroacetic acid to give white solid **6** (25.0 mg, 81.1% yield). m.p. 219-221 °C. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 8.37 (s, 2H, -NH_2), 7.32-7.34 (m, 5H, 5×Ar-H), 5.89 (d, J = 8.26 Hz, 1H, 16-H), 5.18 (d, J = 12.13 Hz, 1H, CHAr), 4.97 (d, J = 12.14 Hz, 1H, CHAr), 4.93 (d, J = 2.03 Hz, 1H, 11-OH), 4.36 (s, 1H, 11-H), 3.12 (q, J = 7.33 Hz, 2H, -CH_2-), 2.99-3.01 (m, 3H, 13-H and -CH_2-), 2.31-2.46 (m, 5H, 12-H, -CH_2- and 2×22-H), 2.00-2.25 (m, 5H, 1-H, 5-H, 15-H and 2×23-H), 1.93 (s, 3H, $OCOCH_3$), 1.72-1.87 (m, 4H, 2×2-H, 7-H and 12-H), 1.51-1.70 (m, 4H, 1-H, 4-H, 6-H and 9-H), 1.47 (s, 3H, 27-CH_3), 1.44 (s, 3H, 26-CH_3), 1.34 (s, 3H, 30-CH_3), 1.26-1.33 (m, 1H, 15-H), 1.04-1.18 (m, 2H, 6-H and 7-H), 0.98 (s, 3H, 19-CH_3), 0.91 (s, 3H, 18-CH_3), 0.81 (d, J = 6.58 Hz, 3H, 28-CH_3). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 173.90, 170.73, 169.78, 161.59, 161.22, 156.39, 155.98, 149.67, 135.62, 129.85, 128.60, 128.49, 128.39, 123.00, 112.97, 89.12, 74.33, 68.03, 66.43, 48.84, 48.77, 46.08, 44.01, 40.08, 39.80, 39.38, 37.90, 36.91, 34.65, 34.62, 32.88, 28.60, 28.47, 27.26, 25.91, 25.68, 25.24, 24.82, 24.40, 24.37, 22.31, 20.94, 20.49, 18.01, 15.61, 8.46. HR-MS (ESI) *m/z*: calcd. for $C_{46}H_{69}NO_7$ $[M+H]^+$: 748.5147, found: 748.5131.

3β-(11-aminoundecyloxy)-21-fusidic acid (benzyl) ester (**7**)

According to the synthesis method of **4**, **g** was reacted with trifluoroacetic acid to give white solid **7** (27.0 mg, 78.8% yield). m.p. 223-225 °C. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.32-7.34 (m, 5H, 5×Ar-H), 6.17 (s, 2H, -NH_2), 5.88 (d, J = 8.25 Hz, 1H, 16-H), 5.18 (d, J = 12.15 Hz, 1H, CHAr), 4.95-4.98 (m, 2H, 11-OH and CHAr), 4.33 (s, 1H, 11-H), 2.97-3.05 (m, 3H, 13-H and -CH_2-), 2.39-2.45 (m, 3H, 12-H and 2×22-H), 2.33 (t, J = 7.46 Hz, 2H, -CH_2-), 2.04-2.24 (m, 5H, 1-H, 5-H, 15-H and 2×23-H), 1.92 (s, 3H, $OCOCH_3$), 1.78-1.86 (m, 4H, 2×2-H, 7-H and 12-H), 1.52-1.75 (m, 4H, 1-H, 4-H, 6-H and 9-H), 1.48 (s, 3H, 27-CH_3), 1.44 (s, 3H, 26-CH_3), 1.36 (s, 3H, 30-CH_3), 1.26-1.30 (m, 1H, 15-H), 1.03-1.18 (m, 2H, 6-H and 7-H), 0.98 (s, 3H, 19-CH_3), 0.91 (s, 3H, 18-CH_3), 0.82 (d, J =

6.58 Hz, 3H, 28-CH_3). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 173.74, 171.05, 169.70, 156.39, 155.98, 149.76, 135.59, 128.89, 128.61, 128.48, 128.41, 115.81, 112.96, 89.04, 74.39, 74.13, 68.05, 66.46, 48.77, 44.05, 40.21, 39.78, 39.36, 38.92, 38.03, 37.01, 35.64, 35.00, 34.53, 33.24, 31.03, 29.40, 29.25, 29.16, 29.11, 28.79, 27.55, 27.40, 26.41, 25.69, 25.29, 25.12, 24.71, 24.34, 22.12, 20.91, 20.37, 18.08, 15.70. HR-MS (ESI) *m/z*: calcd. for $C_{49}H_{75}NO_7$ $[M+H]^+$: 790.5616, found: 790.5603.

3β-(L-lysinyloxy)-21-fusidic acid (benzyl) ester (**8**)

According to the synthesis method of **4**, **h** was reacted with trifluoroacetic acid to give white solid **8** (28.0 mg, 80.5% yield). m.p. 221-222 °C. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 8.53 (s, 2H, -NH_2), 8.19 (s, 2H, -NH_2), 7.29-7.33 (m, 5H, 5×Ar-H), 5.86 (d, *J* = 6.38 Hz, 1H, 16-H), 5.16 (d, *J* = 12.14 Hz, 1H, CHAr), 4.94-4.99 (m, 2H, 11-OH and CHAr), 4.31 (s, 1H, 11-H), 2.97-3.04 (m, 3H, 13-H and -CH_2-), 2.39-2.41 (m, 2H, 2×22-H), 2.20-2.28 (m, 1H, 12-H), 2.03-2.12 (m, 5H, 1-H, 5-H, 15-H and 2×23-H), 1.90 (s, 3H, $OCOCH_3$), 1.70-1.84 (m, 4H, 2×2-H, 7-H and 12-H), 1.54-1.62 (m, 4H, 1-H, 4-H, 6-H and 9-H), 1.45 (s, 3H, 27-CH_3), 1.42 (s, 3H, 26-CH_3), 1.31 (s, 3H, 30-CH_3), 1.26-1.30 (m, 1H, 15-H), 1.05-1.13 (m, 2H, 6-H and 7-H), 0.97 (s, 3H, 19-CH_3), 0.89 (s, 3H, 18-CH_3), 0.80 (d, *J* = 4.30 Hz, 3H, 28-CH_3). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 170.80, 169.91, 169.13, 161.43, 156.37, 155.96, 149.66, 135.66, 129.81, 128.59, 128.39, 128.34, 117.48, 115.84, 114.59, 112.98, 89.24, 78.76, 74.28, 67.67, 67.09, 66.37, 52.69, 48.97, 48.66, 46.08, 43.86, 39.86, 39.34, 38.86, 36.42, 34.94, 31.64, 28.83, 28.23, 25.58, 25.02, 24.60, 23.64, 22.89, 20.90, 17.75, 15.27, 8.44. HR-MS (ESI) *m/z*: calcd. for $C_{44}H_{66}N_2O_7$ $[M+H]^+$: 735.4943, found: 735.4931.

General procedure for the synthesis of 9-11

4-[21-fusidic acid (benzyl) ester-3β-oxy]-4-oxo-butyric acid (**9**)

X1 (420.0 mg, 0.7 mmol) was dissolved in anhydrous dichloromethane (20.0 mL), Succinic anhydride (346.0 mg, 3.5 mmol) and DMAP (254.0 mg, 2.1 mmol) were added with stirring respectively, reacted at room temperature for 10 hours. It was washed successively with 10% hydrochloric acid, water and saturated brine, dried over anhydrous sodium sulfate, filtered, and the solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography ($V_{chloroform}$: $V_{methanol}$ = 150:1) to give white solid **9** (397.0 mg, 81.1% yield). m.p. 217-220 °C. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.28-7.36 (m, 5H, 5×Ar-H), 5.88 (d, *J* = 8.36 Hz, 1H, 16-H), 5.21 (d, *J* = 12.20 Hz, 1H, CHAr), 5.06 (t, *J* = 7.15 Hz, 1H, 24-H), 4.93 (d, *J* = 12.14 Hz, 1H, CHAr), 4.92 (d, *J* = 2.34 Hz, 1H, 11-OH), 4.38 (s, 1H, 11-H), 3.04 (d, *J* = 11.30 Hz, 1H, 13-H), 2.52-2.78 (m, 4H, 2×-CH_2-), 2.38-2.48 (m, 2H, 2×22-H), 2.28-2.31 (m,

1H, 12-H), 2.10-2.22 (m, 3H, 15-H and 2×23-H), 1.96-2.04 (m, 2H, 1-H and 5-H), 1.93 (s, 3H, $OCOCH_3$), 1.74-1.77 (m, 2H, 2-H and 12-H), 1.66-1.72 (m, 2H, 2-H and 7-H), 1.63 (s, 3H, 27-CH_3), 1.52 (s, 3H, 26-CH_3), 1.39-1.48 (m, 4H, 1-H, 4-H, 6-H and 9-H), 1.37 (s, 3H, 30-CH_3), 1.27-1.27 (m, 1H, 15-H), 1.08-1.16 (m, 2H, 6-H and 7-H), 0.98 (s, 3H, 19-CH_3), 0.92 (s, 3H, 18-CH_3), 0.82 (d, J = 6.66 Hz, 3H, 28-CH_3). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 175.53, 170.96, 170.04, 169.48, 147.73, 135.21, 132.09, 129.81, 128.06, 127.98, 127.76, 122.49, 74.50, 73.86, 68.14, 65.92, 48.63, 48.11, 43.43, 39.02, 38.42, 36.11, 35.84, 35.39, 35.09, 30.62, 29.84, 29.79, 29.58, 29.21, 28.64, 28.48, 27.91, 26.38, 25.23, 23.29, 22.44, 20.70, 20.45, 17.22, 16.93, 15.07. HR-MS (ESI) *m/z*: calcd. for $C_{42}H_{58}O_9$ $[M+Na]^+$: 729.39785, found: 729.39569.

2-[21-fusidic acid (benzyl) ester-3β-oxy]-2-oxo-benzoic acid (**10**)

According to the synthesis method of **9**, **X1** was reacted with *o*-phthalic anhydride to give white solid **10** (410.0 mg, 73.2% yield). m.p. 227-228 °C. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.97 (d, J = 7.40 Hz, 1H, Ar-H), 7.47-7.56 (m, 3H, 3×Ar-H), 7.30-7.36 (m, 5H, 5×Ar-H), 5.86 (d, J = 8.39 Hz, 1H, 16-H), 5.26 (d, J = 2.12 Hz, 1H, 11-OH), 5.20 (d, J = 12.20 Hz, 1H, CHAr), 5.04 (t, J = 7.10 Hz, 1H, 24-H), 4.93 (d, J = 12.19 Hz, 1H, CHAr), 4.53 (s, 1H, 11-H), 3.05 (d, J = 10.89 Hz, 1H, 13-H), 2.41-2.44 (m, 1H, 22-H), 2.24-2.34 (m, 2H, 12-H and 22-H), 2.17-2.22 (m, 1H, 23-H), 2.04-2.13 (m, 2H, 5-H and 23-H), 1.96-2.02 (m, 2H, 1-H and 5-H), 1.92 (s, 3H, $OCOCH_3$), 1.80-1.90 (m, 2H, 2-H and 12-H), 1.73-1.75 (m, 2H, 2-H and 7-H), 1.65-1.68 (m, 4H, 1-H, 4-H, 6-H and 9-H), 1.63 (s, 3H, 27-CH_3), 1.51 (s, 3H, 26-CH_3), 1.40 (s, 3H, 30-CH_3), 1.26-1.29 (m, 1H, 15-H), 1.08-1.13 (m, 2H, 6-H and 7-H), 0.94 (s, 3H, 19-CH_3), 0.93 (s, 3H, 18-CH_3), 0.81 (d, J = 6.66 Hz, 3H, 28-CH_3). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 170.63, 169.98, 169.48, 165.76, 147.27, 135.17, 133.64, 132.17, 131.28, 130.14, 129.78, 129.43, 128.74, 128.08, 128.00, 127.79, 126.56, 122.39, 75.77, 73.74, 69.17, 65.95, 48.66, 48.09, 43.40, 39.24, 38.36, 38.20, 36.24, 35.73, 35.34, 34.85, 29.80, 29.26, 29.22, 28.44, 27.94, 26.40, 25.23, 24.47, 21.40, 21.12, 20.45, 17.23, 16.36, 14.88. HR-MS (ESI) *m/z*: calcd. for $C_{46}H_{58}O_9$ $[M+Na]^+$: 777.39785, found: 777.39504.

2-[21-fusidic acid (benzyl) ester-3β-oxy]-2-oxo-pyrazine acid (**11**)

According to the synthesis method of **9**, **X1** was reacted with 2,3-pyrazinedicarboxylic anhydride to give white solid **11** (250.0 mg, 83.7% yield). m.p. 232-234 °C. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 8.69-8.72 (m, 2H, 2×Ar-H), 7.32-7.36 (m, 5H, 5×Ar-H), 5.84 (d, J = 7.76 Hz, 1H, 16-H), 5.71 (s, 1H, 11-OH), 5.23 (s, 1H, 11-H), 5.17 (d, J = 12.07 Hz, 1H, CHAr), 5.02 (t, J = 7.11 Hz, 1H, 24-H), 4.94 (d, J = 12.12 Hz, 1H, CHAr), 3.18 (s, 1H, 3-H), 2.95 (d, J = 10.98 Hz, 1H,

13-H), 2.63-2.66 (m, 3H, 12-H and 2×22-H), 2.36-2.46 (m, 5H, 1-H, 5-H, 15-H and 2×23-H), 2.05-2.11 (m, 4H, 2×2-H, 7-H and 12-H), 1.92 (s, 3H, $OCOCH_3$), 1.79-1.83 (m, 4H, 1-H, 4-H, 6-H and 9-H), 1.54 (s, 3H, 27-CH_3), 1.43 (s, 3H, 26-CH_3), 1.26-1.31 (m, 1H, 15-H), 1.19 (s, 3H, 30-CH_3), 1.11-1.14 (m, 2H, 6-H and 7-H), 1.05 (s, 3H, 19-CH_3), 0.96 (s, 3H, 18-CH_3), 0.87 (d, J = 4.40 Hz, 3H, 28-CH_3). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 170.55, 169.98, 166.73, 165.01, 164.53, 147.50, 146.13, 145.92, 144.82, 144.57, 135.57, 132.71, 131.01, 128.59, 128.35, 122.92, 77.75, 74.22, 73.94, 66.52, 39.35, 38.84, 37.84, 37.74, 36.72, 34.63, 32.81, 32.24, 32.13, 30.09, 30.02, 29.69, 28.99, 28.20, 26.35, 25.61, 24.16, 24.11, 22.36, 20.92, 20.29, 18.19, 17.58, 15.38. HR-MS (ESI) *m/z*: calcd. for $C_{44}H_{56}N_2O_9$ $[M+H]^+$: 755.3913, found: 755.3941.

General procedure for the synthesis of 12-18

4-[21-fusidic acid (benzyl) ester-3β-oxy]-4-oxo-butyrylmorpholine (**12**)

9 (50.0 mg, 70.0 μmol) was dissolved in anhydrous dichloromethane, morpholine (12.0 μL, 140.0 μmol) and EDCI (21.0 mg, 110.0 μmol) were added with stirring, reacted at room temperature for 6 hours. It was washed successively with water and saturated brine, dried over anhydrous sodium sulfate, filtered, and the solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography ($V_{chloroform}$: $V_{methanol}$ = 150:1) to give white solid **12** (41.0 mg, 74.7%). m.p. 227-229 °C. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.45-7.32 (m, 5H, 5×Ar-H), 5.92 (d, J = 8.40 Hz, 1H, 16-H), 5.25 (d, J = 12.20 Hz, 1H, CHAr), 5.10 (t, J = 7.20 Hz, 1H, 24-H), 5.00-4.93 (m, 2H, CHAr and 11-OH), 4.35 (s, 1H, 11-H), 3.75-3.47 (m, 8H, mor-H), 3.06 (d, J = 11.50 Hz, 1H, 13-H), 2.77-2.60 (m, 4H, 2×-CH_2-), 2.56-2.42 (m, 2H, 2×22-H), 2.37-2.28 (m, 1H, 12-H), 2.26-2.00 (m, 5H,15-H, 2×23-H, 1-H and 5-H), 1.97 (s, 3H, $OCOCH_3$), 1.93-1.76 (m, 4H, 2×2-H, 12-H and 7-H), 1.67 (s, 3H, 27-CH_3), 1.65-1.57 (m, 4H, 1-H, 4-H, 6-H and 9-H), 1.56 (s, 3H, 26-CH_3),1.39 (s, 3H, 30-CH_3), 1.36-1.27 (m, 3H,15-H, 6-H and 7-H), 1.01 (s, 3H, 19-CH_3), 0.95 (s, 3H, 18-CH_3), 0.86 (d, J = 6.70 Hz, 3H, 28-CH_3). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 172.64, 170.52, 170.02, 169.98, 148.50, 135.83, 132.59, 130.48, 128.62, 128.55, 128.31, 123.12, 74.59, 74.47, 68.18, 66.92, 66.59, 66.46, 49.19, 48.77, 45.78, 44.02, 42.15, 39.51, 39.10, 37.71, 36.90, 35.83, 34.99, 32.61, 30.99, 30.64, 29.76, 29.71, 29.08, 28.41, 28.03, 27.28, 25.79, 24.38, 22.78, 21.02, 20.68, 18.03, 17.79, 15.69. HR-MS (ESI) *m/z*: calcd. for $C_{46}H_{65}NO_9$ $[M+Na]^+$: 798.4552, found: 798.4558.

4-[21-fusidic acid (benzyl) ester-3β-oxy]-4-oxo-butyryl(4'-methyl)piperazine (**13**)

According to the synthesis method of **12**, **9** was reacted with *N*-methyl piperazine to give white solid **13** (45.0 mg,

80.6% yield). m.p. 225-227 °C. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.43-7.31 (m, 5H, 5×Ar-H), 5.90 (d, J = 8.40 Hz, 1H, 16-H), 5.23 (d, J = 12.20 Hz, 1H, CHAr), 5.08 (t, J = 7.10 Hz, 1H, 24-H), 4.98-4.91 (m, 2H, CHAr and 11-OH), 4.33 (s, 1H, 11-H), 3.73-3.48 (m, 4H, $CON(CH_2)_2$), 3.04 (d, J = 11.60 Hz, 1H, 13-H), 2.75-2.61 (m, 4H, 2×-CH_2-), 2.51-2.35 (m, 7H, $N(CH_2)_2$, 2×22-H and 12-H), 2.32 (s, 3H, N-CH_3), 2.25-1.98 (m, 6H, 15-H, 2×23-H, 1-H, 5-H and 12-H), 1.95 (s, 3H, $OCOCH_3$), 1.90-1.67 (m, 6H, 2×2-H, 1-H, 4-H, 6-H and 7-H), 1.65 (s, 3H, 27-CH_3), 1.63-1.58 (m, 1H, 9-H), 1.54 (s, 3H, 26-CH_3), 1.37 (s, 3H, 30-CH_3), 1.35-1.08 (m, 3H, 15-H, 6-H and 7-H), 0.98 (s, 3H, 19-CH_3), 0.92 (s, 3H, 18-CH_3), 0.83 (d, J = 6.70 Hz, 3H, 28-CH_3). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 174.01, 171.86, 171.32, 171.07, 149.87, 137.19, 133.93, 131.83, 129.96, 129.89, 129.65, 124.48, 75.82, 69.50, 67.79, 56.81, 56.39, 56.06, 55.64, 54.84, 50.56, 50.11, 47.42, 46.50, 45.37, 43.02, 40.86, 40.45, 39.05, 38.25, 37.17, 36.34, 33.94, 32.33, 31.17, 30.42, 29.75, 29.55, 28.61, 27.12, 25.71, 24.13, 22.36, 22.03, 19.37, 19.13, 17.02. HR-MS (ESI) *m/z*: calcd. for $C_{47}H_{68}N_2O_8$ $[M+H]^+$: 789.5049, found: 789.5049.

4-[21-fusidic acid (benzyl) ester-3β-oxy]-4-oxo-butyrylpyrazole (**14**)

According to the synthesis method of **12**, **9** was reacted with pyrazole to give white solid **14** (40.0 mg, 74.7% yield). m.p. 233-236 °C. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 8.26 (d, J = 2.60 Hz, 1H, pyr-H), 7.73 (s, 1H, pyr-H), 7.41-7.30 (m, 5H, 5×Ar-H), 6.46 (dd, J = 2.8, 1.5 Hz, 1H, pyr-H), 5.90 (d, J = 8.30 Hz, 1H, 16-H), 5.23 (d, J = 12.20 Hz, 1H, CHAr), 5.08 (t, J = 7.20 Hz, 1H, 24-H), 4.99 (d, J = 2.40 Hz, 1H, 11-OH), 4.94 (d, J = 12.20 Hz, 1H, CHAr), 4.35 (s, 1H, 11-H), 3.54-3.47 (m, 2H, -CH_2-), 3.02 (d, J = 11.10 Hz, 1H, 13-H), 2.88-2.81 (m, 2H,-CH_2-), 2.56-2.40 (m, 2H, 2×22-H), 2.35-2.27 (m, 1H, 12-H), 2.23-2.00 (m, 5H, 15-H, 2×23-H, 1-H and 5-H), 1.95 (s, 3H, $OCOCH_3$), 1.90-1.79 (m, 3H, 2×2-H and 12-H), 1.66 (s, 3H, 27-CH_3), 1.63-1.56 (m, 1H, 7-H), 1.55 (s, 3H, 26-CH_3), 1.28 (s, 3H, 30-CH_3), 1.18-1.02 (m, 2H, 6-H and 7-H), 0.98 (s, 3H, 19-CH_3), 0.92 (s, 3H, 18-CH_3), 0.84 (d, J = 6.70 Hz, 3H, 28-CH_3). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 171.69, 171.06, 170.56, 170.00, 148.49, 144.24, 135.83, 132.61, 130.50, 128.64, 128.58, 128.44, 128.34, 123.12, 109.75, 74.90, 74.47, 68.24, 66.48, 49.16, 48.76, 44.02, 39.45, 39.08, 37.81, 36.92, 34.96, 32.60, 31.01, 30.65, 29.56, 29.09, 28.91, 28.41, 27.31, 25.79, 24.27, 22.75, 21.03, 20.67, 19.26, 18.00, 17.80, 15.64, 13.81. HR-MS (ESI) *m/z*: calcd. for $C_{45}H_{60}N_2O_8$ $[M+Na]^+$: 779.4242, found: 779.4247.

2-[21-fusidic acid (benzyl) ester-3β-oxy]-2-oxo-benzoylmorpholine (**15**)

According to the synthesis method of **12**, **10** was reacted with morpholine to give white solid **15** (44.0 mg, 80.6% yield). m.p. 240-243 °C. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.99 (d, J = 7.40 Hz, 1H, Ar-H), 7.60-7.52 (m, 1H, Ar-H),

7.48-7.41 (m, 1H, Ar-H), 7.36-7.26 (m, 6H, Ar-H), 5.87 (d, *J* = 8.30 Hz, 1H, 16-H), 5.19 (m, 2H, 11-OH and CHAr), 5.03 (t, *J* = 7.20 Hz, 1H, 24-H), 4.92 (d, *J* = 12.20 Hz, 1H, CHAr), 4.32 (s, 1H, 11-H), 4.00-3.08 (m, 8H, mor-H), 3.04 (d, *J* = 11.20 Hz, 1H, 13-H), 2.53-2.35 (m, 2H, 2×22-H), 2.33-1.92 (m, 6H, 1-H, 2×5-H, 12-H and 2×23-H), 1.90 (s, 3H, $OCOCH_3$), 1.89-1.82 (m, 4H, 2×2-H, 7-H and 12-H), 1.81-1.63 (m, 4H, 1-H, 4-H, 6-H and 9-H), 1.61 (s, 3H, 27-CH_3), 1.50 (s, 3H, 26-CH_3), 1.41 (s, 3H, 30-CH_3), 1.19-1.03 (m, 2H, 6-H and 7-H), 1.00 (s, 3H, 19-CH_3), 0.91 (s, 3H, 18-CH_3), 0.85 (d, *J* = 6.70 Hz, 3H, 28-CH_3). ^{13}C-NMR ($CDCl_3$, 100 MHz) *δ*: 170.55, 170.02, 169.97, 165.03, 148.54, 135.86, 132.92, 132.60, 130.99, 130.49, 130.17, 129.09, 128.63, 128.54, 128.32, 128.27, 127.16, 123.12, 75.65, 74.47, 68.13, 66.63, 66.45, 66.36, 49.32, 48.81, 47.24, 44.05, 42.22, 39.57, 39.11, 36.91, 35.87, 32.00, 31.13, 30.65, 29.77, 29.73, 29.43, 29.08, 28.46, 27.49, 25.78, 22.77, 21.02, 20.82, 17.97, 17.80, 15.86, 14.20. HR-MS (ESI) *m/z*: calcd. for $C_{50}H_{65}NO_9$ $[M+H]^+$: 824.4732, found: 824.4730.

2-[21-fusidic acid (benzyl) ester-3β-oxy]-2-oxo-benzoyl(4'-methyl)piperazine (**16**)

According to the synthesis method of **12**, **10** was reacted with *N*-methyl piperazine to give white solid **16** (45.0 mg, 81.2% yield). m.p. 235-238 °C. ^{1}H-NMR ($CDCl_3$, 400 MHz) *δ*: 7.99 (d, *J* = 7.80 Hz, 1H, Ar-H), 7.59-7.53 (m, 1H, Ar-H), 7.48-7.42 (m, 1H, Ar-H), 7.36-7.26 (m, 6H, Ar-H), 5.88 (d, *J* = 8.30 Hz, 1H, 16-H), 5.25-5.15 (m, 2H, 11-OH and CHAr), 5.05 (t, *J* = 7.20 Hz, 1H, 24-H), 4.93 (d, *J* = 12.20 Hz, 1H, CHAr), 4.34 (s, 1H, 11-H), 4.20-3.15 (m, 4H, $CON(CH_2)_2$), 3.06 (d, *J* = 11.50 Hz, 1H, 13-H), 2.55-2.40 (m, 4H, $N(CH_2)_2$), 2.35 (s, 3H, N-CH_3), 2.32-1.96 (m, 8H, 2×22-H, 1-H, 2×5-H, 12-H and 2×23-H), 1.91 (s, 3H, $OCOCH_3$), 1.90-1.72 (m, 8H, 2×2-H, 7-H, 12-H, 1-H, 4-H, 6-H and 9-H), 1.62 (s, 3H, 27-CH_3), 1.51 (s, 3H, 26-CH_3), 1.41 (s, 3H, 30-CH_3), 1.20-1.05 (m, 2H, 6-H and 7-H), 1.00 (s, 3H, 19-CH_3), 0.91 (s, 3H, 18-CH_3), 0.85 (d, *J* = 6.70 Hz, 3H, 28-CH_3). ^{13}C-NMR ($CDCl_3$, 100 MHz) *δ*: 170.55, 170.00, 167.80, 165.20, 148.56, 135.87, 132.79, 132.60, 132.39, 130.99, 130.44, 128.99, 128.92, 128.63, 128.54, 128.31, 127.17, 123.14, 74.49, 68.09, 66.44, 65.65, 54.53, 54.40, 48.80, 46.49, 45.97, 44.06, 41.45, 39.58, 39.10, 36.85, 35.81, 32.00, 30.65, 29.77, 29.08, 28.47, 27.45, 25.79, 23.28, 22.74, 21.03, 20.92, 19.26, 17.91, 17.80, 16.64, 15.83, 14.20, 13.80. HR-MS (ESI) *m/z*: calcd. for $C_{51}H_{68}N_2O_8$ $[M+H]^+$: 837.5049, found: 837.5047.

2-[21-fusidic acid (benzyl) ester-3β-oxy]-2-oxo-benzoylpyrazole (**17**)

According to the synthesis method of **12**, **10** was reacted with pyrazole to give white solid **17** (39.0 mg, 73.2% yield). m.p. 239-242 °C. ^{1}H-NMR ($CDCl_3$, 400 MHz) *δ*: 8.48 (d, *J* = 2.70 Hz, 1H, pyr-H), 8.08 (d, *J* = 8.40 Hz, 1H, Ar-H), 7.72-7.62 (m, 3H, Ar-H), 7.58 (m, 1H, pyr-H), 7.42-7.32 (m, 5H, Ar-H), 6.50 (dd, *J* = 2.70, 1.40 Hz, 1H, pyr-H),

5.92 (d, *J* = 8.20 Hz, 1H, 16-H), 5.24 (d, *J* = 12.20 Hz, 1H, CHAr), 5.13-5.05 (m, 2H, 24-H and 11-OH), 4.96 (d, *J* = 12.20 Hz, 1H, CHAr), 4.37 (s, 1H, 11-H), 3.10 (d, *J* = 12.20 Hz, 1H, 13-H), 2.58-2.43 (m, 2H, 22-H), 2.40-1.98 (m, 6H, 1-H, 2×5-H, 12-H and 2×23-H), 1.95 (s, 3H, $OCOCH_3$), 1.92-1.69 (m, 4H, 2×2-H, 7-H and 12-H), 1.66 (s, 3H, 27-CH_3), 1.65-1.60 (m, 4H, 1-H, 4-H, 6-H and 9-H), 1.55 (s, 3H, 26-CH_3), 1.45 (s, 3H, 30-CH_3), 1.22-1.05 (m, 2H, 6-H and 7-H), 0.99 (s, 3H, 19-CH_3), 0.94 (s, 3H, 18-CH_3), 0.80 (d, *J* = 6.70 Hz, 3H, 28-CH_3). ^{13}C-NMR ($CDCl_3$, 100 MHz) *δ*: 170.54, 169.99, 168.41, 165.32, 148.47, 144.63, 135.86, 135.07, 132.63, 132.24, 130.99, 130.72, 130.53, 129.53, 129.25, 128.92, 128.64, 128.55, 128.32, 123.13, 109.92, 74.48, 68.26, 66.46, 65.65, 49.41, 48.79, 44.02, 39.51, 39.10, 37.68, 36.82, 35.54, 32.18, 31.26, 30.65, 29.10, 28.47, 27.14, 25.79, 24.05, 23.08, 21.03, 20.80, 19.26, 17.93, 17.81, 15.55, 13.81. HR-MS (ESI) *m/z*: calcd. for $C_{49}H_{60}N_2O_8$ $[M+Na]^+$: 827.4242, found: 827.4240.

2-[21-fusidic acid (benzyl) ester-3β-oxy]-2-oxo-benzoylimidazole (**18**)

According to the synthesis method of **12**, **10** was reacted with imidazole to give white solid **18** (40.0 mg, 75.0% yield). m.p. 235-237 °C. ^{1}H-NMR ($CDCl_3$, 400 MHz) *δ*: 8.19-8.12 (m, 1H, IMZ-H), 7.84 (s, 1H, Ar-H), 7.80-7.70 (m, 2H, IMZ-H and Ar-H), 7.62-7.54 (m, 1H, Ar-H), 7.48 (s, 1H, Ar-H), 7.43-7.33 (m, 5H, Ar-H), 7.15 (s, 1H, IMZ-H), 5.94 (d, *J* = 8.40 Hz, 1H, 16-H), 5.25 (d, *J* = 12.20 Hz, 1H, CHAr), 5.16-5.05 (m, 2H, 24-H and 11-OH), 4.97 (d, *J* = 12.20 Hz, 1H, CHAr), 4.38 (s, 1H, 11-H), 3.10 (d, *J* = 11.40 Hz, 1H, 13-H), 2.58-2.43 (m, 2H, 22-H), 2.27-2.01 (m, 6H, 1-H, 2×5-H, 12-H and 2×23-H), 1.97 (s, 3H, $OCOCH_3$), 1.94-1.69 (m, 6H, 2×2-H, 4-H, 6-H, 7-H and 12-H), 1.67 (s, 3H, 27-CH_3), 1.64-1.59 (m, 2H, 1-H and 9-H), 1.56 (s, 3H, 26-CH_3), 1.46 (s, 3H, 30-CH_3), 1.25-1.05 (m, 2H, 6-H and 7-H), 1.02 (s, 3H, 19-CH_3), 0.96 (s, 3H,18-CH_3), 0.84 (d, *J* = 6.70 Hz, 3H, 28-CH_3). ^{13}C-NMR ($CDCl_3$, 100 MHz) *δ*: 170.56, 169.97, 166.48, 164.72, 148.45, 135.85, 134.72, 132.88, 132.64, 131.44, 131.15, 130.54, 130.31, 129.84, 128.66, 128.64, 128.56, 128.33, 128.19, 123.91, 123.11, 74.46, 74.14, 68.14, 66.47, 49.30, 48.79, 44.02, 39.55, 39.11, 37.82, 36.81, 35.96, 35.47, 32.19, 32.00, 31.13, 29.77, 29.09, 28.45, 27.12, 25.78, 24.06, 23.06, 21.03, 20.83, 17.92, 17.81, 15.67. HR-MS (ESI) *m/z*: calcd. for $C_{49}H_{60}N_2O_8$ $[M+H]^+$: 805.4423, found: 805.4423.

Biology

Cell culture and treatment

The cancer cell lines Hela, KBV, MKN45 and U87 were kindly provided by Dr. Xiaoguang Chen (Institute Materia Medica, Chinese Academy of Medical Sciences) and cultured in our lab [14]. All the cells were maintained in DMEM (Hyclone) with 10% heat-inactivated fetal calf serum (Life Technology), and 100 U/mL penicillin and l00 g/mL

streptomycin in a humidified atmosphere of 5% CO_2 at 37 °C. Cells in the logarithmic growth phase were used for further experiments [14].

MTT assay

Cytotoxicity was detected using a MTT assay following our previous protocol [15,16]. Briefly, cells were seeded in 96-well plates and treated with the tested compounds at the desired concentration for 72 h. The MTT solution was then added into the wells and incubated, and then DMSO was added, the optical density was determined at 570 nm, and the cell survival rate was calculated. Data are expressed as average values from at least three independent experiments.

Protein synthesis assay

Hela cells at 70-80% confluence were treated with 10 μg/mL puromycin for 10 min. After washing twice with ice-cold PBS, cells were lysed with SDS-PAGE sample buffer, and proteins were separated on SDS-PAGE and transferred to Immobilon-P membranes. Membranes were blocked for 1 h in TBS containing 5% non-fat milk and 0.1% Tween-20, followed by incubation with puromycin antibodies (1:100 dilution) overnight at 4 °C. After incubation with HRP-conjugated anti-mouse IgG (1:5000 dilution) for 1 h at room temperature, the membrane was visualized using enhanced chemiluminescence.

Cell cycle analysis

A flow cytometry assay was used to analyze the cell apoptosis as previously reported [15]. Briefly, Hela cells (1×10^5 cells/well) were seeded in 6-well plates. After 24 h incubation, the cells were treated with compound **4** or **FA** (5 μM) for 48 h and 72 h. The cells were fixed in 80% alcohol and overnight at 4 °C. After washing with PBS, cells were treated with PI solution (20 mg/mL PI and 20 mg/mL RNaseA in PBS) for 1 h. Cells were detected in FACS Calibur (Becton-Dickinson, C6, USA), and the cell cycle distribution was analyzed.

TUNEL staining

Cellular apoptosis was assessed by TUNEL staining using the In Situ Cell Death Detection Kit (Roche, Mannheim, Germany) as previously described [17]. Briefly, Hela cells (2×10^4 cells/well) were seeded in 6-well plates. After 24 h incubation, the cells were treated with compound **4** or **FA** (2 μM) for 24 h, and then were fixed and permeabilized. The cells were incubated with 50 mL of reaction mixture containing the labeling enzyme and the TMR green labeled-dUTP at 37 °C for 1 h. After extensive washing, the cells were counterstained with DAPI and observed under a fluorescence microscope (Nikon, Japan).

Xenograft model

Athymic nude mice (6-8 weeks old, BALB/c, female) were used to establish the xenograft tumors following our published Protocol [18]. In brief, Hela cells (5×10^6) were inoculated subcutaneously in right frank regions. When the relative tumor volume reached around 100-200 mm^3 in size, the mice were randomly divided into three groups, control, **4** (25 mg/kg), and **4** (50 mg/kg) with five mice per group. The mice were intra-gastric administration twice a day with compound **4**, and the tumor appearance and size were measured every 3 days. Animals were sacrificed and the tumors were stripped and weighed. The use of animals was approved by the Animal Experimentation Ethics Committee of Yantai University (protocol number 20180607) in accordance with the guidelines for ethical conduct in the care and use of animals.

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REFERENCES

[1] R. Beaglehole, R. Bonita, R. Magnusson, Global cancer prevention: an important pathway to global health and development, Public Health 125 (2011) 821-831.

[2] F. Bray, J.S. Ren, E. Masuver, J. Ferlay, Estimates of global cancer prevalence for 27 sites in the adult population in 2008, Int. J. Cancer 132 (2013) 1133-1145.

[3] D.K. Lokwani, A.P. Sarkate, D.B. Shinde, 3D-QSAR and docking studies of benzoyl urea derivatives as tubulin-binding agents for antiproliferative activity, Med. Chem. Res. 22 (2013) 1415-1425.

[4] K.M. Amin, A.A.M. Eissa, S.M. Abou-Seri, F.M. Awadallah, G.S. Hassan, Synthesis and biological evaluation of novel coumarin-pyrazoline hybrids endowed with phenylsulfonyl moiety as antitumor agents, Eur. J. Med. Chem. 60 (2013) 187-198.

[5] C.Y. Chen, N.Y. Liu, H.C. Lin, Synthesis and bioevaluation of novel benzodipyranone derivatives as P-glycoprotein inhibitors for multidrug resistance reversal agents, Eur. J. Med. Chem. 118 (2016) 219-229.

[6] E. Cohen, The Royal Marsden Hospital Handbook of Cancer Chemotherapy, J. Clin. Nurs. 15 (2006) 1478-1478.

[7] K. Singh, M. Espinoza-Moraga, M. Njoroge, G. Kaur, J. Okombo, C.D. Kock, P.J. Smith, S. Wittlin, K. Chibale, Synthesis and biological characterisation of ester and amide derivatives of fusidic acid as antiplasmodial agents, Bioorg. Med. Chem. Lett. 27 (2017) 658-661.

[8] W.O. Godtfredsen, S. Jahnsen, H. Lorck, K. Roholt, L. Tybring, Fusidic acid: a new antibiotic, Nature 193 (1962) 987-987.

[9] C.N. Kraus, B.W. Burnstead, The safety record of fusidic acid in non-US markets: A focus on skin infections, Clin. Infect. Dis. 52 (2011) S527-537.

[10] M.A. Pfaller, M. Castanheira, H.S. Sader, R.N. Jones, Evaluation of the activity of fusidic acid tested against contemporary Gram-positive clinical isolates from the USA and Canada, Int. J. Antimicrob. Agents 35 (2010) 282-287.

[11] M. Whitby, Fusidic acid in the treatment of methicillin-resistant *Staphylococcus aureus*, Int. J. Antimicrob. Agents 12 (1999) S67-71.

[12] S. Schwarz, R. Csuk, Synthesis and antitumour activity of glycyrrhetinic acid derivatives, Bioorgan. Med. Chem. 18 (2010) 7458-7474.

[13] R. Csuk, S. Schwarz, B. Siewert, R. Kluge, D. Ströhl, Synthesis and antitumor activity of ring A modified glycyrrhetinic acid derivatives, Eur. J. Med. Chem. 46 (2011) 5356-5369.

[14] Y.T. Yang, D.K. Guan, L. Lei, J. Lu, J.Q. Liu, G.Q. Yang, C.H. Yan, R. Zhai, J.W. Tian, Y. Bi, F.H. Fu, H.B. Wang, H6, a novel hederagenin derivative, reverses multidrug resistance *in vitro* and *in vivo*, Toxicol. Appl. Pharm. 341 (2018) 98-105.

[15] G.Y. Lv, D.J. Sun, J.W. Zhang, X.X. Xie, X.Q. Wu, W.S. Fang, J.W. Tian, C.H. Yan, H.B. Wang, F.H. Fu, Lx2-32c, a novel semi-synthetic taxane, exerts antitumor activity against prostate cancer cells *in vitro* and *in vivo*, Acta Pharm. Sin. B. 7 (2017) 52-58.

[16] X.X. Liu, Y.T. Yang, X. Wang, K.Y. Wang, J.Q. Liu, L. Lei, X.M. Luo, R. Zhai, F.H. Fu, H.B. Wang, Y. Bi, Design, synthesis and biological evaluation of novel α-hederagenin derivatives with anticancer activity, Eur. J. Med. Chem. 141 (2017) 427-439.

[17] H.B. Wang, X.J. Ma, S.M. Ren, J.K. Buolamwini, C.H. Yan, A small-molecule inhibitor of MDMX activates p53 and induces apoptosis, Mol. Cancer Ther. 10 (2011) 69-79.

[18] Y.T. Ma, Y.T. Yang, P. Cai, D.Y. Sun, P.A. Sánchez-Murcia, X.Y. Zhang, W.Q. Jia, L. Lei, M.Q. Guo, F. Gago, H.B. Wang, W.S. Fang, A series of enthalpically optimized docetaxel analogues exhibiting enhanced antitumor activity and water solubility, J. Nat. Prod. 81 (2018) 524-533.

Discovery, synthesis of novel fusidic acid derivatives possessed amino-terminal groups at the 3-hydroxyl position with anticancer activity

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Highlights：

- Fusidic acid (FA) derivatives with anti-tumor activity were first discovered.
- A preliminary structure-activity relationship of the anti-tumor activity was described.
- One novel derivative **4** exhibited good anti-tumor activity both *in vitro* and *in vivo*.
- The mechanism that compound **4** induced Hela cells to undergo apoptosis was first proved.