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Research paper

Design and synthesis of parthenolide and 5-fluorouracil conjugates as potential anticancer agents against drug resistant hepatocellular carcinoma

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ABSTRACT

A series of twenty-three parthenolide-5-fluorouracil (5-FU) conjugates ware synthesized and evaluated for their anti-cancer activities against human hepatocellular carcinoma cell line Bel-7402 and 5-fluorouracil resistant human hepatocellular carcinoma cell line Bel-7402/5-FU. The preliminary structure-activity relationships were discussed. The most active compound **15d** showed high activity against Bel-7402/5-FU cell line with IC_{50} value of 2.25 μM, which demonstrated 5.8-fold improvement compared to that of the parent compound parthenolide ($IC_{50} = 12.98$ μM). The investigation of preliminary molecular mechanism of **15d** revealed that **15d** could reverse drug resistance by inhibiting MDR1, ABCC1 and ABCG2 to increase the intracellular drug accumulation and induce apoptosis of Bel-7402/5-FU cells through mitochondria mediated pathway. On the base of these results, compound **15d** is deserved to be further investigated as a potential anti-HCC lead compound for ultimate discovery of pathenolide-based anti-cancer drug.

1. Introduction

Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer with a high global prevalence [1,2]. HCC is highly fatal with only 18% 5-year relative survival rates [3]. In china, HCC is the third leading causes of cancer death [4]. In the United States, HCC is the fifth and eighth leading cause of cancer death in men and women, respectively [3,5]. Most HCC cases are reported in the developing countries, but there has been an alarming increase in United States. Death rates of HCC are increasing at a faster pace than any other cancer [1–5].

Surgical resection and liver transplant are curative treatments for HCC, particularly for HCC at early stage [6,7]. However, most cases of HCC are diagnosed in an advanced stage because of the poor overall prognosis for HCC [8]. The currently used chemotherapeutics for the treatment of HCC include doxorubicin, cisplatin, 5-fluorouracil (5-FU), gemcitabine, methotrexate agents, sorafenib, regorafenib [8–11]. 5-FU was used in clinical practice as first-line drug for the treatment of HCC. However, this chemotherapy comes with severe drug resistance, which led to limited or no efficiency [8,9]. Thus, there is an urgent need to investigate novel potential drugs which may be effective in the control of drug resistant HCC.

Parthenolide (PTL, Fig. 1), a prominent naturally occurring germacranolide originally isolated from *Tanacetum parthenium* L., showed cytotoxicity against various human cancer cells, while sparing normal cells [12]. Even though PTL showed potent anticancer activity, its poor pharmacologic properties limit its clinical application [13]. To overcome this problem, many studies have been devoted to investigation of its structure-activity relationships (SARs) [14–30], which led to discovery of a PTL analogue dimethylaminoparthenolide (DMAPT) [14]. DMAPT showed an oral bioavailability of 70% and was evaluated in a clinical trial for treatment of AML [31,32]. Recently, we developed the other PTL

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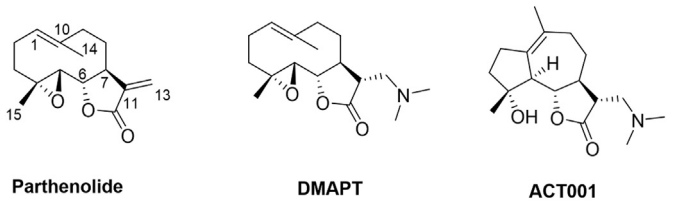


Fig. 1. Structures of PTL, DMAPT, and ACT001.

derivative, ACT001 [26], to be in clinical trial in Australia and China for treatment of glioblastoma [33].

Molecular combination strategy is a rational design of a new chemical entity based structures of two or more known bioactive molecules. The conjugates are commonly generated by connection of the individual molecules via a chemically stable linker. Generally, the conjugates could offer several advantages such as an improvement of affinity and efficacy, an increase in the solubility, multiple-targets in one molecule, or countering balance the known side effects comparing to the one or both conjugated molecules. Selection of two chemical entities for fusion is usually based on their observed synergistic or additive pharmacological activities [34–36].

Recently, it was reported that combination therapy with PTL and 5-FU had a synergistic anti-cancer effect both in vitro and in vivo [37,38], which inspired us to evaluate the anti-cancer efficacy of PTL and 5-FU conjugates. In our continuing efforts to develop PTL-based potential anti-cancer drugs [23–30], we report herein the design (Fig. 2) and synthesis of a series of conjugates of PTL and 5-FU, and evaluation of their anti-HCC activities against human hepatocellular carcinoma cell line Bel-7402 and 5-FU resistant human hepatocellular carcinoma cell line Bel-7402/5-FU. The preliminary structure-activity relationship was discussed, which led to the discovery of the most potent compound **15d**. The preliminary molecular mechanism of compound **15d** was also investigated.

2. Results and discussion

2.1. Chemistry

The synthesis of the conjugates was illustrated in Schemes 1–4. As shown in Scheme 1, the key intermediates **1**, **2** and **3** were prepared with 5-FU as starting material according to reported procedures [39–41]. Conjugation of **3** with 1-Boc-1,6-diaminohexane or 1-Boc-piperazine generated **4a** or **4b**, respectively [42]. Compound **5** was readily obtained from PTL following our previously reported route [25]. Reaction of compound **5** with acid **3** in the presence of *N*,*N*-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) in dichloromethane afforded

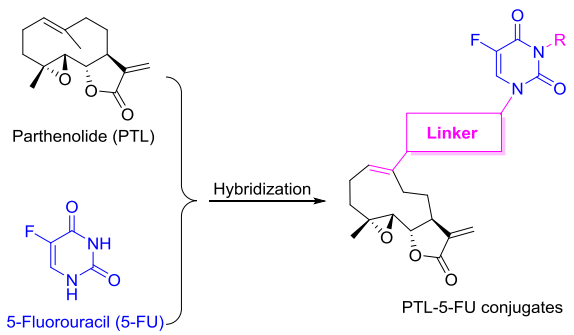


Fig. 2. Design of PTL-5-FU conjugates.

ester **6** (Scheme 2). Azide **7** was synthesized by reaction of compound **5** and DPPA with the presence of DBU [23]. Then, azide **7** was subjected to azide-alkyne cycloaddition with terminal alkyne **1** catalyzed by Cu_2O nanoparticles (Cu_2O-NPs), our previously developed efficient catalyst for azide-alkyne cycloaddition [43], to produce PTL-5-FU conjugate **8**. Acid **9** was prepared by esterifaction of alcohol **5** and Succinic anhydride catalyzed by DMAP in dichloromethane. Acid **9** was treated with alcohol **2** in the presence of DCC and DMAP to yield ester **10**. Removal of Boc group of compound **4a** or **4b** with trifluoroacetic acid, followed by conjugation with acid **9** afforded the desired amide **11a** or **11b**, respectively. Conjugates **12**, **13a**, **13b**, **14a** and **14b** were synthesized through the route outlined in Scheme 3. Alkylation of **6**, **8**, **11a** and **11b** with benzyl bromide or 4-fluorobenzyl bromide provided conjugates **12**, **13a**, **13b**, **14a** and **14b**, respectively. Compound **6** was treated with appropriate different patterns of substituted benzyl halides or alkyl halides with K_2CO_3 as base in DMF to afford conjugates **15a**–**15m** (Scheme 4).

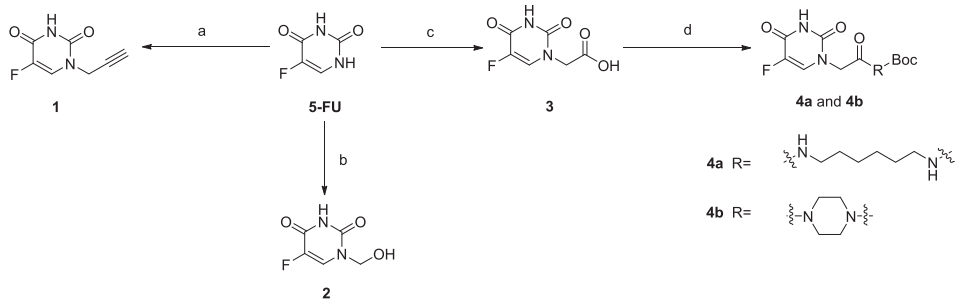
2.2. Biological activities against HCC cell lines

All twenty-three synthesized conjugates were evaluated for their anti-HCC activity against proliferation of human hepatocellular carcinoma cell line Bel-7402 and 5-FU resistant human hepatocellular carcinoma cell line Bel-7402/5-FU. PTL and 5-FU was also included for comparison. Clinically used drug adriamycin, ADR, was used as a positive control. These results were shown in Table 1 and Table 2.

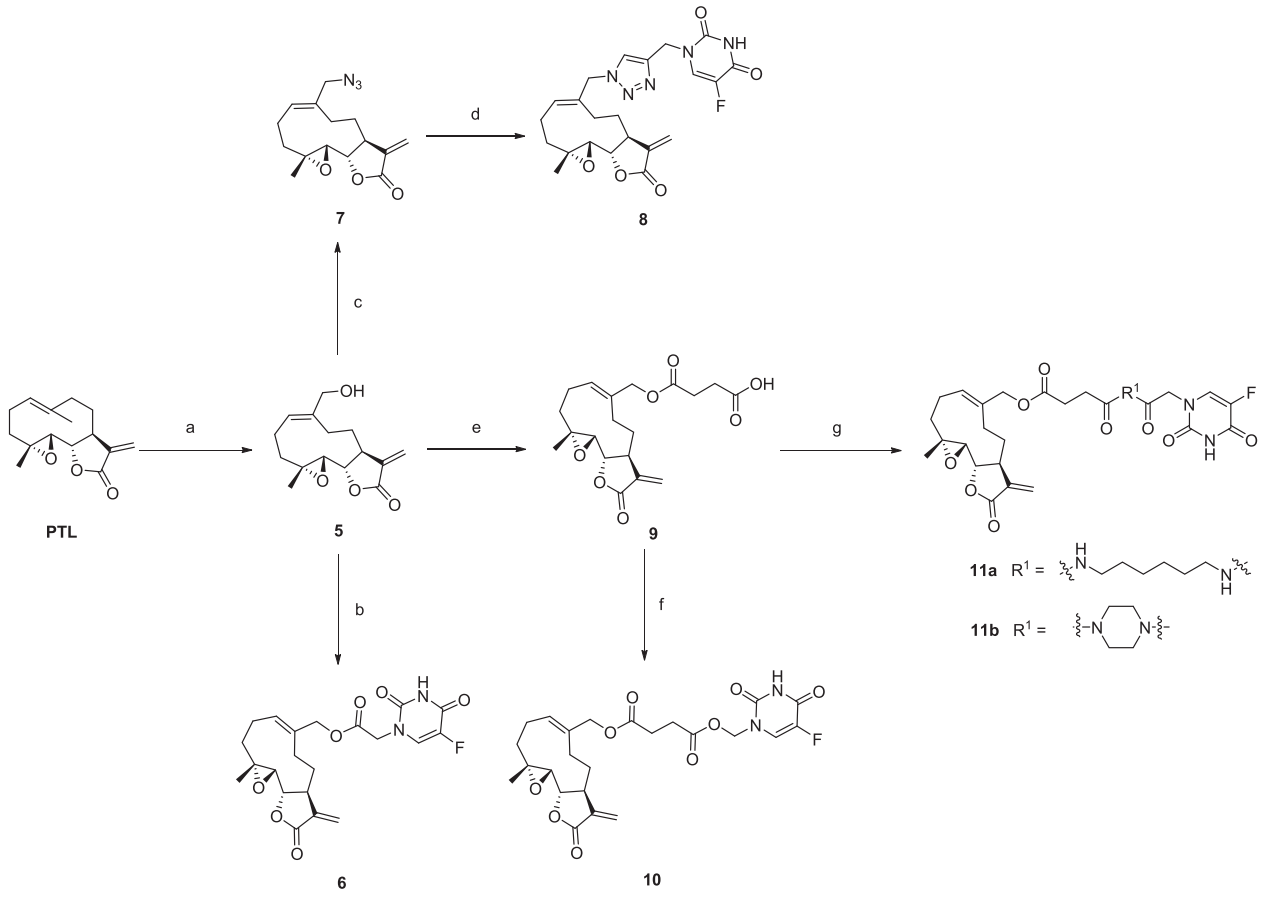
5-FU showed potent activity against Bel-7402 cells with IC_{50} value of 7.36 μM. However, 5-FU had no potency against Bel-7402/5-FU cells ($IC_{50} > 400$ μM). The parent compound PTL was potent against Bel-7402 cells ($IC_{50} = 8.62$ μM), and maintained antiproliferative activity against Bel-7402/5-FU cells ($IC_{50} = 12.98$ μM). Among twenty-three synthesized PTL-5-FU conjugates, sixteen compounds showed more potent activity against Bel-7402/5-FU cells than their parent compound PTL except for compounds **6**, **8**, **10**, **11a**, **11b**, **14a** and **14**. Moreover, most of the conjugates were more or comparable sensitive to Bel7402 and Bel-7402/5-FU cell lines in contrast to 5-FU that was only active for Bel7402 while had no efficacy to Bel-7402/5-FU cells.

As shown in Table 1, for subseries of **6**, **8**, **10**, **11a** and **11b** with no substituent on 3-*N*-position of 5-FU, they lost activity against both of Bel-7402 and Bel-7402/5-FU cell lines. Fortunately, when a benzyl group was introduced to 3-*N*-5-FU moiety of conjugates, the anti-HCC activity of conjugates **12**, **13a**, **14a** and **14b** was greatly potentiated comparing to their counterparts **6**, **8**, **11a** and **11b**. The result suggested that substituent on 3-*N*-5-FU moiety was significant for their anti-HCC activity. Compound **13b**, introducing of 4-fluorobenzyl group to 3-*N*-5-FU moiety, was found to be approximately 1.86- and 1.82-fold more potent against Bel-7402 and Bel-7402/5-FU cells, respectively, than compound **13a**. For the subseries **12**, **13a**, **14a** and **14b**, compound **12** was most potent with IC_{50} values of 3.98 μM and 4.17 μM for Bel-7402 and Bel-7402/5-FU cell lines, respectively. Considering compound **12** showed the most potent activity, more studies were devoted to explore its SAR.

To further investigate the effect of substituents at 3-*N*-5-FU moiety on the anti-HCC activity and explore the SAR, a series of compounds **15a**–**15m** with different patterns of substituents on the benzyl group or alkyl group at 3-*N*-5-FU moiety was synthesized and evaluated for their anti-HCC activity. Generally, all the conjugates with 3-*N*-substitution of 5-FU moiety were more active than parent compound PTL for Bel-7402/5-FU cells, and the conjugates with substituted benzyl groups (**15a**–**15k**) with IC_{50} values ranging from 2.25 μM to 4.5 μM for Bel-7402 cells and ranging from 2.25 μM to 5.01 μM for Bel-7402/5-FU cells were more active than the



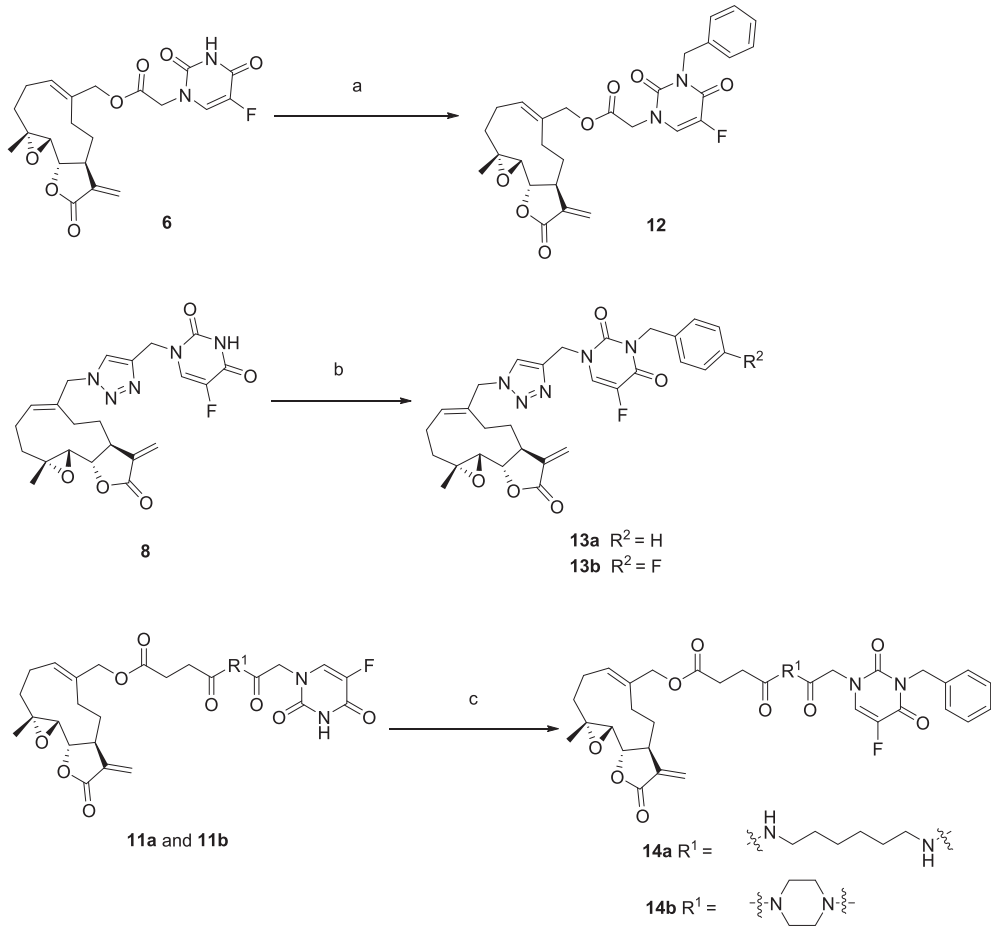
Scheme 1. Synthesis of intermediates **1**–**4**.
Reagents and conditions: (a) K_2CO_3, DMF, propargyl bromide, room temperature, 6 h, 31%; (b) formaldehyde, 60 °C, 2 h, 87%; (c) KOH, bromoacetic acid, H_2O, 40 °C-60 °C, 5 h, 72%; d) EDCI, HOBt, *N*-Boc-1,6-diaminohexane or 1-Boc-piperazine, DMF, room temperature, 12 h, 65% for **4a**, 56% for **4b**.



Scheme 2. Synthesis of conjugates **6**, **8**, **10**, **11a** and **11b**.
Reagents and conditions: (a) SeO_2, *t*-BuOOH, CH_2Cl_2, room temperature, 4 d, 72%; (b) DCC, DMAP, compound **3**, room temperature, 12 h, 50%; (c) DPPA, DBU, THF, 0 °C, 12 h, 69%; (d) Cu_2O-NPs, THF-H_2O, compound **1**, room temperature, 12 h, 37%; (e) Succinic anhydride, DMAP, DCM, room temperature, 12 h, 88%; (f) compound **2**, DCC, DMAP, DCM, room temperature, 12 h, 44%; (g) step 1: compound **4a** or **4b**, TFA, DCM, room temperature, 3 h; step 2: EDCI, HOBt, DIPEA, DCM, room temperature, 12 h, 44% over two steps for **11a**, 50% over two steps for **11b**.

aliphatic groups (**15l** and **15m**). These results suggested that the benzyl group at 3-*N*-5-FU moiety could accommodate diverse patterns of substituents, and the substituted benzyl groups were more favored than aliphatic groups. Among the subseries **15a**–**15m**, compound **15d** exhibited the highest activity against Bel-7402/5-FU cells with IC_{50} value of 2.25 μM, demonstrating 5.8-fold improvement compared to that of the parent compound PTL (IC_{50} = 12.98 μM), moreover, which indicated a comparable potency with the clinically used anti-cancer drug ADR (IC_{50} = 1.14 μM). Moreover, as shown in Fig. 3, compounds **5** (IC_{50} = 8.56 μM) and **16** (IC_{50} > 50 μM) or combination of **5** and **16** (1:1) (IC_{50} = 7.82 μM) were less potent than **15d** (IC_{50} = 2.25 μM). These results suggest that the anti-HCC activity of **15d** may be attributed to the synergic effects of two moieties. The most potent compound **15d** was further evaluated for its toxicity against normal 3T3 cells (mouse embryonic fibroblast cell line). As shown in Table 2, **15d** had selective cytotoxicity against HCC cells (IC_{50} = 2.25 μM) being compared with 3T3 cells (IC_{50} = 5.2 μM) with therapeutic index (TI) value of 2.3.

Taking account that compound **15d** showed the most potent



Scheme 3. Synthesis of conjugates **12**, **13a**, **13b**, **14a** and **14b**.
Reagents and conditions: (a) K_2CO_3, benzyl bromide, DMF, room temperature, 6 h, 78%; (b) K_2CO_3, CH_3CN, 4-fluorobenzyl bromide or benzyl bromide, room temperature, 6 h, 90% yield for **13a**, 90% yield for **13b**; (c) K_2CO_3, benzyl bromide, DMF, room temperature, 6 h, 78% for **14a**, 55% for **14b**.

anti-HCC activity and did not significantly affect the normal cells, **15d** was further selected for investigation of its preliminary mechanism.

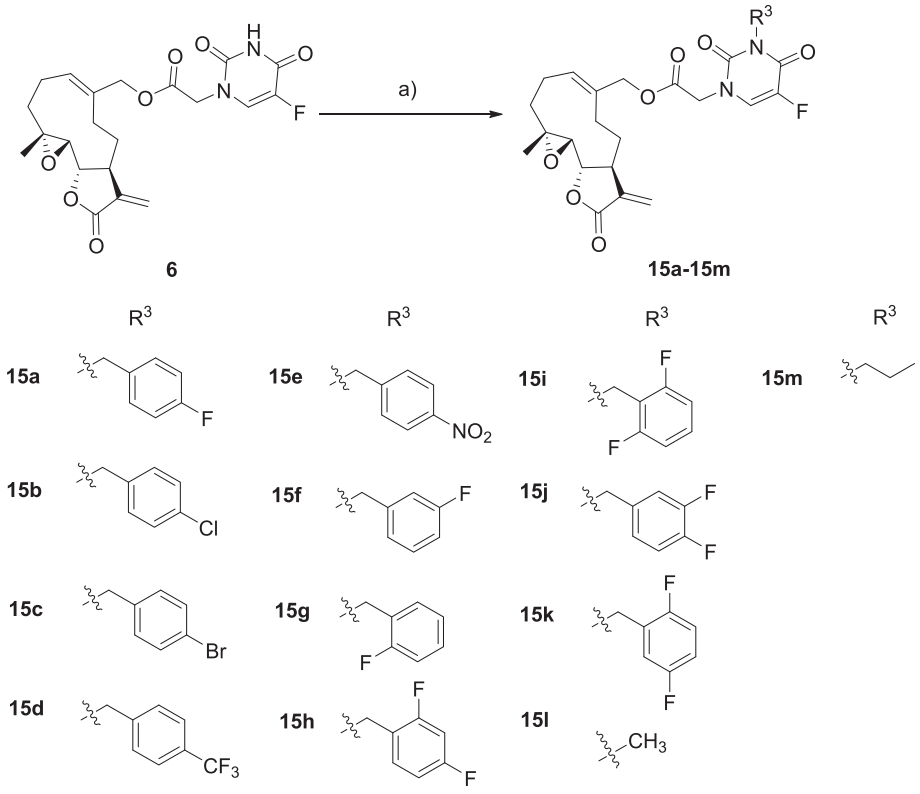
2.3. Preliminary mechanism study of compound *15d*

From the results of the MTT assay, **15d** showed preferably activity against 5-FU resistant Bel-7402/5-FU cells. To explore the mechanism of **15d** in Bel-7402/5-FU cells, we detected the expression level of multi-drug resistant proteins including MDR1, ABCC1 and ABCG2 in Bel-7402 and Bel-7402/5-FU cells, which were recognized as efflux transporters and played important roles in drug resistance. As shown in Fig. 4A, Bel-7402/5-FU cells showed a marked up-regulation of MDR1, ABCC1 and ABCG2 than that of Bel-7402 cells. As shown in Fig. 4B–D, after the treatment of increasing concentrations of **15d** for 24 h, the level of MDR1, ABCC1 and ABCG2 were significantly decreased in a dose-dependent manner. Moreover, from the result of flow cytometry in Fig. 5, the level of p-gp was significantly decreased after the treatment of **15d** for 24 h at 0.5 μM. These results indicated that **15d** could reverse drug resistance by downregulated expression of MDR1, ABCC1 and ABCG2 in Bel-7402/5-FU cells.

It was reported that the over expression of MDR1, ABCC1 and ABCG2 reduced the accumulation of drugs in cells [44]. To determine whether **15d** could increase drug accumulation in Bel-7402/5-FU cells to reverse drug resistance, we performed the accumulation assay. As shown in Fig. 6, after the treatment of **15d** for 24 h in Bel-7402/5-FU cells, the intracellular accumulation of ADR was significantly enhanced with a dose-dependent manner. This result suggested that **15d** could reverse drug resistance by increasing the intracellular drug accumulation.

To further explore the mechanism of **15d** in Bel-7402/5-FU cells, cell apoptosis assay was performed. As shown in Fig. 7, the percentage of apoptosis was 3.37 ± 0.47, 3.97 ± 0.32, 4.90 ± 0.98, 14.43 ± 2.48 and 77.97 ± 2.94 after the treatment of **15 d** at a concentration of 0, 0.5 μM, 1 μM, 2.5 μM and 5 μM, respectively. Moreover, compound **15d** exhibited stronger effect on induction of cell apoptosis compared with that of PTL and 5-FU (Fig. 7). Meanwhile, the DAPI staining experiment showed that the percentage of irregular nuclear morphology and apoptotic bodies were significantly increased after the treatment of **15d** with a dose-dependent manner (Fig. 8). These results indicated that **15d** could induce the apoptosis of Bel-7402/5-FU cells in a dose dependent manner.

In order to further elucidate the mechanism of **15d** for induction of cell apoptosis, the mitochondria mediated apoptosis pathway related proteins were analyzed by Western blot experiment. As shown in Fig. 9, the expression levels of pro-apoptotic proteins Bax and Bim were up-regulated, while the levels of anti-apoptosis proteins Bcl-2 and Bcl-xl were greatly down-regulated after the treatment of **15d**. Then the level of cytochrome C which activated the cleavage of caspase-3 and caspase-9 was clearly increased. Moreover, the caspase-3 and caspase-9 were activated and



Scheme 4. Synthesis of conjugates **15a**–**15m**.
Reagents and conditions: (a) K_2CO_3, DMF, different substituted halides, room temperature, 6 h, 45%–78%.

Table 1
Anti-HCC activities of PTL-5-FU conjugates.

Compounds	IC_{50}[a](μM)	
	Bel-7402[b]	Bel-7402/5-FU[c]
6	>20	>20
8	>20	>20
10	>20	>20
11a	>20	>20
11b	>20	>20
12	3.98 ± 2.39	4.27 ± 1.29
13a	9.16 ± 1.02	8.84 ± 2.49
13b	4.91 ± 1.41	4.86 ± 0.64
14a	18.29 ± 0.83	16.16 ± 1.57
14b	>20	>20
PTL	8.62 ± 1.68	12.98 ± 3.96
5-FU	7.36 ± 1.58	>400
PTL+5-FU (1:1)	–	8.36 ± 3.63
ADR[d]	0.73 ± 0.28	1.14 ± 0.59

[a] All values are the mean of three independent experiments.
[b] Bel-7402: human hepatocellular carcinoma cell line.
[c] Bel-7402/5-FU: 5-FU resistant human hepatocellular carcinoma cell line.
[d] ADR, adriamycin, used as a positive control.

eventually induced apoptosis of Bel-7402/5-FU cells. These results suggested that **15d** could induce apoptosis of Bel-7402/5-FU cells through mitochondria medicated pathway (Fig. 10).

3. Conclusion

In summary, a series of twenty-three new parthenolide-5-fluorouracil conjugates ware synthesized and evaluated for their anti-HCC activities. Among twenty-three synthesized PTL-5-FU conjugates, sixteen compounds showed higher activity against Bel-7402/5-FU cells than their parent compound PTL. It is worth to note that most of the conjugates were more or comparable sensitive to Bel7402 and Bel-7402/5-FU cell lines in contrast to 5-FU that was only active for Bel7402 while had no efficacy to Bel-7402/5-FU cells. Based on the above structure and activity results, the following preliminary SARs can be drawn: (1) substituent on 3-*N*-5-FU moiety of PTL-5-FU conjugates was significant for their anti-HCC activity; (2) the benzyl group at 3-*N*-5-FU moiety can accommodate diverse patterns of substituents, and the substituted benzyl groups were more favored than aliphatic groups.

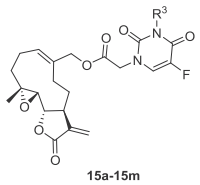
The above investigation led to discovery of the most active compound **15d**, which demonstrated 5.8-fold improvement compared to that of the parent compound PTL, and showed a comparable potency with the clinically used anti-cancer drug ADR. Compound **15d** could induce apoptosis of Bel-7402/5-FU cells through mitochondria mediated pathway and reverse drug resistant by inhibiting MDR1, ABCC1 and ABCG2 to increase the intracellular drug accumulation. On the base of these results, compound **15d** is deserved to be further investigated as a potential anti-HCC lead compound for ultimate discovery of pathenolide-based anti-cancer drug.

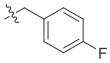
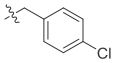
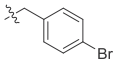
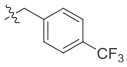
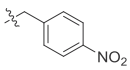
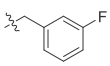
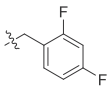
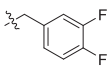
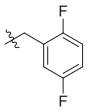
4. Experimental

4.1. Chemistry

Unless otherwise mentioned, all reactions were carried out under a nitrogen atmosphere with dry solvents under anhydrous conditions. Reactions were monitored by thin-layer chromatography carried out on 0.25 mm Tsingdao silica gel plates (60F-254). Visualization was achieved using UV light, phosphomolybdic acid in 1+1ethanol or potassium permanganate in water, each followed

Table 2
Anti-HCC activities of conjugates 15a–15m.



Compounds	R^3	IC_{50}[a](μM)	
		Bel-7402[b]	Bel-7402/5-FU[c]
6[d]	H	>20	>20
12[d]		3.98 ± 2.39	4.27 ± 1.29
15a		2.73 ± 0.86	2.90 ± 0.88
15b		2.56 ± 0.98	2.42 ± 0.11
15c		2.67 ± 1.10	3.24 ± 0.89
15d[e]		2.25 ± 0.25	2.25 ± 0.16
15e		4.07 ± 0.64	4.34 ± 1.00
15f		2.53 ± 0.38	3.70 ± 0.69
15g		3.77 ± 0.11	3.83 ± 0.64
15h		3.67 ± 0.62	5.01 ± 1.17
15i		4.50 ± 1.20	4.98 ± 0.79
15j		2.70 ± 1.10	4.48 ± 0.46
15k		3.06 ± 0.65	4.61 ± 0.39
15l		9.40 ± 1.43	11.73 ± 1.58
15m		8.58 ± 0.19	9.11 ± 1.40
PTL	–	8.62 ± 1.68	12.98 ± 3.96
5-FU	–	7.36 ± 1.58	>400
PTL+5-FU (1:1)	–	–	8.36 ± 3.63
ADR[f]	–	0.73 ± 0.28	1.14 ± 0.59

[a] All values are the mean of three independent experiments.
[b] Bel-7402: human hepatocellular carcinoma cell line.
[c] Bel-7402/5-FU: 5-FU resistant human hepatocellular carcinoma cell line.
[d] Data from Table 1.
[e] IC_{50} value for 3T3 cell line was 5.2 ± 0.45 μM.
[f] ADR, adriamycin, used as a positive control.

by heating. Tsingdao silica gel (60, particle size 0.040–0.063 mm) was used for flash column chromatography. NMR spectra were recorded with a 400 MHz (^{1}H: 400 MHz, ^{13}C: 100 MHz)

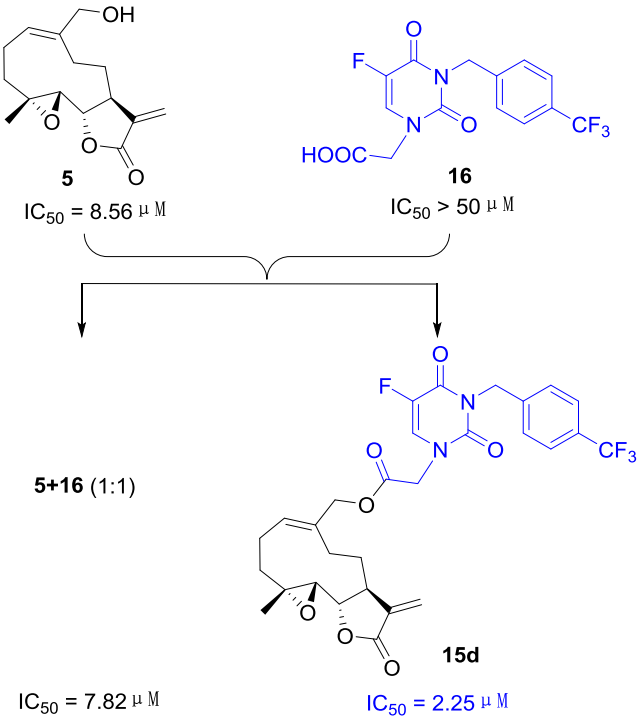


Fig. 3. Comparison of anti-HCC activity of **15d** with that of **5**, **16**, and an equimolar mixture of **5** and **16** against Bel-7402/5-FU cells.

spectrometer. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br. = broad, m = multiplet), coupling constants and integration.

*4.1.1. ((1aR,7aS,10aS,10bS,E)-1a-Methyl-8-methylene-9-oxo-1a,2,3,6,7,7a,8,9,10a,10b-decahydrooxireno[2′,3′:9,10]cyclodeca[1,2-b]furan-5-yl)methyl 2-(5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetate (**6**)*

A mixture of compound **3** (80.00 mg, 0.30 mmol), compound **5** (72.7 mg, 0.38 mmol), DCC (79.00 mg, 0.38 mmol) and 4-dimethylaminopyridine (4.00 mg, 0.03 mmol) was stirred in anhydrous dichloromethene (3.2 mL) for 12 h at room temperature. The reaction mixture was filtered and filtrate was evaporated in vacuo. The resulting crude product was further purified by column chromatography on silica gel to give compound **6** (white ceraceous solid 78 mg, yield 50%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.14 (s, 1H), 7.38 (d, J = 5.3 Hz, 1H), 6.17 (d, J = 2.9 Hz, 1H), 5.71 (t, J = 8.1 Hz, 1H), 5.58 (d, J = 2.2 Hz, 1H), 4.75 (d, J = 12.3 Hz, 1H), 4.56 (d, J = 12.3 Hz, 1H), 4.52–4.38 (m, 2H), 3.83 (t, J = 9.3 Hz, 1H), 2.93–2.77 (m, 2H), 2.47–2.09 (m, 6H), 1.70–1.63 (m, 1H), 1.51 (s, 3H), 1.07 (t, J = 12.7 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.6, 167.2, 157.4 (d, J = 26.2 Hz), 149.8, 140.4 (d, J = 237.6 Hz), 138.6, 134.1, 131.9, 129.2 (d, J = 33.2 Hz), 120.6, 81.1, 68.6, 63.2, 60.2, 49.2, 42.6, 36.4, 25.5, 24.4, 23.8, 17.9. ^{19}F NMR (376 MHz, $CDCl_3$) δ −163.23. HRMS (ESI) calcd for $C_{21}H_{23}FN_2NaO_7^+$ $[M+Na]^+$457.1382, found 457.1385.

*4.1.2. (1aR,7aS,10aS,10bS,E)-5-(Azidomethyl)-1a-methyl-8-methylene-2,3,6,7,7a,8,10a,10b-octahydrooxireno[2′,3′:9,10] cyclodeca[1,2-b]furan-9(1aH)-one (**7**)*

To a solution of compound **5** (1.0 g, 3.80 mmol) in THF (37.6 mL) was added diphenylphosphorous azidophosphate (1.6 g, 5.70 mmol) and 1,8-diazabicyclo[rho]el-7-ene (0.9 g, 5.70 mmol) at 0 °C. The reaction mixture was stirred for 12 h at room temperature, and then filtered. The filtrate was evaporated in vacuo, and the

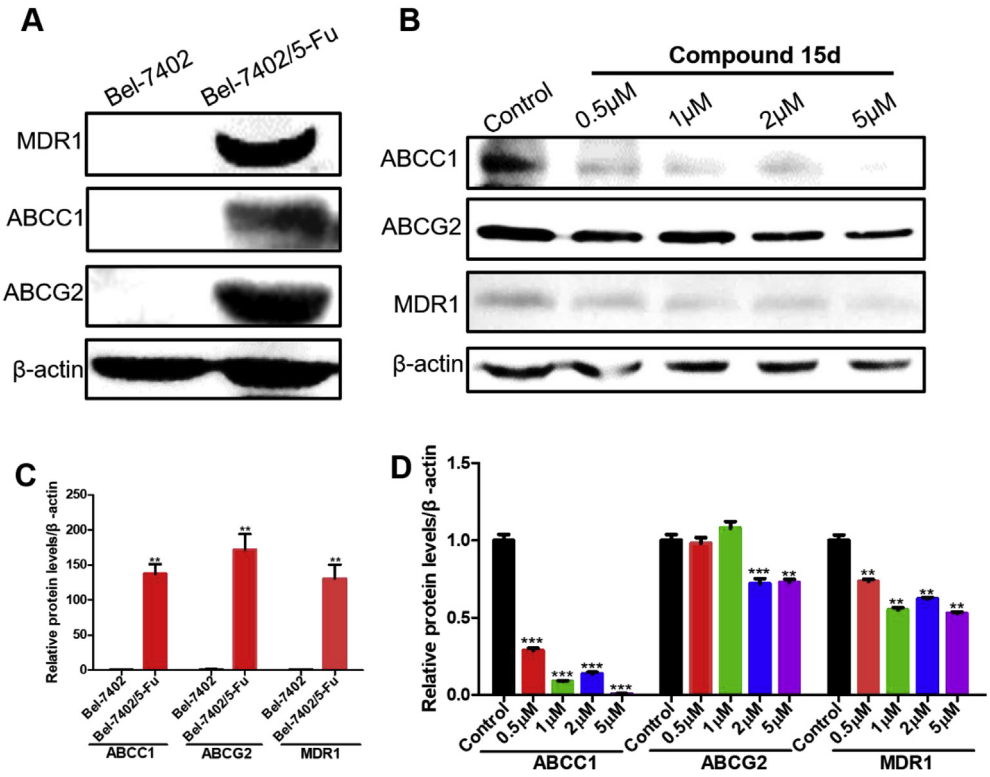


Fig. 4. Compound 15d reduced the expression level of MDR1, ABCC1 and ABCG2 in Bel-7402/5-FU cells. (A) The expression of MDR1, ABCC1 and ABCG2 in Bel7402 and Bel-7402/5-Fu cells. (B) The expression of MDR1, ABCC1 and ABCG2 in Bel-7402/5-Fu cells after the treatment of **15 d** at different concentrations. (C, D) The quantitative analysis of protein expression and statistical analysis. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

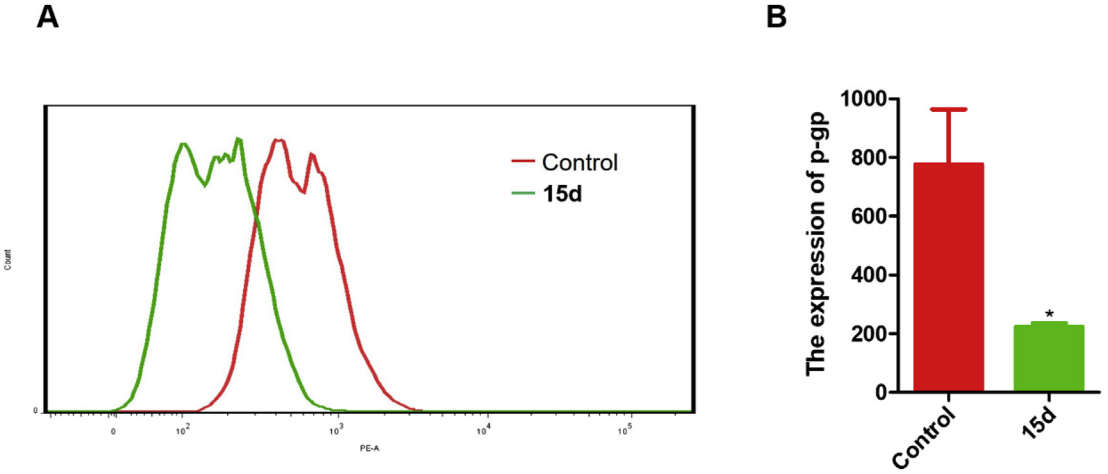


Fig. 5. Compound 15d inhibited the expression of p-gp. (A) The fluorescence intensity of p-gp with the treatment of **15 d** at a concentration of 0.5 μM in Bel-7402/5-FU cells. (B) The statistics of the fluorescence intensity after the treatment of **15d** in Bel7402/5-FU cells. $^{*}P < 0.05$.

resulting crude product was further purified by column chromatography on silica gel to yield compound **7** was obtained as white ceraceous solid 750.0 mg. Yield 69%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.15 (d, $J = 3.2$ Hz, 1H), 5.61 (t, $J = 8.3$ Hz, 1H), 5.50 (d, $J = 2.6$ Hz, 1H), 3.83–3.73 (m, 2H), 3.64 (d, $J = 13.3$ Hz, 1H), 2.80–2.63 (m, 2H), 2.43–2.34 (m, 1H), 2.34–2.31 (m, 1H), 2.27 (dd, $J = 14.7$, 10.1 Hz, 2H), 2.22–2.14 (m, 1H), 2.10 (dd, $J = 12.8$, 6.0 Hz, 1H), 1.70–1.59 (m, 1H), 1.48 (s, 3H), 1.03 (t, $J = 13.0$ Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.2, 138.6, 134.6, 131.0, 120.2, 80.9, 63.2, 59.9, 55.4, 42.5, 36.5, 25.2, 24.1, 23.7, 17.8. HRMS (ESI) calcd for $C_{15}H_{19}N_3NaO_3$ $[M+Na]^+$ 312.1319, found 312.1322.

*4.1.3. 5-Fluoro-1-((1-(((1aR,7aS,10aS,10bS,E)-1a-methyl-8-methylene-9-oxo-1a,2,3,6,7,7a,8,9,10a,10b-decahydrooxireno [2′,3′:9,10]cyclodeca[1,2-b]furan-5-yl)methyl)-1H-1,2,3-triazol-4-yl)methyl)pyrimidine-2,4(1H,3H)-dione (**8**)*

To s stirred solution of compound **7** (117.7 mg, 0.70 mmol) in THF (0.8 mL) was added compound **1** (100.0 mg, 0.35 mmol), H_2O (0.5 mL) and Cu_2O-NPs (0.5 mL) under Ar at room temperature. After being stirred for overnight, the reaction mixture was washed with cold saturated NaCl and then water. The extract was dried over Na_2SO_4, filtered and evaporated in vacuo. The crude product was further purified by column chromatography on silica gel to give

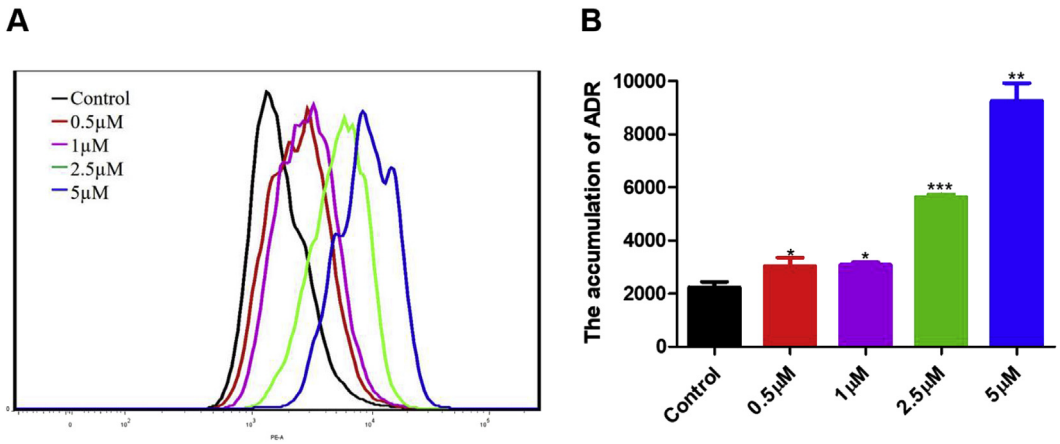


Fig. 6. Compound 15d could increase the accumulation of ADR in Bel-7402/5-FU cells. (A) The accumulation of ADR in Bel-7402/5-FU cells after the treatment of **15 d** at different concentrations of 0.5 μM, 1 μM, 2.5 μM, 5 μM, respectively. (B) The statistics of ADR accumulation in Bel-7402/5-FU cells after the treatment of **15 d** at different concentrations of 0.5 μM, 1 μM, 2.5 μM, 5 μM, respectively. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

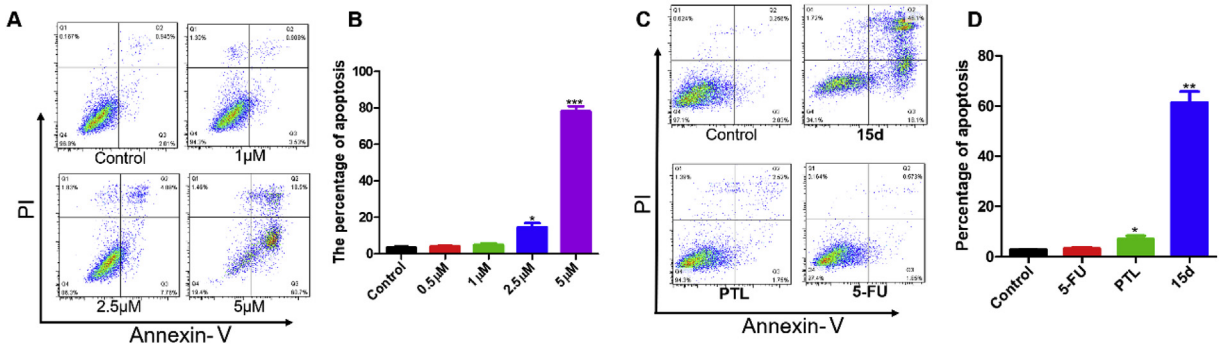


Fig. 7. Compound 15d induced the apoptosis of Bel-7402/5-FU cells. (A) The representative pictures of apoptosis after the treatment of **15 d** at different concentrations by flow cytometry. (B) The statistic of the apoptosis of Bel-7402/5-FU cells after the treatment of **15 d** at different concentrations by flow cytometry. (C) The representative pictures of apoptosis after the treatment of **15d,** PTL and 5-FU at 5 μM, respectively. (D) The statistic of the apoptosis of Bel-7402/5-FU cells after the treatment of **15d,** PTL and 5-FU at 5 μM, respectively. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

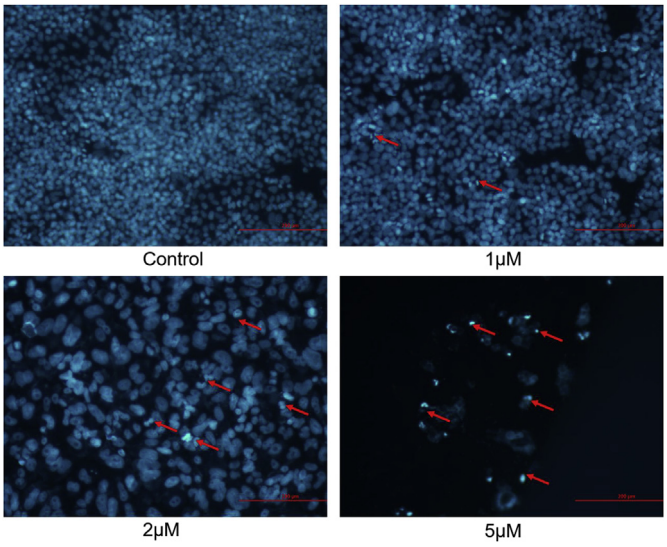


Fig. 8. The nuclear morphology of Bel-7402/5-FU cells after the treatment of 15 d at different concentrations of 1 μM, 2 μM and 5 μM.

compound **8** (colorless oil, 68 mg, yield 37%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (d, $J = 3.0$ Hz, 1H), 7.64 (dd, $J = 5.3$, 1.7 Hz, 1H), 6.25 (d, $J = 3.2$ Hz, 1H), 5.74 (t, $J = 8.3$ Hz, 1H), 5.63 (d, $J = 2.9$ Hz, 1H), 5.12 (d, $J = 14.5$ Hz, 1H), 4.99 (d, $J = 15.0$ Hz, 1H), 4.90 (d, $J = 15.0$ Hz, 1H), 4.77 (d, $J = 14.5$ Hz, 1H), 3.83 (t, $J = 9.3$ Hz, 1H), 2.85 (d, $J = 9.4$ Hz, 1H), 2.71 (t, $J = 8.7$ Hz, 1H), 2.61–2.38 (m, 2H), 2.37–2.14 (m, 3H), 1.99 (d, $J = 15.1$ Hz, 1H), 1.66 (t, $J = 12.5$ Hz, 1H), 1.52 (s, 3H), 1.13 (t, $J = 12.1$ Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.2, 159.8 (d, $J = 25.5$ Hz), 156.2, 148.6, 140.8, 138.4, 135.3 (d, $J = 378.5$ Hz), 131.8, 127.6 (d, $J = 33.1$ Hz), 122.7, 119.9, 79.8, 62.2, 58.9, 53.8, 42.4, 41.5, 35.4, 28.7, 23.0, 22.9, 16.9. ^{19}F NMR (376 MHz, $CDCl_3$) δ −163.60, −163.62. HRMS (ESI) calcd for $C_{22}H_{24}FN_5NaO_5^{+}$ $[M+Na]^{+}$ 480.1654, found 480.1658.

*4.1.4. 4-(((1aR,7aS,10aS,10bS,E)-1a-Methyl-8-methylene-9-oxo-1a,2,3,6,7,7a,8,9,10a,10b-decahydrooxireno[2′,3′:9,10]cyclodeca[1,2-b]furan-5-yl)methoxy)-4-oxobutanoic acid (**9**)*

A mixture of succinic anhydride (454.6 mg, 4.54 mmol), compound **5** (1 g, 3.78 mmol), and DMAP (462.5 mg, 3.78 mmol) was stirred in anhydrous DCM (50 mL) for 12 h at room temperature. The mixutre was adjusted to pH 3 with hydrochloric acid. The extract was dried over Na_2SO_4, filtered and the filtrate was evaporated in vacuo. The crude product was further purified by column chromatography on silica gel to afford compound **9** as white

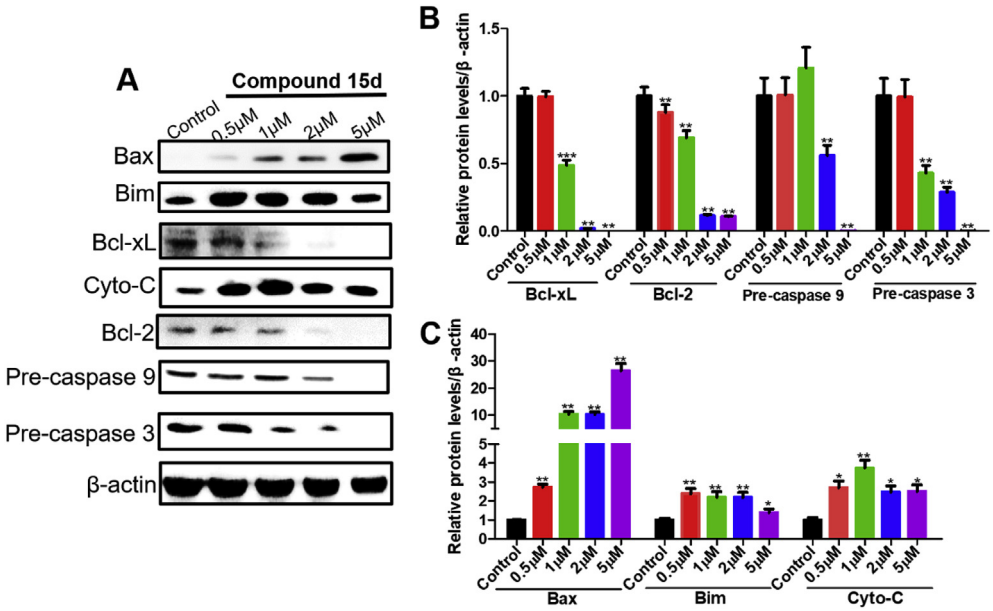


Fig. 9. The Western blot analysis of apoptosis related proteins of mitochondrial pathway in Bel-7402/5-FU cells with the treatment of 15 d at different concentrations. (A) The Western blot analysis of apoptosis related proteins. (B, C) The quantitative analysis of protein expression and statistical analysis. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

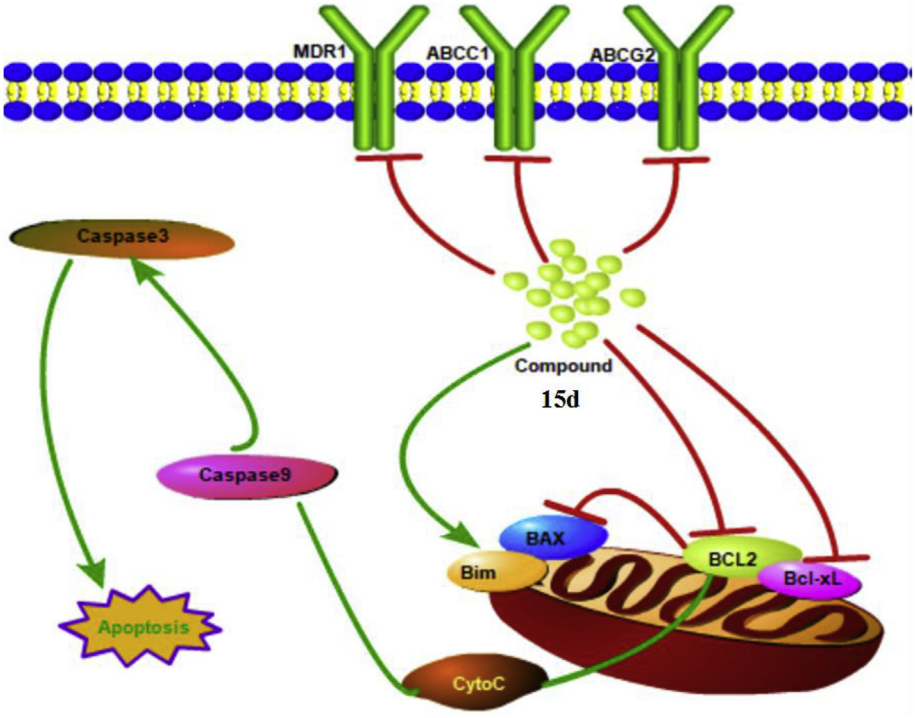


Fig. 10. The preliminary anti-HCC mechanism of compound **15d**.

amorphous solid (1.2 g, yield 88%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.23 (s, 1H), 5.69 (s, 1H), 5.55 (s, 1H), 4.69 (d, $J = 12.4$ Hz, 1H), 4.48 (d, $J = 12.3$ Hz, 1H), 3.85 (t, $J = 9.1$ Hz, 1H), 2.86 (t, $J = 10.6$ Hz, 2H), 2.65 (d, $J = 24.1$ Hz, 4H), 2.53−2.10 (m, 6H), 1.66 (t, $J = 12.1$ Hz, 1H), 1.54 (s, 3H), 1.10 (t, $J = 12.6$ Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.7, 172.0, 169.6, 138.9, 134.8, 130.9, 120.5, 81.2, 67.3, 63.4, 60.2, 42.8, 36.7, 28.9, 28.8, 25.8, 24.6, 23.9, 18.1. HRMS (ESI) calcd for $C_{19}H_{23}O_7^-$ $[M-H]^-$ 363.1449, found 363.1445.

*4.1.5. (5-Fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl (((1aR,7aS,10aS,10bS,E)-1a-methyl-8-methylene-9-oxo-1a,2,3,6,7,7a,8,9,10a,10b-decahydrooxireno[2′,3′:9,10]cyclodeca[1,2-b]furan-5-yl)methyl) succinate (**10**)*

To a stirred solution of compound **2** (122.0 mg, 0.75 mmol) in MeCN (2 mL), compound **9** (325.0 mg, 0.90 mmol), DCC (186.0 mg, 0.90 mmol) and DMAP (8.0 mg, 0.056 mmol) were added under Ar at room temperature. After being stirred for overnight, the reaction

mixture was washed with cold saturated NaCl and then water. The extract was dried over Na_2SO_4, filtered and evaporated in vacuo. The crude product was further purified by column chromatography on silica gel to provide compound **10** as white amorphous solid (130.0 mg, yield 44%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (d, J = 5.1 Hz, 1H), 6.22 (d, J = 2.3 Hz, 1H), 5.70 (t, J = 7.8 Hz, 1H), 5.64 (s, 2H), 5.56 (s, 1H), 4.67 (d, J = 12.5 Hz, 1H), 4.48 (d, J = 12.5 Hz, 1H), 3.85 (t, J = 9.3 Hz, 1H), 2.93 (t, J = 8.7 Hz, 1H), 2.86 (d, J = 9.4 Hz, 1H), 2.69 (d, J = 4.7 Hz, 2H), 2.63 (d, J = 4.3 Hz, 2H), 2.50–2.12 (m, 7H), 1.67 (t, J = 11.6 Hz, 1H), 1.54 (s, 3H), 0.87 (t, J = 6.6 Hz, 1H).^{13}C NMR (100 MHz, $CDCl_3$) δ 172.6, 171.9, 169.7, 157.1 (d, J = 27.0 Hz), 149.3, 140.2 (d, J = 239.5 Hz), 139.0, 134.7, 131.2, 128.6 (d, J = 33.8 Hz), 120.3, 81.1, 70.1, 67.4, 63.3, 60.1, 42.7, 36.6, 29.7, 28.8, 25.8, 24.8, 23.9, 18.1.^{19}F NMR (376 MHz, $CDCl_3$) δ −164.60. HRMS (ESI) calcd for $C_{24}H_{27}FN_2NaO_9$ $[M+Na]^+$ 529.1593, found 529.1596.

4.1.6. ((1aR,7aS,10aS,10bS,E)-1a-methyl-8-methylene-9-oxo-1a,2,3,6,7,7a,8,9,10a,10b-decahydrooxireno[2′,3′:9,10]cyclodeca[1,2-b]furan-5-yl)methyl 4-((6-(2-(5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetamido)hexyl)amino)-4-oxobutanoate (**11a**)

The protective Boc group of compound **4a** (98.3 mg, 0.27 mmol) were removed using trifluoroacetic acid (0.4 mL) in DCM (4 mL) for 3 h at room temperature. The reaction mixture was filtered and the filtrate was evaporated under reduced pressure. The product in dry dichloromethane (2 mL) was added compound **9** (137.7 mg, 0.18 mmol), DIPEA (94.1 μL,0.54 mmol), EDCI (52.3 mg,0.27 mmol) and HOBt (36.0 mg, 0.27 mmol). The reaction mixture was stirred for 12 h at room temperature, filtered, and evaporated under reduced pressure. The crude product was further purified by column chromatography on silica gel to yield compound **11a** as white ceraceous solid (50 mg, yield 44%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.82 (s, 1H), 8.10 (s, 1H), 8.00 (d, J = 4.2 Hz, 1H), 7.81 (s, 1H), 6.03 (s, 1H), 5.65 (s, 1H), 5.57 (s, 1H), 4.58 (d, J = 11.7 Hz, 1H), 4.38 (d, J = 12.8 Hz, 1H), 4.23 (s, 2H), 4.08 (t, J = 8.3 Hz, 1H), 3.16 (s, 1H), 3.11–2.91 (m, 5H), 2.83 (d, J = 9.0 Hz, 1H), 2.41–2.00 (m, 8H), 1.61 (t, J = 10.3 Hz, 1H), 1.46 (s, 3H), 1.36 (s, 4H), 1.23 (s, 5H), 0.92 (t, J = 12.3 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.3, 170.4, 169.5, 166.2, 157.6 (d, J = 25.6 Hz), 149.7, 140.4, 139.3 (d, J = 228.1 Hz), 135.0, 131.1 (d, J = 33.9 Hz), 128.9, 119.4, 80.7, 66.4, 62.7, 60.0, 49.7, 48.6, 41.8, 38.6, 38.5, 36.3, 29.8, 29.1, 29.0, 26.1, 26.0, 24.7, 23.8, 23.2, 17.5. ^{19}F NMR (376 MHz, $CDCl_3$) δ −163.85. HRMS (ESI) calcd for $C_{31}H_{41}FN_4NaO_9$ $[M+Na]^+$ 655.2750, found 655.2755.

4.1.7. ((1aR,7aS,10aS,10bS,E)-1a-Methyl-8-methylene-9-oxo-1a,2,3,6,7,7a,8,9,10a,10b-decahydrooxireno[2′,3′:9,10]cyclodeca[1,2-b]furan-5-yl)methyl 4-(4-(2-(5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetyl)piperazin-1-yl)-4-oxobutanoate (**11b**)

The Boc group of compound **4b** (84 mg, 0.24 mmol) was removed using trifluoroacetic acid (0.4 mL) in DCM (4 mL) for 3 h at room temperature. The reaction mixture was filtered and evaporated under reduced pressure.The product in dry dichloromethane (2 mL) was added compound **9** (131.1 mg, 0.36 mmol), DIPEA (125 μL,0.72 mmol), EDCI (69 mg,0.36 mmol) and HOBt (48 mg, 0.36 mmol). The reaction mixture was stirred for 12 h at room temperature, filtered and evaporated in vacuo. The crude product was further purified by column chromatography on silica gel to yield compound **11b** as white ceraceous solid (70 mg, yield 50%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (d, J = 4.1 Hz, 1H), 6.17 (d, J = 2.8 Hz, 1H), 5.66 (d, J = 7.5 Hz, 1H), 5.57 (d, J = 2.4 Hz, 1H), 4.70–4.54 (m, 3H), 4.55–4.46 (m, 1H), 3.84 (t, J = 9.3 Hz, 1H), 3.79–3.39 (m, 8H), 3.04 (s, 1H), 2.89–2.82 (m, 1H), 2.63 (s, 4H), 2.40 (d, J = 6.6 Hz, 2H), 2.30–2.09 (m, 4H), 1.69–1.60 (m, 1H), 1.51 (s, 3H), 0.85 (t, J = 6.6 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.1, 170.3, 169.9, 165.2, 157.7 (d, J = 26.0 Hz), 150.1 (d, J = 4.5 Hz), 140.3 (d, J = 235.8 Hz), 139.1, 134.8, 130.1 (d, J = 33.5 Hz), 129.8, 120.4, 81.4, 81.3, 66.9, 63.2, 60.3, 42.6, 42.1, 41.4, 36.6, 32.0, 27.8, 25.8, 24.7, 23.9, 18.1. ^{19}F NMR (376 MHz, $CDCl_3$) δ −163.8. HRMS (ESI) calcd for $C_{29}H_{35}FN_4NaO_9^+$ $[M+Na]^+$ 625.2280, found 625.2283.

4.1.8. General procedure for synthesis of compounds **12**, **13a**, **13b**, **14a**, **14b**, and **15a**–**15o**

To s stirred solution of compound **6**, **8**, **11a** or **11b** (0.05 mmol) in DMF (1 mL) was added different substituted benzyl bromide (13.7 μL, 0.12 mmol) and K_2CO_3 (0.18 mmol) at room temperature. After stirring for 6 h, the reaction mixture was washed with cold saturated NH_4Cl and then water. The extract was dried over Na_2SO_4, filtered and the filtrate was evaporated in vacuo. The resulting crude product was further purified by column chromatography on silica gel to provide desired compounds.

4.1.8.1. 1((1aR,7aS,10aS,10bS,E)-1a-methyl-8-methylene-9-oxo-1a,2,3,6,7,7a,8,9,10a,10b-decahydrooxireno[2′,3′:9,10]cyclodeca[1,2-b]furan-5-yl)methyl 2-(3-benzyl-5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetate (**12**).

Yield 78%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (dd, J = 7.7, 1.4 Hz, 2H), 7.29 (d, J = 7.4 Hz, 2H), 7.24 (d, J = 4.9 Hz, 1H), 6.22 (d, J = 3.5 Hz, 1H), 6.22 (d, J = 3.5 Hz, 1H), 5.71 (t, J = 7.9 Hz, 1H), 5.55 (d, J = 3.1 Hz, 1H), 5.11 (s, 2H), 4.79 (d, J = 12.2 Hz, 1H), 4.57–4.47 (m, 1H), 4.40 (t, J = 15.4 Hz, 2H), 3.83 (t, J = 9.3 Hz, 1H), 2.81 (d, J = 9.4 Hz, 2H), 2.42–2.13 (m, 6H), 1.68–1.59 (m, 1H), 1.53 (s, 3H), 1.09 (t, J = 12.3 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.4, 167.8, 167.1, 157.3 (d, J = 25.6 Hz), 150.1, 140.4 (d, J = 237.1 Hz), 138.7, 135.9, 134.2, 132.4, 131.1, 129.3, 129.0, 128.7, 126.9 (d, J = 33.2 Hz), 120.6, 81.1, 71.9, 68.6, 63.4, 50.3, 45.4, 42.7, 36.6, 25.6, 24.4, 24.0, 19.3. ^{19}F NMR (376 MHz, $CDCl_3$) δ −163.37. HRMS (ESI) calcd for $C_{28}H_{29}FN_2NaO_7^+$ $[M+Na]^+$ 547.1851, found 547.1855.

4.1.8.2. 3-Benzyl-5-fluoro-1-((1-(((1aR,7aS,10aS,10bS,E)-1a-methyl-8-methylene-9-oxo-1a,2,3,6,7,7a,8,9,10a,10b-decahydrooxireno[2′,3′:9,10]cyclodeca[1,2-b]furan-5-yl)methyl)-1H-1,2,3-triazol-4-yl)methyl)pyrimidine-2,4(1H,3H)-dione (**13a**).

Yield 90%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (s, 1H), 7.58 (d, J = 5.0 Hz, 1H), 7.47–7.41 (m, 2H), 7.33–7.26 (m, 3H), 6.28 (d, J = 3.3 Hz, 1H), 5.76 (t, J = 8.0 Hz, 1H), 5.64 (d, J = 3.0 Hz, 1H), 5.15 (d, J = 14.4 Hz, 1H), 5.10 (d, J = 3.1 Hz, 2H), 4.98 (d, J = 15.0 Hz, 1H), 4.92 (d, J = 15.0 Hz, 1H), 4.69 (d, J = 14.4 Hz, 1H), 3.85 (t, J = 9.3 Hz, 1H), 2.84 (d, J = 9.4 Hz, 1H), 2.79–2.68 (m, 1H), 2.61–2.16 (m, 6H), 1.91 (d, J = 15.1 Hz, 1H), 1.53 (s, 3H), 1.18–1.10 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.2, 157.3 (d, J = 25.3 Hz), 150.0, 142.2, 140.4 (d, J = 236.6 Hz), 138.3, 136.1, 134.7, 133.1, 129.3, 128.6, 128.1, 126.7 (d, J = 33.0 Hz), 123.3, 121.1, 80.9, 63.4, 59.9, 55.0, 45.2, 44.2, 42.7, 36.6, 25.2, 24.2, 23.7, 18.0. ^{19}F NMR (376 MHz, $CDCl_3$) δ −163.17. HRMS (ESI) calcd for $C_{29}H_{30}FN_5NaO_5^+$ $[M+Na]^+$ 570.2123, found 570.2128.

4.1.8.3. 5-Fluoro-3-(4-fluorobenzyl)-1-((1-(((1aR,7aS,10aS,10bS,E)-1a-methyl-8-methylene-9-oxo-1a,2,3,6,7,7a,8,9,10a,10b-decahydrooxireno[2′,3′:9,10]cyclodeca[1,2-b]furan-5-yl)methyl)-1H-1,2,3-triazol-4-yl)methyl)pyrimidine-2,4(1H,3H)-dione (**13b**).

Yield 90%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (s, 1H), 7.59 (d, J = 5.0 Hz, 1H), 7.43 (dd, J = 8.4, 5.5 Hz, 2H), 6.95 (t, J = 8.6 Hz, 2H), 6.25 (d, J = 3.3 Hz, 1H), 5.74 (t, J = 8.1 Hz, 1H), 5.63 (d, J = 3.0 Hz, 1H), 5.14 (d, J = 14.5 Hz, 1H), 5.07 (d, J = 13.7 Hz, 1H), 5.02 (d, J = 13.9 Hz, 1H), 4.98 (d, J = 15.1 Hz, 1H), 4.92 (d, J = 15.0 Hz, 1H), 4.70 (d, J = 14.5 Hz, 1H), 3.84 (t, J = 9.3 Hz, 1H), 2.84 (d, J = 9.4 Hz, 1H), 2.80–2.67 (m, 1H), 2.59–2.14 (m, 6H), 1.63 (t, J = 12.0 Hz, 1H), 1.52 (s, 3H), 1.12 (t, J = 11.6 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.3, 163.7, 162.5 (d, J = 246.6 Hz), 157.2 (d, J = 25.2 Hz), 149.9, 142.0, 141.4, 140.2 (d, J = 236.4 Hz), 138.3, 131.9 (d, J = 3.2 Hz), 131.3 (d, J = 8.2 Hz), 126.9

(d, $J = 32.9$ Hz), 121.0, 115.5, 115.3 (d, $J = 21.4$ Hz), 80.9, 63.3, 59.9, 54.9, 44.5, 44.1, 42.5, 36.5, 29.7, 24.1, 23.7, 17.9. ^{19}F NMR (376 MHz, $CDCl_3$) δ −113.92, −163.32. HRMS (ESI) calcd for $C_{29}H_{29}F_2N_5NaO_5$ $[M+Na]^+$ 588.2029, found 588.2032.

*4.1.8.4. ((1aR,7aS,10aS,10bS,E)-1a-methyl-8-methylene-9-oxo-1a,2,3,6,7,7a,8,9,10a,10b-decahydrooxireno[2′,3′:9,10]cyclodeca[1,2-b]furan-5-yl)methyl 4-((6-(2-(3-benzyl-5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetamido)hexyl)amino)-4-oxobutanoate (**14a**).* Yield 78%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 (d, $J = 7.2$ Hz, 2H), 7.38 (d, $J = 4.9$ Hz, 1H), 7.28 (d, $J = 6.7$ Hz, 1H), 7.23 (d, $J = 6.9$ Hz, 1H), 6.72 (t, $J = 4.4$ Hz, 1H), 6.20 (d, $J = 1.5$ Hz, 1H), 5.86 (t, $J = 4.6$ Hz, 1H), 5.63 (t, $J = 7.9$ Hz, 1H), 5.58 (s, 1H), 5.10 (s, 2H), 4.58 (d, $J = 12.8$ Hz, 1H), 4.51 (d, $J = 12.8$ Hz, 1H), 4.38−4.27 (m, 2H), 3.85 (t, $J = 9.3$ Hz, 1H), 3.26−3.16 (m, 4H), 3.02 (t, $J = 9.9$ Hz, 1H), 2.85 (d, $J = 9.4$ Hz, 1H), 2.62 (d, $J = 6.3$ Hz, 2H), 2.47−2.40 (m, 3H), 2.38−2.10 (m, 5H), 1.65 (t, $J = 11.7$ Hz, 1H), 1.52 (s, 3H), 1.50−1.41 (m, 4H), 1.29 (s, 3H), 1.09 (t, $J = 12.7$ Hz, 1H).^{13}C NMR (100 MHz, $CDCl_3$) δ 173.0, 171.4, 170.0, 166.2, 157.5 (d, $J = 25.3$ Hz), 150.3, 140.1 (d, $J = 233.4$ Hz), 136.1, 134.6, 129.6, 129.1, 128.6, 128.1, 127.9 (d, $J = 33.0$ Hz), 120.6, 81.4, 66.8, 63.2, 60.3, 51.4, 45.2, 42.6, 39.3, 38.9, 36.7, 30.7, 29.4, 29.4, 28.9, 25.9, 25.6, 25.6, 24.7, 23.8, 18.1. ^{19}F NMR (376 MHz, $CDCl_3$) δ −164.56. HRMS (ESI) calcd for $C_{38}H_{47}FN_4NaO_9^+$ $[M+Na]^+$ 745.3219, found 745.3223.

*4.1.8.5. ((1aR,7aS,10aS,10bS,E)-1a-methyl-8-methylene-9-oxo-1a,2,3,6,7,7a,8,9,10a,10b-decahydrooxireno[2′,3′:9,10]cyclodeca[1,2-b]furan-5-yl)methyl 4-(4-(2-(3-benzyl-5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetyl)piperazin-1-yl)-4-oxobutanoate (**14b**).* Yield 55%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (d, $J = 7.0$ Hz, 2H), 7.31 (d, $J = 6.1$ Hz, 2H), 7.28 (d, $J = 2.8$ Hz, 1H), 6.22 (d, $J = 3.4$ Hz, 1H), 5.75−5.61 (m, 1H), 5.57 (d, $J = 3.0$ Hz, 1H), 5.18−5.06 (m, 2H), 4.68−4.43 (m, 4H), 3.84 (t, $J = 9.4$ Hz, 1H), 3.78−3.39 (m, 8H), 3.12 (t, $J = 9.9$ Hz, 1H), 2.86 (t, $J = 8.2$ Hz, 1H), 2.73−2.54 (m, 4H), 2.49−2.11 (m, 6H), 1.68 (d, $J = 10.0$ Hz, 2H), 1.53 (s, 3H), 1.19−1.10 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.9, 170.1, 169.7, 164.9, 157.4 (d, $J = 25.2$ Hz), 150.3, 140.0 (d, $J = 234.3$ Hz), 139.0, 136.0, 134.7, 129.8, 129.7, 128.7 (d, $J = 33.2$ Hz), 128.2, 127.9, 120.3, 81.3, 66.7, 63.1, 60.2, 49.3, 45.2, 44.8, 44.6, 42.5, 41.9, 41.3, 36.6, 29.1, 27.7, 25.8, 24.6, 23.8, 18.0. ^{19}F NMR (376 MHz, $CDCl_3$) δ −164.69. HRMS (ESI) calcd for $C_{36}H_{41}FN_4NaO_9^+$ $[M+Na]^+$ 715.2750, found 715.2753.

*4.1.8.6. ((1aR,7aS,10aS,10bS,E)-1a-methyl-8-methylene-9-oxo-1a,2,3,6,7,7a,8,9,10a,10b-decahydrooxireno[2′,3′:9,10]cyclodeca[1,2-b]furan-5-yl)methyl 2-(5-fluoro-3-(4-fluorobenzyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetate (**15a**).* Yield 64%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (dd, $J = 5.7$, 3.3 Hz, 1H), 7.53 (dd, $J = 5.7$, 3.3 Hz, 1H), 7.45 (dd, $J = 8.6$, 5.4 Hz, 1H), 7.23 (d, $J = 4.9$ Hz, 1H), 6.98 (t, $J = 8.7$ Hz, 2H), 6.23 (d, $J = 3.5$ Hz, 1H), 5.72 (t, $J = 7.9$ Hz, 1H), 5.55 (d, $J = 3.1$ Hz, 1H), 4.79 (d, $J = 12.2$ Hz, 1H), 4.54 (d, $J = 12.3$ Hz, 1H), 4.45 (d, $J = 17.3$ Hz, 1H), 4.37 (d, $J = 17.3$ Hz, 1H), 4.08 (d, $J = 6.7$ Hz, 2H), 3.84 (t, $J = 9.3$ Hz, 1H), 2.91−2.78 (m, 2H), 2.44−2.13 (m, 6H), 1.54 (s, 3H), 1.09 (t, $J = 12.2$ Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.40, 167.08, 162.65 (d, $J = 246.8$ Hz), 157.22 (d, $J = 25.4$ Hz), 150.10, 140.38 (d, $J = 237.1$ Hz), 138.89, 134.14, 132.59, 131.75 (d, $J = 3.3$ Hz), 131.41 (d, $J = 8.2$ Hz), 126.90 (d, $J = 33.3$ Hz), 120.42, 115.52 (d, $J = 21.5$ Hz), 81.07, 68.68, 63.39, 60.00, 50.32, 44.68, 42.77, 36.64, 25.73, 24.52, 24.04, 18.09. ^{19}F NMR (376 MHz, $CDCl_3$) δ −113.80, −113.84, −163.42, −163.46. HRMS (ESI) calcd for $C_{28}H_{28}F_2N_2NaO_7$ $[M+Na]^+$ 565.1757, found 565.1760.

*4.1.8.7. ((1aR,7aS,10aS,10bS,E)-1a-methyl-8-methylene-9-oxo-1a,2,3,6,7,7a,8,9,10a,10b-decahydrooxireno[2′,3′:9,10]cyclodeca[1,2-b]furan-5-yl)methyl 2-(3-(4-chlorobenzyl)-5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetate (**15b**).* Yield 54%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (d, $J = 8.4$ Hz, 2H), 7.32−7.27 (m, 3H), 6.23 (d, $J = 3.4$ Hz, 1H), 5.73 (t, $J = 7.9$ Hz, 1H), 5.56 (d, $J = 3.1$ Hz, 1H), 5.08 (s, 2H), 4.80 (d, $J = 12.2$ Hz, 1H), 4.55 (d, $J = 12.2$ Hz, 1H), 4.43 (q, $J = 17.3$ Hz, 2H), 3.85 (t, $J = 9.3$ Hz, 1H), 2.92−2.78 (m, 2H), 2.49−2.11 (m, 6H), 1.65 (dd, $J = 14.4$, 11.9 Hz, 1H), 1.55 (s, 3H), 1.09 (t, $J = 12.3$ Hz, 1H).^{13}C NMR (100 MHz, $CDCl_3$) δ 169.5, 167.1, 157.2 (d, $J = 25.4$ Hz), 150.0, 140.2 (d, $J = 236.5$ Hz), 138.8, 134.4, 134.1, 134.0, 132.4, 130.8, 128.8, 127.3 (d, $J = 33.2$ Hz), 120.5, 81.1, 68.7, 63.3, 60.1, 50.3, 44.6, 42.7, 36.6, 25.7, 24.5, 24.0, 18.1.^{19}F NMR (376 MHz, $CDCl_3$) δ −163.57. HRMS (ESI) calcd for $C_{28}H_{28}ClFN_2NaO_7^+$ $[M+Na]^+$ 581.1461, found 581.14.

*4.1.8.8. ((1aR,7aS,10aS,10bS,E)-1a-methyl-8-methylene-9-oxo-1a,2,3,6,7,7a,8,9,10a,10b-decahydrooxireno[2′,3′:9,10]cyclodeca[1,2-b]furan-5-yl)methyl 2-(3-(4-bromobenzyl)-5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetate (**15c**).* Yield 45%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 (d, $J = 8.4$ Hz, 2H), 7.32 (d, $J = 8.4$ Hz, 2H), 7.28 (d, $J = 5.0$ Hz, 1H), 6.21 (d, $J = 3.4$ Hz, 1H), 5.70 (t, $J = 7.9$ Hz, 1H), 5.54 (d, $J = 3.1$ Hz, 1H), 5.04 (s, 2H), 4.78 (d, $J = 12.2$ Hz, 1H), 4.52 (d, $J = 12.3$ Hz, 1H), 4.41 (q, $J = 17.3$ Hz, 2H), 3.83 (t, $J = 9.3$ Hz, 1H), 2.88−2.75 (m, 2H), 2.47−2.06 (m, 6H), 1.68−1.57 (m, 1H), 1.53 (s, 3H), 1.07 (t, $J = 12.4$ Hz, 1H).^{13}C NMR (100 MHz, $CDCl_3$) δ 169.5, 167.1, 157.2 (d, $J = 25.4$ Hz), 150.0, 140.2 (d, $J = 236.4$ Hz), 138.8, 134.9, 134.1, 132.5, 131.7, 131.1, 127.3 (d, $J = 33.3$ Hz), 122.2, 120.5, 81.1, 68.6, 63.3, 60.1, 50.3, 44.7, 42.7, 36.6, 25.6, 24.5, 24.0, 18.1.^{19}F NMR (376 MHz, $CDCl_3$) δ −163.60. HRMS (ESI) calcd for $C_{28}H_{28}BrFN_2NaO_7^+$ $[M+Na]^+$ 625.0956, found 625.0959.

*4.1.8.9. ((1aR,7aS,10aS,10bS,E)-1a-methyl-8-methylene-9-oxo-1a,2,3,6,7,7a,8,9,10a,10b-decahydrooxireno[2′,3′:9,10]cyclodeca[1,2-b]furan-5-yl)methyl 2-(5-fluoro-2,4-dioxo-3-(4-(trifluoromethyl) benzyl)-3,4-dihydropyrimidin-1(2H)-yl)acetate (**15d**).* Yield 63%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 (s, 4H), 7.22 (d, $J = 4.8$ Hz, 1H), 6.25 (d, $J = 3.5$ Hz, 1H), 5.74 (t, $J = 8.0$ Hz, 1H), 5.55 (d, $J = 3.1$ Hz, 1H), 5.17 (s, 2H), 4.80 (d, $J = 12.2$ Hz, 1H), 4.55 (d, $J = 12.2$ Hz, 1H), 4.41 (q, $J = 17.3$ Hz, 2H), 3.85 (t, $J = 9.3$ Hz, 1H), 2.89−2.79 (m, 2H), 2.50−2.11 (m, 6H), 1.58 (s, 1H), 1.55 (s, 3H), 0.96 (t, $J = 7.4$ Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.4, 167.1, 157.2 (d, $J = 25.5$ Hz), 150.0, 140.2 (d, $J = 237.3$ Hz), 139.7, 138.9, 134.1, 132.4, 131.1, 129.5, 129.0, 127.2 (d, $J = 33.2$ Hz), 125.6 (d, $J = 3.8$ Hz), 120.4, 81.0, 68.7, 63.3, 60.0, 50.3, 44.9, 42.7, 36.6, 25.7, 24.5, 24.0, 18.1. ^{19}F NMR (376 MHz, $CDCl_3$) δ −62.60, −163.10. HRMS (ESI) calcd for $C_{29}H_{28}F_4N_2NaO_7$ $[M+Na]^+$ 615.1725, found 615.1728.

*4.1.8.10. ((1aR,7aS,10aS,10bS,E)-1a-methyl-8-methylene-9-oxo-1a,2,3,6,7,7a,8,9,10a,10b-decahydrooxireno[2′,3′:9,10]cyclodeca[1,2-b]furan-5-yl)methyl 2-(5-fluoro-3-(4-nitrobenzyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetate (**15e**).* Yield 60%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (d, $J = 7.5$ Hz, 2H), 7.59 (d, $J = 7.6$ Hz, 2H), 7.28 (d, $J = 4.8$ Hz, 1H), 6.22 (s, 1H), 5.74 (t, $J = 7.8$ Hz, 1H), 5.54 (s, 1H), 5.25−5.13 (m, 2H), 4.80 (d, $J = 12.3$ Hz, 1H), 4.55 (d, $J = 12.3$ Hz, 1H), 4.46 (d, $J = 17.3$ Hz, 1H), 4.37 (d, $J = 17.2$ Hz, 1H), 2.88 (s, 1H), 2.81 (d, $J = 9.4$ Hz, 1H), 2.49−2.13 (m, 7H), 1.72−1.65 (m, 1H), 1.54 (s, 3H), 1.08 (t, $J = 12.6$ Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.5, 167.1, 157.1 (d, $J = 25.5$ Hz), 148.9 (d, $J = 228.4$ Hz), 142.9, 139.0, 134.0, 132.8, 131.1, 130.1, 127.4 (d, $J = 33.2$ Hz), 124.0, 120.3, 81.1, 68.9, 65.8, 63.3, 60.1, 50.4, 44.6, 42.7, 36.6, 25.8, 24.8, 24.0, 18.1.^{19}F NMR (376 MHz, $CDCl_3$) δ −163.20. HRMS (ESI) calcd for $C_{28}H_{28}FN_3NaO_9^+$ $[M+Na]^+$ 592.1702, found 592.1706.

*4.1.8.11. ((1aR,7aS,10aS,10bS,E)-1a-methyl-8-methylene-9-oxo-1a,2,3,6,7,7a,8,9,10a,10b-decahydrooxireno[2′,3′:9,10]cyclodeca[1,2-b]furan-5-yl)methyl 2-(5-fluoro-3-(3-fluorobenzyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetate (**15f**).* Yield 60%. ^{1}H NMR

(400 MHz, $CDCl_3$) δ 7.29 (d, $J = 7.8$ Hz, 1H), 7.23 (dd, $J = 8.7$, 6.3 Hz, 2H), 7.13 (d, $J = 9.6$ Hz, 1H), 7.01–6.90 (m, 1H), 6.23 (d, $J = 3.5$ Hz, 1H), 5.72 (t, $J = 7.9$ Hz, 1H), 5.55 (d, $J = 3.1$ Hz, 1H), 5.10 (s, 2H), 4.80 (d, $J = 12.2$ Hz, 1H), 4.54 (d, $J = 12.2$ Hz, 1H), 4.42 (q, $J = 17.3$ Hz, 2H), 3.84 (t, $J = 9.3$ Hz, 1H), 2.82 (t, $J = 7.1$ Hz, 2H), 2.47–2.11 (m, 6H), 1.71–1.64 (m, 1H), 1.54 (s, 3H), 1.09 (t, $J = 12.1$ Hz, 1H).^{13}C NMR (100 MHz, $CDCl_3$) δ 169.4, 167.0, 162.8 (d, $J = 246.1$ Hz), 157.1 (d, $J = 25.5$ Hz), 150.0, 140.2 (d, $J = 236.8$ Hz), 138.7, 138.1 (d, $J = 7.4$ Hz), 132.3, 130.1 (d, $J = 8.2$ Hz), 127.1 (d, $J = 33.2$ Hz), 124.9, 120.6, 116.0 (d, $J = 22.0$ Hz), 115.1 (d, $J = 20.9$ Hz), 81.0, 68.6, 63.3, 60.1, 50.3, 44.7, 42.7, 36.5, 29.8, 25.6, 24.3, 23.9, 18.0.^{19}F NMR (376 MHz, $CDCl_3$) δ −112.70, −163.41. HRMS (ESI) calcd for $C_{28}H_{28}F_2N_2NaO_7$ $[M+Na]^+$565.1757, found 565.176.

*4.1.8.12. ((1aR,7aS,10aS,10bS,E)-1a-methyl-8-methylene-9-oxo-1a,2,3,6,7,7a,8,9,10a,10b-decahydrooxireno[2′,3′:9,10]cyclodeca[1,2-b]furan-5-yl)methyl 2-(5-fluoro-3-(2-fluorobenzyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetate (**15g**).* Yield 56%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.28 (d, $J = 6.4$ Hz, 1H), 7.37–7.27 (m, 1H), 7.23–7.15 (m, 1H), 7.15–7.05 (m, 2H), 6.02 (d, $J = 3.4$ Hz, 1H), 5.67–5.60 (m, 1H), 5.58 (d, $J = 3.1$ Hz, 1H), 5.13–4.98 (m, 2H), 4.73 (d, $J = 12.5$ Hz, 1H), 4.66–4.54 (m, 2H), 4.51 (d, $J = 12.6$ Hz, 1H), 4.09 (t, $J = 9.3$ Hz, 1H), 3.04–2.91 (m, 1H), 2.83 (d, $J = 9.5$ Hz, 1H), 2.34–2.20 (m, 3H), 2.16–2.02 (m, 3H), 1.60 (t, $J = 10.4$ Hz, 1H), 1.47 (s, 3H), 0.95–0.86 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.4, 167.5, 159.9 (d, $J = 245.0$ Hz), 156.6 (d, $J = 28.7$ Hz), 149.7, 140.0, 139.6, 137.7, 134.4, 130.0, 129.6 (d, $J = 39.4$ Hz), 128.3, 124.4, 123.0 (d, $J = 13.9$ Hz), 119.3, 115.3 (d, $J = 20.6$ Hz), 80.6, 67.8, 62.6, 59.9, 49.9, 41.7, 38.3, 36.2, 24.4, 23.6, 23.2, 17.4. ^{19}F NMR (376 MHz, DMSO-d_6) δ −118.15, −167.69. HRMS (ESI) calcd for $C_{28}H_{28}F_2N_2NaO_7^+$ $[M+Na]^+$ 565.1757, found 565.1761.

*4.1.8.13. ((1aR,7aS,10aS,10bS,E)-1a-methyl-8-methylene-9-oxo-1a,2,3,6,7,7a,8,9,10a,10b-decahydrooxireno[2′,3′:9,10]cyclodeca[1,2-b]furan-5-yl)methyl 2-(3-(2,4-difluorobenzyl)-5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetate (**15h**).* Yield 73%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33–7.27 (m, 1H), 7.25 (s, 1H), 6.89–6.73 (m, 2H), 6.23 (d, $J = 3.5$ Hz, 1H), 5.72 (t, $J = 7.9$ Hz, 1H), 5.56 (d, $J = 3.1$ Hz, 1H), 5.16 (s, 2H), 4.78 (d, $J = 12.2$ Hz, 1H), 4.55 (d, $J = 12.3$ Hz, 1H), 4.43 (q, $J = 17.3$ Hz, 2H), 3.84 (t, $J = 9.3$ Hz, 1H), 2.87–2.75 (m, 2H), 2.48–2.14 (m, 6H), 1.71–1.64 (m, 1H), 1.54 (s, 3H), 1.09 (t, $J = 12.2$ Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.4, 167.1, 162.6 (dd, $J = 249.1$, 12.1 Hz), 161.0 (dd, $J = 251.2$, 12.1 Hz), 157.2 (d, $J = 25.5$ Hz), 149.9, 140.2 (d, $J = 237.2$ Hz), 138.7, 134.1, 132.4, 131.2 (dd, $J = 14.0$, 4.3 Hz), 127.2 (d, $J = 33.3$ Hz), 120.6, 118.8 (dd, $J = 14.7$, 3.8 Hz), 111.5 (dd, $J = 21.2$, 3.8 Hz), 104.1 (t, $J = 25.5$ Hz), 81.1, 68.7, 63.3, 60.1, 50.3, 42.7, 38.7 (d, $J = 3.6$ Hz), 36.6, 25.7, 24.4, 24.0, 18.1. ^{19}F NMR (376 MHz, $CDCl_3$) δ −110.18, −112.37. HRMS (ESI) calcd for $C_{28}H_{27}F_3N_2NaO_7$ $[M+Na]^+$ 583.1663, found 583.1668.

*4.1.8.14. ((1aR,7aS,10aS,10bS,E)-1a-methyl-8-methylene-9-oxo-1a,2,3,6,7,7a,8,9,10a,10b-decahydrooxireno[2′,3′:9,10]cyclodeca[1,2-b]furan-5-yl)methyl 2-(3-(2,6-difluorobenzyl)-5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetate (**15i**).* Yield 64%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25–7.15 (m, 2H), 6.83 (t, $J = 7.7$ Hz, 2H), 6.19 (s, 1H), 5.68 (t, $J = 7.8$ Hz, 1H), 5.54 (s, 2H), 5.22 (s, 1H), 4.74 (d, $J = 12.3$ Hz, 1H), 4.52 (d, $J = 12.3$ Hz, 1H), 4.41 (q, $J = 17.3$ Hz, 2H), 3.81 (t, $J = 9.1$ Hz, 1H), 2.79 (d, $J = 9.4$ Hz, 2H), 2.48–2.06 (m, 6H), 1.64 (t, $J = 12.2$ Hz, 1H), 1.51 (s, 3H), 1.07 (t, $J = 12.6$ Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.5, 167.1, 161.6 (dd, $J = 250.2$, 7.6 Hz), 156.8 (d, $J = 25.5$ Hz), 149.7, 140.1 (d, $J = 236.3$ Hz), 138.6, 134.1, 132.0, 129.7 (t, $J = 10.4$ Hz), 127.2 (d, $J = 33.3$ Hz), 120.7, 111.5 (d, $J = 25.2$ Hz), 111.5 (d, $J = 9.6$ Hz), 81.1, 68.5, 63.3, 60.1, 50.2, 42.7, 36.6, 34.6 (t, $J = 4.0$ Hz), 34.8–34.4 (m), 25.6, 24.3, 24.0, 18.1. ^{19}F NMR (376 MHz, $CDCl_3$) δ −114.09, −163.73. HRMS (ESI) calcd for $C_{28}H_{27}F_3N_2NaO_7^+$ $[M+Na]^+$ 583.1663, found 583.1667.

*4.1.8.15. ((1aR,7aS,10aS,10bS,E)-1a-methyl-8-methylene-9-oxo-1a,2,3,6,7,7a,8,9,10a,10b-decahydrooxireno[2′,3′:9,10]cyclodeca[1,2-b]furan-5-yl)methyl 2-(3-(3,4-difluorobenzyl)-5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetate (**15j**).* Yield 76%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.22 (d, $J = 8.6$ Hz, 2H), 7.14 (s, 1H), 7.09–6.97 (m, 1H), 6.16 (s, 1H), 5.66 (d, $J = 7.8$ Hz, 1H), 5.50 (s, 1H), 4.99 (s, 2H), 4.75 (d, $J = 12.1$ Hz, 1H), 4.49 (d, $J = 12.2$ Hz, 1H), 4.45–4.29 (m, 2H), 3.79 (t, $J = 9.4$ Hz, 1H), 2.77 (t, $J = 10.3$ Hz, 2H), 2.43–2.05 (m, 6H), 1.61 (t, $J = 11.4$ Hz, 1H), 1.48 (s, 3H), 1.03 (t, $J = 12.5$ Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.5, 167.1, 157.1 (d, $J = 25.5$ Hz), 150.2 (dd, $J = 248.4$, 6.7 Hz), 150.1 (dd, $J = 248.3$, 6.8 Hz), 150.0, 140.2 (d, $J = 236.7$ Hz), 138.8, 134.1, 132.7 (dd, $J = 5.5$, 4.1 Hz), 132.5, 127.3 (d, $J = 33.3$ Hz), 125.8 (dd, $J = 6.5$, 3.7 Hz), 120.5, 118.5 (d, $J = 17.5$ Hz), 117.3 (d, $J = 17.3$ Hz), 81.1, 68.7, 63.3, 60.1, 50.3, 44.3, 42.7, 36.5, 25.6, 24.5, 24.0, 18.0. ^{19}F NMR (376 MHz, $CDCl_3$) δ −137.31, −138.44, −163.49.HRMS (ESI) calcd for $C_{28}H_{27}F_3N_2NaO_7^+$ $[M+Na]^+$ 583.1663, found 583.1666.

*4.1.8.16. ((1aR,7aS,10aS,10bS,E)-1a-methyl-8-methylene-9-oxo-1a,2,3,6,7,7a,8,9,10a,10b-decahydrooxireno[2′,3′:9,10]cyclodeca[1,2-b]furan-5-yl)methyl 2-(3-(2,5-difluorobenzyl)-5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetate (**15k**).* Yield 64%. ^{1}H NMR (400 MHz, CDCl3) δ 7.32 (d, $J = 4.9$ Hz, 1H), 7.08–6.96 (m, 1H), 6.96–6.84 (m, 2H), 6.21 (d, $J = 3.3$ Hz, 1H), 5.71 (t, $J = 7.8$ Hz, 1H), 5.55 (d, $J = 2.9$ Hz, 1H), 5.18 (s, 2H), 4.80 (d, $J = 12.2$ Hz, 1H), 4.49 (dt, $J = 47.0$, 14.8 Hz, 3H), 3.83 (t, $J = 9.3$ Hz, 1H), 2.82 (t, $J = 10.4$ Hz, 2H), 2.54–2.09 (m, 6H), 1.91 (d, $J = 9.4$ Hz, 1H), 1.53 (s, 3H), 1.33 (d, $J = 12.7$ Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.5, 167.1, 158.7 (dd, $J = 242.5$, 2.2 Hz), 157.1 (d, $J = 25.3$ Hz), 156.7 (dd, $J = 242.4$, 2.4 Hz), 150.0, 140.1 (d, $J = 237.0$ Hz), 138.6, 134.1, 132.3, 127.5 (d, $J = 33.3$ Hz), 124.4 (dd, $J = 17.0$, 7.8 Hz), 120.7, 116.7 (dd, $J = 24.6$, 8.6 Hz), 116.0 (dd, $J = 18.3$, 8.5 Hz), 115.7 (dd, $J = 14.7$, 3.9 Hz), 81.1, 68.6, 63.3, 60.1, 50.4, 42.7, 39.0 (d, $J = 3.8$ Hz), 36.6, 25.6, 24.3, 24.0, 18.1. ^{19}F NMR (376 MHz, CDCl3) δ −118.37, −123.16, −163.41.HRMS (ESI) calcd for $C_{28}H_{27}F_3N_2NaO_7$+ [M+Na]+ 583.1663, found 583.1668.

*4.1.8.17. ((1aR,7aS,10aS,10bS,E)-1a-methyl-8-methylene-9-oxo-1a,2,3,6,7,7a,8,9,10a,10b-decahydrooxireno[2′,3′:9,10]cyclodeca[1,2-b]furan-5-yl)methyl 2-(5-fluoro-3-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetate (**15l**).* Yield 66%, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 (s, 1H), 6.24 (s, 1H), 5.74 (t, $J = 8.0$ Hz, 1H), 5.58 (s, 1H), 4.78 (d, $J = 12.2$ Hz, 1H), 4.58 (d, $J = 12.2$ Hz, 1H), 4.52–4.37 (m, 2H), 3.85 (t, $J = 9.2$ Hz, 1H), 3.37 (s, 3H), 2.90–2.78 (m, 2H), 2.52–2.14 (m, 6H), 1.69 (t, $J = 11.2$ Hz, 1H), 1.54 (s, 3H), 1.10 (t, $J = 12.8$ Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.5, 167.3, 157.5 (d, $J = 25.4$ Hz), 150.3, 140.3(d, $J = 236.1$ Hz), 139.1, 138.9, 134.2, 126.8 (d, $J = 33.3$ Hz), 120.6, 81.2, 68.7, 63.4, 60.1, 50.3, 42.8, 36.7, 28.7, 25.8, 24.5, 24.1, 18.2. ^{19}F NMR (376 MHz, $CDCl_3$) δ −163.85. HRMS (ESI) calcd for $C_{22}H_{25}FN_2NaO_7^+$ $[M+Na]^+$ 471.1538, found 471.1543.

*4.1.8.18. ((1aR,7aS,10aS,10bS,E)-1a-methyl-8-methylene-9-oxo-1a,2,3,6,7,7a,8,9,10a,10b-decahydrooxireno[2′,3′:9,10]cyclodeca[1,2-b]furan-5-yl)methyl 2-(5-fluoro-2,4-dioxo-3-propyl-3,4-dihydropyrimidin-1(2H)-yl)acetate (**15m**).* Yield 78%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21 (d, $J = 4.8$ Hz, 1H), 6.25 (d, $J = 3.4$ Hz, 1H), 5.73 (t, $J = 8.0$ Hz, 1H), 5.58 (d, $J = 3.0$ Hz, 1H), 4.80 (d, $J = 12.2$ Hz, 1H), 4.56 (d, $J = 12.3$ Hz, 1H), 4.42 (q, $J = 17.2$ Hz, 2H), 3.98–3.89 (m, 2H), 3.85 (t, $J = 9.3$ Hz, 1H), 2.90–2.80 (m, 2H), 2.48–2.16 (m, 6H), 1.68–1.64 (m, 2H), 1.55 (s, 3H), 1.14–1.07 (m, 2H), 0.94 (t, $J = 7.4$ Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.4, 167.2, 157.2 (d, $J = 24.9$ Hz), 150.0, 140.3 (d, $J = 236.2$ Hz), 138.7, 134.1, 132.3, 126.7 (d,

$J = 33.2$ Hz), 120.5, 81.0, 68.6, 63.3, 60.0, 50.3, 43.7, 42.7, 36.6, 34.0, 29.8, 25.0, 20.8, 18.1, 11.3.^{19}F NMR (376 MHz, $CDCl_3$) δ −163.61. HRMS (ESI) calcd for $C_{24}H_{29}FN_2NaO_7^+$ $[M+Na]^+$ 499.1851, found 499.1855.

4.2. Cell culture

The human liver cancer cell line Bel-7402 and its corresponding drug resistance cell line Bel-7402/5-FU were purchased from KeyGen Biotech (Nanjing, China) and maintained in RPMI-1640 supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin in a humidified environment containing 5% CO_2 at a constant temperature of 37 °C.

4.3. MTT assay

Bel-7402 and Bel-7402/5-FU cells were seeded into 96-well plate respectively with 5000 cells each well. After 12 h, compounds with different concentrations were added and incubated for 72 h. Then 20 μL solution of MTT (5 mg/mL) was added into each well and incubated for 4 h. The supernatant was discarded and the crystal violet was dissolved with 200 μL DMSO. The observance of OD 570 nm was measured using a micro-plate reader. The IC_{50} values were calculated by GraphPad Prism 5.

4.4. Cell apoptosis assay

Bel-7402/5-FU cells were seeded in 24 well plate with 1×10^5 cells/well/1 mL. After 12 h, **15d** was added at the concentration of 0.5 μM, 1 μM, 2.5 μM, 5 μM, respectively, and incubated for 48 h. Cell apoptosis was evaluated using a FITC-Annexin V apoptosis detection kit. Briefly, the Bel-7402/5-FU cells were harvested and washed with cold PBS, then the cells were resuspended in binding buffer and incubated with 5 μL of a FITC-conjugated Annexin V and 5 μL PI for 10 min at room temperature in the dark. The samples were analyzed by flow cytometry in 1 h.

4.5. Western blot assay

Bel-7402/5-FU cells were seeded in 6 well plate with 5×10^5 cells/well/1 mL. After 12 h, **15d** was added with the concentration of 0.5 μM, 1 μM, 2 μM and 5 μM and incubated for 24 h. Then cells were harvested, washed three times by PBS and lysed in RIPA buffer for 30 min on ice. The sample was resuspended in loading buffer and denatured at 100 °C for 5 min. Proteins (50 μg) were separated with SDS-PAGE gel electrophoresis and transferred to PVDF membranes. The membranes were then blocked in 5% fat-free milk for 1 h at room temperature. After that primary antibodies were incubated according to the manufacturer's instructions. Then the membranes were washed for 5 times with PBST and incubated with the anti-HRP secondary antibody for 2 h at room temperature. Subsequently, the membranes were washed with PBST and visualized by Tanon Chemiluminescent Imaging System.

4.6. Nuclear morphology assay

The nuclei of Bel-7402/5-FU cells were stained with DAPI to detect the nuclear morphology changes after incubation with **15d**. Briefly, Bel-7402/5-FU cells were seeded in 6 well plate with 5×10^5 cells/well/1 mL. After 12 h, the compound was added with the concentration of 1 μM, 2 μM and 5 μM and incubated for 48 h. Then the cells were washed with PBS buffer for 3 times and incubated with DAPI (10 μg/mL) for 10 min at room temperature. Then the nuclear morphology was observed with fluorescence microscope.

4.7. The drug accumulation assay

Bel-7402/5-FU cells were seeded in 24 well plate with 1×10^5 cells/well/1 mL. After 12 h, **15d** was added with the concentration of 0.5 μM, 1 μM, 2.5 μM and 5 μM and incubated for 48 h. Then the cells were harvested, washed with PBS for 2 times and incubated with ADR (1 μM) for 1 h at 37 °C. After that the cells were collected, resuspended with PBS and analyzed with flow cytometry.

4.8. P-gp expression assay

Bel-7402/5-FU cells with or without **15d** treatment were collected and washed with PBS buffer for 3 times. Then the cells were resuspended and incubated with 5 μL p-gp-PE for 30 min at room temperature. After that the cells were analyzed with flow cytometry.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2019.111706.

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