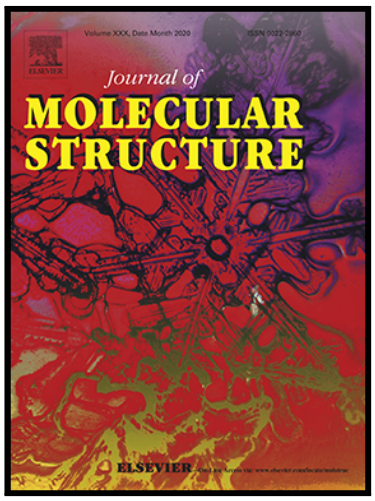

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Highlights

1. More than 34 new analogues of Chaetominine have been synthesized;
2. These new analogues feature versatile electrophilic warheads;
3. Some analogues show improved antitumor activities in cancer cell lines compared to Chaetominine

Synthesis and Bioactivity Studies of Covalent Inhibitors Derived from (-)-Chaetominine

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Abstract

We reported herein the synthesis and bioactivity studies of compounds derived from the natural product (-)-Chaetominine (**6**). The key feature of these compounds is the incorporation of electrophilic groups that are capable of forming covalent bonds with the cysteine or threonine residues of cellular proteins. The cell growth inhibition activities of these derivatives of **6** were tested in four cancer cell lines, i.e. a leukemia cell line K562, a multiple myeloma cell line MM1.S, an acute myeloid leukemia cell line MV4-11, and a colon cancer cell line RKO. The data show that cellular growth inhibition IC_{50} values of **29**, an acrylamide-containing molecule, are 9-17 folds more potent than that of **6**, while other acrylamide-containing compounds are much less potent, suggesting **29** might have covalent interactions with cellular target proteins. Collectively, incorporation of an acrylamide moiety into **6** is a good strategy to improve its cell growth inhibition activity in cancer cell lines.

Key words: Chaetominine, Cellular growth inhibition, Cancer cell line, Covalent warhead

1. Introduction

Nature products feature tremendously structural diversity and often overwhelming three-dimensional complexity, yet in many cases they consist of reactive electrophilic functional groups such as acrylamide, α,β-unsaturated ketone/ester, epoxide, *β*-lactam, and *β*-lactone, among others.[1-6] The natural products containing electrophilic functional groups are capable of forming covalent bonds with the cysteine,[7,8] lysine,[9] threonine,[10] and histidine[6] residues of target proteins in cells, therefore manifesting their multifaceted biological activities. These unique molecules have enlightened and motivated many drug discovery campaigns, in which their synthetic analogues were produced and their bioactivities were evaluated in vitro and in vivo.

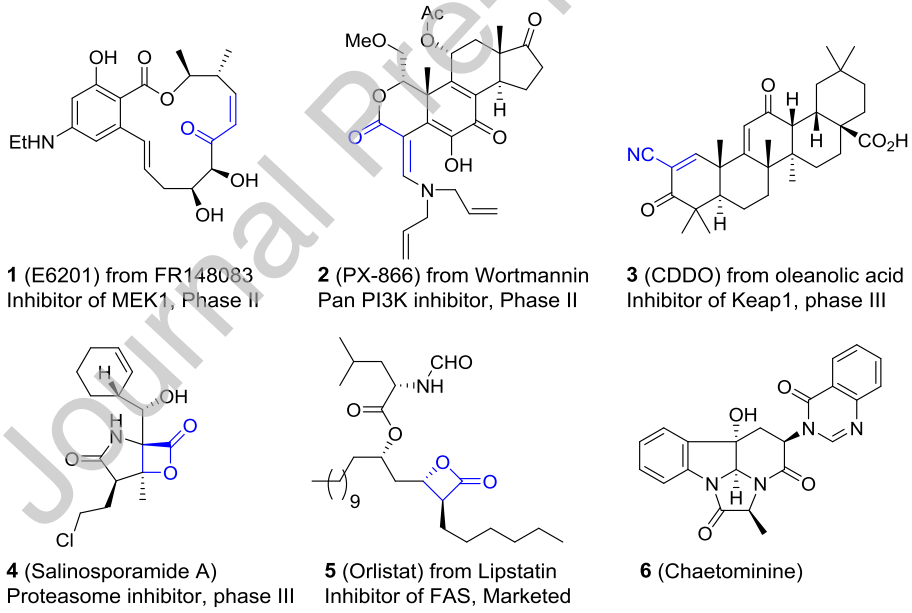


Figure 1: representative examples of targeted covalent inhibitors (TCIs) derived from natural products and (-)-Chaetominine

For example, E6201 (**1**, Figure 1), a MEK1 inhibitor was synthesized based on natural product FR148083 and it was found that the MEK1 Cys207 residue and the conjugated enone moiety of E6201 formed C-S bond in vitro (PDB: 5HZE).[8,11] E6201

(**1**) showed inhibitory activity in several cell-based systems[12] and is currently tested in human for the treatment of metastases melanoma.[13] In addition, the natural product Wortmannin formed a C-N bond with the Lys-833 residue of PI3Kγ through the conjugated ester moiety, and therefore inhibited the PI3K kinases.[9] PX-866 (**2**) was a synthetic analogue of Wortmannin[14] and had been advanced into phase II clinical trials for treatment of several types of cancers.[15-17] CDDO (**3**) was a synthetic analogue of oleanolic acid,[18,19] which inhibited Keap1/Nrf2 through covalently modification of the Cyc151 residue of BTB, a subdomain of Keap1.[7] **3** is currently undergoing two phase III clinical trials for the treatment of kidney diseases.[20] Both the natural product **4**[21] and the lipstatin-derived 5[22] consist of a *β*-lactone moiety. It has been shown that **4** and **5** formed C-O bond with the Thr1 residue of 20S proteasome subunit β5[10] and the Ser2308 residue of human fatty acid synthase (FAS)[23] respectively and therefore inhibited the corresponding target proteins. Currently, **4** is undergoing late-stage clinical trials for the treatment of glioblastoma and ependymoma,[24] and **5** has been approved for the treatment of obesity. Evidently, the so-called targeted covalent inhibitors (TCIs) derived from natural products are of compelling therapeutic potentials for the treatment of human diseases.[1,3,25-31]

(-)-Chaetominine (**6**) is a natural product firstly isolated from an endophytic fungus on the Adenophora axilliflora leaves[32] and potently inhibited cell growth of the human leukemia K562 and colon cancer SW1116 cell line.[32,33] **6** features an interesting tetracyclic ring system and to the best of our knowledge, covalent structural modifications and medicinal chemistry studies of **6** are underexplored. As our ongoing

interest in targeted covalent inhibitors,[34] we incorporated a series of covalent warheads[2,26,35] into the tetracyclic ring system of **6**, made a series of analogues, and studied their bioactivities. Herein, we report the synthesis and bioactivity studies of covalent-warhead-containing compounds derived from **6**.

2. Results and Discussion

Two design strategies were undertaken to modify structures of **6** as shown in Fig. 2. Strategy 1 aims to modify the quinazolin-4(3H)-one moiety and strategy 2 aims to modify the methyl group.

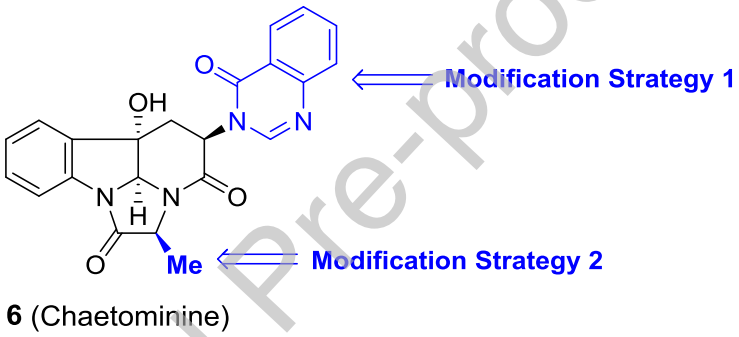


Figure 2: Modification strategies of **6**

Previous studies have confirmed that the acrylamide and the propionate moieties of BTK[36,37] and EGFR[38,39] inhibitors selectively formed covalent bonds with the cysteine residues in the kinase active sites. In addition, chloroacetyl and vinyl sulfonyl groups are electrophilic warheads that are capable of forming covalent bonds with active cysteine residues[35]. The oxirane-2-carboxamide moiety is also an electrophilic groups in the design of a protease inhibitor[40], which forms a covalent bond with the active site Thr residue. Therefore, the aforementioned electrophilic warheads were adopted in our studies and incorporated into the core structure of **6**.

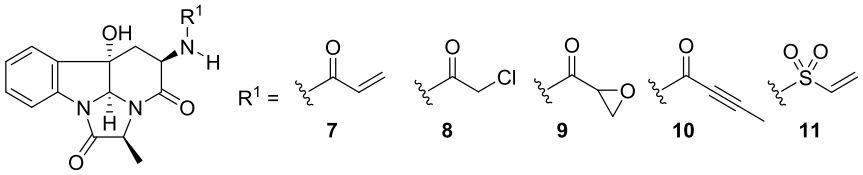
First, the strategy 1 was explored and the quinazolin-4(3H)-one moiety was

replaced by electrophilic warheads as shown in Table 1. Compounds **7-11** were thus synthesized and their cell growth inhibition activities were tested in leukemia cell line K562, which was previously adopted as a model cell line to assay the activity of **6**[32]. In addition, a multiple myeloma cell line MM1.S, an acute myeloid leukemia cell line MV4-11, and a colon cancer cell line RKO were also included to assay the cell growth inhibition activities of **7-11**. As shown in Table 1, **6** has an IC_{50} value of 98.62 μM in the K562 cell line, which is different from previous reported result (IC_{50} = 21 nM) using naturally isolated **6**[32]. In order to examine this discrepancy, a FDA approved drug Decitabine was included as a positive control, which inhibited K562 cell growth with IC_{50} value of 30 nM and is in line with previously reported data[41,42]. The structure of in-house synthesized **6** was confirmed by X-ray crystallography studies (SI, Table S1)[43]. In addition, the isolated **6** from natural source has a specific optical rotation value of -70 (c 0.48 in MeOH)[32], while the synthetic **6** has that of -49.7 (c 0.48 in MeOH)[44] and -48 (c 0.45, MeOH)[45] in literatures and of -51.3 (c 0.48 in MeOH) for our in-house data. The specific optical rotation value of natural **6** is different from all synthetic ones and this inconsistency may partially explain the discrepancy of their cellular activities.

In addition, **6** has IC_{50} values of 65.48, 79.23, and 169.53 μM in MM1.S, MV4-11, and RKO cell lines, respectively. Generally, compounds **7-11** have similar cell growth inhibition activities as that of **6**. In particular, **8** was 3 folds more potent than **6** in MM1.S cell line, whereas **11** was 5 folds and 3 folds more potent than **6** in K562 and MM1.S cell lines, respectively. Compounds comprising highly electrophilic

acrylamide, chloroacetyl, and vinyl sulfonyl groups did not have strong toxicity but comparable activity as **6**, suggesting non-specific toxicity of these electrophilic groups is minimal in these four cell lines.

Table 1: Cell growth inhibition activities of the derivatives of **6**



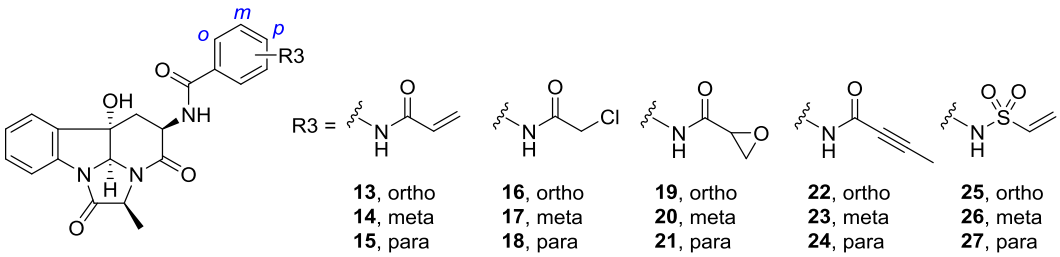
	IC_{50} (μM)[a]			
	K562	MM.1S	MV4-11	RKO
6 (Chaetominine)	98.62±13.56	65.48±9.52	79.23±1.06	169.53±6.39
7	106.80±1.12	52.23±3.92	64.47±5.97	196.70±45.99
8	98.19±1.67	22.41±1.57	49.07±10.82	128.30±19.47
9	86.95±5.54	59.84±1.39	63.68±5.70	132.47±9.44
10	104.12±4.20	88.04±0.79	72.98±5.55	165.05±13.55
11	17.69±4.00	21.54±0.54	42.33±11.23	89.76±9.77
12 (Decitabine)	0.03±0.02	1.12±0.23	0.21±0.16	16.07±7.24

[a] All the values are means of three independent experiments.

Next, the quinazolin-4(3H)-one moiety of **6** was degraded, while compounds **13-27** were synthesized and their cell growth inhibition activities were tested, the results are summarized as shown in Table 2. In general, **15**, **16**, **17**, **18**, **20**, **21**, **24**, **26**, and **27** had lower cell growth inhibition IC_{50} values compared to that of **6**. Among these, **15**, **18**, **21**, **24**, and **27** had para substitution pattern, while **17**, **20**, and **26** had meta substitution pattern, indicating these two positions are optimal for improved potency. The most potent compound is **18**, featuring a chloroacetyl group at the para position. However, the other two chloroacetyl-group-containing compounds also have

very low IC_{50} values, suggesting the activities may be non-specific.

Table 2: Cell growth inhibition activities of compounds derived from **6**



13, ortho **14**, meta **15**, para | **16**, ortho **17**, meta **18**, para | **19**, ortho **20**, meta **21**, para | **22**, ortho **23**, meta **24**, para | **25**, ortho **26**, meta **27**, para

	IC_{50} (μM) [a]			
	K562	MM.1S	MV4-11	RKO
13	106.17±3.42	54.09±1.77	73.75±1.88	116.80±3.71
14	67.88±2.08	66.97±4.64	58.61±14.01	107.83±7.22
15	**30.15±7.94**	**61.30±4.21**	**9.79±2.80**	**88.67±3.41**
16	**22.22±1.365**	**13.98±0.941**	**20.28±4.347**	**36.04±3.649**
17	**7.04±0.59**	**3.89±0.12**	**3.39±0.13**	**18.02±1.38**
18	**4.92±0.23**	**4.80±0.67**	**4.40±0.36**	**14.59±0.93**
19	60.23±25.97	17.68±3.90	29.40±24.16	101.68±7.53
20	17.69±5.02	34.47±2.09	25.13±1.48	93.94±2.43
21	24.46±0.51	24.14±2.62	16.04±0.98	90.09±2.33
22	93.52±5.04	82.57±10.32	77.23±2.43	96.11±7.43
23	55.34±13.83	56.83±10.62	66.30±2.33	97.56±5.85
24	40.87±7.09	47.25±4.27	24.77±5.29	94.07±8.31
25	29.50±1.89	26.09±0.75	41.72±21.24	66.48±5.52
26	39.02±2.33	22.80±0.92	17.75±1.09	83.87±1.10
27	36.07±2.34	21.23±1.00	15.36±2.48	83.82±0.94

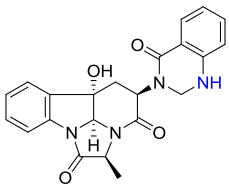
[a] All the values are means of three independent experiments.

The acrylamide moiety containing compound **15** has improved IC_{50} values in K562 and MM1.S cell line, while its close analogues **13** and **14** have equal IC_{50} values as that of **6**, suggesting the improved cellular activity of **15** requires optimal orientation

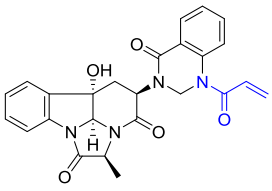
of the acrylamide moiety.

During our synthesis of **6**, we found that its C=N bond was reduced to a C-N bond in the presence of $NaBH_3CN$ and **28** was obtained in good yield. Therefore, three derivatives of **28** were made and tested in cell growth inhibition assays, the results are summarized in Table 3.

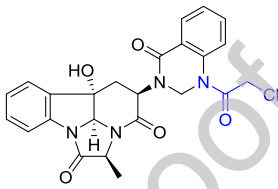
Table 3: Cell growth inhibition activities of compounds derived from over-reduced **6**



28



29



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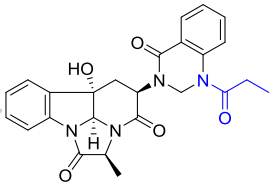


31

	IC_{50} (μM) [a]			
	K562	MM.1S	MV4-11	RKO
28	100.70±6.19	59.59±13.40	64.54±24.59	152.26±43.78
29	**5.61±0.33**	**7.18±0.37**	**8.54±2.45**	**18.80±0.76**
30	1.95±0.57	3.31±0.03	2.12±0.59	5.46±0.51
31	97.18±2.98	58.61±2.01	74.83±4.59	124.50±5.34

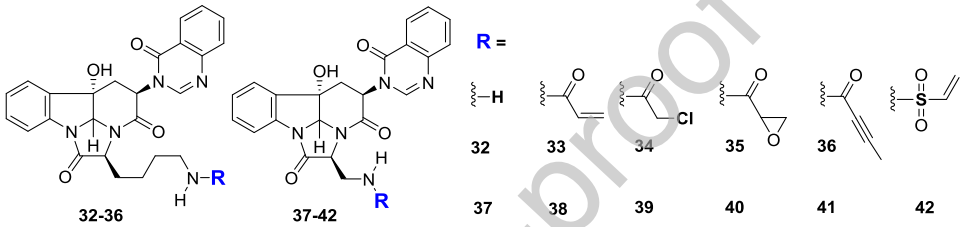
[a] All the values are means of three independent experiments.

Interestingly, the cellular growth inhibition IC_{50} values of **29** are 5.61, 7.18, 8.54, and 18.80 μM in K562, MM1.S, MV4-11, and RKO cell lines, respectively. These numbers account for 17, 9, 9, and 9 folds improvement compared to that of **6**. The cellular growth inhibition IC_{50} values of **30** are 1.95, 3.31, 2.12, and 5.46 μM in K562, MM1.S, MV4-11, and RKO cell lines, respectively, accounting for 50, 19, 37, and 31 folds improvement compared to that of **6**. On the contrary, both **28** and **31** have similar cell growth inhibition activities as that of **6**. It is worthy of note that the acrylamide-containing compounds **7**, **13**, **14**, and **15** inhibit cell growth with much

greater IC_{50} values, i.e. much less potent. The significant difference between **29** and its analogues **7**, **13**, **14**, **15** suggests that the activity of **29** may be target-specific rather than non-specific toxicity.

In addition to the strategy 1, additional modifications of **6** were performed on the methyl group according to the strategy 2. Compounds **32-42** were synthesized and tested in cell growth inhibition assay, the results are summarized in Table 4.

Table 4: Cell growth inhibition activities of compounds based on the strategy 2



	IC_{50} (μM) [a]			
	K562	MM.1S	MV4-11	RKO
32	123.06±7.48	84.15±7.55	70.89±11.26	138.4±16.85
33	163.35±102.47	87.64±7.94	64.42±10.26	159.4±4.40
34	**29.18±3.89**	**13.49±0.86**	**24.63±5.76**	**43.84±3.85**
35	103.63±6.74	88.43±4.96	70.35±15.45	131.4±8.05
36	102.67±6.16	82.37±3.26	70.72±11.76	163.8±34.38
37	**14.85±8.88**	**51.53±21.79**	**61.30±5.92**	**85.23±13.78**
38	94.79±9.83	83.56±15.76	92.42±11.98	177.77±33.32
39	38.02±3.13	35.69±13.00	43.16±11.17	245.55±22.27
40	96.97±11.15	90.45±13.31	95.70±21.36	148.60±8.63
41	100.08±11.39	86.10±1.54	86.89±13.18	143.75±13.51
42	45.77±14.03	88.23±25.43	61.02±18.98	116.31±22.27

[a] All the values are means of three independent experiments.

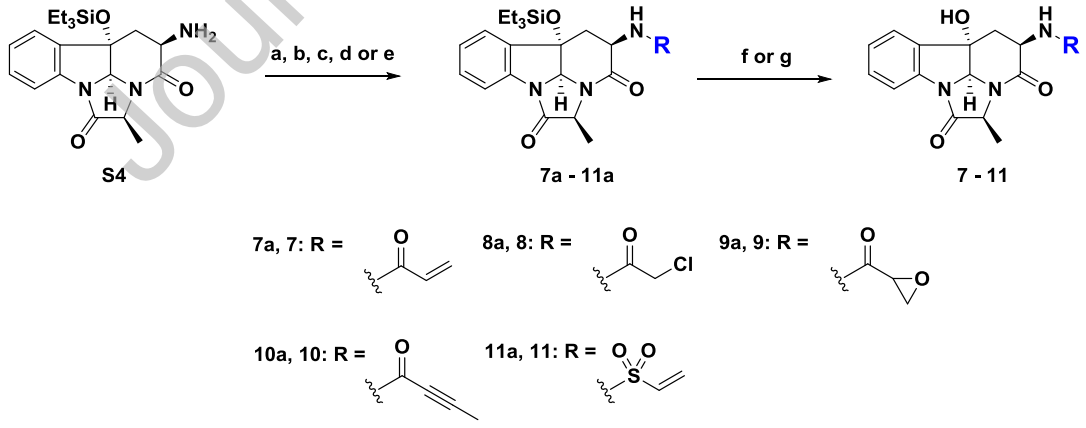
In this series of compounds, a chloroacetyl group-containing compound **34** was

more potent than **6** in the cell growth inhibition assays, which is in line with the facts that **16**, **17**, **18**, and **30**, all chloroacetyl-containing compounds, generally have higher cell potency. **37** has no electrophilic warhead and it inhibited K562 growth with an IC_{50} value of 14.85 μM, 4 folds more potent than that of **6**. The other compounds have similar cell growth inhibition activity as that of **6**. Taken together, the modifications according to the strategy 2 is less successful in term of improve cellular antitumor potency in cancer cell lines.

3. Chemistry

The syntheses of final compounds **7-11** have been summarized in Scheme 1. A previously reported compound **S4**[45,46] was used as the starting material and electrophilic warhead moieties were incorporated at the NH_2 group, furnishing **7a-11a**. Removal of the TES group of **7a-11a** yielded the final compounds **7-11** in moderate to good yields.

Scheme 1: Syntheses of final compounds **7-11**

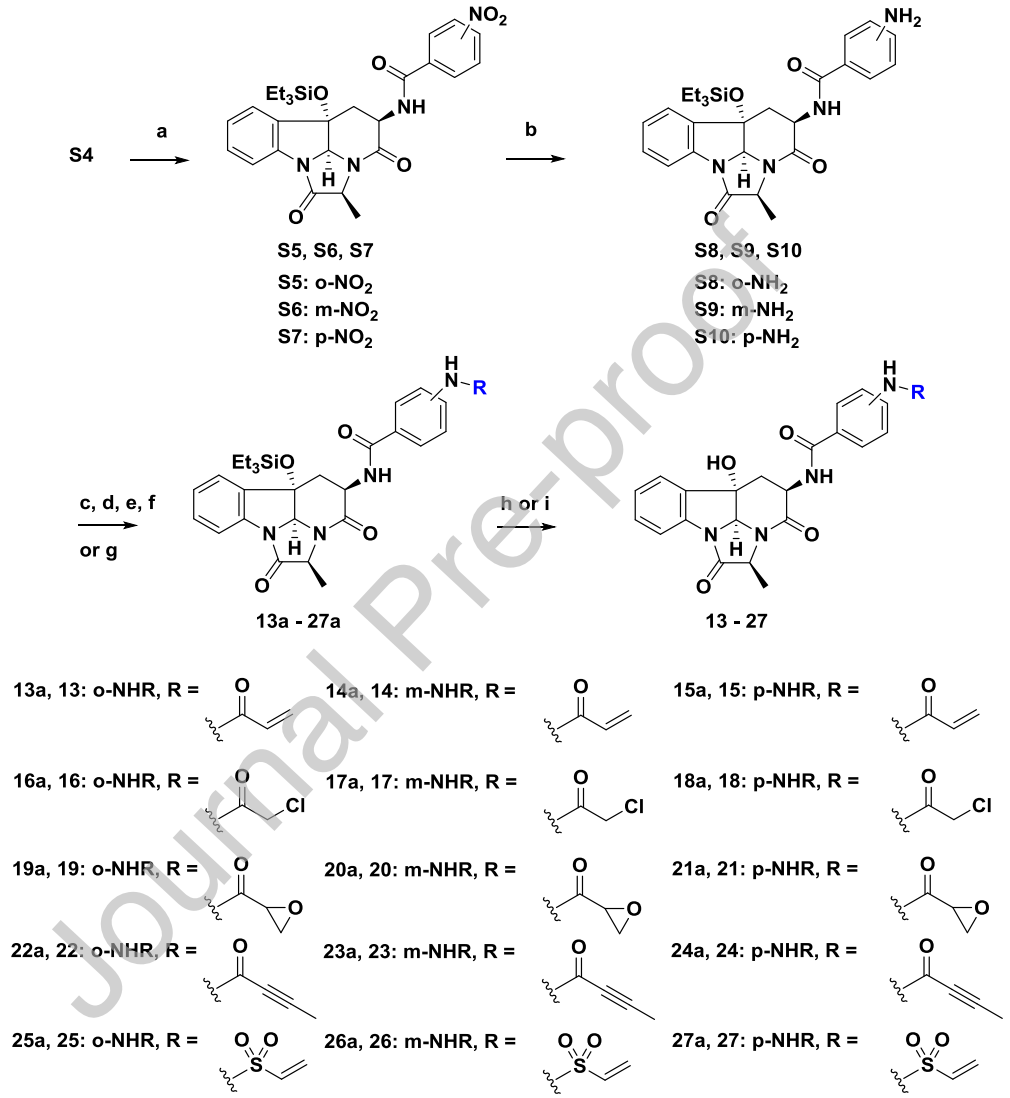


Reaction Conditions: (a) Acryloyl chloride, Et_3N, THF, 2h; (b) Chloroacetyl chloride, Et_3N, THF, 2h; (c) Oxirane-2-carboxylic acid , Isobutyl chloroformate, NMM, THF, overnight; (d) 2-Butynoic acid, Isobutyl chloroformate, NMM,THF, overnight; (e)

(2-Chloroethyl)sulphonyl chloride, Et_3N, THF, 2h; (f) HF, MeCN, overnight; (g) CsF, DMSO, 2h.

The syntheses of **13-27** have been summarized in Scheme 2.

Scheme 2: Syntheses of final compounds **13-27**



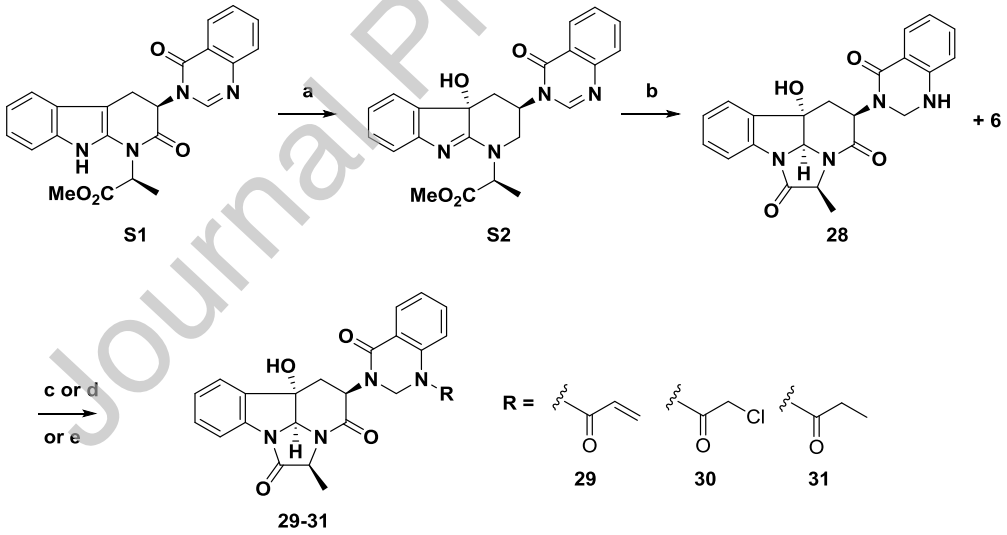
Reaction conditions: (a) Nitrobenzoyl chloride, Et_3N, THF, 2h; (b) Pd/C, H_2, THF, overnight; (c) Acryloyl chloride, Et_3N, THF, 2h; (d) 2-Chloroacetyl chloride, Et_3N, THF, 2h; (e) Oxirane-2-carboxylic acid, isobutyl chloroformate, NMM, THF, overnight; (f) 2-Butynoic acid, isobutyl chloroformate, NMM, THF, overnight; (g) (2-Chloroethyl)sulphonyl chloride, Et_3N, THF, 2h; (h) HF, MeCN, overnight; (i) CsF,

DMSO, 2h.

In addition, **S5**-**S7** were synthesized in good yields using **S4**[45,46] and three nitrobenzoyl chlorides as the starting materials. Pd-catalyzed hydrogenation of the -NO_2 groups of **S5**-**S7** yielded aniline-containing compounds **S8**-**S10**. Subsequently, **S8**-**S10** were allowed to react with acryloyl chloride, 2-chloroacetyl chloride, oxirane-2-carboxylic acid, 2-butynoic acid, or (2-chloroethyl)sulphonyl chloride accordingly, affording **13a**-**27a**. Finally, the TES groups of **13a**-**27a** were removed and the final compounds **13**-**27** were obtained in moderate to good yields.

The final compounds **28**-**31** were synthesized from a previously reported compound **S1**[47] as outlined in Scheme 3.

Scheme 3: Syntheses of final compounds **28**-**31**



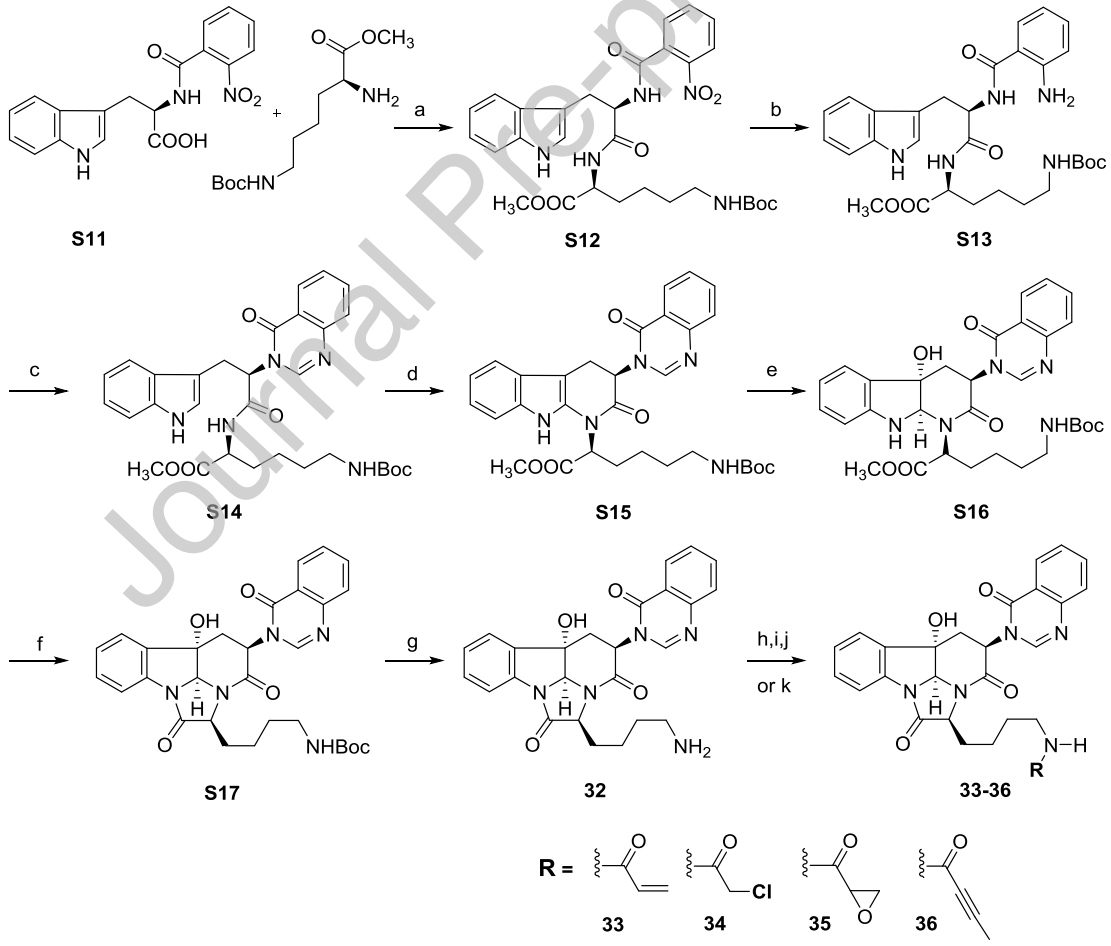
Reaction conditions: (a) O_2, CH_2Cl_2, overnight; (b) $NaBH_3CN$, AcOH, MeOH, 5h; then silica gel, $NH_3{\cdot}H_2O$:EtOH:Acetone:CH_2Cl_2=0.5:1:4:50, overnight. (c) Acryloyl chloride, Et_3N, THF, 2h; (d) Chloroacetyl chloride, Et_3N, THF, 2h; (e) Propionyl chloride, Et_3N, THF, 2 h.

Using a previously reported oxidation-reduction method,[48] **S1** was converted into **6**,

while **28** was also obtained as a minor product, apparently through reduction of the C=N bond of the quinazolin-4(3*H*)-one moiety. The structure and stereochemistry of **6** have been confirmed by X-ray crystallography analysis of its single crystals[43]. Finally, different electrophilic and the propionyl groups were incorporated onto **28** through the corresponding condensation reactions with the NH group, providing final compounds **29**-**31**.

The final compounds **37**-**42** were synthesized from a previously reported compound **S11**[49,50] over 8 linear steps as shown in Scheme 4.

Scheme 4: Syntheses of final compounds **32**-**36**



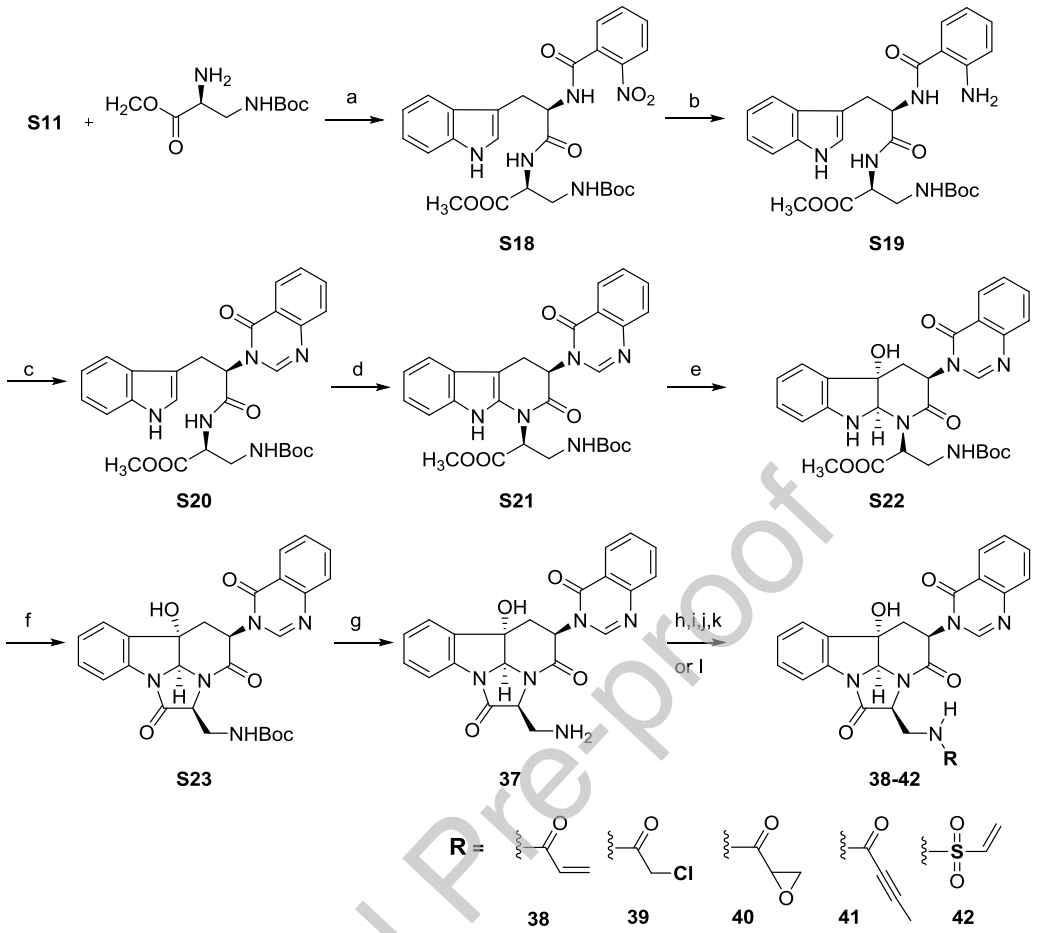
Reaction conditions: (a) Cl-COOiBu, NMM,THF, -30 °C, rt, overnight; (b) Pd/C, MeOH, rt, overnight; (c) $(C_2H_5O)_3CH$, p-TsOH, 30 °C, overnight; (d) NCS, Et_3N,

CH_2Cl_2, 0 ^oC-rt, overnight; (e) O_2, CH_2Cl_2, rt, overnight; (f) $NaBH_3CN$, AcOH, MeOH,35 ^oC, 5h; then silica gel, $NH_3{\cdot}H_2O$:EtOH:Acetone:CH_2Cl_2 = 0.5 :1 :4 :50, 35 ^oC, overnight; (g) CH_2Cl_2/TFA (v/v=4/1), rt, 2h; (h) Acryloyl chloride, Et_3N, THF, rt, 2h; (i) 2-Chloroacetyl chloride, Et_3N, THF, rt, 2h; (j) Oxirane-2-carboxylic acid, isobutyl chloroformate, NMM, THF, 0 ^oC-rt, overnight; (k) 2-Butynoic acid, isobutyl chloroformate, NMM, THF, rt, overnight.

The amide condensation of **S11** and ε-Boc-L-lysine methyl ester provided **S12**, which was reduced to aniline-containing **S13**. In the presence of p-TsOH and triethyl orthoformate,[46,48] cyclization of **S13** led to formation of the quinazolin-4(3H)-one moiety of **S14**. In the presence of NCS, further cyclization of **S14** led to formation **S15**,[48] which underwent oxidation-reduction cascade reactions and provided **S16** as the key intermediate. Subsequent intra-molecular cyclization of **S16** was promoted by the presence of wet silica gel,[48] providing **S17** in good yield. The Boc protecting group of **S17** was removed facilitated by treatment of TFA, furnishing **32**. Finally, the electrophilic warheads were installed using similar methods showing in Scheme 2 and the desired products **33-36** were obtained accordingly.

As shown in Scheme 5, the final compounds **37-42** were synthesized from **S11** and (2*S*)-2-amino-3-(Boc-amino)propionic acid methyl ester through practically same synthetic sequences as shown in Scheme 4. The key difference is (2*S*)-2-amino-3-(Boc-amino)propionic acid methyl ester was used instead of ε-Boc-L-lysine methyl ester, which enables **37-42** with a shorter side chain compared to that of **32-36**.

Scheme 5: Syntheses of final compounds **37**-**42**



Reaction conditions: (a) Cl-CO_2(i-Bu), NMM, THF, -30 ^oC, rt, overnight; (b) Pd/C, MeOH, rt, overnight; (c) $(C_2H_5O)_3CH$, p-TsOH, 30 ^oC, overnight; (d) NCS, Et_3N, CH_2Cl_2, 0 ^oC-rt, overnight; (e) O_2, CH_2Cl_2, rt, overnight; (f) $NaBH(OAc)_3$, AcOH, DCE, 35 ^oC, overnight; (g) CH_2Cl_2/TFA (v/v= 4/1), rt, 2h; (h) Acryloyl chloride, Et_3N, THF, rt, 2h; (i) 2-Chloroacetyl chloride, Et_3N, THF, rt, 2h; (j) Oxirane-2-carboxylic acid, isobutyl chloroformate, NMM, THF, 0 ^oC-rt, overnight; (k) 2-Butynoic acid, isobutyl chloroformate, NMM, THF, 0 ^oC-rt, overnight; (l) 2-Chloroethanesulfonyl chloride, Et_3N, THF, rt, 2h.

4. Conclusions

In summary, we have reported modifications of the natural product (-)-Chaetominine (**6**) through two strategies. The key feature of these modifications is incorporation of electrophilic groups that are capable of forming covalent bonds with the cysteine or threonine residues of cellular proteins. The cell growth inhibition activities of compounds derived from **6** were tested in four cancer cell lines, i.e. a leukemia cell line K562, a multiple myeloma cell line MM1.S, an acute myeloid leukemia cell line MV4-11, and a colon cancer cell line RKO. The data show that cellular growth inhibition IC_{50} values of **29**, an acrylamide-containing molecule, are 9-17 folds more potent than that of **6**, while other acrylamide-containing compounds are much less potent, suggesting **29** might have covalent interactions with cellular proteins. In addition, chloroacetyl-containing compounds are generally more potent than **6** in cell growth inhibition assays, indicating chloroacetyl is over-reactive and less selective. Collectively, the incorporation of an acrylamide moiety into **6** is a good strategy to improve its antitumor activity in cancer cell lines. Further identification of the cellular target proteins of **29** is underway and will be reported in due course.

5. Experimental sections

5.1 General Methods

All the final products were purified on a preparative HPLC (Waters 2545) with a C18 reverse phase column. The mobile phase used here was a gradient flow of solvent A (water, 0.1% of TFA) and solvent B (CH_3CN, 0.1% of TFA) at a flow rate of 40 mL/min. The analytical UPLC model was Waters Acquity H class (UV detection at 230 nm and 254 nm)

and the reverse phase column used was the Acquity UPLC® BEH (C18-1.7 µm, 2.1 × 50 mm). All final compounds were purified to ≥ 95% purity as determined by analytical UPLC analysis. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker Advance NMR 400 spectrometer and a Bruker Advance NMR 500 spectrometer. ^{1}H NMR spectra were calibrated with tetramethylsilane (δ = 0 ppm), DMSO-d_6 (δ = 2.50 ppm), MeOH-d_4 (δ = 3.31 ppm), or $CDCl_3$ (δ = 7.26 ppm). ^{13}C NMR spectra were calibrated with DMSO-d_6 (δ = 39.5 ppm), MeOH-d_4 (δ = 49.0 ppm) or $CDCl_3$ (δ = 77.2 ppm). Multiplicities are given as follows: s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), dt (doublet of triplets), and m (multiplets). High-resolution mass spectral (HRMS) analysis was performed on UHPLC-QTOF (Agilent Corporation) and Thermo DFS (Thermo Fisher Scientific).

a) Procedure for the synthesis of **7a** (general **Method A**). **S4** (49 mg, 0.13 mmol, 1.0 eq) and triethylamine (28 mg, 0.26 mmol, 2.0 eq) were dissolved in CH_2Cl_2 (5 mL). Acryloyl chloride (13 mg, 0.14 mmol, 1.1 eq) was added dropwise. The reaction was stirred at room temperature for 2 h. The mixture was then diluted with water (20 mL) and the aqueous layer was extracted with ethyl acetate (20 mL × 2). The combined organic layers were dried over anhydrous sodium sulfate. The solvent was removed on a rotary evaporator, and the remaining residues were purified by flash column chromatography to provide **7a** (39 mg, 71% yield). The syntheses of **13a**, **14a**, **15a**, **29**, **33**, **38**, and **S25** were similar to that of **7a**.

b) Procedure for the synthesis of **8a** (general **Method B**). **S4** (50 mg, 0.13 mmol, 1.0 eq) and triethylamine (28 mg, 0.26 mmol, 2.0 eq) were dissolved in CH_2Cl_2 (5 mL).

2-Chloroacetyl chloride (18 mg, 0.15 mmol, 1.1 eq) was added dropwise. The reaction was stirred at room temperature for 2 h. The mixture was then diluted with water (20 mL) and the aqueous layer was extracted with ethyl acetate (20 mL×2). The combined organic layers were dried over anhydrous sodium sulfate. The solvent was removed on a rotary evaporator and the remaining residues were purified by flash column chromatography to provide **8a** (40 mg, 67% yield). The syntheses of **16a**, **17a**, **18a**, **30**, **34**, and **39** were similar to that of **8a**.

c) Procedure for the synthesis of **9a** (general **Method C**). *N*-Methyl morpholine (26 mg, 0.24 mmol, 2.0 eq) and isobutyl chlorocarbonate (41 mg, 0.3 mmol, 2.5 eq) were sequentially added to a solution of oxirane-2-carboxylic acid (26 mg, 0.24 mmol, 2.0 eq) in anhydrous THF (5 mL) at 0 ^{o}C. After being stirred at 0 ^{o}C for 1 h, the suspension was slowly added to a solution of **S4** (46 mg, 0.12 mmol, 1.0 eq) and N-methyl morpholine (26 mg, 0.24 mmol, 2.0 eq) in anhydrous THF (5 mL) at 0 ^{o}C. The reaction was stirred at room temperature for 12 h. The mixture was then diluted with water (20 mL) and the aqueous layer was extracted with ethyl acetate (20 mL×2). The combined organic layers were dried over anhydrous sodium sulfate. The solvent was removed on a rotary evaporator and the remaining residues were purified by flash column chromatography to provide **9a** (37 mg, 69% yield). The syntheses of **19a**, **20a**, **21a**, **35**, and **40** were similar to that of **9a**.

d) Procedure for the synthesis of **10a** (general **Method D**). *N*-Methyl morpholine (30 mg, 0.52 mmol, 2.0 eq) and isobutyl chlorocarbonate (35 mg, 0.24 mmol, 0.9 eq) were successively added to a solution of but-2-ynoic acid (22 mg, 0.26 mmol, 1.0 eq)

in anhydrous THF (5 mL) at 0 ^{o}C. After being stirred at 0 ^{o}C for 1 h, the suspension was slowly added to a solution of **S4** (100 mg, 0.26 mmol, 1.0 eq) and N-methyl morpholine (22 mg, 0.26 mmol, 1.0 eq) in anhydrous THF (5 mL) at 0 ^{o}C. The reaction was stirred at room temperature for 12 h. The mixture was then diluted with water (20 mL) and the aqueous layer was extracted with ethyl acetate (20 mL×2). The combined organic layers were dried over anhydrous sodium sulfate. The solvent was removed on a rotary evaporator and the remaining residues were purified by flash column chromatography to provide **10a** (46 mg, 40% yield). The syntheses of **22a**, **23a**, **24a**, **36**, and **41** were similar to that of **10a**.

e) Procedure for the synthesis of **11a** (general **Method E**). **S4** (35 mg, 0.09 mmol, 1.0 eq) was dissolved in CH_2Cl_2 (5 mL). 2-Chloroethane-1-sulfonyl chloride (1.8 mg, 0.11 mmol, 1.2 eq) and triethylamine (14 mg, 0.14 mmol, 1.5 eq) were added sequentially. The reaction was stirred at room temperature for 12 h. The mixture was then diluted with water (20 mL) and the aqueous layer was extracted with ethyl acetate (20 mL×2). The combined organic layers were dried over anhydrous sodium sulfate. The solvent was removed on a rotary evaporator and the remaining residues were purified by flash column chromatography to provide **11a** (18 mg, 42% yield). The syntheses of **25a**, **26a**, **27a**, and **42** were similar to that of **11a**.

f) Procedure for deprotection of triethylsilyl using hydrofluoric acid (general **Method F**). **7a** (60 mg, 0.14 mmol, 1.0 eq) was dissolved in acetonitrile (5 mL). Hydrofluoric acid (49% in water, 0.29 mL, 5.0 eq) was added and the reaction was stirred at room temperature for 12 h. The solvent was removed on a rotary evaporator and the

remaining residues were purified by reverse phase HPLC to provide **7** (23 mg, 53% yield). The syntheses of **8**, **9**, **10**, **13**, **14**, **16**, **17**, **19**, **20**, **22**, **23**, and **25** were similar to that of **7**.

g) Procedure for deprotection of triethylsilyl using cesium fluoride (general **Method G**). **11a** (18 mg, 0.04 mmol, 1.0 eq) was dissolved in DMSO (2 mL). Cesium fluoride (9 mg, 0.06 mmol, 1.5 eq) was added and the reaction was stirred at room temperature for 2 h. The water (3 mL) was added and the solution was purified by reverse phase HPLC to provide **11** (7.8 mg, 57% yield). The syntheses of **15**, **18**, **21**, **24**, **26**, and **27** were similar to that of **11**.

5.2 Synthesis of Final Compounds

(2*S*,2a^1*S*,4*R*,5a*S*)-5a-hydroxy-2-methyl-4-(4-oxoquinazolin-3(4H)-yl)-2a^1,4,5,5a-tetrahydro-3H-2a,9b-diazacyclopenta[*jk*]fluorene-1,3(2H)-dione (1, (-)-Chaetomi-nine) (6). **S1** (1.6g, 3.8 mmol) was dissolved in CH_2Cl_2 (10 mL). The reaction system was degassed, filled with oxygen and stirred at room temperature for 12 h. The solvent was removed on a rotary evaporator and the remaining residues were purified by flash column chromatography. **S2** was isolated in 55% yield (910 mg). Then **S2** was dissolved in methanol (10 mL), followed by acetic acid (0.6mL) and sodium cyanoborohydride (264 mg, 4.2 mmol). The reaction was stirred at 35 ^oC for 5 h, and quenched by saturated sodium bicarbonate solution (20 mL). The mixture was extracted with ethyl acetate (20 mL×2). The combined organic layers were dried over anhydrous sodium sulfate. The solvent was removed on a rotary evaporator. The remaining residues were dissolved in the mixed solvent (10 mL, NH_3-H_2O : EtOH :

Acetone : CH_2Cl_2 = 0.5:1:4:50) followed by addition of silica gel (2.7 g). The reaction was stirred at 35 ^oC for 12 h. The solvent was removed on a rotary evaporator and the remaining residues were purified by flash column chromatography. **6** was obtained as the major product in 36%, while **28** was isolated in 18% yield (151 mg). ^1H NMR (400 MHz, DMSO-d_6): δ 8.29 (s, 1H), 8.18 (d, *J* = 7.9 Hz, 1H), 7.90-7.82 (m, 1H), 7.69 (d, *J* = 8.1 Hz, 1H), 7.58 (t, *J* = 7.6 Hz, 1H), 7.50 (t, *J* = 7.9 Hz, 2H), 7.43 (t, *J* = 7.7 Hz, 1H), 7.25 (t, *J* = 7.4 Hz, 1H), 6.69 (s, 1H), 5.91 (s, 1H), 5.60 (s, 1H), 4.61 (q, *J* = 6.9 Hz, 1H), 2.93 (t, *J* = 12.8 Hz, 1H), 2.58-2.50 (m, 1H), 1.60 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 172.1, 160.0, 147.4, 138.7, 136.7, 134.7, 129.9, 127.3, 127.2, 126.4, 125.5, 124.9, 114.5, 82.5, 76.4, 59.6, 38.2, 14.0. HRMS (ESI) calculated for $C_{22}H_{19}N_4O_4$ $[M+H]^+$ = 403.1401, obtained: 403.1401. $[a]_D^{20\,^oC}$ = - 51.3 ^{o} (c = 0.48, MeOH).

(2*S*,2a^1*S*,4*R*,5a*S*)-5a-Hydroxy-2-methyl-4-(4-oxo-1,4-dihydroquinazolin-3(2H)-yl)-2a^1,4,5,5a-tetrahydro-3H-2a,9b-diazacyclopenta[*jk*]fluorene-1,3(2H)-dione (28)

^1H NMR (400 MHz, DMSO-d_6): δ 7.80 (dd, *J* = 7.9, 1.6 Hz, 1H), 7.57 (d, *J* = 7.9 Hz, 1H), 7.50 (dd, *J* = 7.7, 1.2 Hz, 1H), 7.42 (td, *J* = 7.8, 1.3 Hz, 1H), 7.33 (ddd, *J* = 8.5, 7.3, 1.6 Hz, 1H), 7.26 (td, *J* = 7.6, 1.1 Hz, 1H), 6.88-6.76 (m, 2H), 5.52 (s, 1H), 4.65 (d, *J* = 9.4 Hz, 1H), 4.56 (q, *J* = 6.9 Hz, 1H), 2.63-2.44 (m, 2H), 1.70 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 172.4, 166.3, 163.4, 149.3, 138.8, 137.1, 133.1, 129.7, 128.2, 125.4, 124.9, 117.6, 115.8, 114.7, 114.5, 82.3, 76.4, 59.2, 37.2, 14.2. HRMS (ESI) calculated for $C_{22}H_{19}N_4O_4$ $[M-H]^-$ = 403.1412, obtained: 403.1403. $[a]_D^{20\,^oC}$ = - 32.0 ^{o} (c = 0.7, MeOH).

***N*-((2*S*,2a^1*S*,4*R*,5a*S*)-5a-Hydroxy-2-methyl-1,3-dioxo-1,2,2a^1,4,5,5a-hexahydro-3H-2a,9b-diazacyclopenta[*jk*]fluoren-4-yl)acrylamide (7)** Method F using **7a** (60 mg, 0.14 mmol): 23 mg, 53% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.39 (d, *J* = 8.5 Hz, 1H), 7.50-7.43 (m, 2H), 7.39 (t, *J* = 7.7 Hz, 1H), 7.22 (t, *J* = 7.5 Hz, 1H), 6.34 (dd, *J* = 17.1, 10.2 Hz, 1H), 6.15 (dd, *J* = 17.1, 2.2 Hz, 1H), 5.65 (dd, *J* = 10.1, 2.2 Hz, 1H), 5.46 (s, 1H), 4.85-4.69 (m, 1H), 4.56 (q, *J* = 6.9 Hz, 1H), 2.42 (dd, *J* = 12.9, 3.3 Hz, 1H), 1.93 (t, *J* = 12.8 Hz, 1H), 1.54 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (125 MHz, MeOD-d_4): δ 174.2, 169.8, 168.0, 140.5, 137.5, 131.8, 131.1, 127.4, 126.9, 125.6, 116.2, 84.4, 77.9, 61.0, 40.7, 15.1. HRMS (ESI) calculated for $C_{17}H_{16}N_3O_4$ $[M-H]^-$ = 326.1146, obtained: 326.1141.$[a]_D^{20\,^oC}$ = - 9.4 ^{o} (c = 0.6, MeOH).

2-Chloro-*N*-((2*S*,4*R*,5a*S*)-5a-hydroxy-2-methyl-1,3-dioxo-1,2,2a^1,4,5,5a-hexahydro-3H-2a,9b-diazacyclopenta[*jk*]fluoren-4-yl)acetamide (8). Method F using **8a** (40 mg, 0.09 mmol): 18 mg, 61% yield. ^{1}H NMR (400 MHz, MeOD-d_4): δ 7.55 (d, *J* = 7.9 Hz, 1H), 7.47 (d, *J* = 7.5 Hz, 1H), 7.41 (t, *J* = 7.7 Hz, 1H), 7.26 (t, *J* = 7.6 Hz, 1H), 5.50 (s, 1H), 4.85 (dd, *J* = 12.7, 3.4 Hz, 1H), 4.56 (q, *J* = 6.9 Hz, 1H), 4.17 (s, 2H), 2.59 (dd, *J* = 13.1, 3.4 Hz, 1H), 2.07 (t, *J* = 12.8 Hz, 1H), 1.68 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (125 MHz, MeOD-d_4): δ 174.1, 169.5, 169.2, 140.5, 137.5, 131.1, 126.9, 125.6, 116.2, 84.4, 77.8, 61.0, 43.2, 40.3, 15.1. HRMS (ESI) calculated for $C_{16}H_{15}{}^{35}ClN_3O_4$ $[M-H]^-$ = 348.0757, obtained: 348.0748. $[a]_D^{20\,^oC}$ = - 7.4 ^{o} (c = 1.0, MeOH).

***N*-((2*S*,4*R*,5a*S*)-5a-Hydroxy-2-methyl-1,3-dioxo-1,2,2a^1,4,5,5a-hexahydro-3H-2a,9b-diazacyclopenta*[jk]*fluoren-4-yl)oxirane-2-carboxamide (9)**. Method F using **9a** (37 mg, 0.09 mmol): 10 mg, 35% yield. ^{1}H NMR (400 MHz, MeOD-d_4): δ 7.55 (d, *J*

= 7.9 Hz, 1H), 7.47 (d, *J* = 7.6 Hz, 1H), 7.41 (t, *J* = 7.7 Hz, 1H), 7.25 (t, *J* = 7.6 Hz, 1H), 5.49 (s, 1H), 4.88-4.81 (m, 1H), 4.54 (q, *J* = 6.9 Hz, 1H), 3.48 (dd, *J* = 4.5, 2.5 Hz, 1H), 3.01 (dd, *J* = 6.1, 4.4 Hz, 1H), 2.96 (dd, *J* = 6.1, 2.5 Hz, 1H), 2.53 (dd, *J* = 13.1, 3.4 Hz, 1H), 2.13-2.00 (m, 1H), 1.67 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (125 MHz, MeOD-d_4): δ 174.1, 171.7, 169.5, 140.5, 137.5, 131.1, 126.9, 125.6, 116.2, 84.4, 77.9, 61.0, 49.9, 47.5, 40.3, 15.0. HRMS (ESI) calculated for $C_{17}H_{16}N_3O_5$ $[M-H]^-$ = 342.1095, obtained: 342.1088. $[\alpha]_D^{20\,°C}$ = - 24.9 ^{o} (c = 0.3, MeOH).

***N*-((2*S*,4*R*,5a*S*)-5a-Hydroxy-2-methyl-1,3-dioxo-1,2,2a1,4,5,5a-hexahydro-3H-2a, 9b-diazacyclopenta[*jk*]fluoren-4-yl)but-2-ynamide (10)**. Method F using **10a** (46 mg, 0.10 mmol): 23 mg, 67% yield. ^{1}H NMR (400 MHz, MeOD-d_4): δ 7.55 (d, *J* = 7.9 Hz, 1H), 7.47 (d, *J* = 7.6 Hz, 1H), 7.41 (t, *J* = 7.8 Hz, 1H), 7.25 (t, *J* = 7.6 Hz, 1H), 5.48 (s, 1H), 4.84 (dd, *J* = 12.7, 3.8 Hz, 1H), 4.54 (q, *J* = 6.9 Hz, 1H), 2.52 (dd, *J* = 13.1, 3.4 Hz, 1H), 2.06 (dd, *J* = 12.4, 8.6 Hz, 1H), 2.01 (s, 3H), 1.67 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (125 MHz, MeOD-d_4): δ 174.1, 171.2, 169.2, 140.5, 137.4, 131.1, 126.9, 125.6, 116.1, 85.7, 84.3, 77.8, 75.1, 61.0, 40.4, 15.1, 3.1. HRMS (ESI) calculated for $C_{18}H_{16}N_3O_4$ $[M-H]^-$ = 338.1146, obtained: 338.1138. $[\alpha]_D^{20\,°C}$ = + 1.3^o (c = 0.5, MeOH).

***N*-((2*S*,2a^1*S*,4*R*,5a*S*)-5a-hydroxy-2-methyl-1,3-dioxo-1,2,2a1,4,5,5a-hexahydro-3H -2a,9b-diazacyclopenta[*jk*]fluoren-4-yl)ethenesulfonamide (11)**. Method G using **11a** (49 mg, 0.13 mmol): 39 mg, 71% yield.^{1}H NMR (500 MHz, MeOD-d_4): δ 7.52 (d, *J* = 7.9 Hz, 1H), 7.47-7.44 (d, *J* = 7.5 Hz, 1H), 7.39 (t, *J* = 7.8 Hz, 1H), 7.23 (t, *J* = 7.5 Hz, 1H), 6.78 (dd, *J* = 16.6, 9.9 Hz, 1H), 6.22 (d, *J* = 16.6 Hz, 1H), 5.96 (d, *J* = 9.9 Hz,

1H), 5.42 (s, 1H), 4.50 (q, *J* = 7.0 Hz, 1H), 4.23 (dd, *J* = 12.5, 3.4 Hz, 1H), 2.66 (dd, *J* = 13.2, 3.4 Hz, 1H), 1.97 (t, *J* = 12.9 Hz, 1H), 1.65 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, MeOD-d_4): δ 174.0, 169.4, 140.5, 138.3, 137.4, 131.1, 127.0, 126.2, 125.6, 116.1, 84.2, 77.9, 61.0, 52.8, 42.7, 15.1. HRMS (ESI) calculated for $C_{16}H_{18}N_3O_5S$ $[M+H]^+$ = 364.0962, obtained: 364.0959. $[a]_D^{20\,°C}$ = + 15.3 ^{o}(c = 0.4, MeOH).

2-Acrylamido-*N*-((2*S*,2a^1*S*,4*R*,5a*S*)-5a-hydroxy-2-methyl-1,3-dioxo-1,2,2a^1,4,5,5a-hexahydro-3H-2a,9b-diazacyclopenta[*jk*]fluoren-4-yl)benzamide (13). Method F using **13a** (23 mg, 0.04 mmol): 8 mg, 44% yield. ^{1}H NMR (500 MHz, Methanol-d_4): δ 8.50 (d, *J* = 8.3 Hz, 1H), 7.73 (dd, *J* = 7.8, 1.6 Hz, 1H), 7.60-7.37 (m, 4H), 7.29-7.20 (m, 2H), 6.51 (dd, *J* = 17.0, 10.2 Hz, 1H), 6.38 (dd, *J* = 17.1, 1.5 Hz, 1H), 5.83 (dd, *J* = 10.2, 1.5 Hz, 1H), 5.08 (dd, *J* = 12.8, 3.4 Hz, 1H), 4.61 (q, *J* = 6.9 Hz, 1H), 2.63 (dd, *J* = 13.2, 3.4 Hz, 1H), 2.19 (t, *J* = 12.9 Hz, 1H), 1.72 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, Methanol-d_4): δ 172.6, 169.5, 168.7, 164.7, 139.1, 137.6, 136.1, 131.8, 131.5, 127.6, 126.5, 125.6, 124.3, 123.5, 121.2, 114.8, 83.1, 76.6, 60.1, 59.7, 48.3, 38.8, 13.7, 13.1. HRMS (ESI) calculated for $C_{24}H_{23}N_4O_5$ $[M+H]^+$ = 447.1663, obtained: 447.1666. $[a]_D^{20\,°C}$ = - 4.0°(c = 0.3, MeOH).

3-Acrylamido-*N*-((2*S*,2a^1*S*,4*R*,5a*S*)-5a-hydroxy-2-methyl-1,3-dioxo-1,2,2a^1,4,5,5a-hexahydro-3H-2a,9b-diazacyclopenta[*jk*]fluoren-4-yl)benzamide (14). Method F using **14a** (13 mg, 0.02 mmol): 7 mg, 68% yield. ^{1}H NMR (500 MHz, Methanol-d_4): δ 8.15 (t, *J* = 2.0 Hz, 1H), 7.86 (dd, *J* = 8.1, 2.1 Hz, 1H), 7.64 (dt, *J* = 7.7, 1.3 Hz, 1H), 7.57 (d, *J* = 7.9 Hz, 1H), 7.52-7.39 (m, 3H), 7.27 (td, *J* = 7.5, 1.0 Hz, 1H), 6.52-6.37 (m, 2H), 5.82 (dd, *J* = 9.7, 2.2 Hz, 1H), 5.55 (s, 1H), 5.08 (dd, *J* = 12.7, 3.4 Hz, 1H),

4.58 (q, *J* = 6.9 Hz, 1H), 2.65 (dd, *J* = 13.1, 3.4 Hz, 1H), 2.19 (t, *J* = 12.9 Hz, 1H), 1.71 (d, *J* = 7.0 Hz, 3H).^{13}C NMR (125 MHz, Methanol-d_4): δ 172.8, 168.6, 168.4, 164.8, 139.1, 138.7, 136.2, 134.9, 130.9, 129.7, 128.7, 126.8, 125.6, 124.3, 123.1, 122.7, 119.1, 114.8, 83.0, 76.6, 59.6, 48.4, 39.3, 13.7. HRMS (ESI) calculated for $C_{24}H_{23}N_4O_5$ $[M+H]^+$ = 447.1663, obtained: 447.1667. $[a]_D^{20\,ºC}$ = - 5.3°(c = 0.3, MeOH).

4-Acrylamido-*N*-((2*S*,2a^1*S*,4*R*,5a*S*)-5a-hydroxy-2-methyl-1,3-dioxo-1,2,2a^1,4,5,5a-hexahydro-3H-2a,9b-diazacyclopenta[*jk*]fluoren-4-yl)benzamide (15). Method G using **15a** (26 mg, 0.0 5mmol): 3.5 mg, 16% yield. ^{1}H NMR (500 MHz, Methanol-d_4): δ 7.88 (d, *J* = 8.5 Hz, 2H), 7.79-7.74 (m, 2H), 7.55 (d, *J* = 7.9 Hz, 1H), 7.47 (d, *J* = 7.5 Hz, 1H), 7.40 (td, *J* = 7.7, 1.3 Hz, 1H), 7.24 (td, *J* = 7.5, 1.1 Hz, 1H), 6.45 (dd, *J* = 16.9, 9.6 Hz, 1H), 6.39 (dd, *J* = 16.9, 2.2 Hz, 1H), 5.80 (dd, *J* = 9.6, 2.3 Hz, 1H), 5.53 (s, 1H), 5.09-5.03 (m, 1H), 4.56 (q, *J* = 6.9 Hz, 1H), 2.61 (dd, *J* = 13.1, 3.4 Hz, 1H), 2.17 (t, *J* = 12.9, 1.7 Hz, 1H), 1.69 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 172.9, 168.0, 165.6, 163.4, 141.7, 139.0, 136.8, 131.6, 129.7, 128.8, 128.3, 127.5, 125.4, 124.8, 118.5, 114.4, 82.4, 76.3, 58.8, 47.7, 14.6. HRMS (ESI) calculated for $C_{24}H_{21}N_4O_5$ $[M-H]^-$ = 445.1517, obtained: 445.1515. $[a]_D^{20\,ºC}$ = + 5.2 ^{o} (c = 0.25, DMSO).

2-(2-Chloroacetamido)-*N*-((2*S*,2a^1*S*,4*R*,5a*S*)-5a-hydroxy-2-methyl-1,3-dioxo-1,2,2a^1,4,5,5a-hexahydro-3H-2a,9b-diazacyclopenta[*jk*]fluoren-4-yl)benzamide (16). Method F using **16a** (23 mg, 0.04 mmol): 4.3 mg, 23% yield. 1H NMR (500 MHz, Methanol-d_4): δ 8.44 (dd, *J* = 8.4, 1.1 Hz, 1H), 7.77 (dd, *J* = 7.9, 1.5 Hz, 1H),

7.63-7.47 (m, 3H), 7.43 (td, *J* = 7.7, 1.3 Hz, 1H), 7.29-7.20 (m, 2H), 5.57 (s, 1H), 5.08 (dd, *J* = 12.7, 3.3 Hz, 1H), 4.60 (d, *J* = 6.9 Hz, 2H), 4.32 (s, 2H), 2.64 (dd, *J* = 13.1, 3.4 Hz, 1H), 2.20 (t, *J* = 12.9 Hz, 1H), 1.72 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, MeOD-d_4): δ 172.7, 169.2, 168.6, 139.1, 131.7, 129.8, 127.7, 125.6, 124.3, 123.8, 121.2, 114.8, 83.1, 76.6, 59.7, 42.7, 38.9, 13.7, HRMS (ESI) calculated for $C_{23}H_{22}{}^{35}ClN_4O_5$ $[M+H]^+$ = 469.1273, obtained: 469.1272. $[a]_D^{20\,ºC}$ = - 4.0°(c = 0.15, MeOH).

3-(2-Chloroacetamido)-*N*-((2*S*,2a^1*S*,4*R*,5a*S*)-5a-hydroxy-2-methyl-1,3-dioxo-1,2,2a1,4,5,5a-hexahydro-3H-2a,9b-diazacyclopenta[*jk*]fluoren-4-yl)benzamide (17). Method F using **17a** (27 mg, 0.05 mmol): 8.1 mg, 37% yield. ^{1}H NMR (500 MHz, Methanol-d_4): δ 8.10 (t, *J* = 1.9 Hz, 1H), 7.81 (dd, *J* = 8.1, 2.2 Hz, 1H), 7.69-7.64 (m, 1H), 7.57 (d, *J* = 8.0 Hz, 1H), 7.52-7.40 (m, 3H), 7.27 (td, *J* = 7.6, 1.1 Hz, 1H), 5.55 (s, 1H), 5.08 (dd, *J* = 12.6, 3.4 Hz, 1H), 4.58 (q, *J* = 6.9 Hz, 1H), 4.23 (s, 2H), 2.64 (dd, *J* = 13.1, 3.4 Hz, 1H), 2.19 (t, *J* = 12.9 Hz, 1H), 1.71 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, Acetone-d_6): δ 171.1, 166.9, 166.6, 164.6, 137.4, 136.5, 134.5, 133.2, 128.0, 127.1, 123.9, 122.6, 121.4, 117.5, 113.1, 81.3, 74.9, 58.0, 46.7, 45.4, 40.9, 37.5, 27.6, 12.0. HRMS (ESI) calculated for $C_{23}H_{22}{}^{35}ClN_4O_5$ $[M+H]+$ = 469.1273, obtained: 469.1277. $[a]_D^{20\,ºC}$ =-11.2 ° (c = 0.3, MeOH).

4-(2-Chloroacetamido)-*N*-((2*S*,2a^1*S*,4*R*,5a*S*)-5a-hydroxy-2-methyl-1,3-dioxo-1,2,2a^1,4,5,5a-hexahydro-3H-2a,9b-diazacyclopenta[*jk*]fluoren-4-yl)benzamide (18). Method G using **18a** (28 mg, 0.06 mmol): 14 mg, 50 % yield. ^{1}H NMR (500 MHz, Methanol-d_4): δ 7.87 (d, *J* = 8.7 Hz, 2H), 7.71 (d, *J* = 8.7 Hz, 2H), 7.54 (d, *J* = 7.9 Hz,

1H), 7.46 (d, *J* = 7.5 Hz, 1H), 7.40 (t, *J* = 7.8 Hz, 1H), 7.24 (t, *J* = 7.5 Hz, 1H), 5.52 (s, 1H), 5.12-4.98 (m, 1H), 4.55 (q, *J* = 6.9 Hz, 1H), 4.20 (s, 2H), 2.60 (dd, *J* = 13.1, 3.4 Hz, 1H), 2.16 (t, *J* = 12.9 Hz, 1H), 1.68 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (125 MHz, Methanol-d_4): δ 174.1, 170.1, 169.3, 167.6, 142.7, 140.5, 137.6, 131.1, 130.9, 129.5, 126.9, 125.7, 120.5, 116.2, 84.4, 78.0, 61.0, 49.7, 44.1, 40.7, 15.1. HRMS (ESI) calculated for $C_{23}H_{22}{}^{35}ClN_4O_5$ $[M+H]^+$ = 469.1273, obtained: 469.1277. $[a]_D^{20\,°C}$ = - 0.6 ^{o} (c = 1.0, MeOH).

***N*-(2-(((2*S*,2a^1*S*,4*R*,5a*S*)-5a-Hydroxy-2-methyl-1,3-dioxo-1,2,2a1,4,5,5a-hexahydro-3H-2a,9b-diazacyclopenta[*jk*]fluoren-4-yl)carbamoyl)phenyl)oxirane-2-carboxamide (19)**. Method F using **19a** (12 mg, 0.02 mmol): 3.8mg, 41% yield. ^{1}H NMR (500 MHz, Methanol-d_4): δ 8.46 (d, *J* = 8.4 Hz, 1H), 7.78 (dd, *J* = 7.9, 1.5 Hz, 1H), 7.62-7.48 (m, 3H), 7.47-7.39 (m, 1H), 7.29-7.20 (m, 2H), 5.56 (s, 1H), 5.03 (dd, *J* = 12.7, 3.4 Hz, 1H), 4.60 (q, *J* = 7.0 Hz, 1H), 3.58-3.54 (m, 1H), 3.09 (dd, *J* = 5.8, 4.4 Hz, 1H), 3.00-2.95 (m, 1H), 2.67-2.58 (m, 1H), 2.23 (t, *J* = 12.9 Hz, 1H), 1.71 (d, *J* = 7.0, 3H). ^{13}C NMR (125 MHz, Methanol-d_4): δ 172.7, 168.5, 139.1, 137.4, 136.2, 131.9, 129.8, 127.7, 125.6, 124.3, 123.6, 121.2, 114.8, 83.0, 76.6, 59.7, 49.3, 46.5, 38.9, 13.7. HRMS (ESI) calculated for $C_{24}H_{23}N_4O_6$ $[M+H]^+$ = 463.1612, obtained: 463.1603. $[a]_D^{20\,°C}$ = - 5.9°(c = 0.3, MeOH).

***N*-(3-(((2*S*,2a^1*S*,4*R*,5a*S*)-5a-hydroxy-2-methyl-1,3-dioxo-1,2,2a^1,4,5,5a-hexahydro-3H-2a,9b-diazacyclopenta[*jk*]fluoren-4-yl)carbamoyl)phenyl)oxirane-2-carboxamide (20)**. Method F using **20a** (21 mg, 0.04 mmol): 4.5 mg, 27% yield. ^{1}H NMR (500 MHz, Methanol-d_4): δ 8.10 (d, *J* = 2.0 Hz, 1H), 7.83 (dd, *J* = 8.2, 2.1 Hz, 1H),

7.66 (d, *J* = 7.7 Hz, 1H), 7.58 (d, *J* = 8.0 Hz, 1H), 7.53-7.40 (m, 3H), 7.27 (t, *J* = 7.5 Hz, 1H), 5.55 (s, 1H), 5.08 (dd, *J* = 12.5, 6.1 Hz, 1H), 4.59 (q, *J* = 7.0 Hz, 1H), 3.58 (dd, *J* = 4.4, 2.4 Hz, 1H), 3.11-2.95 (m, 2H), 2.65 (dd, *J* = 13.1, 3.5 Hz, 1H), 2.19 (td, *J* = 12.9, 1.7 Hz, 1H), 1.71 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, Methanol-d_4): δ 174.2, 170.0, 169.7, 140.6, 139.33, 137.61, 136.29, 131.1, 130.2, 127.0, 125.66, 124.87, 124.47, 120.78, 116.19, 84.46, 77.99, 61.1, 50.2, 49.8, 47.3, 40.7, 15.2. HRMS (ESI) calculated for $C_{23}H_{24}N_4O_6$ $[M+H]^+$ = 463.1612, obtained: 463.1608. $[a]_D^{20\,°C}$ = - 6.9 ^{o} (c = 0.3, MeOH).

***N*-(4-(((2*S*,2a^1*S*,4*R*,5a*S*)-5a-Hydroxy-2-methyl-1,3-dioxo-1,2,2a^1,4,5,5a-hexahydro-3H-2a,9b-diazacyclopenta[*jk*]fluoren-4-yl)carbamoyl)phenyl)oxirane-2-carboxamide (21)**. Method G using **21a** (36 mg, 0.06 mmol): 4.2 mg, 15% yield. ^{1}H NMR (500 MHz, Methanol-d_4): δ 7.87 (d, *J* = 8.6 Hz, 2H), 7.74 (d, *J* = 8.6 Hz, 2H), 7.55 (d, *J* = 7.9 Hz, 1H), 7.47 (d, *J* = 7.5 Hz, 1H), 7.40 (t, *J* = 7.7 Hz, 1H), 7.24 (t, *J* = 7.5 Hz, 1H), 5.52 (s, 1H), 5.06 (dd, *J* = 12.6, 3.4 Hz, 1H), 4.56 (q, *J* = 6.9 Hz, 1H), 3.58-3.53 (m, 1H), 3.05-3.00 (m, 1H), 2.95 (dd, *J* = 6.1, 2.4 Hz, 1H), 2.60 (dd, *J* = 13.1, 3.4 Hz, 1H), 2.17 (t, *J* = 12.9 Hz, 1H), 1.68 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (125 MHz, Methanol-d_4): δ 174.2, 170.1, 169.7, 169.3, 142.4, 140.5, 137.6, 131.1, 130.9, 129.4, 126.9, 125.7, 120.8, 116.2, 84.4, 78.0, 61.0, 50.2, 49.7, 47.3, 40.7, 15.1. HRMS (ESI) calculated for $C_{24}H_{23}N_4O_6$ $[M+H]^+$ = 463.1612, obtained: 463.1613. $[a]_D^{20\,°C}$ = - 18.3 ^{o} (c = 0.24, MeOH).

2-(But-2-ynamido)-*N*-((2*S*,2a^1*S*,4*R*,5a*S*)-5a-Hydroxy-2-methyl-1,3-dioxo-1,2,2a1,4,5,5a-hexahydro-3H-2a,9b-diazacyclopenta[*jk*]fluoren-4-yl)benzamide (22).

Method F using **22a** (12 mg, 0.02 mmol): 4.5 mg, 49% yield.^{1}H NMR (500 MHz, Methanol-d_4): δ 8.36 (d, *J* = 8.4 Hz, 1H), 7.79 (dd, *J* = 7.8, 1.6 Hz, 1H), 7.60 – 7.48 (m, 3H), 7.43 (td, *J* = 7.7, 1.3 Hz, 1H), 7.31 – 7.17 (m, 2H), 5.57 (s, 1H), 5.09 (dd, *J* = 12.7, 3.4 Hz, 1H), 4.59 (q, *J* = 7.0 Hz, 1H), 2.66 (dd, *J* = 13.1, 3.4 Hz, 1H), 2.19 (t, *J* = 12.9 Hz, 1H), 2.07 (s, 3H), 1.72 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, MeOD-d_4): δ 172.7, 168.6, 158.9, 139.1, 137.6, 136.1, 131.9, 129.8, 127.7, 125.6, 124.3, 123.7, 121.4, 114.8, 83.0, 76.6, 59.7, 39.0, 27.2, 13.7, 1.8. HRMS (ESI) calculated for $C_{25}H_{23}N_4O_5$ $[M+H]^+$ = 459.1663, obtained: 459.1668. $[a]_D^{20\,ºC}$ = - 13.3°(c = 0.3, MeOH).

3-(But-2-ynamido)-*N*-((2*S*,2a^{1}*S*,4*R*,5a*S*)-5a-hydroxy-2-methyl-1,3-dioxo-1,2,2a^{1},4,5,5a-hexahydro-3H-2a,9b-diazacyclopenta[*jk*]fluoren-4-yl)benzamide (23). Method F using **23a** (21 mg, 0.04 mmol): 6.2 mg, 37% yield. ^{1}H NMR (500 MHz, Methanol-d_4): δ 8.06 (d, *J* = 2.3 Hz, 1H), 7.78 (dd, *J* = 8.0, 2.1 Hz, 1H), 7.64 (d, *J* = 7.8 Hz, 1H), 7.57 (d, *J* = 7.9 Hz, 1H), 7.50 (d, *J* = 7.6 Hz, 1H), 7.44 (dt, *J* = 11.9, 7.9 Hz, 2H), 7.27 (t, *J* = 7.5 Hz, 1H), 5.55 (s, 1H), 5.07 (dd, *J* = 12.7, 3.4 Hz, 1H), 4.58 (q, *J* = 7.0 Hz, 1H), 2.64 (dd, *J* = 13.1, 3.3 Hz, 1H), 2.19 (t, *J* = 12.8 Hz, 1H), 2.06 (s, 3H), 1.71 (d, *J* = 7.0 Hz, 3H).^{13}C NMR (125 MHz, Methanol-d_4): δ 172.3, 168.1, 167.8, 151.8, 138.7, 137.9, 135.72, 134.39, 129.2, 128.2, 125.0, 123.8, 122.5, 122.4, 118.4, 114.3, 84.2, 82.5, 76.1, 73.9, 6.1, 47.9,47.7, 38.7, 13.2, 1.3. HRMS (ESI) calculated for $C_{25}H_{23}N_4O_5$ $[M+H]^+$ = 459.1663, obtained: 459.1670. $[a]_D^{20\,ºC}$ = - 7.6°(c = 0.5, MeOH).

4-(But-2-ynamido)-*N*-((2*S*,2a^{1}*S*,4*R*,5a*S*)-5a-hydroxy-2-methyl-1,3-dioxo-1,2,2a1,4

,5,5a-hexahydro-3H-2a,9b-diazacyclopenta[*jk*]fluoren-4-yl)benzamide (24). Method G using **24a** (29 mg, 0.05 mmol): 5 mg, 21% yield. ^{1}H NMR (500 MHz, Methanol-d_4): δ 7.90-7.83 (m, 2H), 7.68 (d, *J* = 8.6 Hz, 2H), 7.55 (d, *J* = 7.9 Hz, 1H), 7.47 (d, *J* = 7.5 Hz, 1H), 7.40 (t, *J* = 7.7 Hz, 1H), 7.24 (t, *J* = 7.5 Hz, 1H), 5.52 (s, 1H), 5.11-5.01 (m, 1H), 4.56 (q, *J* = 6.9 Hz, 1H), 2.60 (dd, *J* = 13.1, 3.4 Hz, 1H), 2.16 (t, *J* = 12.8 Hz, 1H), 2.04 (s, 3H), 1.68 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (125 MHz, Methanol-d_4): δ 174.2, 170.1, 169.3, 153.6, 142.9, 140.5, 137.6, 131.1, 130.7, 129.4, 126.9, 125.7, 120.4, 116.2, 86.3, 84.4, 78.0, 75.9, 61.0, 49.7, 40.7, 15.1, 3.2. HRMS (ESI) calculated for $C_{25}H_{23}N_4O_5$ $[M+H]^+$ = 459.1663, obtained: 459.1670. $[a]_D^{20\,ºC}$ = - 2.1 ^{o} (c = 0.29, MeOH).

***N*-((2*S*,2a^{1}*S*,4*R*,5a*S*)-5a-Hydroxy-2-methyl-1,3-dioxo-1,2,2a1,4,5,5a-hexahydro-3 H-2a,9b-diazacyclopenta[*jk*]fluoren-4-yl)-2-(vinylsulfonamido)benzamide (25)**. Method F using **25a** (40 mg, 0.13 mmol): 6.9 mg, 11% yield.^{1}H NMR (500 MHz, Methanol-d_4): δ 7.78 (dd, *J* = 7.8, 1.5 Hz, 1H), 7.65-7.39 (m, 5H), 7.28-7.17 (m, 2H), 6.78 (dd, *J* = 16.5, 10.0 Hz, 1H), 6.21 (d, *J* = 16.5 Hz, 1H), 6.01 (d, *J* = 10.0 Hz, 1H), 5.56 (s, 1H), 5.12-5.03 (m, 1H), 4.59 (q, *J* = 7.0 Hz, 1H), 2.63 (dd, *J* = 13.1, 3.4 Hz, 1H), 2.19 (t, *J* = 12.9 Hz, 1H), 1.71 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, Methanol-d_4): δ 172.7, 169.2, 168.4, 139.1, 137.9, 136.1, 135.6, 132.1, 129.8, 127.9, 127.5, 125.6, 124.3, 123.6, 122.5, 120.6, 114.8, 83.0, 76.6, 59.7, 38.9, 13.7. HRMS (ESI) calculated for $C_{23}H_{23}N_4O_6S$ $[M+H]^+$ = 483.1333, obtained: 483.1334. $[a]_D^{20\,ºC}$ = - 5.4°(c = 0.5, MeOH).

***N*-((2*S*,2a^1*S*,4*R*,5a*S*)-5a-hydroxy-2-methyl-1,3-dioxo-1,2,2a^1,4,5,5a-hexahydro-3H-2a,9b-diazacyclopenta[*jk*]fluoren-4-yl)-3-(vinylsulfonamido)benzamide (26)**. Method G using **26a** (20 mg, 0.03 mmol): 5.1mg, 35.2% yield. ^{1}H NMR (500 MHz, Methanol-d_4): δ 7.71 (d, *J* = 2.1 Hz, 1H), 7.63 (dt, *J* = 7.0, 1.8 Hz, 1H), 7.57 (d, *J* = 7.9 Hz, 1H), 7.50 (dd, *J* = 7.6, 1.2 Hz, 1H), 7.46-7.40 (m, 3H), 7.27 (td, *J* = 7.5, 1.0 Hz, 1H), 6.72 (dd, *J* = 16.5, 10.0 Hz, 1H), 6.22 (d, *J* = 16.5 Hz, 1H), 6.00 (d, *J* = 10.0 Hz, 1H), 5.55 (s, 1H), 5.07 (dd, *J* = 12.6, 3.4 Hz, 1H), 4.59 (q, *J* = 7.0 Hz, 1H), 2.63 (dd, *J* = 13.1, 3.4 Hz, 1H), 2.19 (t, *J* = 12.9 Hz, 1H), 1.71 (d, *J* = 7.0 Hz, 3H).^{13}C NMR (125 MHz, Methanol-d_4): δ 172.3, 168.1, 167.6, 138.6, 137.7, 135.7, 135.2, 134.7, 129.2, 128.6, 126.4, 125.1, 123.8, 122.8, 122.2, 118.9, 114.3, 82.5, 76.1, 59.2, 47.9, 38.7, 13.2. HRMS (ESI) calculated for $C_{23}H_{23}N_4O_6S[M+H]^+$ = 483.1333, obtained: 483.1338. $[a]_D^{20\,°C}$ = - 8.0°(c = 0.3, MeOH).

***N*-((2*S*,2a^1*S*,4*R*,5a*S*)-5a-hydroxy-2-methyl-1,3-dioxo-1,2,2a^1,4,5,5a-hexahydro-3H-2a,9b-diazacyclopenta[*jk*]fluoren-4-yl)-4-(vinylsulfonamido)benzamide (27)**. Method G using **27a** (13 mg, 0.02 mmol): 4.3 mg, 40% yield. ^{1}H NMR (500 MHz, Methanol-d_4): δ 7.84 (d, *J* = 8.6 Hz, 2H), 7.55 (d, *J* = 7.9 Hz, 1H), 7.46 (d, *J* = 7.5 Hz, 1H), 7.40 (t, *J* = 7.7 Hz, 1H), 7.31-7.21 (m, 3H), 6.70 (dd, *J* = 16.5, 9.9 Hz, 1H), 6.23 (d, *J* = 16.5 Hz, 1H), 6.00 (d, *J* = 9.9 Hz, 1H), 5.52 (s, 1H), 5.11-5.00 (m, 1H), 4.55 (q, *J* = 6.9 Hz, 1H), 2.60 (dd, *J* = 13.1, 3.4 Hz, 1H), 2.15 (t, *J* = 12.9 Hz, 1H), 1.68 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (125 MHz, Methanol-d_4): δ 174.2, 170.1, 169.3, 142.6, 140.5, 137.6, 137.1, 131.1, 130.4, 129.8, 128.4, 126.9, 125.6, 119.8, 116.2, 84.4, 78.0, 61.0, 49.7, 40.7, 15.1. HRMS (ESI) calculated for $C_{23}H_{23}N_4O_6S$ $[M+H]^+$ = 483.1333,

obtained: 483.1334. $[a]_D^{20\,°C}$ = - 3.7 ^{o} (c = 0.3, MeOH).

(2*S*,2a^{1}*S*,4*R*,5a*S*)-4-(1-Acryloyl-4-oxo-1,4-dihydroquinazolin-3(2H)-yl)-5a-hydroxy-2-methyl-2a^{1},4,5,5a-tetrahydro-3H-2a,9b-diazacyclopenta[*jk*]fluorene-1,3(2H)-dione (29). Method A using **28** (20 mg, 0.05 mmol): 8.9 mg, 51% yield. ^{1}H NMR (400 MHz, Methanol-d_4): δ 8.05 (d, *J* = 7.7 Hz, 1H), 7.66 (td, *J* = 7.8, 1.6 Hz, 1H), 7.58 (d, *J* = 7.9 Hz, 1H), 7.52 - 7.39 (m, 4H), 7.27 (td, *J* = 7.5, 1.1 Hz, 1H), 6.74 (dd, *J* = 16.7, 10.4 Hz, 1H), 6.45 (dd, *J* = 16.7, 1.8 Hz, 1H), 5.89 (dd, *J* = 10.4, 1.8 Hz, 1H), 5.55 (s, 1H), 5.38 (d, *J* = 12.2 Hz, 1H), 5.07 (d, *J* = 12.2 Hz, 1H), 4.56 (q, *J* = 7.0 Hz, 1H), 2.45 (dd, *J* = 12.8, 3.4 Hz, 1H), 1.71 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, Methanol-d_4): δ 172.4, 167.0, 139.7, 139.0, 136.1, 132.7, 129.8, 128.4, 127.5, 126.1, 125.6, 124.3, 123.4, 114.8, 83.0, 76.5, 59.9, 13.5. HRMS (ESI) calculated for $C_{25}H_{23}N_4O_5$ $[M+H]^+$ = 459.1663, obtained: 459.1669. $[a]_D^{20\,°C}$ = - 25.5 ^{o} (c = 0.4, MeOH).

(2*S*,2a^{1}*S*,4*R*,5a*S*)-4-(1-(2-Chloroacetyl)-4-oxo-1,4-dihydroquinazolin-3(2H)-yl)-5a-hydroxy-2-methyl-2a^{1},4,5,5a-tetrahydro-3H-2a,9b-diazacyclopenta[*jk*]fluorene-1,3(2H)-dione (30). Method B using **28** (21 mg, 0.05 mmol): 9 mg, 51% yield. ^{1}H NMR (500 MHz, Methanol-d_4): δ 8.05 (d, *J* = 7.8 Hz, 1H), 7.77-7.65 (m, 2H), 7.58 (d, *J* = 7.9 Hz, 1H), 7.55-7.40 (m, 2H), 7.28 (td, *J* = 7.6, 1.1 Hz, 1H), 5.72-5.57 (m, 1H), 5.55 (s, 1H), 5.39-5.01 (m, 2H), 4.63-4.45 (m, 3H), 2.50 (d, *J* = 12.5 Hz, 1H), 1.72 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, Methanol-d_4): δ 172.3, 139.7, 139.0, 136.1, 129.8, 125.6, 124.4, 114.8, 83.0, 76.6, 59.9, 13.5. HRMS (ESI) calculated for $C_{24}H_{21}{}^{35}ClN_4O_5Na$ $[M+Na]^+$ = 503.1093, obtained: 503.1093.$[a]_D^{20\,°C}$ = - 42.8 ^{o} (c =

0.6, MeOH).

(2S,2a1S,4R,5aS)-5a-hydroxy-2-methyl-4-(4-oxo-1-propionyl-1,4-dihydroquinazolin-3(2H)-yl)-2a1,4,5,5a-tetrahydro-3H-2a,9b-diazacyclopenta[*jk*]fluorene-1,3(2H)-dione(31). **28** (40mg, 0.1 mmol, 1.0 eq) and triethylamine (0.1mL) were dissolved in dichloromethane (5 mL). Propionyl chloride (11 mg, 0.12 mmol, 1.2 eq) was added dropwise. The reaction was stirred at room temperature for 2 h. The water (2 mL) was added and the solution was purified by reverse phase HPLC to afford **31** (12mg,28.6%).^{1}H NMR (500 MHz, Methanol-d_4): δ 8.00 (d, J = 7.8 Hz, 1H), 7.59 (dd, J = 27.3, 7.6 Hz, 3H), 7.49 (dd, J = 7.6, 1.2 Hz, 1H), 7.41 (td, J = 7.8, 1.3 Hz, 2H), 7.25 (td, J = 7.5, 1.0 Hz, 1H), 5.62 (s, 1H), 5.51 (d, J = 16.3 Hz, 1H), 5.24 (d, J = 29.2 Hz, 1H), 5.04 (s, 1H), 4.54 (q, J = 6.9 Hz, 1H), 2.62 (s, 3H), 2.45 (dd, J = 13.0, 3.3 Hz, 1H), 1.69 (d, J = 7.0 Hz, 3H), 1.12 (t, J = 7.3 Hz, 3H). HRMS (ESI) calculated for $C_{25}H_{25}N_4O_5$ $[M+H]^+$ = 461.1819, obtained: 461.1828.

(4*R*,5a*S*)-2-(4-Aminobutyl)-5a-hydroxy-4-(4-oxoquinazolin-3(4H)-yl)-2a1,4,5,5a-tetrahydro-3H-2a,9b-diazacyclopenta[*jk*]fluorene-1,3(2H)-dione (32). **S15** (2.6 g, 4.5 mmol) was dissolved in dichloromethane (40 mL). The reaction system was degassed, filled with oxygen, and stirred at room temperature for 12 h. The solvent was removed on a rotary evaporator and the remaining residues were purified by flash column chromatography. **S16** was isolated as a white solid in 35% yield (942 mg). Then, **S16** (942 mg, 1.6 mmol, 3.0 equiv.) was dissolved in methanol (20 mL), followed by addition of AcOH (287 mg, 4.8 mmol, 3.0 equiv.) and sodium cyanoborohydride (204 mg, 3.2 mmol, 2 equiv.). The reaction was stirred at 35 ^{o}C for

5 h and quenched by addition of saturated sodium bicarbonate solution (20 mL). The mixture was extracted with ethyl acetate (20 mL×2). The combined organic layers were dried over anhydrous sodium sulfate. The solvent was removed on a rotary evaporator. The remaining residues were dissolved in the mixed solvent (10 mL, NH_3-H_2O : EtOH : Acetone : CH_2Cl_2 = 0.5:1:4:50), followed by addition of silica gel (2.8 g). The reaction was stirred at 35 ^oC for 12 h. The solvent was removed on a rotary evaporator, and the remaining residues were purified by flash column chromatography. **S17** was isolated as a white solid in 24% yield (214 mg).

Finally, **S17** (214 mg, 0.4mmol, 1.0 equiv.) was dissolved in the CH_2Cl_2/TFA mixture (10 mL, 4/1 ratio) and stirred at ambient temperature for 2 h. The solvent was removed on a rotary evaporator, and the remaining residues were purified by reverse phase HPLC to provide **32** (186 mg, 87%, a white solid).^{1}H NMR (500 MHz, Methanol-d_4): δ 8.35 (s, 1H), 8.20 (d, *J* = 8.0 Hz, 1H), 7.88 (ddd, *J* = 1.5, 7.2, 8.4 Hz, 1H), 7.75 (dd, *J* = 1.0, 8.3 Hz, 1H), 7.63 – 7.55 (m, 2H), 7.49 (dd, *J* = 1.2, 7.7 Hz, 1H), 7.44 (td, *J* = 1.2, 7.8 Hz, 1H), 7.26 (td, *J* = 1.0, 7.5 Hz, 1H), 5.60 (s, 1H), 5.25 (s, 1H), 4.70 (t, *J* = 3.7 Hz, 1H), 3.26 – 2.99 (m, 1H), 2.93 (dt, *J* = 4.5, 9.8 Hz, 2H), 2.72 – 2.53 (m, 2H), 2.01 (d, *J* = 13.2 Hz, 1H), 1.75 – 1.62 (m, 1H), 1.57 (dd, *J* = 8.2, 15.4 Hz, 2H), 1.15 (s, 1H). ^{13}C NMR (125 MHz, Methanol-d_4): δ 173.17, 162.20, 159.02, 158.73, 147.94, 140.43, 137.05, 136.14, 131.42, 128.92, 127.87, 127.47, 127.03, 125.84, 123.07, 118.62, 116.34, 116.02, 84.53, 78.14, 65.11, 40.51, 39.24, 29.65, 27.59. HRMS (ESI) calculated for $C_{25}H_{26}N_5O_4$ $[M+H]^+$ = 460.1979, obtained: 460.1985. $[a]_D^{20\ ^oC}$= -43.8 ^{o} (c = 1.0, MeOH).

N-(4-((2*R*,2a1*S*,4*R*,5a*S*)-5a-Hydroxy-1,3-dioxo-4-(4-oxoquinazolin-3(4H)-yl)-1,2,2a1,4,5,5a-hexahydro-3H-2a,9b-diazacyclopenta[*jk*]fluoren-2-yl)butyl)acrylamide (33). Method A using **32** (40 mg, 0.09 mmol) as the substrate: 7.2 mg, 16% yield, a white solid. ^{1}H NMR (500 MHz, Methanol-d_4): δ 8.42 (s, 1H), 8.25 (d, *J* = 8.0 Hz, 1H), 7.88 (ddd, *J* = 1.5, 7.2, 8.5 Hz, 1H), 7.73 (d, *J* = 8.1 Hz, 1H), 7.59 (dd, *J* = 8.2, 17.1 Hz, 2H), 7.48 (dd, *J* = 1.2, 7.6 Hz, 1H), 7.42 (td, *J* = 1.3, 7.8 Hz, 1H), 7.25 (td, *J* = 1.1, 7.6 Hz, 1H), 6.16 – 6.00 (m, 2H), 5.58 (s, 1H), 5.50 (dd, *J* = 3.4, 8.6 Hz, 1H), 4.63 (dd, *J* = 3.1, 4.9 Hz, 1H), 3.22 (dtd, *J* = 7.4, 14.1, 14.8, 21.4 Hz, 2H), 2.98 (s, 1H), 2.68 (dd, *J* = 3.2, 13.0 Hz, 1H), 2.62 – 2.46 (m, 1H), 2.09 – 1.93 (m, 1H), 1.48 (dd, *J* = 4.6, 11.0 Hz, 3H), 1.17 – 0.97 (m, 1H). ^{13}C NMR (125 MHz,Methanol-d_4): δ 173.14, 168.03, 162.00, 160.16, 159.85, 147.21, 140.44, 137.02, 136.31, 132.06, 131.45, 129.11, 127.98, 127.03, 126.36, 125.86, 116.04, 115.49, 84.52, 78.13, 65.18, 40.03, 30.04, 30.04, 27.63. HRMS (ESI) calculated for $C_{28}H_{27}N_5O_5$ $[M+H]^+$ = 514.2085, obtained: 514.2086. $[\alpha]_D^{20\,^oC}$ = -9.7 ^{o} (c =0.6, MeOH).

2-Chloro-N-(4-((2*R*,2a1*S*,4*R*,5a*S*)-5a-hydroxy-1,3-dioxo-4-(4-oxoquinazolin-3(4H)-yl)-1,2,2a1,4,5,5a-hexahydro-3H-2a,9b-diazacyclopenta[*jk*]fluoren-2-yl)butyl)acetamide (34). Method B using **32** (40 mg, 0.09 mmol): 6.4 mg, 14% yield, a white solid.^{1}H NMR (500 MHz, Methanol-d_4): δ 8.33 (s, 1H), 8.26 (d, *J* = 8.0 Hz, 1H), 7.87 (ddd, *J* = 1.5, 7.2, 8.4 Hz, 1H), 7.76 – 7.69 (m, 1H), 7.64 – 7.55 (m, 2H), 7.49 (d, *J* = 7.5 Hz, 1H), 7.43 (td, *J* = 1.3, 7.8 Hz, 1H), 7.26 (td, *J* = 1.0, 7.5 Hz, 1H), 5.58 (s, 1H), 5.01 – 4.90 (m, 1H), 4.64 (dd, *J* = 3.0, 4.8 Hz, 1H), 3.89 (d, *J* = 7.9 Hz, 2H), 3.21 (dd, *J* = 13.0, 19.6 Hz, 2H), 2.67 (dd, *J* = 3.2, 12.9 Hz, 1H), 2.56 (t, *J* = 13.2 Hz, 1H), 2.05

– 1.93 (m, 1H), 1.54 – 1.38 (m, 3H), 1.29 (s, 1H), 1.05 (d, *J* = 11.0 Hz, 1H). ^{13}C NMR (125 MHz, Methanol-d_4): δ 173.27, 169.16, 162.33, 148.25, 140.51, 137.07, 136.09, 131.44, 128.88, 127.81, 127.73, 127.02, 125.88, 116.04, 84.53, 78.19, 65.11, 43.16, 40.41, 29.98, 27.66. HRMS (ESI) calculated for $C_{27}H_{26}ClN_5O_5$ $[M+H]^+$ = 536.1695, obtained: 536.1698. $[a]_D^{20\,ºC}$ = -10.3 ^{o} (c =0.4, $CHCl_3$).

N-(4-((2*R*,2a1*S*,4*R*,5a*S*)-5a-Hydroxy-1,3-dioxo-4-(4-oxoquinazolin-3(4H)-yl)-1,2,2a1,4,5,5a-hexahydro-3H-2a,9b-diazacyclopenta[*jk*]fluoren-2-yl)butyl)oxirane-2-carboxamide (35). Method C using **32** (40 mg, 0.09 mmol): 8.8 mg, 18% yield, a white solid.^{1}H NMR (500 MHz, Methanol-d_4): δ 8.40 – 8.22 (m, 1H), 7.87 (ddd, *J* = 1.6, 7.2, 8.6 Hz, 1H), 7.73 (dd, *J* = 2.6, 7.7 Hz, 1H), 7.65 – 7.55 (m, 2H), 7.50 (dd, *J* = 3.8, 7.6 Hz, 1H), 7.44 (tdt, *J* = 1.5, 3.0, 7.9 Hz, 1H), 7.33 – 7.20 (m, 1H), 5.59 (d, *J* = 2.6 Hz, 1H), 4.63 (t, *J* = 4.1 Hz, 1H), 3.28 – 3.21 (m, 1H), 3.21 – 3.14 (m, 1H), 3.06 – 2.82 (m, 1H), 2.78 (dd, *J* = 4.4, 6.1 Hz, 1H), 2.68 (d, *J* = 13.0 Hz, 1H), 2.64 – 2.48 (m, 2H), 2.08 – 1.84 (m, 1H), 1.55 – 1.34 (m, 2H), 1.29 (s, 1H), 1.04 (d, *J* = 12.7 Hz, 1H). HRMS (ESI) calculated for $C_{29}H_{27}N_5O_5$ $[M+H]^+$ = 530.2034,obtained: 530.2039. $[a]_D^{20\,ºC}$ = -3.9 ^{o} (c =0.3, MeOH).

N-(4-((2*R*,2a1*S*,4*R*,5a*S*)-5a-Hydroxy-1,3-dioxo-4-(4-oxoquinazolin-3(4H)-yl)-1,2,2a1,4,5,5a-hexahydro-3H-2a,9b-diazacyclopenta[*jk*]fluoren-2-yl)butyl)but-2-ynamide (36). Mehod D using **32** (40 mg, 0.09 mmol): 7.8 mg, 17% yield, a white solid. ^{1}H NMR (500 MHz, Methanol-d_4): δ 8.36 (s, 1H), 8.28 (dd, *J* = 1.4, 8.0 Hz, 1H), 7.87 (ddd, *J* = 1.5, 7.2, 8.5 Hz, 1H), 7.71 (dd, *J* = 1.0, 8.2 Hz, 1H), 7.63 – 7.55 (m, 2H), 7.48 (dd, *J* = 1.2, 7.6 Hz, 1H), 7.42 (td, *J* = 1.3, 7.8 Hz, 1H), 7.25 (td, *J* = 1.0, 7.5 Hz,

1H), 5.57 (s, 1H), 4.62 (dd, *J* = 3.1, 4.9 Hz, 1H), 3.13 (d, *J* = 6.3 Hz, 2H), 2.67 (dd, *J* = 3.2, 13.0 Hz, 1H), 2.53 (d, *J* = 4.3 Hz, 1H), 1.98 (ddt, *J* = 4.1, 13.5, 16.6 Hz, 1H), 1.85 (s, 3H), 1.42 (q, *J* = 7.3 Hz, 2H), 1.05 (s, 1H). ^{13}C NMR (125 MHz, Methanol-d_4): δ 173.19, 167.30, 162.14, 155.84, 147.73, 140.44, 137.02, 136.18, 131.43, 128.98, 127.95, 127.35, 127.02, 125.89, 125.86, 123.00, 116.03, 84.62, 84.51, 78.14, 75.42, 65.10, 44.07, 40.36, 39.23, 31.36, 29.89, 27.67, 3.10. HRMS (ESI) calculated for $C_{29}H_{27}N_5O_5$ $[M+H]^+$ = 526.2085,obtained: 526.2086. $[a]_D^{20\,°C}$ = 3.2 ^{o} (c =1.0, MeOH).

(2*R*,2a1*S*,4*R*,5a*S*)-2-(Aminomethyl)-5a-hydroxy-4-(4-oxoquinazolin-3(4H)-yl)-2a 1,4,5,5a-tetrahydro-3H-2a,9b-diazacyclopenta[*jk*]fluorene-1,3(2H)-dione (37). The synthesis of **S21** from **S20** was similar to that of **S15**. 4.3 g of **S20** was used and **S21** was obtained as a white solid in 4.1 g, 95.8% yield. The synthesis of **S22** from **S21** was similar to that of **S16**. 4.1 g of **S21** was used and **S22** was obtained as a white solid in 1.3 g, 32% yield.

S22 (446 mg, 0.8 mmol, 1.0 equiv.) was dissolved in MeOH (10 mL), followed by addition of AcOH (0.1mL) and $NaBH(OAc)_3$ (346 mg, 1.6 mmol, 2.0 equiv.). The reaction was stirred at 35 ^{o}C for 5 h, and quenched by addition of saturated sodium bicarbonate solution (20 mL). The mixture was extracted with dichloromethane (20 mL×2). The combined organic layers were dried over anhydrous sodium sulfate. The solvent was removed on a rotary evaporator and the remaining residues were purified by flash column chromatography. **S23** was isolated as a white solid in 40% yield (167 mg).

Finally, **S23** (167 mg, 0.32 mmol) was dissolved in the CH_2Cl_2/TFA mixture (10 mL, 4/1 ratio) and the solution was stirred at ambient temperature for 1 h. The solvent was removed on a rotary evaporator and the remaining residues were purified by reverse phase HPLC to provide **37** as a white solid (118 mg, 88%).^{1}H NMR (500 MHz, Methanol-d_4): δ 8.30 (s, 1H), 8.22 (d, *J* = 7.9 Hz, 1H), 7.87 (ddd, *J* = 1.6, 7.1, 8.5 Hz, 1H), 7.72 (d, *J* = 8.2 Hz, 1H), 7.62 – 7.54 (m, 2H), 7.51 (dd, *J* = 1.1, 7.6 Hz, 1H), 7.44 (td, *J* = 1.3, 7.8 Hz, 1H), 7.28 (td, *J* = 1.1, 7.6 Hz, 1H), 5.70 (s, 1H), 4.91 (dd, *J* = 2.4, 8.2 Hz, 1H), 3.88 – 3.68 (m, 1H), 3.62 (dd, *J* = 8.4, 14.2 Hz, 1H), 2.96 (d, *J* = 18.8 Hz, 1H), 2.67 (dd, *J* = 3.4, 13.1 Hz, 1H). HRMS (ESI) calculated for $C_{22}H_{20}N_5O_4$ $[M+H]^+$ = 418.1510, obtained: 418.1509. $[\alpha]_D^{20\,°C}$= -38.7 ^{o} (c =0.1, MeOH).

N-(((2*R*,2a1*S*,4*R*,5a*S*)-5a-Hydroxy-1,3-dioxo-4-(4-oxoquinazolin-3(4H)-yl)-1,2,2a 1,4,5,5a-hexahydro-3H-2a,9b-diazacyclopenta[*jk*]fluoren-2-yl)methyl)acrylamide (38). Method A using **37** (38 mg, 0.07 mmol): 18 mg, 42% yield, a white solid.^{1}H NMR (500 MHz, Methanol-*d*4): δ 8.55 (s, 1H), 8.25 (s, 1H), 7.92 – 7.85 (m, 1H), 7.73 (d, J = 8.1 Hz, 1H), 7.61 (t, J = 7.6 Hz, 1H), 7.56 (d, J = 8.0 Hz, 1H), 7.45 (d, J = 7.5 Hz, 1H), 7.40 (td, J = 1.3, 7.8 Hz, 1H), 7.23 (t, J = 7.5 Hz, 1H), 6.26 (s, 1H), 6.10 (d, J = 15.7 Hz, 1H), 5.61 (s, 1H), 5.54 (s, 1H), 4.67 (t, J = 3.8 Hz, 1H), 4.38 (d, J = 65.7 Hz, 1H), 2.87 (s, 1H), 2.65 (dd, J = 3.5, 12.9 Hz, 1H). HRMS (ESI) calculated for $C_{25}H_{22}N_5O_5$ $[M+H]^+$ = 472.1655, obtained: 472.1622. $[\alpha]_D^{20\,°C}$ = -38.7 ^{o}(c =0.1, MeOH).

2-Chloro-N-(((2*R*,2a1*S*,4*R*,5a*S*)-5a-hydroxy-1,3-dioxo-4-(4-oxoquinazolin-3(4H)-

yl)-1,2,2a1,4,5,5a-hexahydro-3H-2a,9b-diazacyclopenta[*jk*]fluoren-2-yl)methyl)ac etamide (39). Method B using **37** (60 mg, 0.14 mmol): 24.8 mg, 35% yield, a white solid. ^{1}H NMR (500 MHz, Methanol-d_4): δ 8.55 (s, 1H), 8.26 (d, *J* = 8.1 Hz, 1H), 7.72 (d, *J* = 8.2 Hz, 1H), 7.61 (t, *J* = 7.5 Hz, 1H), 7.56 (d, *J* = 8.1 Hz, 1H), 7.46 (d, *J* = 7.5 Hz, 1H), 7.41 (td, *J* = 1.3, 7.7 Hz, 1H), 7.24 (t, *J* = 7.5 Hz, 1H), 5.61 (s, 1H), 4.74 – 4.60 (m, 1H), 4.51 – 4.20 (m,1H), 3.95 (d, *J* = 29.1 Hz, 2H), 2.90 (s, 1H), 2.66 (dd, *J* = 3.4, 12.9 Hz, 1H). HRMS (ESI) calculated for $C_{24}H_{21}ClN_5O_5$ $[M+H]^+$ = 494.1226, obtained: 494.1227. $[a]_D^{20\,°C}$ = -22.8 ^{o} (c =1.0, MeOH).

N-(((2*R*,2a1*S*,4*R*,5a*S*)-5a-Hydroxy-1,3-dioxo-4-(4-oxoquinazolin-3(4H)-yl)-1,2,2a 1,4,5,5a-hexahydro-3H-2a,9b-diazacyclopenta[*jk*]fluoren-2-yl)methyl)oxirane-2-c arboxamide (40). Method C using **37** (65 mg,0.15mmol): 31 mg, 41% yield, a white solid.^{1}H NMR (500 MHz, Methanol-d_4): δ 8.32 (s, 1H), 8.23 (s, 1H), 7.84 (ddd, *J* = 1.6, 7.2, 8.5 Hz, 1H), 7.72 – 7.66 (m, 1H), 7.56 (t, *J* = 8.2 Hz, 2H), 7.48 (dd, *J* = 1.0, 7.4 Hz, 1H), 7.42 (td, *J* = 1.2, 7.8 Hz, 1H), 7.25 (td, *J* = 1.0, 7.5 Hz, 1H), 6.15 (s, 0H), 5.59 (d, *J* = 7.9 Hz, 1H), 4.63 – 4.57 (m, 1H), 4.35 (s, 1H), 3.35 (s, 0H), 2.77 (dd, *J* = 4.5, 6.0 Hz, 1H), 2.65 (dd, *J* = 3.5, 12.9 Hz, 1H), 2.54 – 2.31 (m, 1H). HRMS (ESI) calculated for $C_{25}H_{22}N_5O_6$ $[M+H]^+$ = 488.1565, obtained: 488.1575. $[a]_D^{20\,°C}$ = -18.2 ^{o} (c =1.0, MeOH).

N-(((2*R*,2a1*S*,4*R*,5a*S*)-5a-Hydroxy-1,3-dioxo-4-(4-oxoquinazolin-3(4H)-yl)-1,2,2a 1,4,5,5a-hexahydro-3H-2a,9b-diazacyclopenta[*jk*]fluoren-2-yl)methyl)but-2-yna mide (41). Method D using **37** (45 mg, 0.11mmol): 20.9mg, 40% yield, a white solid.^{1}H NMR (500 MHz, Methanol-d_4): δ 8.48 (s, 1H), 8.26 (d, *J* = 7.8 Hz, 1H), 7.88

(ddd, *J* = 1.5, 7.2, 8.6 Hz, 1H), 7.72 (dd, *J* = 1.0, 8.2 Hz, 1H), 7.64 – 7.59 (m, 1H), 7.57 (d, *J* = 7.9 Hz, 1H), 7.48 (dd, *J* = 1.2, 7.7 Hz, 1H), 7.42 (td, *J* = 1.3, 7.8 Hz, 1H), 7.25 (td, *J* = 1.1, 7.5 Hz, 1H), 6.04 (s, 1H), 5.60 (s, 1H), 4.63 (dd, *J* = 3.0, 5.5 Hz, 1H), 4.41 (dd, *J* = 3.1, 15.0 Hz, 1H), 3.66 (d, *J* = 16.8 Hz, 1H), 2.85 (s, 1H), 2.66 (dd, *J* = 3.5, 12.9 Hz, 1H), 1.87 (s, 3H). HRMS (ESI) calculated for $C_{26}H_{22}N_5O_5$ $[M+H]^+$ = 484.1615, obtained: 484.1617. $[a]_D^{20\,°C}$ = 11.3° (c =1.0, MeOH).

N-(((2*R*,2a1*S*,4*R*,5a*S*)-5a-Hydroxy-1,3-dioxo-4-(4-oxoquinazolin-3(4H)-yl)-1,2,2a 1,4,5,5a-hexahydro-3H-2a,9b-diazacyclopenta[*jk*]fluoren-2-yl)methyl)ethenesulfo namide (42). Method E using **37** (49 mg, 0.12 mmol): 8.4 mg, 14% yield, a white solid.^{1}H NMR (500 MHz, Methanol-d_4): δ 8.43 (s, 1H), 8.27 (d, *J* = 7.9 Hz, 1H), 7.89 (ddd, *J* = 1.5, 7.2, 8.5 Hz, 1H), 7.73 (dd, *J* = 1.1, 8.3 Hz, 1H), 7.65 – 7.56 (m, 2H), 7.48 (dd, *J* = 1.1, 7.3 Hz, 1H), 7.44 (td, *J* = 1.2, 7.7 Hz, 1H), 7.26 (td, *J* = 1.0, 7.6 Hz, 1H), 6.52 (t, *J* = 13.4 Hz, 1H), 6.09 (d, *J* = 16.6 Hz, 1H), 5.80 (s, 1H), 5.63 (s, 1H), 4.69 (t, *J* = 3.5 Hz, 1H), 4.05 (dd, *J* = 3.2, 14.4 Hz, 1H), 3.65 (d, *J* = 28.2 Hz, 1H), 3.05 – 2.80 (m, 1H), 2.65 (d, *J* = 12.8 Hz, 1H). HRMS (ESI) calculated for $C_{24}H_{22}N_5O_6S$ $[M+H]^+$ = 508.1285, obtained: 508.1296. $[a]_D^{20\,°C}$ = -6.1 ° (c =1.0, MeOH).

5.3 Cell Culture

The K562, MM.1S, MV4-11, and RKO cell lines were obtained from National Collection of Authenticated Cell Cultures in Shanghai, China. All cell lines passed STR analysis when received. K562 and MV4-11 cells were cultured in IMDM (Gibco, C12440500BT) supplemented with 10% fetal bovine serum (Gibco, 10091-148),

MM1.S cells were cultured in RPMI (Gibco, C11875500BT) supplemented with 10% fetal bovine serum, and RKO cells were cultured in MEM (Gibco, C11095500BT) supplemented with 10% fetal bovine serum.

The testing compounds were serially diluted in 96-well plates from the highest concentration of 400 uM. Subsequently, the K562 cells or MV4-11 cells were seeded into the wells at the density of 2000 cells/well or 25000 cells/well, respectively. For MM.1S cells or RKO cells, the attached cells were washed with PBS and digested by trypsin. Subsequently, the MM.1S cells or RKO cells were seeded into the wells at the density of 30000 cells/well or 8000 cells/well, respectively. For all the four cell types, the cells in plates were incubated with 5% CO_2 at 37 ^{o}C for 4 days. Thereafter, the reagent of cell counting kit-8 (Dojindo) was added to measure the cell viability according to the manufacturer's protocols. The absorbance of OD_{450} was detected by a multimode microplate reader (TECAN SPARK 10 M). The untreated cells were set as 100% cell viability. The IC_{50} values were calculated by fitting nonlinear regression curve in GraphPad Prism 7.

Credit Author Statement

X. Z., D. W., D. C., G.W., and H. W. designed and synthesized the compounds. X. F., Z. Y., and X. L. developed the methods and tested the cellular activities, X. Z., D. W., D. C., G.W., and X. F. collected the data. J. Y. and Y. Z. designed the experiments, supervised the study and wrote the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this

paper.

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Abbreviation

AcOH: Acetic acid; BTK: Bruton's tyrosine kinase; DCE: 1,2-dichloroethane; EGFR: Epidermal growth factor receptor; Keap1: Kelch-like ECH-associated protein 1; NMM: N-Methyl morpholine; Nrf2: Nuclear factor erythroid 2-related factor 2; MeCN: acetonitrile; MEK1: Dual specificity mitogen-activated protein kinase kinase 1; PI3K: Phosphoinositide 3-kinase; p-TsOH: p-Toluenesulfonic acid; NCS: N-Chlorosuccinimide; STR: Short tandem repeat; TCI: Targeted covalent inhibitor; TES: Triethylsilyl; TFA: Trifluoroacetic acid; THF: Tetrahydrofuran.

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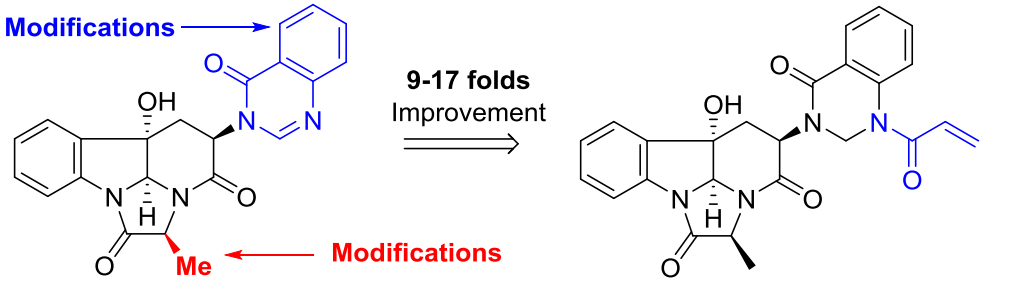
Graphical Abstract



Chaetominine

K562 IC_{50} = 98.62±13.56 μM
MM1.S IC_{50} = 65.48±9.52 μM
MV4;11 IC_{50} = 79.23±1.06 μM
RKO IC_{50} = 169.53±6.39 μM

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K562 IC_{50} = 5.61±0.33 μM
MM1.S IC_{50} = 7.18±0.37 μM
MV4;11 IC_{50} = 8.54±2.45 μM
RKO IC_{50} = 18.80±0.76 μM