

Article

Synthesis of Cucurbitacin B Derivatives as Potential Anti-Hepatocellular Carcinoma Agents

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Abstract: Cucurbitacin B shows potent activity against tumor cells, but its high toxicity limits its application in the clinic. A series of cucurbitacin B derivatives was synthesized and evaluated for their anti-hepatocellular carcinoma (HCC) activities against the HepG-2 cell line. These compounds were also tested for their toxicity against the L-O2 normal cell line. The compound with the most potential, **10b**, exhibited potent activity against the HepG-2 cell line with an IC₅₀ value of 0.63 μM. Moreover, compound **10b** showed the highest TI value (4.71), which is a 14.7-fold improvement compared to its parent compound cucurbitacin B. A preliminary molecular mechanism study of **10b** indicated that **10b** could inhibit P-STAT3 to induce the activation of mitochondrial apoptotic pathways. An in vivo acute toxicity study indicated that the compound **10b** has preferable safety and tolerability compared with cucurbitacin B. These findings indicate that compound **10b** might be considered as a lead compound for exploring effective anti-HCC drugs.

Keywords: Cucurbitacin B; Anti-hepatocellular carcinoma; Derivative; Toxicity; Synthesis

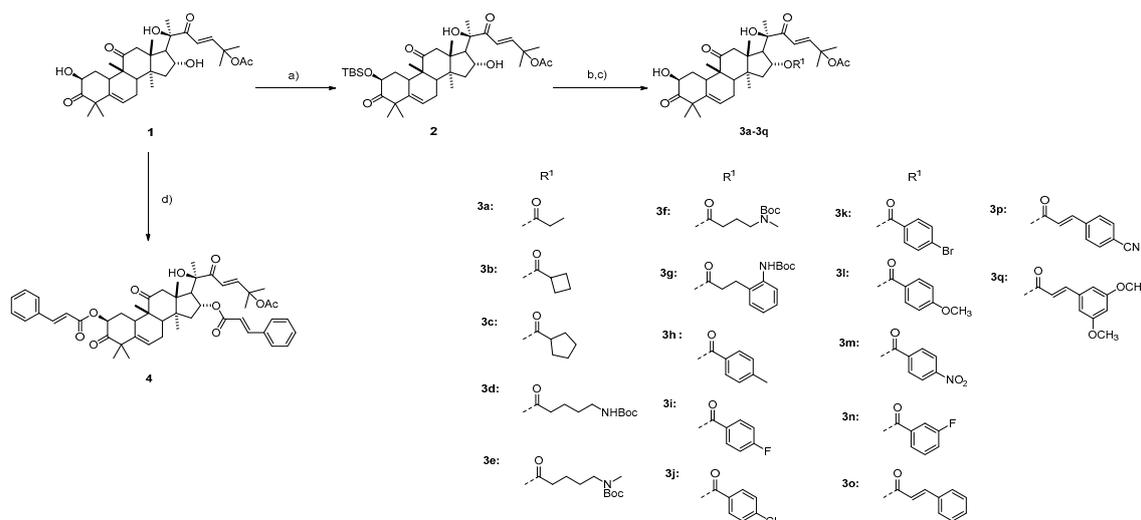
1. Introduction

Cancer is a disease with high morbidity and mortality. It is the second leading cause of death, after cardiovascular disease, worldwide [1,2]. The World Health Organization has reported that there were approximately 17.5 million new cancer cases and more than 8.7 million cancer-related deaths worldwide in 2015, and cancer-related deaths are expected to increase to 13.1 million by 2030 [3–5]. Cancer is a type of abnormal and excessive growth of tissue, in which abnormal body cells begin to divide uncontrollably by ignoring the principles of normal cell division. Cancer cells invade or spread to other healthy parts of the body, resulting in the formation of new tumor; this process is called metastasis. Approximately 90% of cancer-linked deaths are caused by it [5–7]. So far, cancer treatments mostly rely on traditional chemotherapy, especially for late-stage or complex cancers. Unfortunately, side effects and acquired drug resistance limit the effectiveness of traditional chemotherapy. Side effects, such as extreme fatigue and leukopenia, are caused by traditional chemotherapy's low selectivity towards cancer cells and normal cells. These unpleasant side effects seriously reduce the life quality of cancer patients [8–10]. Therefore, the development of new chemotherapeutic drugs with high efficiency and low-toxicity side effects is a great challenge for medicinal chemists. Natural compounds can induce apoptosis by targeting multiple cellular signaling pathways; so, the development of new pharmacologically effective chemotherapeutic agents from natural compounds may be feasible [11–13].

2. Results and Discussion

2.1. Chemistry

The starting material cucurbitacin B was isolated from *Cucumis melo* L. The presence of free hydroxyl groups allowed us to prepare ester derivatives of cucurbitacin B for an evaluation of the influence of the ester side chain on their anti-HCC activities. It was reported that the introduction of an acyl group to a 2-hydroxyl moiety greatly reduced the anticancer activity [41]. We planned to explore the effect of selective acylation on a 16-hydroxyl group on the anti-HCC activity. However, a 2-hydroxyl group is more reactive than a 16-hydroxyl group for acylation [41]. Therefore, we first designed protection for the 2-hydroxyl group. As shown in Scheme 1, the treatment of compound **1** with *t*-butyldimethylsilyl chloride (TBSCl) in the presence of imidazole selectively provided the 2-TBS-protected derivative **2** without the influence of the hydroxyl group linked to C20 of the cucurbitacin B. The hydroxyl group linked to C20 is a tertiary hydroxyl group that shows a higher steric hindrance effect than the C16 hydroxyl group. Therefore, the C16 hydroxyl group was selectively esterified without affecting the hydroxyl group linked to C20 of the cucurbitacin B. Compound **2** was coupled with different patterns of carboxylic acids in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) and 4-dimethylaminopyridine (DMAP), followed by removal of the TBS protection to afford the esters **3a–3q** in yields of 48–86%. Direct conjugation of compound **1** with cinnamic acid provided the derivative **4** in a high yield (90%).



Scheme 1. The synthesis of compounds **3a–3q** and **4**. Reagents and conditions: a) TBSCl, imidazole, DCM, 43%; b) R¹COOH, EDCI, TEA, DMAP, DCM; c) TBAF/AcOH, THF, 47–86% yield in two steps; d) Cinnamic acid, EDCI, TEA, DMAP, DCM, 90%.

It was reported that the introduction of a phenylsulfonyl-substituted furoxan NO-releasing moiety could reduce the toxicity of brusatol [42–46]. Accordingly, 18 cucurbitacin B derivatives, **9a–9c** and **10a–10o**, with the introduction of a phenylsulfonyl-substituted furoxan NO-releasing moiety were designed (Scheme 2). Compound **5** was prepared according to a reported procedure [42]. Reaction of **5** with different diols or alcoholamines gave **6a–6o** or **7a–7c**, respectively [42–44,46]. Compounds **6a–6o** were treated with succinic anhydride in the presence of DMAP to produce the corresponding acids, followed by conjugation with compound **2** and removal of the TBS group to yield **10a–10o** in yields of 61–82% in two steps. The acid **8** was obtained by the reaction of **2** and succinic anhydride. The removal of Boc groups of **7a–7c** gave the corresponding amines, followed by coupling with the acid **8** in the presence of 2-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HATU) and *N,N*-diisopropylethylamine (DIPEA) and deprotection of the TBS group to afford **9a–9c** in yields

Table 1. The inhibitory activity and clog P of **3a–3q** and **4** against the HepG-2 and L-O2 cell lines.

Compound	IC ₅₀ ^a (μM)		TI ^b	Clog P ^d
	HepG-2	L-O2		
1	0.060 ± 0.02	0.019 ± 0.003	0.32	2.96
3a	1.01 ± 0.04	1.02 ± 0.58	1	4.19
3b	0.54 ± 0.04	0.52 ± 0.08	0.96	4.57
3c	2.45 ± 1.75	2.37 ± 0.09	0.97	5.13
3d	4.89 ± 0.92	8.01 ± 0.47	1.64	5.10
3e	9.28 ± 0.37	5.50 ± 2.48	0.59	5.70
3f	0.93 ± 0.06	0.41 ± 0.11	0.44	5.85
3g	0.22 ± 0.11	0.042 ± 0.001	0.19	5.90
3h	9.62 ± 1.34	6.90 ± 1.37	0.72	6.09
3i	7.42 ± 0.40	4.42 ± 0.97	0.60	5.73
3j	4.67 ± 0.28	3.57 ± 2.58	0.76	6.30
3k	2.99 ± 2.06	1.93 ± 0.32	0.65	6.45
3l	3.71 ± 1.69	2.47 ± 0.47	0.67	5.76
3m	0.92 ± 0.44	0.74 ± 0.15	0.8	5.33
3n	4.05 ± 1.39	2.48 ± 0.52	0.61	5.73
3o	3.31 ± 0.24	9.59 ± 2.38	2.90	5.94
3p	2.34 ± 0.91	1.47 ± 0.36	0.63	5.38
3q	3.85 ± 0.63	5.25 ± 1.63	1.36	5.95
4	>20	>20	N.D. ^c	8.78
ADR ^e	0.30 ± 0.05	0.041 ± 0.013	0.14	-

^a All values are the mean of three independent experiments. ^b TI: Therapeutic Index = IC₅₀(L-O2)/IC₅₀(HepG-2).

^c N.D., Not determined. ^d clog P was calculated by ChemBioDraw Ultra 14.0. ^e ADR, adriamycin, was used as a positive control.

Table 2. The inhibitory activity and clog P of **9a–9c**, **10a–10o**, and **11** against the HepG-2 and L-O2 cell lines.

Compound	IC ₅₀ ^a (μM)		TI ^b	Clog P ^d
	HepG-2	L-O2		
1	0.060 ± 0.02	0.019 ± 0.003	0.32	2.96
9a	0.46 ± 0.14	0.69 ± 0.43	1.5	2.06
9b	0.53 ± 0.01	0.80 ± 0.40	1.51	3.29
9c	0.92 ± 0.16	2.61 ± 1.77	2.84	3.73
10a	0.82 ± 0.33	2.04 ± 0.67	2.49	3.57
10b	0.63 ± 0.29	2.97 ± 0.23	4.71	3.97
10c	0.52 ± 0.09	2.10 ± 1.41	4.03	4.09
10d	0.64 ± 0.26	2.62 ± 0.52	4.1	4.62
10e	1.15 ± 0.24	3.21 ± 0.09	2.79	5.15
10f	0.83 ± 0.38	3.25 ± 0.40	3.92	5.68
10g	1.19 ± 0.55	4.29 ± 0.66	3.61	6.21
10h	1.15 ± 0.24	1.17 ± 0.08	1.02	6.74
10i	6.92 ± 0.75	3.27 ± 0.43	0.47	7.27
10j	10.28 ± 1.11	6.69 ± 3.95	0.65	7.79
10k	>20	>20	N.D. ^c	8.32
10l	1.01 ± 0.40	2.72 ± 0.38	2.69	4.01
10m	1.35 ± 0.10	2.94 ± 0.09	2.18	3.85
10n	1.11 ± 0.22	3.31 ± 0.18	0.34	4.77
10o	1.02 ± 0.28	3.00 ± 0.26	2.94	5.08
11	>20	>20	N.D. ^c	3.43
ADR ^e	0.30 ± 0.05	0.041 ± 0.013	0.14	-

^a All values are the mean of three independent experiments. ^b TI: Therapeutic Index = IC₅₀(L-O2)/IC₅₀(HepG-2).

^c N.D., Not determined. ^d clog P was calculated by ChemBioDraw Ultra 14.0. ^e ADR, adriamycin, was used as a positive control.

Considering that the introduction of a phenylsulfonyl-substituted furoxan NO-releasing moiety could reduce the toxicity of brusatol [42–45], derivatives **9a–9c** were synthesized and evaluated for their anti-HCC activity. As shown in Table 2, compounds **9a–9c** were less toxic than the parent compound **1** and showed improved TI values (ranging from 1.5 to 2.84) compared to compound **1** (TI = 0.32). These results prompted us to further synthesize more derivatives with a phenylsulfonyl-substituted furoxan NO-releasing moiety, i.e., compounds **10a–10o**. They featured with a different linker between cucurbitacin B and the phenylsulfonyl-substituted furoxan NO-releasing moiety. Compounds **10a–10o** exhibited potent or moderate activity against HepG-2 cells. Importantly, their cytotoxicity against L-O2 was significantly reduced compared to their parent compound cucurbitacin B, with IC₅₀ values ranging from 1.17 to 3.31 μ M. The compound with the most potential, **10b**, showed the highest TI value (4.71), which is a 14.7-fold improvement compared to its parent compound cucurbitacin B. Moreover, it still maintained potent activity against HepG-2 cells, with an IC₅₀ value of 0.63 μ M. These results suggested that the introduction of a phenylsulfonyl-substituted furoxan NO-releasing moiety could reduce the toxicity against normal cells and maintain potent anti-HCC activity.

The compound with the most potential, **10b**, was selected for a preliminary study on its mechanism of action.

2.3. Preliminary Study of Compound **10b**'s Mechanism of Action

To determine whether the cytotoxic effect of compound **10b** was associated with apoptosis, a cell apoptosis assay was performed by flow cytometry. As shown in Figure 2A, compound **10b** significantly induced cell apoptosis in a dose-dependent manner. To further analyze the mechanism by which compound **10b** induces apoptosis, a Western blot assay was performed. STAT3 is associated with the expression of its downstream mitochondrial proteins, such as Bcl-2, Bax, and Bim, which are critical regulators of cell apoptosis. As a result, we investigated the protein level of STAT3 and P-STAT3 using a Western blot analysis. As shown in Figure 2B, the total expression of STAT3 remained little changed. STAT3 is usually activated by phosphorylation to perform its functions. Inhibition of P-STAT3 plays an important role in cancer therapy. Our data showed that the level of P-STAT3 was clearly inhibited after treatment with compound **10b** in a dose-dependent manner. Moreover, the expression of the apoptotic-related members Bax and Bim were strongly increased, while the expression of the anti-apoptotic-related member Bcl-2 was substantially diminished after treatment with compound **10b**. As shown in Figure 1B, the level of caspase 3 was decreased. Accordingly, we proposed that compound **10b** inhibited P-STAT3 to induce mitochondrial-pathway-mediated apoptosis.

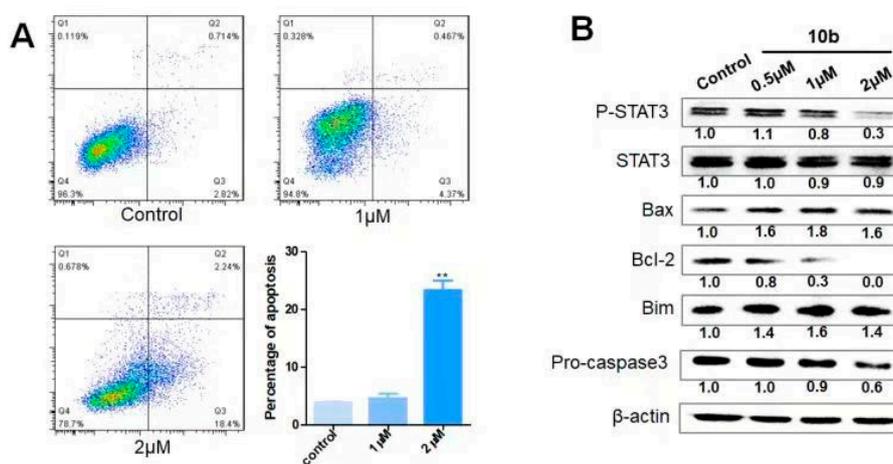


Figure 2. (A) The representative images and the statistical results of flow cytometry after treatment with compound **10b** at 0, 1, and 2 μ M in a cell apoptosis assay. ** $p < 0.01$. (B) The level of STAT3, P-STAT3, Bax, Bim, Bcl-2, and caspase 3 after treatment with compound **10b** at different concentrations by a Western blot assay.

2.4. In Vivo Acute Toxicity Study of 10b

To evaluate the toxicity of compound **10b**, an acute toxicity assay was performed using Balb/C mice. After the administration of cucurbitacin B or compound **10b** by intravenous injection, the clinical symptoms, deaths, and body weight were observed. As shown in Figure 3A, the percentage of death was 3/3, 3/3, 3/3, 3/3, and 3/3 after the administration of cucurbitacin B at a dose of 2 mg/kg, 5 mg/kg, 10 mg/kg, 20 mg/kg, and 50 mg/kg, respectively, while the percentage of death was 0/3, 0/3, 0/3, 0/3, and 1/3 after the administration of compound **10b** at a dose of 2 mg/kg, 5 mg/kg, 10 mg/kg, 20 mg/kg, and 50 mg/kg, respectively. Moreover, as shown in Figure 3B, the body weight did not change after the administration of compound **10b** at different doses as compared to the vehicle group. These results demonstrate that compound **10b** shows preferable safety and tolerability as compared with cucurbitacin B.

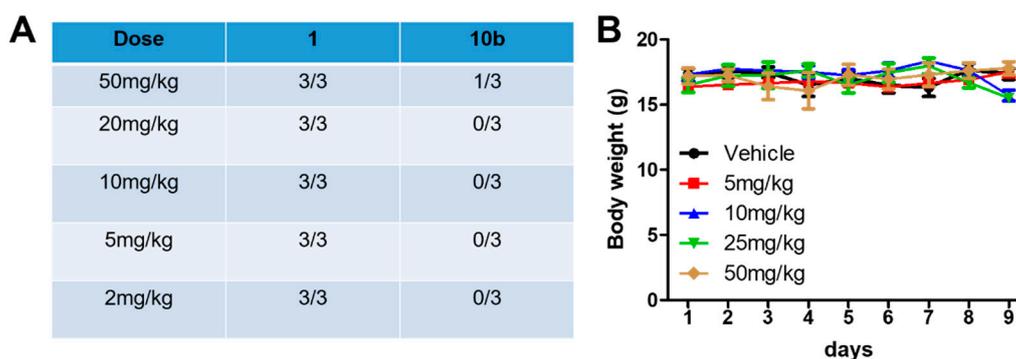


Figure 3. (A) The percentage of death in mice after the administration of cucurbitacin B and compound **10b**. (B) The body weight change after the administration of compound **10b** by intravenous injection.

3. Experimental

3.1. Chemistry

3.1.1. General Methods

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 ethanol, or potassium permanganate in water, each followed 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 (^1H : 400 MHz, ^{13}C : 100 MHz) 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. Purity testing was done by means of analytical HPLC on a Shimadzu LD-20A system with an ODS-C18 column (4.6 × 150 mm, 5 μm) eluted at 1–1.3 mL/min with Milli-Q water and CH_3CN . The purity of all tested compounds was $\geq 95\%$ by HPLC (Supplementary Materials).

3.1.2. Preparation of Cucurbitacin B (1)

The starting material cucurbitacin B (**1**) was obtained from *Pedicellus Melo* in a yield of only 0.3%. (purity: 97%). ^1H NMR (400 MHz, CDCl_3) δ 7.04 (d, J = 15.6 Hz, 1H), 6.46 (d, J = 15.6 Hz, 1H), 5.77 (d, J = 5.3 Hz, 1H), 4.47–4.37 (m, 1H), 4.33 (dd, J = 14.7, 7.4 Hz, 1H), 4.26 (s, 1H), 3.61 (d, J = 3.8 Hz, 1H), 3.23 (d, J = 14.5 Hz, 1H), 2.72 (d, J = 12.8 Hz, 1H), 2.66 (d, J = 14.6 Hz, 1H), 2.48 (d, J = 7.0 Hz, 1H), 2.39 (dd, J = 19.4, 7.9 Hz, 1H), 2.29 (ddd, J = 12.3, 5.7, 3.3 Hz, 1H), 2.07–1.92 (m, 6H), 1.85 (t, J = 10.2 Hz, 2H), 1.55 (s, 3H), 1.53 (s, 3H), 1.45–1.39 (m, 4H), 1.34 (s, 3H), 1.32 (s, 3H), 1.26 (s, 3H), 1.06 (s, 3H), 0.96 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 213.2, 212.3, 202.6, 170.3, 152.1, 140.5, 120.5, 120.4, 79.4, 78.3,

71.7, 71.3, 58.3, 50.8, 50.3, 48.7, 48.5, 48.2, 45.4, 42.5, 36.1, 33.8, 29.5, 26.5, 26.1, 24.0, 23.9, 22.1, 21.4, 20.2, 19.9, 18.9. HRMS (ESI) calcd. for $C_{32}H_{46}NaO_8$ $[M + Na]^+$ 581.3085, found 581.3088.

3.1.3. Procedure for the Synthesis of Compounds 2

(6*R,E*)-6-((2*S,9R,13R,14S,16R*)-2-((*tert*-butyldimethylsilyloxy)-16-Hydroxy-4,4,9,13,14-pentamethyl-3,11-dioxo-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)-6-hydroxy-2-methyl-5-oxohept-3-en-2-yl acetate (2). To a solution of cucurbitacin B (1) (200.0 mg, 0.36 mmol) and TBSCl (80.9 mg, 0.54 mmol) in dry DCM (5 mL) was added imidazole (48.7 mg, 0.72 mmol) at 0 °C. The mixture was stirred for 4 h at room temperature. The reaction was quenched with saturated aqueous NH_4Cl and extracted with CH_2Cl_2 (3 × 15 mL). The combined organic layers were washed with saturated brine, dried over Na_2SO_4 , and concentrated to give an oily crude product, which was purified on a silica gel column to yield compound 2 (104 mg, 43.2%) as a white solid. 1H NMR (400 MHz, $CDCl_3$) δ 7.04 (d, $J = 15.6$ Hz, 1H), 6.45 (d, $J = 15.6$ Hz, 1H), 5.73 (d, $J = 5.1$ Hz, 1H), 4.45 (dd, $J = 12.7, 5.7$ Hz, 1H), 4.34 (dd, $J = 14.3, 7.1$ Hz, 1H), 4.25 (s, 1H), 3.25 (d, $J = 14.5$ Hz, 1H), 2.69 (t, $J = 14.6$ Hz, 2H), 2.49 (d, $J = 7.0$ Hz, 1H), 2.38 (dd, $J = 19.4, 7.8$ Hz, 1H), 2.10–2.02 (m, 1H), 2.01–1.91 (m, 5H), 1.86 (dd, $J = 13.0, 9.3$ Hz, 1H), 1.77 (d, $J = 6.5$ Hz, 1H), 1.54 (d, $J = 7.2$ Hz, 6H), 1.42 (s, 4H), 1.34 (s, 3H), 1.27 (s, 3H), 1.24 (s, 1H), 1.22 (s, 3H), 1.06 (s, 3H), 0.97 (s, 3H), 0.88 (s, 9H), 0.11 (s, 3H), –0.02 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 212.5, 210.7, 202.6, 170.3, 151.9, 140.5, 120.5, 119.9, 79.4, 78.4, 73.8, 71.4, 58.3, 51.1, 50.8, 48.9, 48.7, 48.3, 45.5, 42.6, 36.2, 34.5, 29.4, 26.5, 26.1, 25.9, 24.1, 23.9, 22.1, 21.8, 20.2, 19.9, 19.0, 18.6, –4.4, –5.4. HRMS (ESI) calcd. for $C_{38}H_{60}NaO_8$ $[M + Na]^+$ 695.3950, found 695.3954.

3.1.4. Procedure for the Synthesis of Compounds 3a–3q

To a solution of compound 2 (100 mg, 0.15 mmol), EDCI (85 mg, 0.45 mmol), DMAP (1.2 mg, 0.01 mmol), and the corresponding acid (0.45 mmol, 3 eq) in CH_2Cl_2 (2 mL) was added Et_3N (62.5 μ L, 0.45 mmol) at 0 °C. The mixture was stirred for 8 h at room temperature. The reaction was quenched with saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2 (3 × 15 mL). The combined organic layers were washed with saturated brine, dried over Na_2SO_4 , and concentrated to give an oily crude product, which was simple purified to yield a white solid. The solid was used for the next step directly. The solid was dissolved in THF (2 mL). HOAc (38 μ L, 0.65 mmol) and TBAF (169 mg, 0.65 mmol) were added to the mixture. The mixture was stirred at room temperature for 24 h, and then diluted with ethyl acetate (20 mL). The organic phase was washed with H_2O (3 × 20 mL), dried over Na_2SO_4 , and concentrated. The residue was purified by column chromatography on silica gel to obtain a white solid 3a–3q.

(2*S,9R,13R,14S,16R*)-17-((*R,E*)-6-Acetoxy-2-hydroxy-6-methyl-3-oxohept-4-en-2-yl)-2-hydroxy-4,4,9,13,14-pentamethyl-3,11-dioxo-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-16-yl propionate (3a) (65% yield in two steps). (purity: 97%) 1H NMR (400 MHz, $CDCl_3$) δ 7.12 (dd, $J = 15.5, 2.0$ Hz, 1H), 6.40 (dd, $J = 15.6, 1.8$ Hz, 1H), 5.76 (s, 1H), 5.17 (t, $J = 7.7$ Hz, 1H), 4.49–4.35 (m, 1H), 4.29 (s, 1H), 3.60 (s, 1H), 3.24 (d, $J = 14.5$ Hz, 1H), 2.71 (dd, $J = 20.2, 10.3$ Hz, 3H), 2.46–2.24 (m, 2H), 2.09 (dt, $J = 7.5, 5.6$ Hz, 2H), 2.05–1.97 (m, 5H), 1.71–1.51 (m, 6H), 1.40 (s, 3H), 1.37 (s, 1H), 1.32 (s, 3H), 1.29–1.22 (m, 9H), 1.07 (s, 3H), 1.04 (dd, $J = 7.6, 2.0$ Hz, 2H), 1.01 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 213.0, 211.8, 201.0, 173.9, 169.7, 152.7, 140.5, 120.5, 119.4, 79.3, 77.8, 73.6, 71.7, 54.3, 50.3, 50.1, 48.7, 48.5, 48.1, 43.3, 42.2, 36.1, 33.8, 29.5, 27.3, 26.6, 26.5, 23.9, 23.8, 22.0, 21.4, 20.2, 19.8, 18.9, 9.1. HRMS (ESI) calcd. for $C_{35}H_{50}NaO_9$ $[M + Na]^+$ 637.3347, found 637.3350.

(9*R,13R,14S,16R*)-17-((*R,E*)-6-Acetoxy-2-hydroxy-6-methyl-3-oxohept-4-en-2-yl)-2-hydroxy-4,4,9,13,14-pentamethyl-3,11-dioxo-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-16-yl cyclobutanecarboxylate (3b) (86% yield in two steps). (purity: 95%). 1H NMR (400 MHz, $CDCl_3$) δ 7.08 (d, $J = 15.5$ Hz, 1H), 6.41 (d, $J = 15.5$ Hz, 1H), 5.76 (s, 1H), 5.19 (t, $J = 7.7$ Hz, 1H), 4.40 (d, $J = 8.1$ Hz, 1H), 4.26 (s, 1H), 3.59 (s, 1H), 3.23 (d, $J = 14.5$ Hz, 1H), 2.99–2.83 (m, 1H), 2.71 (dd, $J = 17.2, 10.4$ Hz, 3H), 2.34

(dd, $J = 38.6, 14.7$ Hz, 2H), 2.22–2.12 (m, 2H), 2.08–1.96 (m, 7H), 1.93–1.83 (m, 2H), 1.58 (s, 6H), 1.40 (s, 3H), 1.32 (s, 3H), 1.26 (s, 9H), 1.07 (s, 3H), 1.00 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 213.0, 211.8, 201.1, 174.9, 169.7, 152.4, 140.6, 120.5, 119.6, 79.3, 77.9, 73.6, 71.7, 54.3, 50.3, 50.2, 48.7, 48.5, 47.9, 43.5, 42.2, 37.8, 36.1, 33.8, 29.8, 29.5, 26.5, 25.1, 24.9, 23.9, 23.9, 22.0, 21.4, 20.2, 19.8, 18.8, 18.5. HRMS (ESI) calcd. for $\text{C}_{37}\text{H}_{52}\text{NaO}_9$ $[\text{M} + \text{Na}]^+$ 663.3504 found 663.3508.

(2*S*,9*R*,13*R*,14*S*,16*R*)-17-((*R,E*)-6-Acetoxy-2-hydroxy-6-methyl-3-oxohept-4-en-2-yl)-2-hydroxy-4,4,9,13,14-pentamethyl-3,11-dioxo-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-16-yl cyclopentanecarboxylate (**3c**) (52% yield in two steps). (purity: 98%). ^1H NMR (400 MHz, CDCl_3) δ 7.08 (d, $J = 15.6$ Hz, 1H), 6.42 (d, $J = 15.6$ Hz, 1H), 5.75 (dd, $J = 3.7, 1.9$ Hz, 1H), 5.16 (t, $J = 7.8$ Hz, 1H), 4.39 (ddd, $J = 12.8, 5.8, 3.9$ Hz, 1H), 4.26 (s, 1H), 3.59 (d, $J = 3.9$ Hz, 1H), 3.23 (d, $J = 14.6$ Hz, 1H), 2.71 (dd, $J = 12.8, 11.3$ Hz, 3H), 2.53–2.44 (m, 1H), 2.39 (dd, $J = 19.5, 7.9$ Hz, 1H), 2.28 (ddd, $J = 12.5, 5.8, 3.4$ Hz, 1H), 2.04–1.86 (m, 6H), 1.81–1.59 (m, 6H), 1.56 (d, $J = 2.2$ Hz, 6H), 1.51–1.48 (m, 2H), 1.39 (s, 3H), 1.31 (s, 3H), 1.26 (d, $J = 3.1$ Hz, 6H), 1.23 (s, 2H), 1.06 (s, 3H), 0.99 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 213.0, 211.8, 201.0, 176.1, 169.7, 152.5, 140.5, 120.5, 119.6, 79.3, 77.9, 73.6, 71.7, 54.2, 50.3, 50.1, 48.7, 48.5, 47.9, 43.6, 43.5, 42.2, 30.1, 29.5, 29.5, 26.5, 26.4, 25.8, 25.7, 23.9, 23.8, 21.9, 21.4, 20.1, 19.8, 18.8. HRMS (ESI) calcd. for $\text{C}_{38}\text{H}_{54}\text{NaO}_9$ $[\text{M} + \text{Na}]^+$ 677.3660, found 677.3665.

(2*S*,9*R*,13*R*,14*S*,16*R*)-17-((*R,E*)-6-Acetoxy-2-hydroxy-6-methyl-3-oxohept-4-en-2-yl)-2-hydroxy-4,4,9,13,14-pentamethyl-3,11-dioxo-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-16-yl 5-((*tert*-butoxycarbonyl)amino)pentanoate (**3d**) (67% yield in two steps). (purity: 95%). ^1H NMR (400 MHz, CDCl_3) δ 7.13 (d, $J = 15.6$ Hz, 1H), 6.38 (d, $J = 15.6$ Hz, 1H), 5.76 (d, $J = 5.3$ Hz, 1H), 5.17 (t, $J = 8.0$ Hz, 1H), 4.70 (s, 1H), 4.47–4.32 (m, 1H), 4.26 (s, 1H), 3.59 (d, $J = 3.8$ Hz, 1H), 3.23 (d, $J = 14.6$ Hz, 1H), 3.13–3.04 (m, 2H), 2.70 (dd, $J = 19.5, 11.0$ Hz, 3H), 2.39 (dd, $J = 19.4, 7.7$ Hz, 1H), 2.29 (ddd, $J = 12.4, 5.7, 3.4$ Hz, 1H), 2.10 (t, $J = 7.2$ Hz, 2H), 2.01 (s, 3H), 2.00–1.92 (m, 3H), 1.57 (s, 3H), 1.54 (s, 4H), 1.47–1.40 (m, 12H), 1.39 (s, 3H), 1.35 (s, 1H), 1.32 (s, 3H), 1.27 (d, $J = 4.8$ Hz, 6H), 1.23 (s, 1H), 1.07 (s, 3H), 1.00 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 213.0, 211.7, 201.0, 172.9, 169.8, 156.1, 152.9, 140.5, 120.5, 119.4, 79.3, 77.8, 73.6, 71.7, 54.2, 50.3, 50.0, 48.7, 48.5, 48.1, 43.4, 42.2, 40.1, 36.1, 33.8, 33.3, 29.8, 29.5, 29.3, 28.5, 26.9, 26.4, 23.9, 23.8, 21.9, 21.8, 21.4, 20.2, 19.8, 18.9. HRMS (ESI) calcd. for $\text{C}_{42}\text{H}_{63}\text{NNaO}_{11}$ $[\text{M} + \text{Na}]^+$ 780.4293, found 780.4296.

(2*S*,9*R*,13*R*,14*S*,16*R*)-17-((*R,E*)-6-Acetoxy-2-hydroxy-6-methyl-3-oxohept-4-en-2-yl)-2-hydroxy-4,4,9,13,14-pentamethyl-3,11-dioxo-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-16-yl 5-((*tert*-butoxycarbonyl)(methyl)amino)pentanoate (**3e**) (66% yield in two steps). (purity: 96%). ^1H NMR (400 MHz, CDCl_3) δ 7.11 (d, $J = 15.6$ Hz, 1H), 6.38 (d, $J = 15.6$ Hz, 1H), 5.75 (d, $J = 5.4$ Hz, 1H), 5.18 (t, $J = 7.9$ Hz, 1H), 4.44–4.35 (m, 1H), 4.26 (s, 1H), 3.59 (d, $J = 3.8$ Hz, 1H), 3.28–3.12 (m, 3H), 2.80 (s, 3H), 2.69 (dd, $J = 20.6, 11.0$ Hz, 3H), 2.39 (dd, $J = 19.5, 7.8$ Hz, 1H), 2.28 (ddd, $J = 12.3, 5.6, 3.4$ Hz, 1H), 2.10 (s, 2H), 2.03–1.86 (m, 7H), 1.55 (d, $J = 5.6$ Hz, 6H), 1.51–1.45 (m, 4H), 1.42 (s, 9H), 1.39 (s, 3H), 1.31 (s, 3H), 1.27 (s, 3H), 1.25 (s, 3H), 1.23 (s, 1H), 1.06 (s, 3H), 0.99 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 212.9, 211.7, 200.9, 172.9, 169.6, 155.9, 152.7, 140.6, 120.4, 119.4, 79.2, 77.8, 73.5, 71.7, 54.3, 50.3, 50.0, 48.7, 48.5, 48.1, 47.9, 43.4, 42.2, 36.1, 34.2, 33.8, 33.5, 29.8, 29.4, 28.6, 26.7, 26.5, 23.9, 23.8, 21.9, 21.9, 21.4, 20.1, 19.8, 18.9. HRMS (ESI) calcd. for $\text{C}_{43}\text{H}_{65}\text{NNaO}_{11}$ $[\text{M} + \text{Na}]^+$ 794.4450, found 794.4453.

(2*S*,9*R*,13*R*,14*S*,16*R*)-17-((*R,E*)-6-Acetoxy-2-hydroxy-6-methyl-3-oxohept-4-en-2-yl)-2-hydroxy-4,4,9,13,14-pentamethyl-3,11-dioxo-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-16-yl 4-((*tert*-butoxycarbonyl)(methyl)amino)butanoate (**3f**) (64% yield in two steps). (purity: 98%). ^1H NMR (400 MHz, CDCl_3) δ 7.11 (d, $J = 15.6$ Hz, 1H), 6.39 (d, $J = 15.6$ Hz, 1H), 5.76 (d, $J = 5.3$ Hz, 1H), 5.20 (t, $J = 8.0$ Hz, 1H), 4.44–4.35 (m, 1H), 4.25 (s, 1H), 3.59 (d, $J = 3.7$ Hz, 1H), 3.28–3.12 (m, 3H), 2.82 (s, 3H), 2.70 (dd, $J = 18.3, 11.0$ Hz, 3H), 2.40 (dd, $J = 19.4, 7.5$ Hz, 1H), 2.30 (ddd, $J = 12.3, 5.5, 3.3$ Hz, 1H), 2.08 (s, 2H), 2.01 (s, 3H), 1.99 (d, $J = 5.7$ Hz, 2H), 1.77–1.69 (m, 2H), 1.56 (d, $J = 5.9$ Hz, 6H), 1.43 (s, 9H), 1.40 (s, 3H), 1.32 (s, 3H), 1.28 (s, 3H), 1.26 (s, 3H), 1.24 (s, 3H), 1.07 (s, 3H), 1.00 (s, 3H). ^{13}C NMR (100 MHz,

CDCl_3) δ 212.9, 211.7, 201.1, 172.6, 169.7, 155.9, 152.8, 140.6, 120.5, 119.4, 79.2, 77.8, 73.7, 71.7, 54.3, 50.34, 50.1, 48.7, 48.5, 48.1, 43.3, 42.2, 36.1, 34.4, 33.9, 30.9, 29.8, 29.5, 28.6, 26.5, 23.9, 23.8, 21.9, 21.4, 20.2, 19.8, 18.9. HRMS (ESI) calcd. for $\text{C}_{42}\text{H}_{63}\text{NNaO}_{11}$ $[\text{M} + \text{Na}]^+$ 780.4293, found 780.4295.

(2*S*,9*R*,13*R*,14*S*,16*R*)-17-((*R,E*)-6-Acetoxy-2-hydroxy-6-methyl-3-oxohept-4-en-2-yl)-2-hydroxy-4,4,9,13,14-pentamethyl-3,11-dioxo-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-16-yl 3-(2-((*tert*-butoxycarbonyl)amino)phenyl)propanoate (**3g**) (47% yield in two steps). (purity: 95%). ^1H NMR (400 MHz, CDCl_3) δ 7.65 (d, $J = 7.7$ Hz, 1H), 7.20–7.04 (m, 4H), 7.00 (t, $J = 7.3$ Hz, 1H), 6.34 (d, $J = 15.6$ Hz, 1H), 5.74 (d, $J = 3.5$ Hz, 1H), 5.15 (t, $J = 7.9$ Hz, 1H), 4.38 (d, $J = 9.1$ Hz, 1H), 4.25 (s, 1H), 3.61 (s, 1H), 3.19 (d, $J = 14.5$ Hz, 1H), 2.81 (dt, $J = 23.3, 7.6$ Hz, 2H), 2.69 (d, $J = 14.2$ Hz, 2H), 2.61 (d, $J = 7.3$ Hz, 1H), 2.44 (t, $J = 7.7$ Hz, 2H), 2.36 (dd, $J = 20.4, 7.5$ Hz, 1H), 2.27 (d, $J = 11.7$ Hz, 1H), 1.95 (s, 3H), 1.94–1.78 (m, 3H), 1.53 (d, $J = 9.1$ Hz, 3H), 1.49 (s, 12H), 1.36 (s, 3H), 1.31 (s, 3H), 1.24 (s, 5H), 1.11 (s, 3H), 1.04 (s, 3H), 0.96 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 212.95, 211.70, 200.86, 173.16, 169.85, 153.82, 153.04, 140.37, 136.13, 129.31, 127.00, 124.28, 120.35, 119.09, 80.19, 79.15, 77.67, 74.02, 71.64, 65.61, 54.07, 50.28, 49.93, 48.55, 48.36, 47.92, 42.97, 42.07, 35.99, 34.12, 33.71, 29.36, 28.45, 27.06, 26.18, 25.59, 23.75, 23.57, 21.83, 21.34, 20.08, 19.69, 18.59. HRMS (ESI) calcd. for $\text{C}_{46}\text{H}_{63}\text{NNaO}_{11}$ $[\text{M} + \text{Na}]^+$ 828.4293, found 828.4296.

(2*S*,9*R*,13*R*,14*S*,16*R*)-17-((*R,E*)-6-Acetoxy-2-hydroxy-6-methyl-3-oxohept-4-en-2-yl)-2-hydroxy-4,4,9,13,14-pentamethyl-3,11-dioxo-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-16-yl 4-methylbenzoate (**3h**) (79% yield in two steps). (purity: 97%). ^1H NMR (400 MHz, CDCl_3) δ 7.75 (d, $J = 8.0$ Hz, 2H), 7.16 (d, $J = 7.9$ Hz, 2H), 6.87 (d, $J = 15.5$ Hz, 1H), 6.51 (d, $J = 15.5$ Hz, 1H), 5.74 (d, $J = 4.2$ Hz, 1H), 5.54 (t, $J = 7.8$ Hz, 1H), 4.42 (d, $J = 10.8$ Hz, 1H), 4.19 (s, 1H), 3.61 (d, $J = 2.2$ Hz, 1H), 3.29 (d, $J = 14.5$ Hz, 1H), 2.89 (d, $J = 7.2$ Hz, 1H), 2.74 (d, $J = 18.2$ Hz, 2H), 2.47–2.31 (m, 4H), 2.10 (dd, $J = 13.6, 9.1$ Hz, 1H), 2.03 (d, $J = 12.0$ Hz, 5H), 1.46 (s, 6H), 1.40 (s, 3H), 1.37 (s, 3H), 1.31 (s, 3H), 1.25 (d, $J = 8.9$ Hz, 6H), 1.09 (s, 3H), 1.06 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 213.0, 211.9, 201.3, 169.8, 165.9, 152.5, 143.8, 140.5, 129.7, 129.1, 127.4, 120.5, 119.5, 79.5, 78.2, 74.1, 71.7, 54.8, 50.4, 48.7, 48.5, 48.0, 43.6, 42.3, 36.1, 33.8, 32.0, 29.8, 29.5, 26.6, 25.8, 24.2, 23.9, 22.8, 22.1, 21.8, 21.4, 20.2, 19.8, 18.8. HRMS (ESI) calcd. for $\text{C}_{40}\text{H}_{52}\text{NaO}_9$ $[\text{M} + \text{Na}]^+$ 699.3504, found 699.3508.

(2*S*,9*R*,13*R*,14*S*,16*R*)-17-((*R,E*)-6-Acetoxy-2-hydroxy-6-methyl-3-oxohept-4-en-2-yl)-2-hydroxy-4,4,9,13,14-pentamethyl-3,11-dioxo-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-16-yl 4-fluorobenzoate (**3i**) (81% yield in two steps). (purity: 98%). ^1H NMR (400 MHz, CDCl_3) δ 7.96–7.82 (m, 2H), 7.05 (t, $J = 8.4$ Hz, 2H), 6.90 (d, $J = 15.6$ Hz, 1H), 6.48 (d, $J = 15.6$ Hz, 1H), 5.74 (d, $J = 3.6$ Hz, 1H), 5.55 (t, $J = 7.8$ Hz, 1H), 4.42 (dd, $J = 12.7, 5.7$ Hz, 1H), 4.14 (s, 1H), 3.61 (s, 1H), 3.29 (d, $J = 14.6$ Hz, 1H), 2.87 (d, $J = 7.3$ Hz, 1H), 2.78 (d, $J = 14.7$ Hz, 1H), 2.73 (d, $J = 12.4$ Hz, 1H), 2.41 (dd, $J = 18.9, 6.5$ Hz, 1H), 2.35–2.27 (m, 1H), 2.19–2.07 (m, 1H), 2.03 (d, $J = 10.1$ Hz, 4H), 1.97–1.86 (m, 1H), 1.46 (s, 6H), 1.43 (s, 3H), 1.36 (s, 3H), 1.31 (s, 3H), 1.26 (s, 3H), 1.24 (s, 2H), 1.09 (s, 3H), 1.06 (s, 3H). ^{19}F NMR (376 MHz, CDCl_3) δ –105.30––105.40 (m). ^{13}C NMR (100 MHz, CDCl_3) δ 213.0, 211.7, 201.3, 169.7, 165.9 (d, $J = 254.2$ Hz), 164.9, 152.8, 140.5, 132.2 (d, $J = 9.2$ Hz), 131.1, 128.9, 126.3 (d, $J = 2.7$ Hz), 120.4, 119.3, 115.6 (d, $J = 22.0$ Hz), 79.4, 78.1, 74.5, 71.7, 54.6, 50.4, 50.3, 48.7, 48.5, 48.0, 43.6, 42.2, 36.1, 33.8, 29.4, 26.7, 26.1, 24.1, 23.9, 22.1, 21.4, 20.2, 19.8, 18.9. HRMS (ESI) calcd. for $\text{C}_{39}\text{H}_{49}\text{FNaO}_9$ $[\text{M} + \text{Na}]^+$ 703.3253, found 703.3258.

(2*S*,9*R*,13*R*,14*S*,16*R*)-17-((*R,E*)-6-Acetoxy-2-hydroxy-6-methyl-3-oxohept-4-en-2-yl)-2-hydroxy-4,4,9,13,14-pentamethyl-3,11-dioxo-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-16-yl 4-chlorobenzoate (**3j**) (69% yield in two steps). (purity: 95%). ^1H NMR (400 MHz, CDCl_3) δ 7.79 (d, $J = 8.3$ Hz, 2H), 7.34 (d, $J = 8.3$ Hz, 2H), 6.88 (d, $J = 15.6$ Hz, 1H), 6.48 (d, $J = 15.6$ Hz, 1H), 5.73 (s, 1H), 5.55 (t, $J = 7.8$ Hz, 1H), 4.41 (dd, $J = 12.7, 5.7$ Hz, 1H), 3.28 (d, $J = 14.5$ Hz, 1H), 2.87 (d, $J = 7.2$ Hz, 1H), 2.81–2.67 (m, 2H), 2.50–2.26 (m, 2H), 2.16–2.06 (m, 1H), 2.02 (d, $J = 10.9$ Hz, 4H), 1.97–1.86 (m, 2H), 1.45 (s, 6H), 1.42 (s, 3H), 1.35 (s, 3H), 1.30 (s, 3H), 1.25 (s, 3H), 1.24 (s, 3H), 1.08 (s, 3H), 1.05 (s, 3H). ^{13}C

NMR (100 MHz, CDCl₃) δ 212.9, 211.7, 201.3, 169.7, 165.0, 152.8, 140.5, 139.6, 131.1, 128.8, 120.4, 119.4, 79.4, 78.1, 74.6, 71.7, 54.6, 50.3, 50.3, 48.6, 48.4, 48.0, 43.5, 42.2, 36.1, 33.8, 29.4, 26.6, 26.1, 24.1, 23.9, 22.0, 21.3, 20.2, 19.8, 18.9. HRMS (ESI) calcd. for C₃₉H₄₉ClNaO₉ [M + Na]⁺ 719.2957, found 719.2962.

(2*S*,9*R*,13*R*,14*S*,16*R*)-17-((*R,E*)-6-Acetoxy-2-hydroxy-6-methyl-3-oxohept-4-en-2-yl)-2-hydroxy-4,4,9,13,14-pentamethyl-3,11-dioxo-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-16-yl 4-bromobenzoate (**3k**) (64% yield in two steps). (purity: 95%). ¹H NMR (400 MHz, CDCl₃) δ 7.73 (d, *J* = 8.5 Hz, 2H), 7.52 (d, *J* = 8.5 Hz, 2H), 6.90 (d, *J* = 15.6 Hz, 1H), 6.47 (d, *J* = 15.6 Hz, 1H), 5.74 (d, *J* = 5.4 Hz, 1H), 5.55 (t, *J* = 7.9 Hz, 1H), 4.42 (dd, *J* = 12.9, 5.9 Hz, 1H), 4.13 (d, *J* = 7.0 Hz, 1H), 3.29 (d, *J* = 14.7 Hz, 1H), 2.87 (d, *J* = 7.4 Hz, 1H), 2.76 (dd, *J* = 21.3, 13.9 Hz, 2H), 2.47–2.28 (m, 2H), 2.16–2.07 (m, 1H), 2.06–2.01 (m, 4H), 1.95 (d, *J* = 3.4 Hz, 1H), 1.46 (d, *J* = 4.2 Hz, 6H), 1.44 (s, 3H), 1.36 (s, 3H), 1.31 (s, 3H), 1.27 (s, 3H), 1.24 (s, 3H), 1.09 (s, 3H), 1.06 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 213.0, 211.7, 201.2, 169.8, 165.2, 152.9, 140.5, 131.8, 131.2, 128.9, 128.3, 120.4, 119.3, 79.4, 78.1, 74.7, 71.7, 54.6, 50.4, 50.3, 48.7, 48.5, 48.0, 43.5, 42.2, 36.1, 33.8, 29.4, 26.7, 26.1, 24.1, 23.9, 22.1, 21.4, 20.2, 19.8, 18.9. HRMS (ESI) calcd. for C₃₉H₄₉BrNaO₉ [M + Na]⁺ 763.2452, found 763.2458.

(2*S*,9*R*,13*R*,14*S*,16*R*)-17-((*R,E*)-6-Acetoxy-2-hydroxy-6-methyl-3-oxohept-4-en-2-yl)-2-hydroxy-4,4,9,13,14-pentamethyl-3,11-dioxo-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-16-yl 4-methoxybenzoate (**3l**) (78% yield in two steps). (purity: 99%). ¹H NMR (400 MHz, CDCl₃) δ 7.82 (d, *J* = 8.8 Hz, 2H), 6.87 (t, *J* = 12.0 Hz, 3H), 6.51 (d, *J* = 15.6 Hz, 1H), 5.74 (d, *J* = 5.4 Hz, 1H), 5.54 (t, *J* = 7.9 Hz, 1H), 4.42 (dd, *J* = 7.2, 3.2 Hz, 1H), 4.15 (s, 1H), 3.83 (s, 3H), 3.60 (d, *J* = 3.4 Hz, 1H), 3.29 (d, *J* = 14.6 Hz, 1H), 2.88 (d, *J* = 7.4 Hz, 1H), 2.78 (d, *J* = 14.7 Hz, 1H), 2.47–2.28 (m, 2H), 2.14–2.03 (m, 2H), 2.02 (s, 3H), 1.97–1.88 (m, 1H), 1.46 (d, *J* = 5.3 Hz, 6H), 1.42 (s, 3H), 1.37 (s, 3H), 1.32 (d, *J* = 5.5 Hz, 3H), 1.27 (d, *J* = 3.5 Hz, 3H), 1.25 (s, 3H), 1.09 (s, 3H), 1.06 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 213.1, 211.9, 201.4, 169.8, 165.6, 163.6, 152.5, 140.6, 131.7, 122.6, 120.5, 119.6, 113.7, 79.5, 78.3, 74.0, 71.8, 55.6, 54.9, 50.4, 48.8, 48.5, 48.0, 43.6, 42.3, 36.1, 33.9, 29.8, 29.5, 26.7, 25.9, 24.3, 22.8, 22.1, 21.4, 20.2, 19.8, 18.9. HRMS (ESI) calcd. for C₄₀H₅₂BrNaO₁₀ [M + Na]⁺ 715.3453, found 715.3458.

(2*S*,9*R*,13*R*,14*S*,16*R*)-17-((*R,E*)-6-Acetoxy-2-hydroxy-6-methyl-3-oxohept-4-en-2-yl)-2-hydroxy-4,4,9,13,14-pentamethyl-3,11-dioxo-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-16-yl 4-nitrobenzoate (**3m**) (64% yield in two steps). (purity: 95%). ¹H NMR (400 MHz, CDCl₃) δ 8.24 (d, *J* = 8.7 Hz, 2H), 8.05 (d, *J* = 8.7 Hz, 2H), 6.94 (d, *J* = 15.6 Hz, 1H), 6.47 (d, *J* = 15.6 Hz, 1H), 5.74 (s, 1H), 5.61 (t, *J* = 7.9 Hz, 1H), 4.42 (d, *J* = 10.8 Hz, 1H), 4.12 (s, 1H), 3.60 (d, *J* = 2.8 Hz, 1H), 3.29 (d, *J* = 14.6 Hz, 1H), 2.90 (d, *J* = 7.4 Hz, 1H), 2.80 (d, *J* = 14.6 Hz, 1H), 2.73 (d, *J* = 12.8 Hz, 1H), 2.52–2.27 (m, 2H), 2.16 (dd, *J* = 14.1, 9.0 Hz, 1H), 2.07–1.98 (m, 4H), 1.48 (s, 3H), 1.47 (s, 3H), 1.37 (s, 3H), 1.31 (s, 3H), 1.27 (s, 3H), 1.25 (s, 6H), 1.10 (s, 3H), 1.08 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 212.9, 211.5, 201.1, 169.8, 164.2, 153.4, 150.7, 140.7, 135.4, 130.9, 128.9, 123.7, 120.4, 119.2, 79.3, 77.9, 75.5, 71.7, 54.3, 50.4, 50.3, 48.6, 48.5, 48.1, 43.6, 42.2, 36.1, 33.9, 29.8, 29.5, 26.6, 24.0, 23.9, 22.0, 21.4, 20.2, 19.7, 19.0. HRMS (ESI) calcd. for C₃₉H₄₉NNaO₁₁ [M + Na]⁺ 730.3198, found 730.3210.

(2*S*,9*R*,13*R*,14*S*,16*R*)-17-((*R,E*)-6-Acetoxy-2-hydroxy-6-methyl-3-oxohept-4-en-2-yl)-2-hydroxy-4,4,9,13,14-pentamethyl-3,11-dioxo-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-16-yl 3-fluorobenzoate (**3n**) (80% yield in two steps). (purity: 95%). ¹H NMR (400 MHz, CDCl₃) δ 7.69 (d, *J* = 7.3 Hz, 1H), 7.51 (d, *J* = 8.3 Hz, 1H), 7.42–7.33 (m, 1H), 7.22 (d, *J* = 7.4 Hz, 1H), 6.89 (d, *J* = 15.5 Hz, 1H), 6.49 (d, *J* = 15.6 Hz, 1H), 5.74 (s, 1H), 5.57 (t, *J* = 7.5 Hz, 1H), 4.42 (d, *J* = 7.2 Hz, 1H), 4.19 (s, 1H), 3.60 (s, 1H), 3.30 (d, *J* = 14.4 Hz, 1H), 2.89 (d, *J* = 7.0 Hz, 1H), 2.85–2.70 (m, 2H), 2.41 (d, *J* = 19.3 Hz, 1H), 2.31 (s, 1H), 2.12 (t, *J* = 11.3 Hz, 1H), 2.03 (d, *J* = 15.4 Hz, 3H), 1.93 (d, *J* = 15.3 Hz, 1H), 1.46 (s, 6H), 1.42 (s, 3H), 1.37 (s, 3H), 1.31 (s, 3H), 1.26 (d, *J* = 10.4 Hz, 6H), 1.08 (d, *J* = 10.4 Hz, 6H). ¹³C NMR (100 MHz, CDCl₃) δ 212.9, 211.6, 201.3, 169.7, 164.7, 162.5 (d, *J* = 247.4 Hz), 152.9, 140.6, 132.2 (d, *J* = 7.1 Hz), 130.2 (d, *J* = 7.8 Hz), 125.6 (d, *J* = 2.7 Hz), 120.4, 120.2 (d, *J* = 21.3 Hz), 119.4, 116.4 (d, *J* = 22.7 Hz), 79.4, 78.1, 74.8, 71.7, 54.6, 50.4, 48.7, 48.5, 48.1, 43.6, 42.3, 36.1, 33.9, 29.5, 26.7, 25.7, 24.1, 23.1, 22.1, 21.4, 20.2, 19.8,

18.9. ^{19}F NMR (376 MHz, CDCl_3) δ –112.09 (dd, J = 13.9, 8.1 Hz). HRMS (ESI) calcd. for $\text{C}_{39}\text{H}_{49}\text{FNaO}_9$ [$\text{M} + \text{Na}$] $^+$ 703.3253, found 703.3258.

(2*S*,9*R*,13*R*,14*S*,16*R*)-17-((*R,E*)-6-Acetoxy-2-hydroxy-6-methyl-3-oxohept-4-en-2-yl)-2-hydroxy-4,4,9,13,14-pentamethyl-3,11-dioxo-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-16-yl cinnamate (**3o**) (81% yield in two steps). (purity: 97%). ^1H NMR (400 MHz, CDCl_3) δ 7.60 (d, J = 15.9 Hz, 1H), 7.52 (s, 2H), 7.35 (s, 3H), 7.07 (d, J = 15.5 Hz, 1H), 6.45 (d, J = 15.5 Hz, 1H), 6.32 (d, J = 16.0 Hz, 1H), 5.76 (s, 1H), 5.37 (t, J = 7.5 Hz, 1H), 4.41 (d, J = 7.7 Hz, 1H), 4.29 (s, 1H), 3.61 (s, 1H), 3.28 (d, J = 14.5 Hz, 1H), 2.85–2.66 (m, 3H), 2.48–2.26 (m, 2H), 2.03 (s, 2H), 1.97 (s, 3H), 1.52 (d, J = 11.6 Hz, 6H), 1.43 (s, 3H), 1.34 (s, 3H), 1.32 (s, 3H), 1.27 (s, 3H), 1.25 (s, 3H), 1.09 (s, 3H), 1.04 (s, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 213.0, 211.8, 200.9, 169.7, 166.5, 152.8, 145.4, 140.5, 134.5, 130.4, 128.9, 128.3, 120.5, 119.3, 117.8, 79.2, 77.9, 73.7, 71.7, 54.5, 50.3, 50.2, 48.7, 48.5, 48.1, 43.4, 42.3, 36.1, 33.9, 29.8, 29.4, 26.8, 26.5, 23.9, 21.9, 21.4, 20.2, 19.8, 18.9. HRMS (ESI) calcd. for $\text{C}_{41}\text{H}_{52}\text{NaO}_9$ [$\text{M} + \text{Na}$] $^+$ 711.3504, found 711.3507.

(2*S*,9*R*,13*R*,14*S*,16*R*)-17-((*R,E*)-6-Acetoxy-2-hydroxy-6-methyl-3-oxohept-4-en-2-yl)-2-hydroxy-4,4,9,13,14-pentamethyl-3,11-dioxo-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-16-yl (*E*)-3-(4-cyanophenyl)acrylate (**3p**) (85% yield in two steps). (purity: 97%). ^1H NMR (400 MHz, CDCl_3) δ 7.65 (dd, J = 17.0, 8.1 Hz, 4H), 7.58 (d, J = 15.9 Hz, 1H), 7.11 (d, J = 15.5 Hz, 1H), 6.50 (d, J = 15.8 Hz, 1H), 6.41 (d, J = 15.6 Hz, 1H), 5.75 (d, J = 3.9 Hz, 1H), 5.36 (t, J = 8.1 Hz, 1H), 4.41 (dd, J = 12.4, 5.3 Hz, 1H), 4.29 (s, 1H), 3.61 (s, 1H), 3.27 (d, J = 14.6 Hz, 1H), 2.75 (dd, J = 19.3, 10.9 Hz, 3H), 2.48–2.27 (m, 2H), 2.02 (d, J = 8.0 Hz, 2H), 1.98 (s, 3H), 1.56 (s, 3H), 1.48 (s, 3H), 1.43 (s, 3H), 1.32 (d, J = 5.5 Hz, 6H), 1.26 (s, 3H), 1.24 (s, 3H), 1.09 (s, 3H), 1.05 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 212.9, 211.7, 200.8, 169.7, 165.9, 153.1, 142.9, 140.6, 138.9, 132.6, 128.8, 121.6, 120.4, 119.1, 113.4, 79.2, 77.8, 74.2, 71.7, 54.2, 50.3, 50.1, 48.7, 48.5, 48.1, 43.4, 42.2, 36.1, 33.9, 29.8, 29.4, 27.3, 26.5, 23.9, 23.8, 21.9, 21.4, 20.2, 19.8, 18.9. HRMS (ESI) calcd. for $\text{C}_{42}\text{H}_{51}\text{NNaO}_9$ [$\text{M} + \text{Na}$] $^+$ 736.3456, found 736.3458.

(2*S*,9*R*,13*R*,14*S*,16*R*)-17-((*R,E*)-6-Acetoxy-2-hydroxy-6-methyl-3-oxohept-4-en-2-yl)-2-hydroxy-4,4,9,13,14-pentamethyl-3,11-dioxo-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-16-yl (*E*)-3-(3,5-dimethoxyphenyl)acrylate (**3q**) (82% yield in two steps). (purity: 95%). ^1H NMR (400 MHz, CDCl_3) δ 7.51 (d, J = 15.9 Hz, 1H), 7.07 (d, J = 15.6 Hz, 1H), 6.66 (d, J = 2.2 Hz, 2H), 6.45 (t, J = 9.2 Hz, 2H), 6.28 (d, J = 15.9 Hz, 1H), 5.75 (d, J = 5.5 Hz, 1H), 5.36 (t, J = 7.8 Hz, 1H), 4.41 (dd, J = 12.9, 6.0 Hz, 1H), 3.78 (s, 7H), 3.27 (d, J = 14.7 Hz, 1H), 2.82–2.68 (m, 3H), 2.48–2.26 (m, 2H), 2.11–1.94 (m, 6H), 1.53 (d, J = 3.2 Hz, 6H), 1.43 (s, 3H), 1.34 (s, 3H), 1.31 (s, 3H), 1.26 (s, 3H), 1.24 (s, 3H), 1.08 (s, 3H), 1.04 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 213.0, 211.9, 200.9, 169.8, 166.4, 161.1, 152.9, 145.5, 140.5, 136.3, 120.5, 119.3, 118.2, 106.4, 102.5, 79.2, 77.9, 73.8, 71.7, 55.5, 54.4, 50.3, 50.2, 48.7, 48.5, 48.1, 43.4, 42.2, 36.1, 33.8, 29.8, 29.4, 26.8, 26.7, 23.9, 21.9, 21.4, 20.2, 19.8, 18.8. HRMS (ESI) calcd. for $\text{C}_{43}\text{H}_{56}\text{NaO}_{11}$ [$\text{M} + \text{Na}$] $^+$ 771.3715, found 771.3718.

3.1.5. Procedure for the Synthesis of Compounds 4

(2*S*,9*R*,13*R*,14*S*,16*R*)-17-((*R,E*)-6-Acetoxy-2-hydroxy-6-methyl-3-oxohept-4-en-2-yl)-4,4,9,13,14-pentamethyl-3,11-dioxo-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthrene-2,16-diyl (2*E*,2'*E*)-bis(3-phenylacrylate) (**4**). To a solution of compound **1** (100.0 mg, 0.18 mmol), EDCI (171.6 mg, 0.9 mmol), DMAP (1.2 mg, 0.01 mmol), and cinnamic acid (133.4 mg, 0.9 mmol) in CH_2Cl_2 (2 mL) was added Et_3N (125 μL , 0.9 mmol) at 0 °C. The mixture was stirred for 8 h at room temperature. The reaction was quenched with saturated aqueous NaHCO_3 and extracted with CH_2Cl_2 (3 \times 15 mL). The combined organic layers were washed with saturated brine, dried over Na_2SO_4 , and concentrated to give an oily crude product, which was purified on a silica gel column to yield compound **4** (112 mg, yield: 76%) as a white solid. (purity: 95%). ^1H NMR (400 MHz, CDCl_3) δ 7.74 (d, J = 16.0 Hz, 1H), 7.61 (d, J = 15.9 Hz, 1H), 7.52 (s, 4H), 7.37 (d, J = 8.6 Hz, 6H), 7.08 (d, J = 15.5 Hz, 1H), 6.49 (t, J = 15.0 Hz, 2H), 6.34 (d, J = 16.0 Hz, 1H), 5.79 (s, 1H), 5.64 (dd, J = 13.2, 4.5 Hz, 1H), 5.38 (t, J = 7.5 Hz, 1H), 3.31

(d, $J = 14.5$ Hz, 1H), 2.90 (d, $J = 11.9$ Hz, 1H), 2.79 (t, $J = 10.5$ Hz, 2H), 2.43 (d, $J = 19.1$ Hz, 1H), 2.23 (d, $J = 11.6$ Hz, 1H), 2.07 (t, $J = 10.6$ Hz, 2H), 1.99 (s, 3H), 1.54 (s, 3H), 1.52 (s, 3H), 1.45 (s, 3H), 1.37 (d, $J = 4.4$ Hz, 5H), 1.30 (s, 3H), 1.25 (s, 5H), 1.13 (s, 3H), 1.06 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 212.1, 205.8, 200.9, 169.7, 166.5, 166.0, 152.9, 145.9, 145.4, 139.9, 134.5, 134.4, 130.6, 130.4, 129.0, 128.9, 128.3, 120.6, 119.2, 117.8, 117.4, 79.3, 77.9, 73.7, 73.5, 54.4, 51.4, 50.2, 48.8, 48.6, 48.1, 43.4, 42.3, 34.5, 32.2, 32.0, 29.5, 28.9, 26.7, 26.6, 23.9, 22.8, 21.9, 21.5, 20.1, 19.9, 18.8. HRMS (ESI) calcd. for $\text{C}_{50}\text{H}_{58}\text{NaO}_{10}$ [$\text{M} + \text{Na}$] $^+$ 841.3922, found 841.3928.

3.1.6. Procedure for the Synthesis of Compounds 6a–6o and 7a–7c

Compounds 6a–6o and 7a–7c were synthesized according to the procedure previously reported [42–44,46].

3.1.7. Procedure for the Synthesis of Compound 8

4-(((2*S*,9*R*,13*R*,14*S*,16*R*)-17-((*R,E*)-6-Acetoxy-2-hydroxy-6-methyl-3-oxohept-4-en-2-yl)-2-((*tert*-butyldimethylsilyloxy)-4,4,9,13,14-pentamethyl-3,11-dioxo-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-16-yl)oxy)-4-oxobutanoic acid (**8**). To a solution of compound 2 (50.0 mg, 0.074 mmol) in dry DCM (2 mL) was added succinic anhydride (37.4 mg, 0.37 mmol) and DMAP (10.0 mg, 0.08 mmol). The mixture was stirred for 4 h at room temperature. The reaction was quenched with 1*N* HCl, the pH was adjusted to 2–3, and extraction was performed with CH_2Cl_2 (3×15 mL). The combined organic layers were washed with saturated brine, dried over Na_2SO_4 , and concentrated to give an oily crude product, which was purified on a silica gel column to yield compound 8 (49.0 mg, yield: 86%) as a white solid. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 12.21 (s, 1H), 6.88 (d, $J = 15.7$ Hz, 1H), 6.73 (d, $J = 15.8$ Hz, 1H), 5.65 (s, 1H), 5.32 (s, 2H), 4.72 (d, $J = 11.9$ Hz, 1H), 3.47 (d, $J = 14.2$ Hz, 1H), 3.02 (d, $J = 11.6$ Hz, 1H), 2.60 (d, $J = 6.2$ Hz, 1H), 2.44 (s, 1H), 2.39–2.17 (m, 5H), 1.95 (s, 3H), 1.84 (dd, $J = 26.6, 10.6$ Hz, 4H), 1.46 (s, 6H), 1.26 (s, 3H), 1.20 (s, 7H), 1.12 (d, $J = 15.3$ Hz, 4H), 0.88 (s, 3H), 0.83 (s, 12H), 0.02 (s, 3H), -0.05 (s, 3H). ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$) δ 212.3, 210.7, 203.5, 173.4, 171.3, 169.4, 150.3, 140.5, 120.4, 118.8, 79.3, 78.3, 73.5, 73.0, 55.1, 50.6, 49.8, 48.7, 47.7, 47.3, 42.7, 41.6, 35.9, 32.5, 29.0, 28.9, 28.7, 26.3, 26.2, 25.8, 24.7, 23.4, 21.7, 21.5, 19.8, 19.3, 18.1, 18.0, $-4.5, -5.2$. HRMS (ESI) calcd. for $\text{C}_{42}\text{H}_{64}\text{NaO}_{11}\text{Si}$ [$\text{M} + \text{Na}$] $^+$ 795.4110, found 795.4115.

3.1.8. General Procedure for the Synthesis of Compounds 9a–9c

Compounds 7a–7c (1.5 mmol) were dissolved in dry DCM. TFA (20% in DCM) was dropwise added into the reaction mixture at 0 °C, then the mixture was allowed to warm at 20 °C for 2 h. The solvent was removed by reduced pressure. The residue was purified by flash chromatography to obtain the crude amine as a colored oily matter.

Compound 8 (154 mg, 0.2 mmol) and HATU (95.1 mg, 0.25 mmol) were dissolved in dry DMF (4 mL), then DIPEA (44 μL , 0.25 mmol) was added. The mixture was stirred at 30 °C for 1 h, then the corresponding amine (0.6 mmol, 3 eq.) was added. The reaction was stirred for 2 h at this temperature. Then, the reaction was quenched with saturated aqueous NH_4Cl and extracted with EA (3×15 mL). The combined organic layers were washed with saturated brine, dried over Na_2SO_4 , and concentrated to give an oily crude product, which was purified on a silica gel column (PE/EA = 3:1–1:1) to give compound 7 as a yellow solid, which was simple purified to yield a white solid. The solid was used for the next step directly. The solid was dissolved in THF (2 mL). To the mixture was added HOAc (38 μL , 0.65 mmol) and TBAF (169 mg, 0.65 mmol). The mixture was stirred at room temperature for 24 h, and then diluted with ethyl acetate (20 mL). The organic phase was washed with H_2O (3×20 mL), dried over Na_2SO_4 , and concentrated. The residue was purified by column chromatography on silica gel to obtain a white solid 9a–9c.

3-((11-oxidanyl)(oxo)(phenyl)-15-sulfanyl)-4-(2-(4-(((9R,13R,14S,16R)-17-((R,E)-6-acetoxy-2-hydroxy-6-methyl-3-oxohept-4-en-2-yl)-2-hydroxy-4,4,9,13,14-pentamethyl-3,11-dioxo-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-16-yl)oxy)-4-oxobutanamido)ethoxy)-1,2,5-oxadiazole 2-oxide (**9a**) (67% yield in three steps). (purity: 99%). ¹H NMR (400 MHz, CDCl₃) δ 8.13–8.02 (m, 2H), 7.78 (t, *J* = 7.5 Hz, 1H), 7.65 (t, *J* = 7.8 Hz, 2H), 7.16 (d, *J* = 15.6 Hz, 1H), 6.57 (t, *J* = 5.7 Hz, 1H), 6.40 (d, *J* = 15.6 Hz, 1H), 5.76 (d, *J* = 5.4 Hz, 1H), 5.18 (t, *J* = 7.9 Hz, 1H), 4.52 (t, *J* = 5.8 Hz, 2H), 4.46–4.34 (m, 1H), 4.25 (s, 1H), 3.64–3.42 (m, 3H), 3.24 (d, *J* = 14.6 Hz, 1H), 2.71 (dd, *J* = 17.2, 7.2 Hz, 3H), 2.56–2.27 (m, 6H), 2.16–2.05 (m, 2H), 2.02 (s, 3H), 2.00–1.87 (m, 3H), 1.56 (d, *J* = 7.8 Hz, 6H), 1.40 (s, 3H), 1.33 (s, 3H), 1.26 (s, 3H), 1.24 (s, 3H), 1.07 (s, 3H), 1.00 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 213.1, 211.8, 201.2, 172.5, 172.0, 170.1, 158.9, 153.1, 140.4, 137.8, 136.0, 129.9, 128.7, 120.5, 119.3, 110.6, 79.4, 77.8, 73.9, 71.7, 70.7, 54.2, 50.4, 50.1, 48.7, 48.5, 48.1, 43.1, 42.2, 37.1, 36.1, 33.8, 30.8, 29.4, 28.6, 26.9, 26.5, 23.8, 23.7, 22.1, 21.4, 20.2, 19.8, 18.9. HRMS (ESI) calcd. for C₄₆H₅₉N₃NaO₁₅S [M + Na]⁺ 948.3559, found 948.3562.

4-(3-(4-(((9R,13R,14S,16R)-17-((R,E)-6-Acetoxy-2-hydroxy-6-methyl-3-oxohept-4-en-2-yl)-2-hydroxy-4,4,9,13,14-pentamethyl-3,11-dioxo-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-16-yl)oxy)-4-oxobutanamido)propoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide (**9b**) (73% yield in three steps) (purity: 99%). ¹H NMR (400 MHz, CDCl₃) δ 8.11–8.01 (m, 2H), 7.77 (t, *J* = 7.5 Hz, 1H), 7.64 (t, *J* = 7.9 Hz, 2H), 7.18 (d, *J* = 15.6 Hz, 1H), 6.65 (t, *J* = 5.9 Hz, 1H), 6.40 (d, *J* = 15.6 Hz, 1H), 5.75 (d, *J* = 5.5 Hz, 1H), 5.17 (t, *J* = 7.9 Hz, 1H), 4.50 (t, *J* = 5.1 Hz, 2H), 4.45–4.35 (m, 1H), 4.25 (s, 1H), 3.79–3.66 (m, 2H), 3.61 (d, *J* = 3.8 Hz, 1H), 3.24 (d, *J* = 14.6 Hz, 1H), 2.70 (t, *J* = 11.3 Hz, 3H), 2.57–2.24 (m, 6H), 2.03 (s, 3H), 1.99–1.80 (m, 4H), 1.58 (s, 3H), 1.54 (s, 3H), 1.40 (s, 3H), 1.32 (s, 3H), 1.28 (s, 3H), 1.24 (d, *J* = 4.4 Hz, 6H), 1.07 (s, 3H), 1.00 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 213.1, 211.8, 201.3, 172.3, 172.2, 170.3, 159.1, 153.3, 140.4, 137.9, 135.9, 129.9, 128.7, 120.5, 119.3, 110.7, 79.4, 77.8, 74.0, 71.7, 70.5, 54.0, 50.4, 50.1, 48.6, 48.5, 48.1, 43.1, 42.2, 38.4, 36.1, 33.8, 31.0, 29.8, 29.4, 29.4, 27.2, 26.4, 23.8, 23.6, 22.1, 21.4, 20.1, 19.8, 18.9. HRMS (ESI) calcd. for C₄₇H₆₁N₃NaO₁₅S [M + Na]⁺ 962.3716, found 962.3720.

4-(2-(4-(4-(((9R,13R,14S,16R)-17-((R,E)-6-Acetoxy-2-hydroxy-6-methyl-3-oxohept-4-en-2-yl)-2-hydroxy-4,4,9,13,14-pentamethyl-3,11-dioxo-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-16-yl)oxy)-4-oxobutanoyl)piperazin-1-yl)ethoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide (**9c**) (44% yield in three steps). (purity: 95%). ¹H NMR (400 MHz, CDCl₃) δ 8.04 (d, *J* = 7.6 Hz, 2H), 7.76 (t, *J* = 7.5 Hz, 1H), 7.62 (t, *J* = 7.9 Hz, 2H), 7.16 (d, *J* = 15.6 Hz, 1H), 6.41 (d, *J* = 15.6 Hz, 1H), 5.75 (d, *J* = 5.4 Hz, 1H), 5.26–5.12 (m, 1H), 4.55 (t, *J* = 5.3 Hz, 2H), 4.40 (dd, *J* = 12.8, 5.9 Hz, 1H), 4.29 (s, 1H), 3.68–3.52 (m, 3H), 3.47 (t, *J* = 8.3 Hz, 2H), 3.25 (d, *J* = 14.6 Hz, 1H), 2.88 (t, *J* = 5.3 Hz, 2H), 2.79 (s, 1H), 2.71 (dd, *J* = 11.0, 7.4 Hz, 3H), 2.63 (dt, *J* = 9.5, 6.1 Hz, 2H), 2.54 (dd, *J* = 9.3, 4.9 Hz, 2H), 2.50–2.25 (m, 5H), 2.01 (s, 3H), 1.98 (d, *J* = 9.7 Hz, 2H), 1.57 (d, *J* = 1.3 Hz, 6H), 1.41 (s, 3H), 1.31 (d, *J* = 7.5 Hz, 6H), 1.26 (s, 3H), 1.24 (s, 3H), 1.07 (s, 3H), 1.00 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 213.1, 211.9, 201.2, 172.7, 169.8, 169.7, 159.0, 153.0, 140.4, 138.1, 135.8, 129.8, 128.6, 120.5, 119.4, 110.6, 79.2, 77.8, 73.9, 71.7, 69.4, 56.2, 54.3, 53.5, 53.2, 50.4, 50.1, 48.7, 48.5, 48.1, 45.3, 43.1, 42.2, 41.8, 38.7, 36.1, 33.8, 29.4, 28.9, 27.8, 26.7, 26.6, 23.7, 22.0, 21.4, 20.2, 19.8, 18.9. HRMS (ESI) calcd. for C₅₀H₆₇N₄O₁₅S [M + H]⁺ 995.4318, found 995.4322.

3.1.9. General Procedure for the Synthesis of Compounds **10a–10o**

To a solution of **6a–6o** (0.5 mmol) in dry DCM (20 mL) was added succinic anhydride (75 mg, 0.75 mmol) and DMAP (61 mg, 0.5 mmol). The mixture was stirred for 4 h at room temperature. The reaction was quenched with 1N HCl, the pH was adjusted to 2–3 and extraction was performed with CH₂Cl₂ (3 × 15 mL). The combined organic layers were washed with saturated brine, dried over Na₂SO₄, and concentrated to give an oily crude product, which was purified on a silica gel column to obtain the corresponding acid in a yield of 86–96%.

To a solution of compound **2** (135 mg, 0.2 mmol), EDCI (96 mg, 0.5 mmol), DMAP (1.2 mg, 0.01 mmol), and the corresponding acid (0.4 mmol, 2 eq.) in CH₂Cl₂ (2 mL) was added Et₃N (69.5 μL, 0.5 mmol) at 0 °C. The mixture was stirred for 8 h at room temperature. The reaction was quenched

with saturated aqueous NaHCO₃ and extracted with CH₂Cl₂ (3 × 15 mL). The combined organic layers were washed with saturated brine, dried over Na₂SO₄, and concentrated to give an oily crude product, which was simple purified to yield a white solid. The solid was used for the next step directly. The solid was dissolved in THF (2 mL). HOAc (38 μL, 0.65 mmol) and TBAF (169 mg, 0.65 mmol) were added to the mixture. The mixture was stirred at room temperature for 24 h, and then diluted with ethyl acetate (20 mL). The organic phase was washed with H₂O (3 × 20 mL), dried over Na₂SO₄, and concentrated. The residue was purified by column chromatography on silica gel to obtain a white solid **10a–10o**.

4-(2-((4-(((9R,13R,14S,16R)-17-((R,E)-6-Acetoxy-2-hydroxy-6-methyl-3-oxohept-4-en-2-yl)-2-hydroxy-4,4,9,13,14-pentamethyl-3,11-dioxo-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-16-yl)oxy)-4-oxobutanoyl)oxy)ethoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide (**10a**) (81% yield in two steps). (purity: 95%). ¹H NMR (400 MHz, CDCl₃) δ 8.05 (d, J = 7.7 Hz, 2H), 7.77 (t, J = 7.5 Hz, 1H), 7.63 (t, J = 7.8 Hz, 2H), 7.18 (d, J = 15.6 Hz, 1H), 6.40 (d, J = 15.6 Hz, 1H), 5.75 (d, J = 5.2 Hz, 1H), 5.19 (s, 1H), 4.67–4.56 (m, 2H), 4.55–4.46 (m, 2H), 4.45–4.35 (m, 1H), 4.28 (s, 1H), 3.60 (d, J = 3.7 Hz, 1H), 3.25 (d, J = 14.6 Hz, 1H), 2.78–2.66 (m, 3H), 2.65–2.25 (m, 6H), 2.05–1.85 (m, 6H), 1.58 (s, 3H), 1.55 (s, 3H), 1.40 (s, 3H), 1.32 (s, 3H), 1.29 (s, 3H), 1.25 (s, 3H), 1.24 (s, 2H), 1.07 (s, 3H), 1.00 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 213.1, 211.8, 201.1, 172.2, 171.7, 169.8, 158.8, 153.2, 140.5, 138.0, 135.9, 129.9, 128.7, 120.5, 119.2, 110.5, 79.2, 77.8, 74.1, 71.7, 68.9, 61.5, 54.2, 50.3, 50.1, 48.6, 48.5, 48.1, 43.1, 42.2, 36.1, 33.8, 29.4, 28.8, 28.6, 26.9, 26.3, 23.8, 23.7, 22.0, 21.4, 20.1, 19.8, 18.9. HRMS (ESI) calcd. for C₄₆H₅₈N₂NaO₁₆S [M + Na]⁺ 949.3399, found, 949.3402.

4-(3-((4-(((9R,13R,14S,16R)-17-((R,E)-6-Acetoxy-2-hydroxy-6-methyl-3-oxohept-4-en-2-yl)-2-hydroxy-4,4,9,13,14-pentamethyl-3,11-dioxo-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-16-yl)oxy)-4-oxobutanoyl)oxy)propoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide (**10b**) (74% yield in two steps). (purity: 99%). ¹H NMR (400 MHz, CDCl₃) δ 8.05 (d, J = 8.0 Hz, 2H), 7.76 (t, J = 7.4 Hz, 1H), 7.63 (t, J = 7.7 Hz, 2H), 7.17 (d, J = 15.6 Hz, 1H), 6.39 (d, J = 15.6 Hz, 1H), 5.76 (d, J = 5.0 Hz, 1H), 5.18 (t, J = 8.0 Hz, 1H), 4.51 (t, J = 6.0 Hz, 2H), 4.45–4.36 (m, 1H), 4.36–4.21 (m, 3H), 3.60 (d, J = 3.7 Hz, 1H), 3.24 (d, J = 14.6 Hz, 1H), 2.71 (dd, J = 15.6, 11.0 Hz, 3H), 2.65–2.34 (m, 5H), 2.30 (dd, J = 9.0, 5.7 Hz, 1H), 2.21 (p, J = 5.9 Hz, 2H), 2.01 (s, 3H), 2.00–1.86 (m, 3H), 1.58 (s, 3H), 1.55 (s, 3H), 1.45–1.33 (m, 4H), 1.32 (s, 3H), 1.28 (s, 3H), 1.26 (s, 3H), 1.24 (s, 1H), 1.07 (s, 3H), 1.00 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 213.1, 211.7, 201.0, 172.3, 171.8, 169.8, 159.0, 153.1, 140.5, 138.1, 135.8, 129.8, 128.7, 120.5, 119.3, 110.6, 79.2, 77.8, 74.1, 71.7, 68.1, 60.6, 54.2, 50.3, 50.0, 48.6, 48.5, 48.1, 43.1, 42.2, 36.1, 33.8, 29.4, 28.9, 28.7, 28.1, 26.9, 26.4, 23.8, 23.7, 22.0, 21.4, 20.2, 19.8, 18.9. HRMS (ESI) calcd. for C₄₇H₆₀N₂NaO₁₆S [M + Na]⁺ 963.3556, found 963.3560.

4-(4-((4-(((9R,13R,14S,16R)-17-((R,E)-6-Acetoxy-2-hydroxy-6-methyl-3-oxohept-4-en-2-yl)-2-hydroxy-4,4,9,13,14-pentamethyl-3,11-dioxo-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-16-yl)oxy)-4-oxobutanoyl)oxy)butoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide (**10c**) (78% yield in two steps). (purity: 99%). ¹H NMR (400 MHz, CDCl₃) δ 8.09–8.00 (m, 2H), 7.76 (t, J = 7.5 Hz, 1H), 7.63 (t, J = 7.9 Hz, 2H), 7.17 (d, J = 15.6 Hz, 1H), 6.40 (d, J = 15.6 Hz, 1H), 5.76 (d, J = 5.5 Hz, 1H), 5.19 (t, J = 7.9 Hz, 1H), 4.45 (t, J = 6.3 Hz, 2H), 4.42–4.36 (m, 1H), 4.27 (s, 1H), 4.23–4.11 (m, 2H), 3.60 (d, J = 3.9 Hz, 1H), 3.25 (d, J = 14.6 Hz, 1H), 2.78–2.65 (m, 3H), 2.65–2.24 (m, 6H), 2.01 (s, 3H), 2.00–1.93 (m, 4H), 1.82 (dt, J = 13.0, 6.4 Hz, 2H), 1.67 (s, 1H), 1.58 (s, 3H), 1.55 (s, 3H), 1.41 (s, 3H), 1.37 (d, J = 14.5 Hz, 1H), 1.32 (s, 3H), 1.29 (s, 3H), 1.26 (s, 3H), 1.22 (d, J = 13.6 Hz, 1H), 1.07 (s, 3H), 1.00 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 213.0, 211.7, 201.0, 172.4, 171.8, 169.8, 159.0, 153.1, 140.5, 138.1, 135.8, 129.8, 128.7, 120.5, 119.3, 110.6, 79.2, 77.8, 74.1, 71.7, 71.0, 64.0, 54.2, 50.3, 50.1, 48.6, 48.5, 48.1, 43.1, 42.2, 36.1, 33.8, 29.4, 28.9, 28.7, 26.9, 26.4, 25.3, 25.1, 23.9, 23.7, 22.0, 21.4, 20.2, 19.8, 18.9. HRMS (ESI) calcd. for C₄₈H₆₂N₂NaO₁₆S [M + Na]⁺ 977.3712, found 977.3718.

4-((5-((4-(((9R,13R,14S,16R)-17-((R,E)-6-Acetoxy-2-hydroxy-6-methyl-3-oxohept-4-en-2-yl)-2-hydroxy-4,4,9,13,14-pentamethyl-3,11-dioxo-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-16-yl)oxy)-4-oxobutanoyl)oxy)pentyl)oxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide (**10d**) (82% yield in two steps). (purity: 98%). ¹H NMR (400 MHz, CDCl₃) δ 8.04 (d, J = 7.9 Hz, 2H), 7.76 (t, J = 7.4 Hz, 1H), 7.62 (t,

$J = 7.7$ Hz, 2H), 7.17 (d, $J = 15.6$ Hz, 1H), 6.40 (d, $J = 15.6$ Hz, 1H), 5.76 (d, $J = 4.7$ Hz, 1H), 5.19 (s, 1H), 4.42 (t, $J = 6.3$ Hz, 3H), 4.26 (s, 1H), 4.12 (dd, $J = 9.8, 6.2$ Hz, 2H), 3.59 (d, $J = 3.6$ Hz, 1H), 3.24 (d, $J = 14.6$ Hz, 1H), 2.80–2.66 (m, 3H), 2.64–2.25 (m, 6H), 2.09–1.85 (m, 8H), 1.76–1.61 (m, 3H), 1.58 (s, 3H), 1.55 (d, $J = 7.0$ Hz, 4H), 1.40 (d, $J = 6.5$ Hz, 3H), 1.32 (s, 3H), 1.29 (s, 3H), 1.25 (d, $J = 7.4$ Hz, 5H), 1.07 (s, H), 1.00 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 213.0, 211.7, 201.0, 172.5, 171.8, 169.7, 159.1, 153.0, 140.5, 138.2, 135.8, 129.8, 128.7, 120.5, 119.3, 110.6, 79.2, 77.8, 74.1, 71.7, 71.4, 64.4, 54.3, 50.3, 50.1, 48.7, 48.5, 48.1, 43.2, 42.2, 36.1, 33.8, 29.5, 29.0, 28.8, 28.2, 28.2, 26.8, 26.4, 23.9, 23.7, 22.3, 22.0, 21.4, 20.2, 19.8, 18.9. HRMS (MALDI) calcd. for $\text{C}_{49}\text{H}_{64}\text{N}_2\text{NaO}_{16}\text{S}$ [$\text{M} + \text{Na}$] $^+$ 991.3869, found 991.3875.

4-((6-((4-(((9R,13R,14S,16R)-17-((R,E)-6-Acetoxy-2-hydroxy-6-methyl-3-oxohept-4-en-2-yl)-2-hydroxy-4,4,9,13,14-pentamethyl-3,11-dioxo-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-16-yl)oxy)-4-oxobutanoyl)oxy)hexyl)oxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide (**10e**) (72% yield in two steps). (purity: 96%). ^1H NMR (400 MHz, CDCl_3) δ 8.05 (d, $J = 7.7$ Hz, 2H), 7.76 (t, $J = 7.4$ Hz, 1H), 7.62 (t, $J = 7.8$ Hz, 2H), 7.17 (d, $J = 15.6$ Hz, 1H), 6.40 (d, $J = 15.6$ Hz, 1H), 5.75 (s, 1H), 5.19 (t, $J = 7.9$ Hz, 1H), 4.42 (t, $J = 6.4$ Hz, 3H), 4.28 (s, 1H), 4.08 (dt, $J = 10.7, 6.3$ Hz, 2H), 3.60 (d, $J = 3.7$ Hz, 1H), 3.25 (d, $J = 14.5$ Hz, 1H), 2.71 (dd, $J = 15.1, 11.0$ Hz, 3H), 2.64–2.26 (m, 6H), 2.06–1.93 (m, 6H), 1.91–1.85 (m, 2H), 1.71–1.62 (m, 2H), 1.58 (s, 3H), 1.56 (s, 3H), 1.50–1.39 (m, 7H), 1.33 (s, 3H), 1.29 (s, 3H), 1.26 (s, 3H), 1.24 (s, 2H), 1.07 (s, 3H), 1.00 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 213.0, 211.7, 201.0, 172.5, 171.8, 169.7, 159.2, 153.1, 140.5, 138.2, 135.8, 129.8, 128.7, 120.5, 119.3, 110.6, 79.2, 77.8, 74.0, 71.7, 71.5, 64.6, 54.3, 50.4, 50.1, 48.7, 48.5, 48.1, 43.2, 42.2, 36.1, 33.8, 29.5, 29.0, 28.8, 28.6, 28.4, 26.8, 26.4, 25.6, 25.4, 23.9, 23.7, 22.0, 21.4, 20.2, 19.8, 18.9. HRMS (ESI) calcd. for $\text{C}_{50}\text{H}_{66}\text{N}_2\text{NaO}_{16}\text{S}$ [$\text{M} + \text{Na}$] $^+$ 1005.4025, found 1005.4028.

4-((7-((4-(((9R,13R,14S,16R)-17-((R,E)-6-Acetoxy-2-hydroxy-6-methyl-3-oxohept-4-en-2-yl)-2-hydroxy-4,4,9,13,14-pentamethyl-3,11-dioxo-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-16-yl)oxy)-4-oxobutanoyl)oxy)heptyl)oxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide (**10f**) (76% yield in two steps). (purity: 95%). ^1H NMR (400 MHz, CDCl_3) δ 8.04 (d, $J = 7.9$ Hz, 2H), 7.76 (t, $J = 7.5$ Hz, 1H), 7.62 (t, $J = 7.8$ Hz, 2H), 7.16 (d, $J = 15.6$ Hz, 1H), 6.40 (d, $J = 15.6$ Hz, 1H), 5.75 (d, $J = 5.3$ Hz, 1H), 5.19 (t, $J = 7.9$ Hz, 1H), 4.40 (t, $J = 6.5$ Hz, 3H), 4.27 (s, 1H), 4.07 (td, $J = 6.6, 2.0$ Hz, 2H), 3.59 (d, $J = 3.8$ Hz, 1H), 3.24 (d, $J = 14.6$ Hz, 1H), 2.71 (dd, $J = 16.0, 7.8$ Hz, 3H), 2.63–2.24 (m, 6H), 2.04–1.82 (m, 8H), 1.67–1.60 (m, 2H), 1.58 (s, 3H), 1.55 (s, 3H), 1.44 (dd, $J = 8.3, 5.8$ Hz, 2H), 1.40 (s, 3H), 1.35 (s, 4H), 1.32 (s, 3H), 1.28 (s, 3H), 1.26 (s, 3H), 1.24 (s, 2H), 1.07 (s, 3H), 1.00 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 213.0, 211.7, 201.0, 172.5, 171.8, 169.7, 159.2, 153.1, 140.5, 138.2, 135.8, 129.8, 128.6, 120.5, 119.3, 110.6, 79.2, 77.8, 74.0, 71.7, 71.6, 64.8, 54.2, 50.3, 50.1, 48.6, 48.5, 48.1, 43.1, 42.2, 36.1, 33.8, 29.4, 29.0, 28.8, 28.8, 28.6, 28.4, 26.8, 26.4, 25.9, 25.6, 23.8, 23.7, 22.0, 21.4, 20.2, 19.8, 18.9. HRMS (MALDI) calcd. for $\text{C}_{51}\text{H}_{68}\text{N}_2\text{NaO}_{16}\text{S}$ [$\text{M} + \text{Na}$] $^+$ 1019.4182, found 1019.4185.

4-((8-((4-(((9R,13R,14S,16R)-17-((R,E)-6-Acetoxy-2-hydroxy-6-methyl-3-oxohept-4-en-2-yl)-2-hydroxy-4,4,9,13,14-pentamethyl-3,11-dioxo-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-16-yl)oxy)-4-oxobutanoyl)oxy)octyl)oxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide (**10g**) (66% yield in two steps). (purity: 99%). ^1H NMR (400 MHz, CDCl_3) δ 8.04 (dd, $J = 8.5, 1.2$ Hz, 2H), 7.79–7.72 (m, 1H), 7.61 (dd, $J = 10.8, 5.0$ Hz, 2H), 7.16 (d, $J = 15.6$ Hz, 1H), 6.40 (d, $J = 15.6$ Hz, 1H), 5.75 (d, $J = 5.6$ Hz, 1H), 5.19 (t, $J = 7.8$ Hz, 1H), 4.40 (t, $J = 6.5$ Hz, 3H), 4.26 (s, 1H), 4.06 (td, $J = 6.7, 1.7$ Hz, 2H), 3.58 (d, $J = 3.7$ Hz, 1H), 3.24 (d, $J = 14.7$ Hz, 1H), 2.71 (dd, $J = 15.6, 7.5$ Hz, 3H), 2.60–2.34 (m, 5H), 2.30 (ddd, $J = 9.1, 6.7, 3.4$ Hz, 1H), 2.04–1.92 (m, 6H), 1.90–1.81 (m, 2H), 1.66–1.60 (m, 2H), 1.57 (d, $J = 8.1$ Hz, 6H), 1.45 (dd, $J = 9.4, 5.9$ Hz, 2H), 1.40 (s, 3H), 1.39 (s, 1H), 1.35 (d, $J = 1.4$ Hz, 6H), 1.32 (s, 3H), 1.28 (s, 3H), 1.26 (s, 3H), 1.24 (s, 1H), 1.07 (s, 3H), 1.00 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 212.9, 211.6, 200.9, 172.3, 171.7, 169.6, 159.1, 152.9, 140.4, 138.1, 135.6, 129.6, 128.5, 120.3, 119.2, 110.5, 79.1, 77.7, 73.9, 71.6, 71.6, 64.8, 54.2, 50.2, 50.0, 48.5, 48.4, 48.0, 43.1, 42.1, 36.0, 33.7, 29.3, 29.1, 29.0, 28.9, 28.7, 28.6, 28.4, 26.7, 26.3, 25.8, 25.5, 23.8, 23.6, 21.8, 21.3, 20.0, 19.7, 18.8. HRMS (MALDI) calcd. for $\text{C}_{52}\text{H}_{70}\text{N}_2\text{NaO}_{16}\text{S}$ [$\text{M} + \text{Na}$] $^+$ 1033.4338, found 1033.4342.

4-((9-((4-(((9R,13R,14S,16R)-17-((R,E)-6-Acetoxy-2-hydroxy-6-methyl-3-oxohept-4-en-2-yl)-2-hydroxy-4,4,9,13,14-pentamethyl-3,11-dioxo-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-16-yl)oxy)-4-oxobutanoyl)oxy)nonyl)oxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide (**10h**) (69% yield in two steps). (purity: 97%). ¹H NMR (400 MHz, CDCl₃) δ 8.05 (dd, *J* = 8.4, 1.1 Hz, 2H), 7.76 (dd, *J* = 10.7, 4.3 Hz, 1H), 7.62 (t, *J* = 7.9 Hz, 2H), 7.16 (d, *J* = 15.6 Hz, 1H), 6.41 (d, *J* = 15.6 Hz, 1H), 5.76 (d, *J* = 5.6 Hz, 1H), 5.20 (t, *J* = 7.8 Hz, 1H), 4.41 (t, *J* = 6.6 Hz, 3H), 4.27 (s, 1H), 4.08 (dt, *J* = 6.9, 6.5 Hz, 2H), 3.70 (s, 1H), 3.59 (d, *J* = 3.9 Hz, 1H), 3.25 (d, *J* = 14.7 Hz, 1H), 2.72 (dd, *J* = 15.2, 11.0 Hz, 3H), 2.61–2.35 (m, 5H), 2.35–2.27 (m, 1H), 2.04–1.93 (m, 6H), 1.92–1.81 (m, 2H), 1.61 (d, *J* = 7.5 Hz, 3H), 1.59 (s, 3H), 1.56 (s, 3H), 1.44 (d, *J* = 7.4 Hz, 2H), 1.41 (s, 3H), 1.33 (s, 8H), 1.29 (s, 3H), 1.27 (s, 3H), 1.25 (s, 3H), 1.08 (s, 3H), 1.01 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 213.1, 211.7, 201.0, 172.5, 171.8, 169.8, 159.1, 153.1, 140.5, 138.2, 135.7, 129.8, 128.7, 120.5, 119.3, 110.6, 100.1, 79.2, 77.8, 74.0, 71.7, 65.0, 54.3, 50.4, 50.1, 48.7, 48.5, 48.1, 43.2, 42.2, 36.1, 33.8, 29.8, 29.5, 29.3, 29.2, 29.0, 28.8, 28.7, 28.5, 26.8, 26.4, 26.0, 25.7, 23.9, 23.7, 22.0, 21.4, 20.2, 19.8, 18.9. HRMS (MALDI) calcd. for C₅₃H₇₂N₂NaO₁₆S [M + Na]⁺ 1047.4495, found 1047.4498.

4-((10-((4-(((9R,13R,14S,16R)-17-((R,E)-6-Acetoxy-2-hydroxy-6-methyl-3-oxohept-4-en-2-yl)-2-hydroxy-4,4,9,13,14-pentamethyl-3,11-dioxo-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-16-yl)oxy)-4-oxobutanoyl)oxy)decyl)oxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide (**10i**) (71% yield in two steps). (purity: 95%). ¹H NMR (400 MHz, CDCl₃) δ 8.08–7.98 (m, 2H), 7.74 (t, *J* = 7.5 Hz, 1H), 7.60 (t, *J* = 7.9 Hz, 2H), 7.14 (d, *J* = 15.6 Hz, 1H), 6.40 (d, *J* = 15.6 Hz, 1H), 5.74 (d, *J* = 5.5 Hz, 1H), 5.18 (t, *J* = 7.9 Hz, 1H), 4.44–4.35 (m, 3H), 4.27 (d, *J* = 7.8 Hz, 1H), 4.10–3.99 (m, 2H), 3.58 (d, *J* = 3.9 Hz, 1H), 3.24 (d, *J* = 14.6 Hz, 1H), 2.76–2.66 (m, 3H), 2.60–2.23 (m, 6H), 2.04–1.90 (m, 6H), 1.90–1.79 (m, 2H), 1.65–1.58 (m, 2H), 1.56 (s, 3H), 1.55 (s, 3H), 1.38–1.44 (m, 6H), 1.31 (s, 13H), 1.27 (s, 3H), 1.25 (s, 3H), 1.23 (s, 1H), 1.05 (s, 3H), 0.99 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 213.0, 211.7, 201.0, 172.4, 171.7, 169.7, 159.1, 153.0, 140.5, 138.2, 135.7, 129.7, 128.6, 120.4, 119.3, 110.6, 79.2, 77.8, 74.0, 71.7, 71.7, 64.9, 54.2, 50.3, 50.0, 48.6, 48.5, 48.1, 43.1, 42.2, 36.0, 33.8, 29.5, 29.4, 29.3, 29.1, 29.0, 28.8, 28.7, 28.5, 26.7, 26.4, 25.9, 25.6, 23.8, 23.7, 21.9, 21.3, 20.1, 19.8, 18.8. HRMS (MALDI) calcd. for C₅₄H₇₄N₂NaO₁₆S [M + Na]⁺ 1061.4651, found 1061.4655.

4-((11-((4-(((9R,13R,14S,16R)-17-((R,E)-6-Acetoxy-2-hydroxy-6-methyl-3-oxohept-4-en-2-yl)-2-hydroxy-4,4,9,13,14-pentamethyl-3,11-dioxo-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-16-yl)oxy)-4-oxobutanoyl)oxy)undecyl)oxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide (**10j**) (75% yield in two steps). (purity: 95%). ¹H NMR (400 MHz, CDCl₃) δ 8.05 (d, *J* = 8.1 Hz, 2H), 7.75 (t, *J* = 7.3 Hz, 1H), 7.61 (t, *J* = 7.5 Hz, 2H), 7.16 (d, *J* = 15.5 Hz, 1H), 6.40 (d, *J* = 15.6 Hz, 1H), 5.76 (d, *J* = 4.0 Hz, 1H), 5.20 (t, *J* = 8.0 Hz, 1H), 4.41 (t, *J* = 6.5 Hz, 3H), 4.27 (s, 1H), 4.05 (t, *J* = 6.7 Hz, 2H), 3.59 (s, 1H), 3.24 (d, *J* = 14.6 Hz, 1H), 2.71 (dd, *J* = 14.1, 11.1 Hz, 3H), 2.65–2.26 (m, 6H), 2.05–1.92 (m, 6H), 1.92–1.79 (m, 3H), 1.61 (d, *J* = 6.1 Hz, 1H), 1.58 (s, 3H), 1.56 (s, 3H), 1.48–1.38 (m, 6H), 1.28 (dd, *J* = 21.2, 12.1 Hz, 22H), 1.07 (s, 3H), 1.00 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 213.0, 211.7, 201.0, 172.5, 171.8, 169.7, 159.2, 153.0, 140.6, 138.3, 135.7, 129.8, 128.7, 120.5, 119.3, 110.6, 79.2, 77.8, 74.0, 71.8, 71.7, 65.0, 54.3, 50.4, 50.1, 48.7, 48.5, 48.1, 43.2, 42.2, 36.1, 33.8, 29.8, 29.6, 29.6, 29.5, 29.4, 29.2, 29.0, 28.9, 28.7, 28.5, 26.8, 26.4, 26.0, 25.7, 23.9, 23.7, 22.0, 21.4, 20.2, 19.8, 18.9. HRMS (MALDI) calcd. for C₅₅H₇₆N₂NaO₁₆S [M + Na]⁺ 1075.4808, found 1075.4812.

4-((12-((4-(((9R,13R,14S,16R)-17-((R,E)-6-Acetoxy-2-hydroxy-6-methyl-3-oxohept-4-en-2-yl)-2-hydroxy-4,4,9,13,14-pentamethyl-3,11-dioxo-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-16-yl)oxy)-4-oxobutanoyl)oxy)dodecyl)oxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide (**10k**) (73% yield in two steps). (purity: 96%). ¹H NMR (400 MHz, CDCl₃) δ 8.04 (d, *J* = 7.5 Hz, 2H), 7.75 (t, *J* = 7.5 Hz, 1H), 7.61 (t, *J* = 7.8 Hz, 2H), 7.15 (d, *J* = 15.6 Hz, 1H), 6.41 (d, *J* = 15.6 Hz, 1H), 5.76 (d, *J* = 5.3 Hz, 1H), 5.20 (t, *J* = 7.9 Hz, 1H), 4.40 (t, *J* = 6.6 Hz, 3H), 4.26 (s, 1H), 4.05 (t, *J* = 6.8 Hz, 2H), 3.58 (d, *J* = 3.6 Hz, 1H), 3.24 (d, *J* = 14.7 Hz, 1H), 2.79–2.65 (m, 3H), 2.60–2.34 (m, 5H), 2.30 (ddd, *J* = 12.3, 6.1, 3.2 Hz, 1H), 2.05–1.92 (m, 6H), 1.91–1.81 (m, 2H), 1.61 (d, *J* = 6.2 Hz, 2H), 1.57 (d, *J* = 7.9 Hz, 6H), 1.46–1.37 (m, 6H), 1.32 (s, 6H), 1.28 (s, 12H), 1.26 (s, 3H), 1.24 (s, 3H), 1.07 (s, 3H), 1.00 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 213.0, 211.7, 201.1, 172.4, 171.8, 169.7, 159.2, 153.0, 140.6, 138.3, 135.7, 129.7, 128.7, 120.5, 119.4, 110.6, 79.2, 77.8, 74.0, 71.8, 71.7, 65.0, 54.3, 50.3, 50.1, 48.7, 48.5, 48.1, 43.2, 42.2, 36.1, 33.9, 29.8, 29.7, 29.6, 29.6, 29.5, 29.4, 29.2, 29.0, 28.9, 28.7, 28.5,

26.8, 26.4, 26.0, 25.7, 23.9, 23.8, 22.0, 21.4, 20.2, 19.8, 18.9. HRMS (MALDI) calcd. for C₅₆H₇₈N₂NaO₁₆S [M + Na]⁺ 1089.4964, found 1089.4968.

4-(((E)-4-((4-(((9R,13R,14S,16R)-17-((R,E)-6-Acetoxy-2-hydroxy-6-methyl-3-oxohept-4-en-2-yl)-2-hydroxy-4,4,9,13,14-pentamethyl-3,11-dioxo-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-16-yl)oxy)-4-oxobutanoyl)oxy)but-2-en-1-yl)oxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide (**10l**) (61% yield in two steps). (purity: 99%). ¹H NMR (400 MHz, CDCl₃) δ 8.06 (d, J = 7.5 Hz, 2H), 7.77 (t, J = 7.5 Hz, 1H), 7.63 (t, J = 7.8 Hz, 2H), 7.18 (d, J = 15.6 Hz, 1H), 6.39 (d, J = 15.6 Hz, 1H), 5.91 (t, J = 5.5 Hz, 2H), 5.76 (d, J = 5.4 Hz, 1H), 5.20 (t, J = 8.0 Hz, 1H), 5.05 (d, J = 5.0 Hz, 2H), 4.74 (d, J = 4.9 Hz, 2H), 4.46–4.36 (m, 1H), 4.28 (s, 1H), 3.60 (d, J = 3.8 Hz, 1H), 3.25 (d, J = 14.7 Hz, 1H), 2.72 (dd, J = 19.1, 10.9 Hz, 3H), 2.66–2.27 (m, 7H), 2.04–1.97 (m, 4H), 1.58 (s, 3H), 1.55 (s, 3H), 1.41 (s, 3H), 1.33 (s, 3H), 1.29 (s, 3H), 1.26 (s, 3H), 1.25 (s, 3H), 1.08 (s, 3H), 1.01 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 213.1, 211.7, 201.0, 172.1, 171.8, 169.8, 153.2, 140.5, 135.9, 130.4, 129.9, 128.7, 125.9, 120.5, 119.2, 79.2, 77.8, 74.1, 71.7, 66.7, 60.3, 54.2, 50.4, 50.1, 48.6, 48.5, 48.2, 43.2, 42.2, 36.1, 33.8, 29.8, 29.5, 28.9, 28.7, 26.9, 26.4, 23.9, 23.7, 22.0, 21.4, 20.2, 19.8, 18.9. HRMS (ESI) calcd. for C₄₈H₆₀N₂NaO₁₆S [M+Na]⁺ 975.3556, found 975.3560.

4-((4-((4-(((9R,13R,14S,16R)-17-((R,E)-6-Acetoxy-2-hydroxy-6-methyl-3-oxohept-4-en-2-yl)-2-hydroxy-4,4,9,13,14-pentamethyl-3,11-dioxo-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-16-yl)oxy)-4-oxobutanoyl)oxy)but-2-yn-1-yl)oxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide (**10m**) (77% yield in two steps). (purity: 99%). ¹H NMR (400 MHz, CDCl₃) δ 8.07 (d, J = 7.5 Hz, 2H), 7.77 (t, J = 7.3 Hz, 1H), 7.64 (t, J = 7.6 Hz, 2H), 7.18 (d, J = 14.9 Hz, 1H), 6.40 (d, J = 15.4 Hz, 1H), 5.77 (s, 1H), 5.21 (t, J = 7.5 Hz, 1H), 5.10 (s, 2H), 4.79–4.67 (m, 2H), 4.41 (d, J = 12.7 Hz, 1H), 4.27 (s, 1H), 3.59 (s, 1H), 3.25 (d, J = 14.8 Hz, 1H), 2.74–2.67 (m, 3H), 2.65–2.54 (m, 2H), 2.43 (d, J = 4.5 Hz, 2H), 2.40–2.27 (m, 2H), 2.01 (d, J = 9.7 Hz, 4H), 1.59 (s, 3H), 1.56 (s, 3H), 1.41 (s, 3H), 1.33 (s, 3H), 1.29 (s, 3H), 1.27 (s, 3H), 1.25 (s, 4H), 1.08 (s, 3H), 1.01 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 213.0, 211.7, 201.1, 171.6, 169.8, 158.0, 153.2, 140.6, 137.8, 135.9, 129.9, 128.8, 120.5, 119.3, 100.0, 84.0, 79.2, 78.8, 77.8, 74.2, 71.8, 58.7, 54.3, 52.2, 50.4, 50.1, 48.7, 48.5, 48.2, 43.2, 42.2, 36.1, 33.9, 29.8, 29.5, 28.8, 28.7, 27.0, 26.4, 23.9, 23.8, 22.0, 21.4, 20.2, 19.8, 18.9. HRMS (ESI) calcd. for C₄₈H₅₈N₂NaO₁₆S [M + Na]⁺ 973.3399, found 973.3402.

4-3-((4-(((9R,13R,14S,16R)-17-((R,E)-6-Acetoxy-2-hydroxy-6-methyl-3-oxohept-4-en-2-yl)-2-hydroxy-4,4,9,13,14-pentamethyl-3,11-dioxo-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-16-yl)oxy)-4-oxobutanoyl)oxy)-2,2-dimethylpropoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide (**10n**) (71% yield in two steps). (purity: 95%). ¹H NMR (400 MHz, CDCl₃) δ 8.04 (d, J = 7.9 Hz, 2H), 7.75 (t, J = 7.4 Hz, 1H), 7.62 (t, J = 7.7 Hz, 2H), 7.17 (d, J = 15.6 Hz, 1H), 6.39 (d, J = 15.6 Hz, 1H), 5.76 (d, J = 4.3 Hz, 1H), 5.15 (t, J = 8.0 Hz, 1H), 4.45–4.35 (m, 1H), 4.26 (s, 1H), 4.22–4.14 (m, 2H), 4.09 (d, J = 10.9 Hz, 1H), 3.98 (d, J = 11.0 Hz, 1H), 3.60 (d, J = 3.5 Hz, 1H), 3.24 (d, J = 14.6 Hz, 1H), 2.76–2.26 (m, 9H), 2.04–1.85 (m, 6H), 1.56 (d, J = 11.9 Hz, 6H), 1.40 (s, 3H), 1.32 (s, 3H), 1.28 (s, 3H), 1.26 (s, 3H), 1.24 (s, 2H), 1.09 (s, 6H), 1.07 (s, 3H), 1.00 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 213.1, 211.7, 201.1, 172.2, 171.8, 169.8, 159.3, 153.1, 140.5, 138.3, 135.8, 129.9, 128.7, 120.6, 119.3, 110.6, 79.2, 77.8, 75.8, 74.1, 71.8, 68.8, 54.3, 50.4, 50.1, 48.7, 48.5, 48.2, 43.1, 42.2, 35.6, 33.9, 30.5, 29.5, 28.9, 28.8, 27.0, 26.4, 23.7, 22.0, 21.7, 21.4, 20.2, 19.8, 18.9. HRMS (ESI) calcd. for C₄₉H₆₄N₂NaO₁₆S [M + Na]⁺ 991.3869, found 991.3873.

4-((4-(((4-(((9R,13R,14S,16R)-17-((R,E)-6-Acetoxy-2-hydroxy-6-methyl-3-oxohept-4-en-2-yl)-2-hydroxy-4,4,9,13,14-pentamethyl-3,11-dioxo-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-16-yl)oxy)-4-oxobutanoyl)oxy)methyl)benzyl)oxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide (**10o**) (63% yield in two steps). (purity: 95%). ¹H NMR (400 MHz, CDCl₃) δ 8.07–7.98 (m, 2H), 7.74 (d, J = 7.5 Hz, 1H), 7.59 (t, J = 7.9 Hz, 2H), 7.45 (d, J = 8.1 Hz, 2H), 7.39 (d, J = 8.2 Hz, 2H), 7.17 (d, J = 15.6 Hz, 1H), 7.17 (d, J = 15.6 Hz, 1H), 6.40 (d, J = 15.6 Hz, 1H), 5.76 (d, J = 5.5 Hz, 1H), 5.44 (s, 2H), 5.21 (t, J = 7.9 Hz, 1H), 5.15 (s, 2H), 4.45–4.36 (m, 1H), 4.28 (s, 1H), 3.60 (d, J = 3.9 Hz, 1H), 3.25 (d, J = 14.6 Hz, 1H), 2.78–2.25 (m, 10H), 2.05–1.86 (m, 6H), 1.57 (s, 3H), 1.54 (s, 3H), 1.41 (s, 3H), 1.33 (s, 3H), 1.29 (s, 3H), 1.25 (d, J = 6.5 Hz, 4H), 1.07 (s, 3H), 1.01 (s, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 213.0, 211.7, 201.0, 172.2, 171.8, 169.8, 158.7, 153.2,

140.5, 138.1, 137.0, 135.8, 133.9, 129.8, 128.7, 128.6, 128.5, 120.5, 119.3, 110.6, 79.2, 77.8, 74.1, 72.3, 71.7, 66.0, 54.3, 50.3, 50.1, 48.6, 48.5, 48.1, 43.2, 42.2, 36.1, 33.8, 29.5, 29.0, 28.8, 26.9, 26.4, 23.9, 23.7, 22.0, 21.4, 20.2, 19.8, 18.9. HRMS (ESI) calcd. for $C_{52}H_{62}N_2NaO_{16}S$ $[M + Na]^+$ 1025.3712, found 1025.3718.

3.1.10. Procedure for the Synthesis of Compound 11

4-(((2*S*,9*R*,13*R*,14*S*,16*R*)-17-((*R,E*)-6-Acetoxy-2-hydroxy-6-methyl-3-oxohept-4-en-2-yl)-2-hydroxy-4,4,9,13,14-pentamethyl-3,11-dioxo-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-16-yl)oxy)-4-oxobutanoic acid (**11**). Compound **8** (77 mg, 0.1 mmol) was dissolved in THF (5 mL). HOAc (28 μ L, 0.5 mmol) and TBAF (131 mg, 0.5 mmol) were added to the mixture. The mixture was stirred at room temperature for 24 h, and then diluted with ethyl acetate (20 mL). The organic phase was washed with H_2O (3×20 mL), dried over Na_2SO_4 , and concentrated. The residue was purified by column chromatography on silica gel to obtain a white solid **11** (52 mg, 79% yield). (purity: 95%). 1H NMR (400 MHz, $CDCl_3$) δ 7.16 (d, $J = 15.6$ Hz, 1H), 6.41 (d, $J = 15.6$ Hz, 1H), 5.77 (d, $J = 5.5$ Hz, 1H), 5.22 (t, $J = 7.8$ Hz, 1H), 4.41 (dd, $J = 12.9, 6.0$ Hz, 1H), 3.25 (d, $J = 14.6$ Hz, 1H), 2.77–2.67 (m, 3H), 2.58 (dd, $J = 12.2, 6.5$ Hz, 2H), 2.43 (t, $J = 6.7$ Hz, 2H), 2.38 (d, $J = 7.8$ Hz, 1H), 2.31 (ddd, $J = 12.5, 5.8, 3.4$ Hz, 1H), 2.04–1.93 (m, 6H), 1.59 (s, 3H), 1.55 (s, 3H), 1.41 (s, 3H), 1.39 (s, 1H), 1.33 (s, 3H), 1.28 (d, $J = 4.5$ Hz, 6H), 1.25 (s, 3H), 1.08 (s, 3H), 1.01 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 213.1, 211.8, 201.1, 176.9, 171.7, 170.0, 153.1, 140.5, 120.5, 119.3, 79.4, 77.9, 74.3, 71.8, 54.3, 50.4, 50.1, 48.7, 48.5, 48.1, 43.2, 42.2, 36.09, 33.9, 29.5, 28.9, 28.8, 27.0, 26.3, 23.9, 23.8, 21.9, 21.4, 20.2, 19.9, 18.8. HRMS (ESI) calcd. for $C_{36}H_{50}NaO_{11}$ $[M + Na]^+$ 681.3245, found 681.3250.

3.2. Biological Assay

3.2.1. Cell Culture

HepG-2 human hepatocellular carcinoma cells and L-O2 human normal liver cells were maintained in DMEM culture medium supplemented with 10% FBS at 37 °C under a humidified atmosphere with 5% CO_2 .

3.2.2. MTT Assay

HepG-2 and L-O2 cells in logarithmic growth phase were seeded into a 96-well plate at 5×10^3 /well/200 μ L. After 12 h, compounds at different concentrations were added and incubated for 72 h at 37 °C, 5% CO_2 . Then, 20 μ L MTT (5 mg/mL) was added into each well and incubated for 4 h. The supernatant was discarded, and the precipitate was dissolved with DMSO. The absorbance of the optical density (OD) at 570 nm was detected using a microplate reader and the IC_{50} was calculated with GraphPad Prism 5.

3.2.3. Cell Apoptosis Assay

HepG-2 cells were seeded into a 24-well plate with 5×10^4 /well/1 mL. After 12 h, cells were treated with compound **10b** at different concentrations for 48 h. The cells were collected, washed with cold PBS, and stained with Annexin-V/PI according to the manufacturer's instructions. Then, the cells were analyzed with flow cytometry.

3.2.4. Western Blot Assay

HepG-2 cells were treated with compound **10b** for 24 h, and the cell lysates were collected. Then, the total protein was separated by SDS-PAGE and transferred to PVDF membranes. The membranes were incubated with primary antibodies (1:1000) at 4 °C overnight. After being washed with PBST (PBS + 0.5% Tween 20) 5 times, the membranes were incubated with HRP-conjugated secondary antibodies (1:5000 dilution) for 2 h at room temperature. The membranes were then washed with PBST 5 times and the protein blots were detected by ECL chemiluminescence.

3.2.5. Animal Assay

Five-week-old Balb/C mice were purchased from the Chinese Academy of Sciences and maintained under specific pathogen free (SPF) conditions. The mice were divided into a control group, a cucurbitacin B group, and a compound **10b** group. The control group mice were treated with vehicle buffer intravenously. The cucurbitacin B group mice were treated with cucurbitacin B at doses of 2 mg/kg, 5 mg/kg, 10 mg/kg, 20 mg/kg, and 50 mg/kg, respectively (three mice each dose). The compound **10b** group mice were treated with compound **10b** at doses of 2 mg/kg, 5 mg/kg, 10 mg/kg, 20 mg/kg, and 50 mg/kg respectively (three mice each dose). The clinical symptoms, deaths, and body weight were observed and recorded.

4. Conclusion

In summary, 37 cucurbitacin B derivatives were synthesized and evaluated for their anti-hepatocellular carcinoma activities against the HepG-2 cell line. These compounds were also tested for their toxicity against the L-O2 normal cell line. These studies indicated that the introduction of a phenylsulfonyl-substituted furoxan NO-releasing moiety could reduce the toxicity against normal cells and maintain potent anti-HCC activity to some extent. The compound with the most potential, **10b**, exhibited potent activity against the HepG-2 cell line, with an IC₅₀ value of 0.63 μM. Moreover, compound **10b** showed the highest TI value (4.71), which is a 14.7-fold improvement compared to its parent compound cucurbitacin B (Table 2). The preliminary study of the molecular mechanism of **10b** indicated that **10b** could inhibit P-STAT3 to induce the activation of mitochondrial apoptotic pathways (Figure 2). An in vivo acute toxicity study indicated that compound **10b** has preferable safety and tolerability compared with cucurbitacin B (Figure 3). These findings indicate that compound **10b** might be considered as a lead compound for exploring effective anti-HCC drugs.

Supplementary Materials: Copies of the ¹H/¹³C NMR spectra, HPLC, and dose–response curves of all new compounds.

Author Contributions: Conceptualization and design of the study: Y.C., Y.D., and Q.Z.; Synthesis of the compounds: W.G. and X.C.; Performance of the pharmacological tests: W.M. and Y.D.; Statistical analysis of the data: W.G., X.C., Z.L., T.W., Y.C., Y.D., and Q.Z.; Writing and revising of the manuscript: W.G., Y.C., Y.D., and Q.Z.

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Sample Availability: Samples of the compounds **3**, **4**, **9–11** are available from the authors.



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