Full Paper

Synthesis and Cytotoxicity Studies of New Cryptophycin Analogues

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Two analogues of cryptophycin were synthesized and biologically evaluated for their *in-vitro* cytotoxicities against several solid tumors and leukemia cell lines. The results revealed that both analogues exhibited a broad range of cytotoxic activity with observed IC_{50} values in the μM-range, and compound **4** was more effective than compound **3** in most assays studied.

Keywords: Anticancer / Cryptophycin / Cytotoxicity / Synthesis / Taxol

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Introduction

The cryptophycins are a family of macrocyclic depsipeptides isolated from terrestrial blue-green algae (*Nostoc* sp), which are potent tubulin-binding antimitotic agents with excellent activities against multi-drug resistant (MDR) cancer cell lines and mammary-derived tumors [1, 2]. Cryptophycin-1 (**1**, Fig. 1) is the key cytotoxin in Nostoc sp and displays remarkable cytotoxic activity [3, 4]. More importantly, compared to vinblastine, colchicine, and paclitaxel, the cryptophycin-1 presents reduced susceptibility to P-glycoprotein-mediated multiple drug resistance [5].

By 2005, twenty eight cryptophycin analogues had been isolated, while hundreds of synthetic analogues had been synthesized for the structure-activity relationship (SAR) studies in search for more potent and drugable compounds [1, 6]. Some synthetic cryptophycin analogues have demonstrated superior activities and drugable properties to their natural compounds. The closely related synthetic analogue cryptophycin-52 (**2**, LY355703, Fig. 1) was identified by Eli Lilly as the pharmacologically most promising first-generation clinical candidate, and is currently in clinical studies as an antitumor agent [8 – 11].

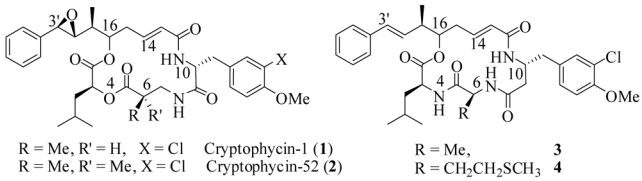

Figure 1. Structures of cryptophycin-1 (**1**), −52 (**2**), and the olefin analogues **3** and **4**.

However, the low bio-availability in *in-vivo* cytotoxic assays of cryptophycin-1 caused by its poor stability and low solubility [12] led some research teams to initiate an attempt to improve it through structure modifications.

In this work, we intend to introduce a lactam on C4 and polar groups (for instants: sulfur element of Met, OH of Ser, lactam of Asp) on C6 of cryptophycin-1 in order to alter its solubility. Two lactam analogues of cryptophycin-1, **3** and **4** (Fig. 1) were synthesized for the first time, and their *in-vitro* cytotoxicities were evaluated by the MTT assay.

Results and discussion

The retrosynthetic analysis of compounds **3** and **4** suggested that they can be derived from two main building

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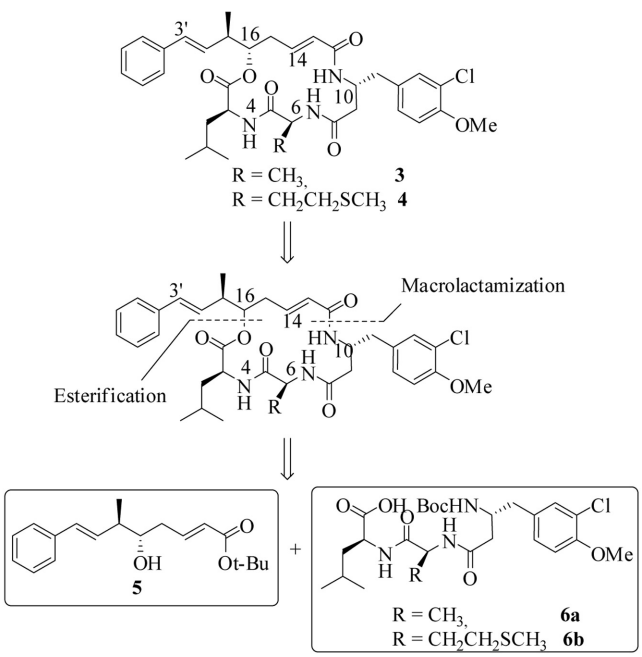
Abbreviations: 2,3-Dichloro-5,6-dicyanobenzoquinone (DDQ); 4-(Dimethylamino)-pyridin (DMAP); 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC · HCl); Hydroxybenzotriazole (HOBT)

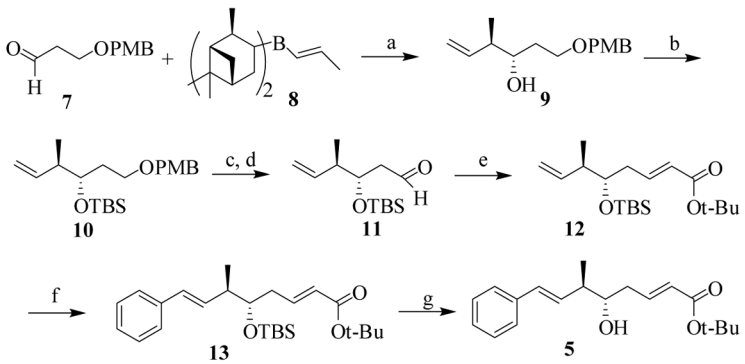


Scheme 1. The retrosynthetic analysis of the olefin analogues **3** and **4**.

blocks, the octadienoate ester **5** and the tripeptide **6** (Scheme 1), respectively.

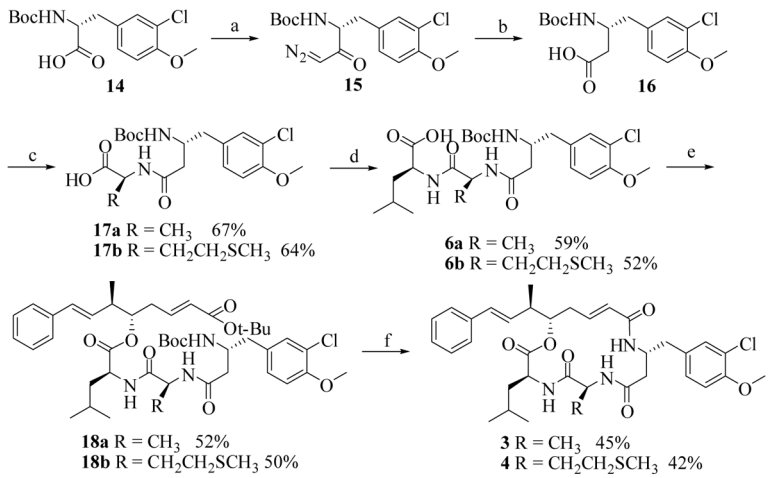
Based on the above retrosynthetic analysis, **5** was prepared using the crotylboration approach [13, 14]. Compound **7** was prepared from 4-methyloxidyl-benanhydride in several steps (Scheme 2). The key step in Scheme 2 utilized the crotylboration with crotyl diisopinocampheylborane **8** (prepared from (+)-**B**-methoxydiisopinacampheyl-borane) to generate the desired stereochemistry at both chiral centers of **9** in 55% yield (91% ee). Silyl protection of the secondary alcohol in **9** with *tert*-butyldimethylsilyl chloride and triethylamine afforded the silyl ether **10** in 92% yield. Deprotection of the *p*-methoxybenzyl ether with (2,3-dichloro-5,6-dicyanobenzoquinone) DDQ followed by Swern Oxidation of the resulting alcohol furnished the aldehyde **11** in 54% yield over two steps. Wittig – Horner olefination of **11** provided the α,β-unsaturated *tert*-butyl ester **12** in 79% yield. Heck coupling on **12** with iodobenzene in the presence of palladium acetate and sodium bicarbonate furnished the required ester **13** in 60% yield. Removal of the silyl ether with tetrabutylammonium fluoride led to the formation of the desired octadienoate ester **5** in 71% yield.

In the second synthon, tripeptides **6a** and **6b**, were synthesized starting from the D-tyrosine derivative (*R*)-2-(*tert*-butoxycarbonylamino)-3-(3-chloro-4-methoxyphenyl) propanoic acid **14** (Scheme 3). Compound **14** was prepared from D-tyrosine, through chlorination by $SOCl_2$, Boc protection of the NH_2 group by $(Boc)_2O$, methylation by Me_2SO_4, and deprotection of the methyl ester. The use of diazomethane afforded the diazoketone derivative **15** in 85% yield, followed by addition of trifluoroacetic acid silver salt; the homo-amino acid derivative (*R*)-3-(*tert*-butoxycarbonylamino)-4-(3-chloro-4-methoxyphenyl) butanoic acid **16** was afforded in 60% yield. Activated by 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC · HCl) and hydroxybenzotriazole (HOBT), **16** was coupled with (*S*)-methyl 2-aminopropanoate and (*S*)-methyl 2-amino-4-(methylthio)butanoate, respectively, subsequently treated with NaOH, this furnished the dipeptide derivatives **17a** (67%) and **17b** in 64% yield over two steps. In a same procedure, **17a** and **17b** were coupled with (*S*)-methyl 2-amino-4-methylpentanoate to obtain the tripeptide derivatives **6a** (59%) and **6b** (52%) over two steps.



Reagents and conditions: a) THF, Et_2O, - 78 °C, 2 h, then NaOH, H_2O_2 , 55%, (91% ee); b) TBSCl, Et_3N, THF, - 78 °C, 10 min, 92%; c) DDQ, CH_2Cl_2, H_2O, 0 °C, 15 min; d) Swern Oxidition, 54% over two steps; e) $(EtO)_2P(O)CH_2C(O)OtBu$, DBU, LiCl, CH_3CN, r. t., 2 h, 79%; f) PhI, $Pd(OAc)_2$, Et_3N, CH_3CN, 83 °C, 18 h, 60%; g) tetrabutylammonium fluoride, 12 h, 71%.

Scheme 2. Synthesis of the octadienoate ester **5**.



Reagents and conditions: a) CH_2N_2, $ClCO_2Et$, 4 h, 85%; b) CF_3CO_2Ag, 6 h, 60%; c) *N*-Boc-L-amino acid-OMe, EDC x HCl, HOBT, Et_3N, overnight, then NaOH, MeOH / H_2O, 1 h; d) L-Leucine-OMe, EDC x HCl, HOBT, Et_3N, overnight, then NaOH, MeOH / H_2O, 1 h; e) octadienoate ester 5, DCC, DMAP, overnight; f) 20% CF_3CO_2H in CH_2Cl_2, 2 h, then EDC x HCl, HOBT, Et_3N, overnight.

Scheme 3. Synthesis of the cryptophycin analogues **3** and **4**.

Table 1. Cytotoxicity of compounds **3** and **4** against fifteen cell lines.

Comp.	Cytotoxicity (IC_{50}, μM)														
	Solid tumor cells									Leukemia cells			Normal cells		
	T47D	H460	Hela	DV145	LoVo	HepG2	HCT116	A549	SGC-7901	L1210	K562	RAW	CHO	VEC	293T
Taxol	1.39	2.42	15.68	66.87	>100	>100	>100	>100	5.36	>100	>100	43.53	>100	5.23	68.71
3	3.50	21.47	29.62	69.84	94.81	43.10	52.66	77.84	38.09	51.44	34.32	38.78	>100	57.99	>100
4	3.78	26.64	24.73	53.73	>100	36.25	64.96	54.30	19.06	37.23	18.34	34.96	>100	34.55	>100

Each experiment was performed independently three times.

The tripeptide derivatives **6a** and **6b** were activated by DCC and a catalytic amount of 4-(dimethylamino)-pyridin (DMAP), addition of the octadienoate ester **5** to the mixed anhydride afforded the intermediate **18a** (52%) and **18b** (50%), respectively (Scheme 3). Simultaneous deprotection of the *tert*-butyl ester and the *N*-Boc with trifluoroacetic acid in CH_2Cl_2 produced the cyclized precursor and by EDC · HCl and HOBT activation provided the desired compounds **3** (45%) and **4** (42%), respectively.

A preliminary *in-vitro* evaluation of the cytotoxic activity of **3** and **4** was performed by MTT assays. The nine solid cancer cell lines (T47D: human breast cancer cell, NCI-H460: human lung cancer cell, Hela: human cervical cancer, HepG2: human liver cancer, LoVo and HCT116: human colon cancer, A549: human lung cancer, DV145: human prostate cancer, SGC-7901: human stomach cancer) and three leukemia cell lines (K562: multi-drug resistant human leukemia cell, L1210: mouse acute lymph leukemia cell, RAW: mouse leukemic monocyte macrophage cell) were employed in the present study. To study the possible cytotoxicity in normal cells, the growth inhibition of compounds **3** and **4** in 293T (human kidney epithelium cell), CHO (china hamster ovary cell), and VEC (human blood vessel bast cell) were evaluated. The IC_{50} values were measured and are listed in Table 1.

As shown in Table 1, compounds **3** and **4** had demonstrated effective growth inhibitory activities in almost all of the cell lines studied, broader than that of Taxol. In the leukemia cell lines studied, Taxol presented poor or no activity, whereas both compounds **3** and **4** were active. Similarly, in the solid tumor cell lines of HepG2, HCT116, and A549 both were active, but Taxol was a poor inhibitor.

In DV145, similar IC_{50} were observed for both Taxol and the compounds synthesized. Taxol presented slightly better growth inhibitory activities in T47D and SGC-7901 cell lines, and much better ones in H460. The inhibitory activities in Hela were comparable to both Taxol and the compounds synthesized. In LoVo, neither Taxol nor our compounds were active.

The IC_{50} values of compounds **3** and **4** in normal cell lines are all larger than that of Taxol, and most of them are above 100 μM except in VEC. This result implies that compounds **3** and **4** are generally safer than Taxol.

In summary, compounds **3** and **4** exhibited a broader cytotoxicity than Taxol both in solid tumors and leukemia cell lines. In most cell lines tested, compound **4** was slightly more active than compound **3**, and the thio-substitution on C6 of compound **4** was believed to produce a positive effect on the cytotoxic activity. Though more evidence is needed, this encouraging observation has led us to design potentially more potent compounds with different polar group at position C6 of compound **4**.

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The authors have declared no conflict of interest.

Experimental

Chemistry

Melting points were recorded on a B-540 melting point apparatus (Büchi Labortechnik, Flawil, Switzerland) and are uncorrected. The ^{1}H-NMR spectra were recorded on a Bruker Avance DMX-400 NMR-spectrometer (400 MHz, 298C; Bruker Bioscience, Billerica, MA, USA). Chemical shifts are given in ppm relative to tetramethylsilane (TMS) as internal standard (0 ppm). Mass spectra were recorded on an Esquire-LC-00075 mass spectrometer (Bruker). All reagents were purchased from commercial suppliers and were purified when necessary.

*(3S,4R)-1-(4-Methoxybenzyloxy)-4-methyl-5-hexen-3-ol **9***

Aldehyde **7** (0.62 g, 3.0 mmol) was dissolved in dry ether (4 mL) and cooled to −78°C in N_2 atmosphere. **8** (3.5 mmol, preparation method as described [13]) was dissolved in dry ether (2 mL) dropwise via syringe at −78°C in a period of 10 min. The mixture was stirred at -78°C for 2 h and heated to room temperature. NaOH (2 N, 3 mL) and H_2O_2 (55%, 6 mL) were added dropwise and the mixture was stirred for another 2 h. The reaction was quenched with saturated aqueous $NaHCO_3$. The aqueous layer was extracted with CH_2Cl_2. The combined organic extracts were dried ($MgSO_4$), filtered, concentrated, and purified by flash column chromatography (hexane/EtOAc, 8:1) to provide a colorless oil (0.41 g, 55%). ^{1}H-NMR ($CDCl_3$) δ: 7.28 – 7.26 (d, *J* = 8.7 Hz, 2H, CH), 6.91 – 6.88 (d, *J* = 8.7 Hz, 2H, CH), 5.87 – 5.78 (m, 1H, CH), 5.08 – 5.06 (br d, *J* = 5.0 Hz, 2H, CH_2), 4.47 (s, 2H, CH_2), 3.82 (s, 3H, OCH_3), 3.74 – 3.61 (m, 3H, CH_2, CH), 2.82 – 2.81 (d, *J* = 3.0 Hz, 1H, CH), 1.76 – 1.69 (m, 2H, CH_2), 1.07 – 1.05 (d, *J* = 6.9 Hz, 3H, CH_3).

*(3S,4R)-3-[(tert-Butyldimethylsilyl)oxy]-1-(4-methoxybenzyloxy)-4-methyl-5-hexene **10***

Alcohol **9** (0.15 g, 0.60 mmol) was dissolved in CH_2Cl_2 (6 mL) and cooled to −78°C. 2, 6-Lutidine (0.14 mL, 1.20 mmol) was added, followed by TBSOTf (0.17 mL, 0.72 mmol) dropwise via a syringe. After 10 min, the reaction was quenched with saturated aqueous $NaHCO_3$. The aqueous layer was extracted with CH_2Cl_2. The combined organic extracts were dried ($MgSO_4$), filtered, concentrated, and purified by flash column chromatography (hexane/EtOAc, 9:1) to provide a colorless oil (0.20 g, 92%).

^{1}H-NMR ($CDCl_3$) δ: 7.14 – 7.06 (d, *J* = 8.6 Hz, 2H, CH), 6.81 – 6.69 (d, *J* = 8.6 Hz, 2H, CH), 5.80 – 5.71 (m, 1H, CH), 5.00 – 4.92 (br d, *J* = 5.0 Hz, 2H, CH_2), 4.44 (s, 2H, CH_2), 3.80 (s, 3H, OCH_3), 3.77 – 3.73 (m, 1H,CH), 3.48 – 3.45 (dt, *J* = 2.1, 7.0 Hz, 2H, CH_2), 2.29 – 2.26 (m, 1H, CH), 1.70 – 1.63 (m, 2H, CH_2), 1.00 – 0.98 (d, *J* = 6.9 Hz, 3H, CH_3), 0.88 (s, 9H, t-Bu-H), 0.08 (s, 3H, $SiCH_3$), 0.04 (s, 3H, $SiCH_3$).

*(3S)-3-[(tert-Butyldimethylsilyl)oxy]-4-methyl-5-hexenal **11***

p-Methoxybenzyl ether **10** (1.60 g, 4.39 mmol) was dissolved in CH_2Cl_2 (20 mL). Water (1.30 mL) and solid DDQ (0.99 g, 4.39 mmol) were added. The mixture was stirred for 1 h, then diluted with CH_2Cl_2 (200 mL), washed with saturated aqueous $NaHCO_3$ (40 mL) and brine, dried ($MgSO_4$), concentrated, and purified by flash column chromatography (hexane/EtOAc, 4:1) to provide a mixture of the desired primary alcohol and *p*-methoxybenzaldehyde which was used without further purification in the next step.

A mixture of CH_2Cl_2 (25 mL) and oxalyl chloride (2.2 mL, 20 mmol) was cooled to −50 to −60°C as DMSO (3.4 mL, 40 mmol) was added. The mixture was stirred for 2 min and the primary alcohol (10 mmol in 10 mL CH_2Cl_2) was added; 15 min later, triethylamine (7.0 mL, 50 mmol) was added. The mixture was stirred for 5 min and then warmed to room temperature. H_2O (50 mL) was added and the aqueous layer was extracted with CH_2Cl_2 (50 mL). The organic layers were combined, washed with brine (100 mL), dried ($MgSO_4$), concentrated, and purified by flash column chromatography (hexane/EtOAc, 10:1) to provide product **11** as an oil (0.57g, 54% over two steps).

^{1}H-NMR ($CDCl_3$) δ: 9.78 – 9.72 (t, *J* = 2.2 Hz, 1H, CHO), 5.79 – 5.70 (dd, *J* = 7.0, 17.1 Hz, 1H, CH), 5.06 – 5.04 (m, 1H, CH_2), 5.05-4.99 (m, 1H, CH_2), 4.20 – 4.17 (m, 1H, CH), 2.50 – 2.43 (dt, *J* = 2.0, 6.2 Hz, 2H, CH_2), 2.39 – 2.33 (m, 1H, CH), 1.03 – 1.01 (d, *J* = 6.9 Hz, 3H, CH_3), 0.87 (s, 9H, t-Bu-H), 0.08 (s, 3H, $SiCH_3$), 0.04 (s, 3H, $SiCH_3$).

*tert-Butyl(5S,6R,2E)-5-[(tert-butyldimethylsilyl)oxy]-6-methyl-2,7-octadienoate **12***

tert-Butyldiethylphosphonoacetate (1.16 mL, 4.95 mmol), DBU (0.44 mL, 2.97 mmol), and LiCl (0.15 g, 3.47 mmol) were stirred vigorously at room temperature for 30 min in anhydrous CH_3CN (12 mL). The solution was added dropwise to a solution of anhydrous aldehyde **11** (0.60 g, 2.48 mmol) in CH_3CN (6 mL). After 2 h, saturated aqueous NH_4Cl solution was added. The organic layers were further washed with brine. The aqueous layers were extracted with EtOAc, dried ($MgSO_4$), concentrated, and purified (hexane/EtOAc, 10:1) to get the product as a colorless oil (0.66 g, 79%).

^{1}H-NMR ($CDCl_3$) δ: 6.82 – 6.75 (dt, *J* = 8.0, 16.2 Hz, 1H, CH), 5.80 – 5.71 (m, 1H, CH), 5.74 – 5.70 (br d, *J* = 16.1 Hz, 1H, CH), 5.03 (br s, 1H, CH_2), 5.01 – 4.99 (br d, *J* = 9.1 Hz, 1H, CH_2), 3.67 – 3.63 (m, 1H, CH), 2.29 – 2.23 (m, 3H, CH_2, CH), 1.46 (s, 9H, t-Bu-H), 1.00 – 0.99

(d, J = 6.8 Hz, 3H, CH_3), 0.88 (s, 9H, t-Bu-H), 0.03 (s, 3H, $SiCH_3$), 0.03 (s, 3H, $SiCH_3$).

tert-Butyl-(5S,6R,2E,7E)-5-[(tert-butyldimethylsilyl)-oxy]-6-methyl-8-phenyl-2,7-octadienoate ***13***

Olefin **12** (75 mg, 0.22 mmol) was dissolved in anhydrous CH_3CN (2 mL) under argon atmosphere. Then, iodobenzene (49 mg, 0.24 mmol), $Pd(OAc)_2$ (2.50 mg, 0.01 mmol), and TEA (0.31 mL, 2.2 mmol) was added. The tube was sealed and heated at 80 to 85°C for 24 h. The mixture was concentrated and subjected directly to column chromatography (hexane/EtOAc, 9:1) to provide product **13** as an oil (55 mg, 60%).

tert-Butyl-(5S,6R,2E,7E)-5-hydroxy-6-methyl-8-phenyl-2,7-octadienoate ***5***

The ester **13** (25 mg, 0.06 mmol) was dissolved in THF (500 µL). TBAF solution (1 M in THF, 66 µL, 0.07 mmol) was added dropwise. After 2 h, saturated aqueous Na_2CO_3 solution was added. The aqueous layers were extracted with EtOAc and the organic layers were further washed with brine, concentrated, and subjected to a short plug of silica (hexane/EtOAc, 4:1), providing a pale yellow oil (13 mg, 71%).

$[\alpha]_{20}^{d}$ = + 66° (c = 0.80, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ: 7.37 – 7.36 (d, J = 7.3 Hz, 2H, CH), 7.32 – 7.28 (dt, J = 7.0 Hz, 2H, CH), 7.25 – 7.20 (t, J = 7.2 Hz, 1H, CH), 6.95 – 6.88 (dt, J = 7.5, 15.6 Hz, 1H, CH), 6.48 – 6.44 (d, J = 16.0 Hz, 1H, CH), 6.17 – 6.11 (dd, J = 8.6, 16.4 Hz, 1H, CH), 5.86 – 5.82 (br d, J = 15.6 Hz, 1H, CH), 3.67 – 3.62 (m, 1H, CH), 2.48 – 2.38 (m, 1H, CH), 2.36 – 2.28 (m, 1H, CH), 1.86 (br s, 1H, CH), 1.47 (s, 9H, t-Bu-H), 1.15 (s, 3H, CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 165.7, 144.0, 137.0, 131.8, 130.9, 128.5 (2C), 127.4, 126.1 (2C), 125.4, 80.2, 73.8, 43.2, 37.1, 28.1 (3C), 16.8; MS (ESI) m/z: 325 [M + Na]. HRMS (FAB, NBA) calcd. for $C_{19}H_{27}O_3$: [M + H] 303.1960; found: 303.1980.

(R)-tert-Butyl 1-(3-chloro-4-methoxyphenyl)-4-diazo-3-oxobutan-2-ylcarbamate ***15***

To a solution of (*R*)-2-(*tert*-butoxycarbonylamino)-3-(3-chloro-4-methoxyphenyl) propanoic acid **14** (329 mg, 1 mmol) in CH_2Cl_2 (8 mL) at 0°C, triethylamine (0.17 mL, 1.2 mmol) and ethyl chloroformate (0.11 mL, 1.1 mmol) was added. The mixture was stirred at 0°C for 10 min. CH_2N_2 ether solution (4 mL, 1.2 mmol) was then added dropwise. The mixture slowly warmed up to room temperature and was then stirred for 1 h. Partition of the mixture between EtOAc and H_2O, successive washing with 0.1 N HCl and saturated $NaHCO_3$, drying over $MgSO_4$, followed by concentration gave the crude product which was further purified by column chromatography (Hex/EtOAc, 2:1) to yield the diazo compound **15** (220 mg, 62%). ^{1}H-NMR ($CDCl_3$) δ: 7.24 (s, 1H, CH), 7.01 – 7.14 (d, J = 8.2 Hz, 1H, CH), 6.84 – 6.92 (d, J = 8.3 Hz, 1H, CH), 5.32 (s, 1H, CH), 4.41 (br s, 1H, CH), 3.92 (s, 3H, OCH_3), 2.83 – 3.05 (m, 2H, CH_2), 1.46 (s, 9H, Boc-H).

(R)-3-(tert-Butoxycarbonylamino)-4-(3-chloro-4-methoxyphenyl)butanoic acid ***16***

The solid diazo compound **15** (220 mg, 0.63 mmol) was dissolved in THF (6 mL) and H_2O (0.3 mL). CF_3CO_2Ag (100 mg, 0.45 mmol) was added and the mixture was stirred at room temperature overnight. Brine was then added and the mixture was stirred for 2 h. The mixture was then filtered through celite and the solid residue was washed with EtOAc. The filtrate was washed with H_2O, dried over $MgSO_4$, and co-evaporation with toulene afforded a solid acid **16** (128 mg, 60%).

^{1}H-NMR ($CDCl_3$) δ: 7.25 (s, 1H, CH), 7.01 – 7.12 (d, J = 8.3 Hz, 1H, CH), 6.87 – 6.94 (d, J = 8.1 Hz, 1H, CH), 3.95 – 4.08 (m, 1H, CH), 3.93 (s, 3H, OCH_3), 2.77 – 2.85 (m, 2H, CH_2), 2.45 – 2.54 (m, 2H, CH_2), 1.45 (s, 9H, Boc-H).

(S)-2-((R)-3-(tert-Butoxycarbonylamino)-4-(3-chloro-4-methoxyphenyl)butanamido)propanoic acid ***17a***

The solid acid **16** (110 mg, 0.32 mmol) was dissolved in CH_2Cl_2 (6 mL). EDC · HCl (92 mg, 0.48 mmol), and HOBT (65 mg, 0.32 mmol) was added and the mixture was stirred for 30 min at room temperature. Then, (*S*)-methyl 2-aminopropanoate (50 mg, 0.48 mmol) and Et_3N (132 µL, 0.96 mmol) were added and the mixture was stirred overnight at room temperature in N_2 atmosphere. The mixture was diluted by CH_2Cl_2 (6 mL), washed by 0.1 N HCl and brine, dried with Na_2SO_4 and purified by column chromatography (Hex/EtOAc, 1:1). The methyl-ester of **17a** was obtained as a pale yellow solid.

The methyl-ester of **17a** was dissolved in MeOH (6 mL) and 2 N NaOH (650 µL, 0.65 mmol) was added dropwise. The solution was stirred for 2 h at room temperature and the excess MeOH was distilled in vacuum. H_2O (50 mL) was added to obtain a solution of the sodium salt of **17a**. In an ice bath, 1 N HCl was added dropwise until pH 2 – 3 and a white suspension was formed. The mixture was extracted with EtOAC (20 mL) twice. EtOAC layer was combined, dried (Na_2SO_4) and distilled *in vacuo* to yield a pale yellow solid **17a** (90 mg, 67%).

^{1}H-NMR ($CDCl_3$) δ: 7.05 – 7.21 (m, 2H, CH), 6.81 – 6.89 (m, 1H, CH), 4.68 (s, 1H, CH), 4.07 (s, 1H, CH), 3.85 (s, 3H, OCH_3), 2.19 – 2.78 (m, 4H, CH_2), 1.52 (s, 3H, CH_3), 1.41 (s, 9H, Boc-H).

(S)-2-((R)-3-(tert-Butoxycarbonylamino)-4-(3-chloro-4-methoxyphenyl)butanamido)-4-(methylthio)butanoic acid ***17b***

The solid acid **16** was treated with (*S*)-methyl 2-amino-4-(methylthio)butanoate according to the same process as **17a** to yield **17b** as a pale yellow wax (100 mg, 64%).

^{1}H-NMR ($CDCl_3$) δ: 7.27 – 7.12 (m, 2H, CH), 6.84 – 6.72 (m, 1H, CH), 4.65 (s, 1H, CH), 3.95 (s, 1H, CH), 3.77 (s, 3H, OCH_3), 2.24 – 2.79 (m, 8H, CH_2), 1.99 (s, 3H, SCH_3), 1.40 (s, 9H, Boc-H).

(6R,10S,13S)-6-(3-Chloro-4-methoxybenzyl)-13-isobutyl-2,2,10-trimethyl-4,8,11-trioxo-3-oxa-5,9,12-triazatetradecan-14-oic acid ***6a***

The peptide **17a** was treated with (*S*)-methyl 2-amino-4-methylpentanoate to yield a pale yellow solid **6a** (68 mg, 59%).

^{1}H-NMR ($CDCl_3$) δ: 7.39 – 7.45 (m, 2H, NH), 7.01 – 7.19 (m, 2H, CH), 6.77 – 6.83 (m, 1H, CH), 4.46 – 4.61 (m, 2H, CH), 4.10 – 4.17 (m, 1H, CH), 3.82 (s, 3H, OCH_3), 2.39 – 2.75 (m, 4H, CH_2), 2.02 (m, 3H, CH_2, CH), 1.62 (s, 3H, CH_3), 1.40 (s, 9H, Boc-H), 0.89 (s, 6H, CH_3).

(6R,10S,13S)-6-(3-Chloro-4-methoxybenzyl)-13-isobutyl-2,2-dimethyl-10-(2-(methylthio)ethyl)-4,8,11-trioxo-3-oxa-5,9,12-triazatetradecan-14-oic acid ***6b***

A white solid **6b** (60 mg, 52%) was obtained from the peptide **17b** according to the same process as for **6a**.

^{1}H-NMR ($CDCl_3$) δ: 7.41(m, 2H, NH), 7.10 – 7.23 (m, 2H, CH), 6.71 – 6.83 (m, 1H, CH), 4.42 – 4.69 (m, 2H, CH), 4.12 – 4.21 (m, 1H, CH), 3.94 (s, 3H, OCH_3), 2.39 – 2.75 (m, 8H, CH_2), 2.08 (m, 3H, CH_2, CH), 1.65 (s, 3H, CH_3), 1.45 (s, 9H, Boc-H), 0.86 (s, 6H, CH_3).

(6R,10S,13S)-((1E,3R,4S,6E)-8-tert-Butoxy-3-methyl-8-oxo-1-phenylocta-1,6-dien-4-yl)6-(3-chloro-4-methoxybenzyl)-13-isobutyl-2,2-dimethyl-10-(2-(methylthio)ethyl)-4,8,11-trioxo-3-oxa-5,9,12-triazatetradecan-14-oate ***18a***

6a (70 mg, 0.13 mmol) was dissolved in CH_2Cl_2 (5 mL). DCC (41 mg, 0.20 mmol) and DMAP (catalyst) was added and stirred for 30 min at room temperature. Then, the octadienoate ester **5** (40 mg, 0.14 mmol) was added and stirred overnight in N_2 atmosphere. The mixture was diluted by CH_2Cl_2 (10 mL), washed by 0.1 N HCl and brine, dried with Na_2SO_4, and purified by silica gel column (Hex/EtOAc, 2:1) to yield **8a** (53 mg, 52%) as a pale yellow solid.

^{1}H-NMR ($CDCl_3$) δ: 7.23 – 7.40 (m, 6H, CH), 6.66 – 6.88 (m, 2H, CH), 6.35 – 6.44 (dd, *J* = 6.1, 12.0 Hz, 1H, CH), 6.24 – 6.31 (d, *J* = 12.3 Hz, 1H, CH), 5.96 – 6.04 (d, *J* = 12.0 Hz, 1H, CH), 5.83 – 5.92 (dd, *J* = 6.4, 12.6 Hz, 1H, CH), 4.65 – 4.74 (m, 1H, CH), 4.37 – 4.43 (m, 1H, CH), 4.17 – 4.22 (m, 1H, CH), 4.02 – 4.11 (m, 1H, CH), 3.75 (s, 3H, OCH_3), 2.28 – 2.68 (m, 6H, CH_2), 1.82 – 1.85 (m, 2H, CH_2), 1.83 – 1.87 (m, 1H, CH), 1.48 (s, 3H, CH_3), 1.45 (s, 9H, t-Bu-H), 1.40 (s, 9H, Boc-H), 0.89 (s, 6H, CH_3).

(6R,10S,13S)-((1E,3R,4S,6E)-8-tert-Butoxy-3-methyl-8-oxo-1-phenylocta-1,6-dien-4-yl)6-(3-chloro-4-methoxybenzyl)-13-isobutyl-2,2-dimethyl-10-(2-(methylthio)ethyl)-4,8,11-trioxo-3-oxa-5,9,12-triazatetradecan-14-oate ***18b***

A white solid **18b** (56 mg, 50%) was obtained from the peptide **6b** according to the same process as for **18a**.

^{1}H-NMR ($CDCl_3$) δ: 7.21 – 7.38 (m, 6H, CH), 6.56 – 6.77 (m, 2H, CH), 6.38 – 6.45 (dd, *J* = 6.2, 12.1 Hz, 1H, CH), 6.17 – 6.25 (d, *J* = 12.4 Hz, 1H, CH), 5.89 – 5.95 (d, *J* = 12.0 Hz, 1H, CH), 5.72 – 5.83 (dd, *J* = 6.1, 12.3 Hz, 1H, CH), 4.63 – 4.91 (m, 1H, CH), 4.22 – 4.32 (m, 1H, CH), 4.10 – 4.16 (m, 1H, CH), 3.96 – 4.05 (m, 1H, CH), 3.68 (s, 3H, OCH_3), 2.24 – 2.72 (m, 10H, CH_2), 1.87 – 1.94 (m, 3H, CH_2, CH), 1.73 (s, 3H, SCH_3), 1.43 (s, 9H, Bu-H), 1.40 (s, 9H, Boc-H), 0.88 (s, 6H, CH_3).

(3S,6S,10R,16S,E)-10-(3-Chloro-4-methoxybenzyl)-3-isobutyl-6-methyl-16-((R,E)-4-phenylbut-3-en-2-yl)-1-oxa-4,7,11-triazacyclohexadec-13-ene-2,5,8,12-tetraone ***3***

18a (50 mg, 0.06 mmol) was dissolved in 20% trifluroacetic acid/CH_2Cl_2 (1 mL). The mixture was stirred at room temperature overnight and then diluted with toluene (2 mL). Evaporation and co-evaporation with 6% HCl/EtOH gave a brownish solid. The solid was dissolved in CH_2Cl_2 (2 mL) and EDCI (18 mg, 0.09 mmol), HOBT (13 mg, 0.09 mmol), and triethylamine (26 μL, 0.19 mmol) were added successively to the mixture. After stirring overnight at room temperature, the mixture was washed with 0.1 N HCl, H_2O, and saturated $NaHCO_3$. The organic layer was dried (Na_2SO_4), concentrated *in vacuo* and purified by flash chromatography (Hex/EtOAC, 1:3) to get **3** as a pale yellow solid (18 mg, 45%).

^{1}H-NMR ($CDCl_3$) δ: 7.16 – 7.27 (m, 6H, CH), 6.57 – 6.74 (m, 2H, CH), 6.26 – 6.37 (dd, *J* = 6.1, 12.0 Hz, 1H, CH), 6.18 – 6.24 (d, *J* = 12.5 Hz, 1H, CH), 6.08 – 6.16 (d, *J* = 12.1 Hz, 1H, CH), 5.66 – 5.73 (dd, *J* =6.4, 12.2 Hz, 1H, CH), 4.70 – 4.85 (m, 1H, CH), 4.33 – 4.49 (m, 1H, CH), 4.05 – 4.18 (m, 1H, CH), 3.92 – 4.15 (m, 1H, CH), 3.65 (s, 3H, OCH_3), 2.21 – 2.66 (m, 6H, CH_2), 1.74 – 1.96 (m, 2H, CH_2), 1.61 – 1.84 (m, 1H, CH), 1.47 (s, 3H, CH_3), 0.89 (s, 6H, CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 171.3, 169.5, 165.7, 164.0, 151.8, 147.5, 137.0, 131.8, 130.9, 128.5 (3C), 127.4, 126.1 (3C), 125.4, 122.7, 114.8, 78.2, 58.3, 52.8 (2C), 46.1, 42.3, 41.8 (2C), 40.6, 39.1, 23.1 (2C), 22.2, 17.7; MS (ESI) *m/z*: 660 [M + Na]. HRMS (FAB, NBA) calcd. for $C_{35}H_{44}ClN_3O_6$: [M + H] 638.2930; found: 638.2910.

(3S,6S,10R,16S,E)-10-(3-Chloro-4-methoxybenzyl)-3-isobutyl-6-(2-(methylthio)ethyl)-16-((R,E)-4-phenylbut-3-en-2-yl)-1-oxa-4,7,11-triazacyclohexadec-13-ene-2,5,8,12-tetraone ***4***

The compound **4** from compound **18b** was obtained as a white solid (17 mg, 42%) according to the same process of for **3**.

^{1}H-NMR ($CDCl_3$) δ: 7.29 – 7.44 (m, 6H, CH), 6.64 – 6.81 (m, 2H, CH), 6.32 – 6.54 (dd, *J* = 6.3, 12.5 Hz, 1H, CH), 6.05 – 6.38 (d, *J* = 12.1 Hz, 1H, CH), 6.01 – 6.21 (d, *J* = 12.3 Hz, 1H, CH), 5.61 – 5.98 (dd, *J* = 6.2, 12.0 Hz, 1H, CH), 4.62 – 4.84 (m, 1H, CH), 4.16 – 4.41 (m, 1H, CH), 3.89 – 4.15 (m, 1H, CH), 3.80 – 4.02 (m, 1H, CH), 3.71 (s, 3H, OCH_3), 2.25 – 2.79 (m, 10H, CH_2), 2.01 – 2.29 (m, 3H, CH_2 CH), 1.69 (s, 3H, SCH_3), 0.91 (s, 6H, CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 173.5, 171.5, 167.4, 164.9, 153.2, 146.5, 134.6, 131.3, 129.9, 127.5 (3C), 126.9, 125.5 (3C), 124.2, 123.7, 115.8, 80.2, 60.5, 54.8 (2C), 47.7, 44.2, 42.8 (2C), 41.5, 40.1, 31.5, 29.8, 22.3 (2C), 21.8, 17.3; MS (ESI) *m/z*: 720 [M + Na]. HRMS (FAB, NBA) calcd. for $C_{37}H_{48}ClN_3O_6S$: [M + H] 698.3420; found: 698.3370.

Cell culture and cytotoxicity assay

All cancer cell lines were cultured in RPMI-1640 medium with heat-inactivated 10% fetal bovine serum at 37°C. Taxol was used as the positive control. All tested compounds were dissolved in DMSO at the concentrations 1.0 mg/100 μL and then diluted to the appropriate concentrations. Cells were seeded in 96-well microtiter plates (5×10^5 cells/well). After 24-hour incubation in appropriate medium, cells were treated with various concentrations (50, 10, 1.0, 0.1, 0.01, 0.001 μg/mL) of test compounds, and incubated for another 48 h. Afterwards, 10 μL of stock 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT, Sigma) solution was added to each well (final: 0.25 mg/mL) for another 4 h of incubation. After 4 h, DMSO (100 μL) was added and the optical density (OD) was read at 570 nm. The IC_{50} values were calculated using the PrismPad computer program (GraphPad Software, Inc. San Diego, CA, USA). All experiments were performed in triplicate and the IC_{50} values were derived from the mean OD values.

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