Synthesis and Antitumor Activity of Mechercharmycin A Analogues

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Several analogues of the cytotoxic thiopeptide IB-01211 or mechercharmycin A (**1**) have been synthesized. The cytotoxicity of **1** and the synthesized analogues were evaluated against a panel of three human tumor cell lines. Thiopeptide **1** and the most active derivatives **2** and **3c** were chosen for further studies on effects on cell cycle progression and induction of apoptosis. Interestingly, the inhibition of cell division and activation of a programmed cell death by apoptosis were detected.

Introduction

Natural products are a rich source for drug discovery and are challenging synthetic targets; hence, they often inspire new synthetic methods.[1] Thiazoles and oxazoles, as well as their reduced variants, are common structural features of many biologically important natural products.[2] Only few concatenated azoles containing substituents on the azole rings have been described. These comprise the methylated bisoxazoles (−)-muscoride A[3] and leucamide A,[4] the cyclic octaazole telomestatin,[5] which features two oxazole rings with methyl substituents at position 5, and finally, the diazonamides,[6] which have a complex structure containing a bis-oxazole. IB-01211 or mechercharmycin A (**1**) is a cyclic thiopeptide[7] containing a phenylpentaazole system, a D-*allo*-Ile-L-Val dipeptide, and an exocyclic methylidene. The synthesis of **1** was recently described by our group.[8] Thiopeptide **1** is closely related in structure to the potent telomerase inhibitor telomestatin[5] and YM-216391.[9]

We sought to prepare analogues of **1** with improved solubility and activity. The first structural modifications comprised substitution on the pentaazole system and modifications of the exocyclic methylidene (Figure 1). We envisaged that introduction of one (**1a**, **b**) or two (**1c**) methyl groups could increase the solubility of the parent compound and allow a conformational change. The second variation consisted of modifying the exocyclic double bond via elimination (**2**) or conjugation of the hydrate form **3** with acids, PEG[a] residues, or amino acids (**3a**−**f**).

Results and Discussion

Chemistry. Compounds **1a**−**c**, **2**, and **3a**−**f** were synthesized using basically the same strategy that we described for the total synthesis of **1**.[8a] This is a convergent method based on macrocyclization with simultaneous formation of the thiazole ring from a peptide-heterocycle. Having previously reported the difficulty of handling the alcohol **3**,[8] we used different conditions for the macrocyclization of the intermediates leading to **2** and the esters **3a**−**e** than for those leading to **1a**−**c**, which feature an exocyclic methylidene. Thus, whereas **3a**−**e** were all prepared from **3** via experimental conditions that avoid dehydration, **1a**−**c** were obtained through concomitant macrocyclization and formation of the thiazole and the double bond.

The syntheses were built around three synthetic intermediates: the bis-oxazoles **4a**−**c**, the phenyl-bis-oxazoles **5a** and **5b**, and the dipeptide **6** (Scheme 1). Compounds **4a** and **5a** have previously been synthesized[8] by a process based on cyclization of Ser and Ph-Ser peptides to give an oxazoline, followed by further oxidation to afford the oxazole. This method was used to prepare oxazoles **4b**, **4c**, and **5b**, using Gly- and Thr-containing peptides, as well as Ser and Ph-Ser, as starting materials (see Experimental Section). The peptide-heterocycles **7a** and **7b** were obtained in excellent yields from the peptide **6** and compounds **5a**, **5b**, respectively, using EDC·HCl (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride) and HOBt (1-hydroxybenzotriazole) as coupling agents. The tetraoxazole peptides **9** were obtained from the corresponding free acid of **7**[10] and the appropriate amine **8** under the conditions described above. Compounds **9a**−**e** feature three key functional groups: a thioamide, an acetal-protected α-bromoketone, and a *tert*-butoxymethyl group (R = CH_2O^tBu for **9a**−**d**). Simultaneous elimination of the O^tBu protecting group and the dimethylacetal of **9** using formic acid under reflux afforded the free alcohol plus the α-bromoketone, which was ready for macrocyclization via thiazole formation. Macrocyclization was achieved by refluxing a dilute ethanolic solution of the crude material for 48 h.[11] For **1a**−**c** (Scheme 1, left), this reaction was followed by dehydration of the crude material using mesyl chloride and triethylamine (TEA) at low temperature. The last

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[a] Abreviations: A-549, human lung cancer cells; DAST, diethylaminosulfur trifluoride; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; DIEA, diisopropylethylamine; DMAP, 4-dimethylaminopyridine; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; EDC·HCl, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; FAM-DEVD-FMK, carboxyfluorescein derivative of fluoromethyl ketone; HOBt, 1-hydroxybenzotriazole; HT-29, human colon cancer cells; HTB, human colorectal adenocarcinoma cell line; LR, Lawesson's reagent; MsCl, mesyl chloride, NSCL, non-small-cell lung; MDA-MB-231, human breast adenocarcinoma cancer cells; PBS, phosphate buffered saline; PEG, poly(ethylene glycol); TEA, triethylamine; TFA, trifluoroacetic acid; THF, tetrahydrofuran.

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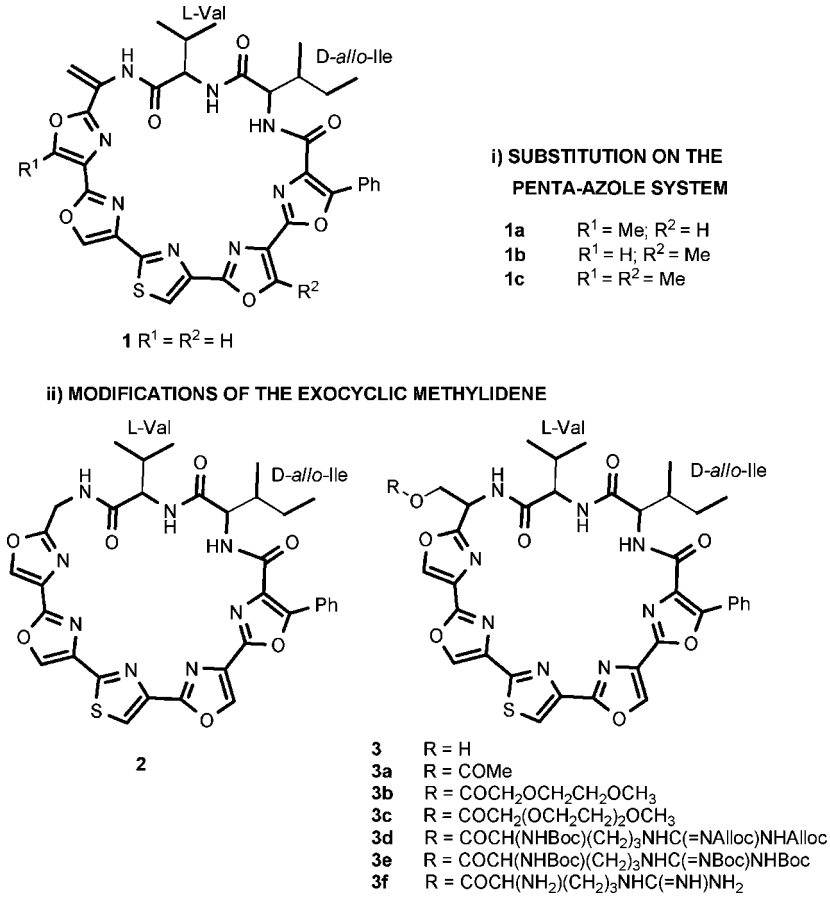


Figure 1. Structures of thiopeptide **1** and analogues **1a**−**c**, **2**, and **3a**−**f**.

step after the macrocyclization was avoided when **9e** was used to give compound **2**. By use of **9a**, this route gave, after deprotection and macrocyclization, alcohol **3**, which was directly acylated to give **3a**−**e**. Acetate **3a** was obtained using acetic anhydride in THF at room temperature. Compounds **3b**−**e** were prepared by reaction of the appropriate acid with the alcohol **3** using EDC·HCl and 4-dimethylaminopyridine (DMAP) as condensation agents. The protection initially used for Arg was N^{α}-Boc, bis-$N^{\gamma,\gamma'}$-Alloc, which allowed isolation of **3d** in 16% yield under the conditions described above. Elimination[12] of the Alloc protecting group gave a crude product in which N^{α}-Boc-**3f** only corresponded to 3% (as detected by HPLC−MS). The deprotection gave better yield using tri-Boc-protected Arg. Simultaneous elimination of the three protecting groups of **3e** using trifluoroacetic acid (TFA) diluted in CH_2Cl_2 allowed the isolation with a 17% yield of purified **3f**.

Biological Results

The cytotoxicity of the thiopeptide **1** analogues was evaluated against a panel of three human tumor cell lines: A-549 lung carcinoma NSCL, HT-29 colon carcinoma, and MDA-MB-231 breast adenocarcinoma.

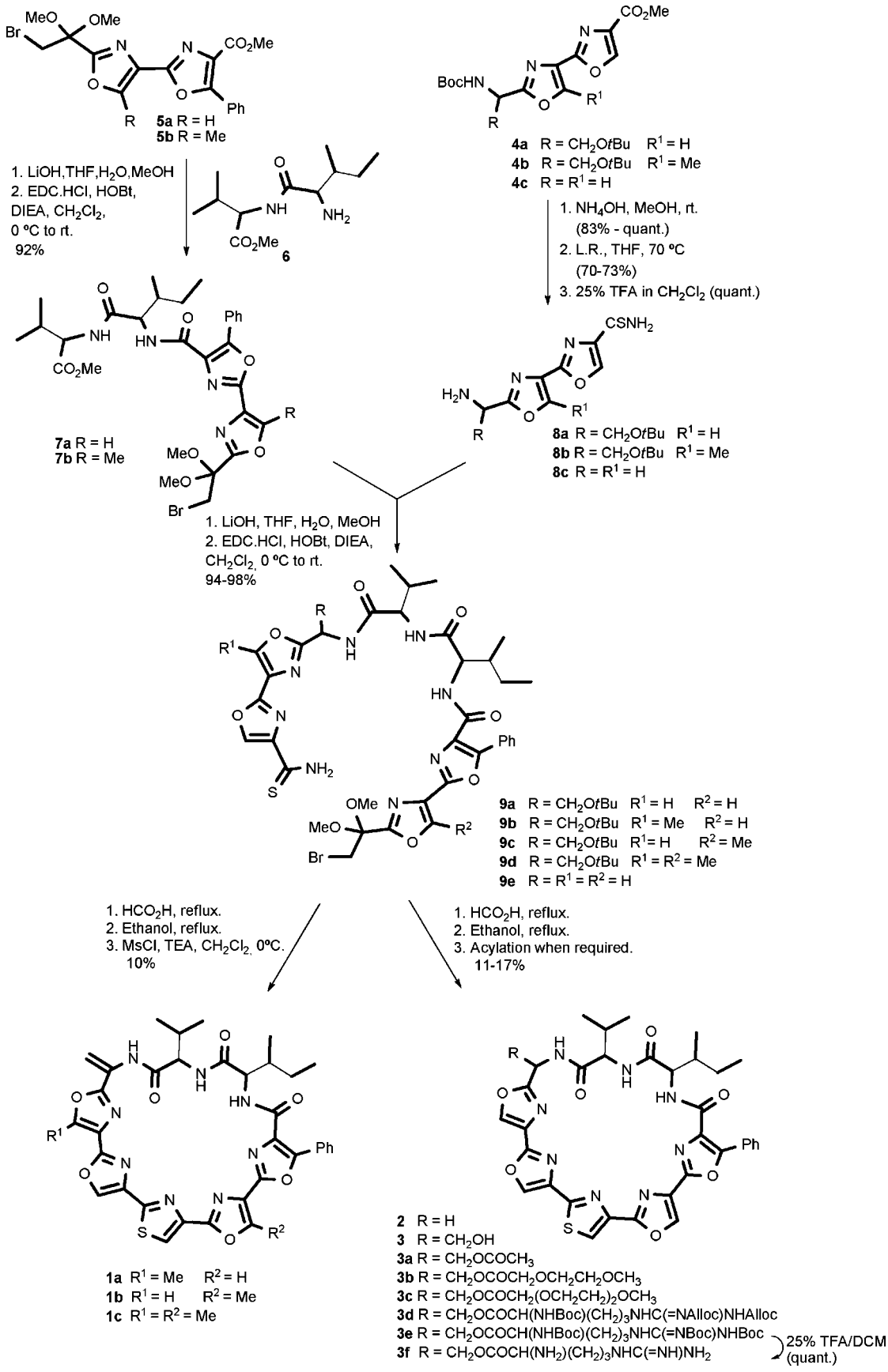
A conventional colorimetric assay was used to estimate values of GI_{50} (defined here as the drug concentration that causes 50% of cell growth inhibition after 72 h of continuous exposure to the test molecule). Thiopeptide **1** was included for comparison. The results are shown in Table 1.

All the cyclic analogues, except **3f**, are active against human tumor cell lines but to a lesser extent compared to **1**. Introduction of one or two methyl substituents (**1a**−**c**) diminishes the GI_{50} by 1 order of magnitude for **1b** (lines A-549 and HT-29) and **1c** (all three lines) and by 2 orders of magnitude for **1a** (all three lines) and **1b** (MDA-MB-231). The greatest cytotoxicity is found in derivatives in which the exocyclic double bond was eliminated (**2**) or substituted by a PEG-carboxymethyl (**3b** and **3c**). Acetate derivative **3a** maintains a modest growth inhibition at micromolar concentrations in the three lines; however, acylation with an Arg residue leads to total loss of activity. Interestingly, substitution of the acetyl residue by a methoxyethoxyacetyl in **3b** increases cytotoxicity to A-549; likewise, the longer polyetheracetyl of **3c** produces the same effect in all three tumor cell lines. Compound **2** is a demethylidene analogue of **1** and also a constitutional isomer of YM-216391. Compound **2** and YM-216391 differ only in the position of the thiazole ring inside the pentaazole structure of the macrocycle.[13] The open ring precursors **9a**−**e** are generally inactive; only **9c** is moderately active against two tumor cell lines at micromolar concentrations. The azole analogues[14] of **1** recently obtained by our group, which do not contain the central thiazole, were also inactive. These results demonstrate the importance of the thiazole for the activity of these macrocyclic compounds.

Thiopeptide **1** and the most active analogues **2** and **3c** were then selected for further biological evaluation.

Effects of 1, 2, and 3c on Cell Cycle Progression of HT-29, A-549, and MDA-MB-231 Cells. Thiopeptide **1** is closely related in structure to potent telomerase inhibitors such as telomestatin. Telomerase generates telomere repeats, specialized structures located at the end of eukaryotic chromosomes. Telomeres are important for maintenance of genomic stability and integrity during cell proliferation. Thiopeptide **1** and their analogues **2** and **3c** were tested for

Scheme 1. Synthesis of Thiopeptide **1** Analogues



effects on cell cycle progression in HT-29, A-549, and MDA-MB-231 tumor cells. The cells were treated with each compound (see Experimental Section) and then analyzed by flow cytometry to determine if the cell cycle had been arrested at a specific phase. A control set of untreated cells was also used.

Table 1. In Vitro Cytotoxicity of **1** and Its Open-Chain and Cyclic Peptide Analogues[a]

	cytotoxicity (GI_{50}, μM)		
compd	A-549	HT-29	MDA-MB-231
$^{-}$1	0.03	0.04	0.09
1a	1.66	2.49	2.91
1b	0.54	0.63	1.02
1c	0.31	0.36	0.70
2	0.17	0.12	0.10
3a	4.68	4.94	4.03
3b	0.89	1.31	1.16
3c	0.12	0.13	0.13
3f	na	na	na
9a	na	na	na
9b	na	na	na
9c	4.99	na	4.46
9d	na	na	na
9e	na	na	na

[a] na: not active at 10 μg/mL.

Compared to the control cells (Figure 2, panels 1a−c), the cells treated with **1** (Figure 2, panels 2a−c) and with compound **2** (Figure 2, panels 3a−c) had higher population stopped at the G2 phase except for A-549 cells treated with compound **2** that showed enlarged arrest in S phase. Nervertheless, cells treated with compound **3c** showed a very pronounced arrest in S phase in all cell lines (Figure 2, panels 4a−c). Hence, these compounds alter cell cycle progression.

Induction of Apoptosis by Thiopeptide 1, 2, or 3c in HT-29, A-549, and MDA-MB-231 Cell Lines. Many anticancer drugs induce cell cycle arrest and apoptosis. Apoptotic cells undergo characteristic morphological changes. Among these, the cell surface often bends and breaks up into membrane-enclosed fragments called apoptotic bodies. This process depends on a cascade of proteolytic enzymes called caspases.[15] They exist in most of the cells as inactive precursors (zymogens) that, once activated, kill cells.

To analyze whether **1**, **2**, and **3c** induce apoptosis, HT-29, A-549, and MDA-MB-231 cell lines were incubated with each compound and then qualitatively measured for apoptosis (see Experimental Section). The analysis consisted of detecting active caspases (caspase-3/7) using a colorimetric test (CaspaTag in situ caspase detection kits, CHEMICON).

All cell lines showed a high level of apoptotic cell death after treatment with **1** (Figure 3, panels 2a−c), compound **2** (Figure 3, panels 3a−c), or compound **3c** (Figure 3, panels 4a−c). A negative control of the assay was performed by analyzing untreated cells under the same experimental conditions (Figure 3, panels 1a−c).

Conclusion

Several analogues of thiopeptide **1** were synthesized using slight variations of a methodology previously described for the preparation of the parent compound. Conditions that allow isolation of the hydroxymethyl derivative have been established, thus enabling preparation of several analogues by acylation. Methyl substituents were introduced onto the pentaazole system by employing different starting materials, whereas the exocyclic double bond was modified in the last synthetic step.

Thiopeptide **1** and analogues **2** and **3c** were shown to inhibit cell cycle progression in cancer cell lines. As previously mentioned, **1** is closely related in structure to YM-216391[9] and the potent telomerase inhibitor telomestatin.[5]

Telomerase activity is important during telomere replication in chromosomes; thus, telomerase inhibition will cause replication faults and DNA damage effect, which would in turn affect cell cycle progression. We observed that **1** and compound **2** caused cells to accumulate mainly in G2 phase, which could activate the G2-checkpoint activation. In contrast, **3c**, though less active than **1**, provoked major S phase arrest in all cell lines, indicating that it has potent biological activity related to DNA replication. Moreover, the effects observed consecutively to the treatment of cells with **3c** resemble those expected for a telomerase inhibitor.

Perturbation of the cell cycle induces activation of apoptosis and leads to cell cycle arrest. Different cancer cell lines treated with **1**, **2**, or **3c** specifically underwent apoptosis, thereby indicating that these compounds do not act by some general toxicity mechanism. These compounds inhibit cell division and activate programmed cell death by apoptosis.

The resemblance among **1**, its analogues **2**, **3c**, and known telomerase inhibitors, together with the fact that most of the human tumors seem to depend on telomerase reactivation to prevent critical telomere loss, is testament to the potential of exploring these compounds as anticancer therapies.

Experimental Section

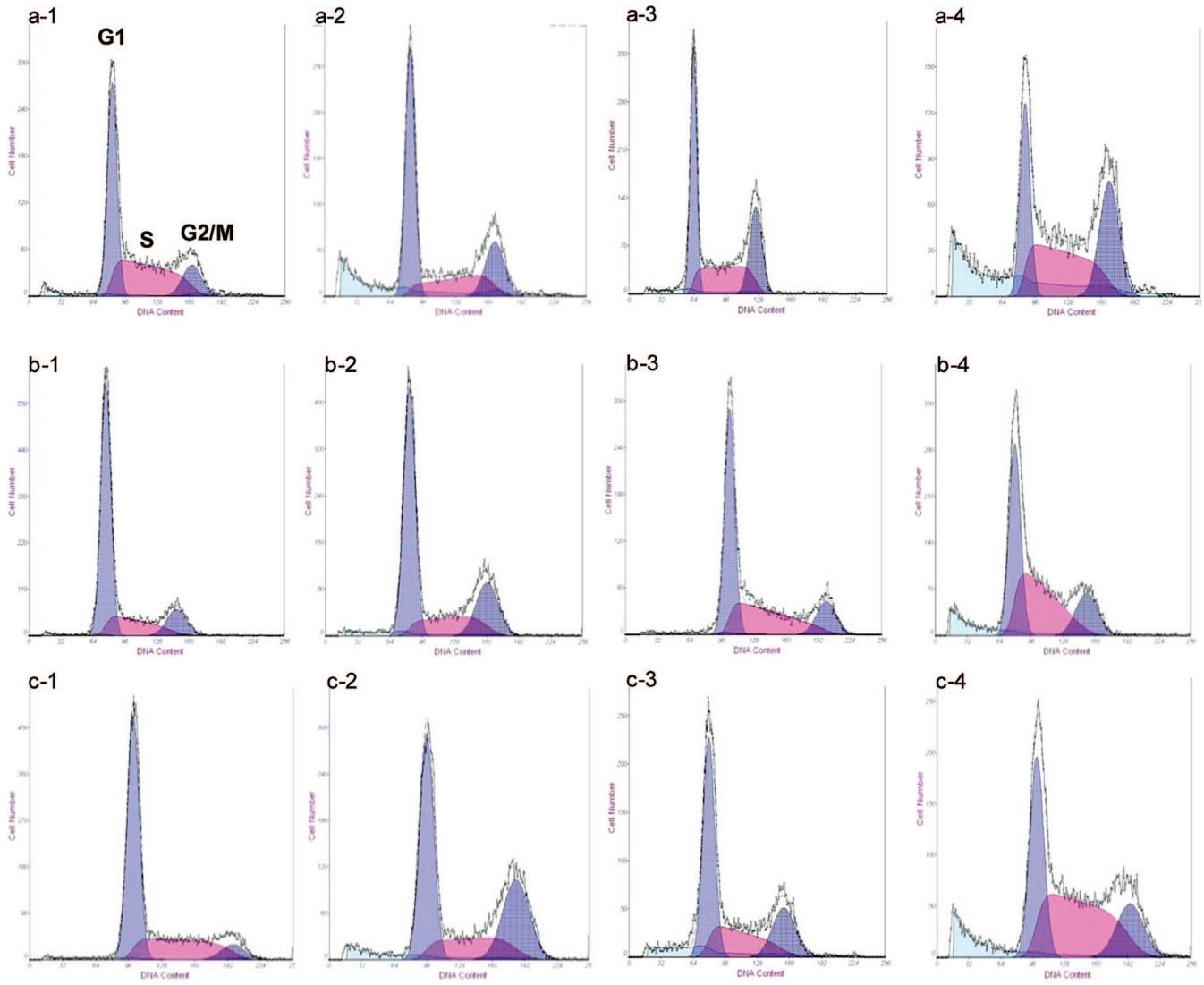
For general data, see the Supporting Information.

Compounds **4a**, **5a**, **6**, **7a**, **8a**, and **9a** were synthesized as previously reported.[8]

Macrocyclization−Elimination Reaction for Preparation of 1a−c. A solution of **9** (1.0 mmol) in 98% HCO_2H (28 mL) was stirred under reflux for 1 h. The reaction mixture was cooled, poured into a solution of $NaHCO_3$, and then extracted with CH_2Cl_2. The organic layer was dried and then concentrated in vacuo to give the keto- and O-deprotected product as a yellow oil. A solution of this oil in EtOH (70 mL) was stirred at 85 °C for 48 h. Concentration in vacuo gave a brown residue, which was purified on the Isco Flash system with a RediSep silica gel disposable flash column. Elution with CH_2Cl_2−MeOH (9:1) gave the O-deprotected product as a yellow oil. This product was dissolved in dry CH_2Cl_2 (20 mL), and the solution was cooled to 0 °C. TEA (2.48 mmol) and MsCl (1.24 mmol) were then added dropwise. The resulting solution was stirred for 5 h at 0 °C, washed with NH_4Cl and water, dried, and concentrated.

Peptide-Heterocycle 1a. The final product was purified by preparative HPLC using H_2O (0.045% TFA) and MeCN (0.036% TFA) as eluents (gradient 40−80% in 25 min; flow rate 3 mL/min) to afford **1a** (65.4 mg, 10%) as a yellow solid. Mp ($CHCl_3$) 165−167 °C. $[\alpha]_D$ +16.8 (*c* 0.25, DMF). ^{1}H NMR (DMF, 400 MHz) δ 0.81−1.07 (m, 12H); 1.22−1.32 (m, 1H); 1.52−1.69 (m, 1H); 1.92−2.02 (m, 1H); 2.04−2.15 (m, 1H); 2.77 (s, 3H); 4.42 (dd, J = 6.8 and 9.6 Hz, 1H); 4.79−4.82 (m, 1H); 5.69 (s, 1H); 6.19 (s, 1H); 7.50−7.61 (m, 3H); 8.50−8.53 (m, 2H); 8.59 (s, 1H); 8.64 (bs, 1H); 8.91 (bs, 1H); 9.16 (s, 1H); 9.19 (s, 1H); 10.22 (bs, 1H). ^{13}C NMR (DMF, 100 MHz) δ 11.4 (q); 11.7 (q); 13.6 (q); 18.7 (q); 19.3 (q); 26.7 (t); 31.8 (d); 39.7 (d); 55.6 (d); 60.6 (d); 104.5 (t); 121.3 (d); 127.0 (s); 127.9 (2d); 128.0 (s); 128.2 (s); 128.6 (2d); 129.3 (s); 130.2 (d); 130.7 (s); 136.0 (s); 138.1 (s); 139.5 (d); 140.4 (d); 143.0 (s); 151.5 (s); 153.6 (s); 155.5 (s); 155.8 (s); 156.5 (s); 157.5 (s); 157.9 (s); 171.0 (s); 172.2 (s). MS (MALDI-TOF) *m*/*z* 761 (M + K, 100), 745 (M + Na, 85). HRMS *m*/*z* calcd for $C_{36}H_{38}N_9O_7S$ (M + NH_4) 740.2609, found 740.2602.

Peptide-Heterocycle 1b. The final product was purified by preparative HPLC using H_2O (0.045% TFA) and MeCN (0.036% TFA) as eluents (gradient 40−80 in 25 min; flow 3 mL/min) to afford **1b** (51.1 mg, 7%) as a yellow solid. Mp ($CHCl_3$) 160−162 °C. $[\alpha]_D$ +18.4 (*c* 0.19, DMF). ^{1}H NMR (DMF, 400 MHz) δ 0.81−1.01 (m, 12H); 1.21−1.33 (m, 1H); 1.48−1.61 (m, 1H); 1.95−2.03 (m, 1H); 2.06−2.16 (m, 1H); 2.94 (s, 3H); 4.41 (dd, J = 6.4 and 9.4 Hz, 1H); 4.81−4.84 (m, 1H); 5.77 (s, 1H); 6.22 (s, 1H); 7.49−7.62 (m, 3H); 8.51 (s, 1H); 8.54−8.56 (m, 2H); 8.64 (bs, 1H); 8.90 (bs, 1H); 9.0 (s, 1H); 9.14 (s, 1H); 10.30 (bs, 1H). ^{13}C NMR (DMF, 100 MHz) δ 11.2 (q); 11.7 (q); 13.6 (q); 18.7



Phase of Cell Cycle	HT-29				A-549				MDA-MB-231			
	a-1	a-2	a-3	a-4	b-1	b-2	b-3	b-4	c-1	c-2	c-3	c-4
G1 (%)	45.5	51.3	39.7	27.2	66.3	55.5	50.4	40.0	60.9	46.4	50.3	33.6
G2/M(%)	12.9	22.4	27.2	31.4	13.0	22.8	14.2	16.7	7.5	31.3	21.8	17.3
S (%)	41.4	26.2	33.0	41.2	20.6	21.6	35.3	43.1	31.5	22.2	27.8	49.0

Figure 2. Cell cycle progression in cancer cell lines treated with **1**, **2** or **3c**; a-1, HT-29 asynchronous colon cell line; a-2, HT-29 with thiopeptide **1**; a-3, HT-29 with compound **2**; a-4, HT-29 with compound **3c**; b-1, A-549 asynchronous lung cell line; b-2, A-549 with thiopeptide **1**; b-3, A-549 with compound **2**; b-4, A-549 with compound **3c**; c-1, MDA-MB-231 asynchronous breast cell line; c-2, MDA-MB-231 with thiopeptide **1**; c-3, MDA-MB-231 with compound **2**; c-4, MDA-MB-231 with compound **3c**.

(q); 19.3 (q); 26.7 (t); 31.7 (d); 39.8 (d); 55.6 (d); 60.7 (d); 105.4 (t); 125.6 (d); 127.2 (s); 127.8 (2d); 128.1 (s); 128.5 (s); 128.7 (2d); 129.3 (s); 130.1 (d); 132.4 (s); 135.4 (s); 136.5 (s); 139.5 (d); 140.7 (d); 143.6 (s); 151.4 (s); 153.3 (s); 156.2 (s); 158.4 (s); 159.0 (s); 159.6 (s); 160.3 (s); 171.1 (s); 172.3 (s). MS (MALDI-TOF) *m/z* 761 (M + K, 100), 745 (M + Na, 85). HRMS *m/z* calcd for $C_{36}H_{38}N_9O_7S$ (M + NH_4) 740.2609, found 740.2585.

Peptide-Heterocycle 1c. The final product was purified by preparative HPLC using H_2O (0.045% TFA) and MeCN (0.036% TFA) as eluents (gradient 50−90% in 25 min; flow rate 3 mL/min) to afford **1c** (75 mg, 8%) as a yellow solid. Mp ($CHCl_3$) 155−157 °C. $[\alpha]_D$ +18.6 (*c* 0.16, DMF). ^{1}H NMR (DMF, 500 MHz) δ 0.81−1.08 (m, 12H); 1.20−1.33 (m, 1H); 1.47−1.59 (m, 1H); 1.95−2.02 (m, 1H); 2.08−2.16 (m, 1H); 2.79 (s, 3H); 2.95 (s, 3H); 4.42 (dd, J = 5.6 and 11.4 Hz, 1H); 4.82 (m, 1H); 5.70 (s, 1H); 6.19 (s, 1H); 7.53−7.61 (m, 3H); 8.51 (s, 1H); 8.56−8.57 (m, 2H); 8.63 (bs, 1H); 8.93 (bs, 1H); 9.16 (s, 1H); 10.26 (bs, 1H). ^{13}C NMR (DMF, 125 MHz) δ 11.6 (q); 11.9 (q); 12.1 (q); 14.3 (q); 14.5 (q); 19.8 (q); 28.9 (t); 31.3 (d); 38.6 (d); 58.0 (d); 61.0 (d); 103.3 (t); 124.9 (d); 128.7 (2d); 129.6 (2d); 130.8 (d); 138.6 (d). MS (MALDI-TOF) *m/z* 775 (M + K, 100), 759 (M + Na, 85). HRMS *m/z* calcd for $C_{37}H_{40}N_9O_7S$ (M + NH_4) 754.2765, found 754.2764.

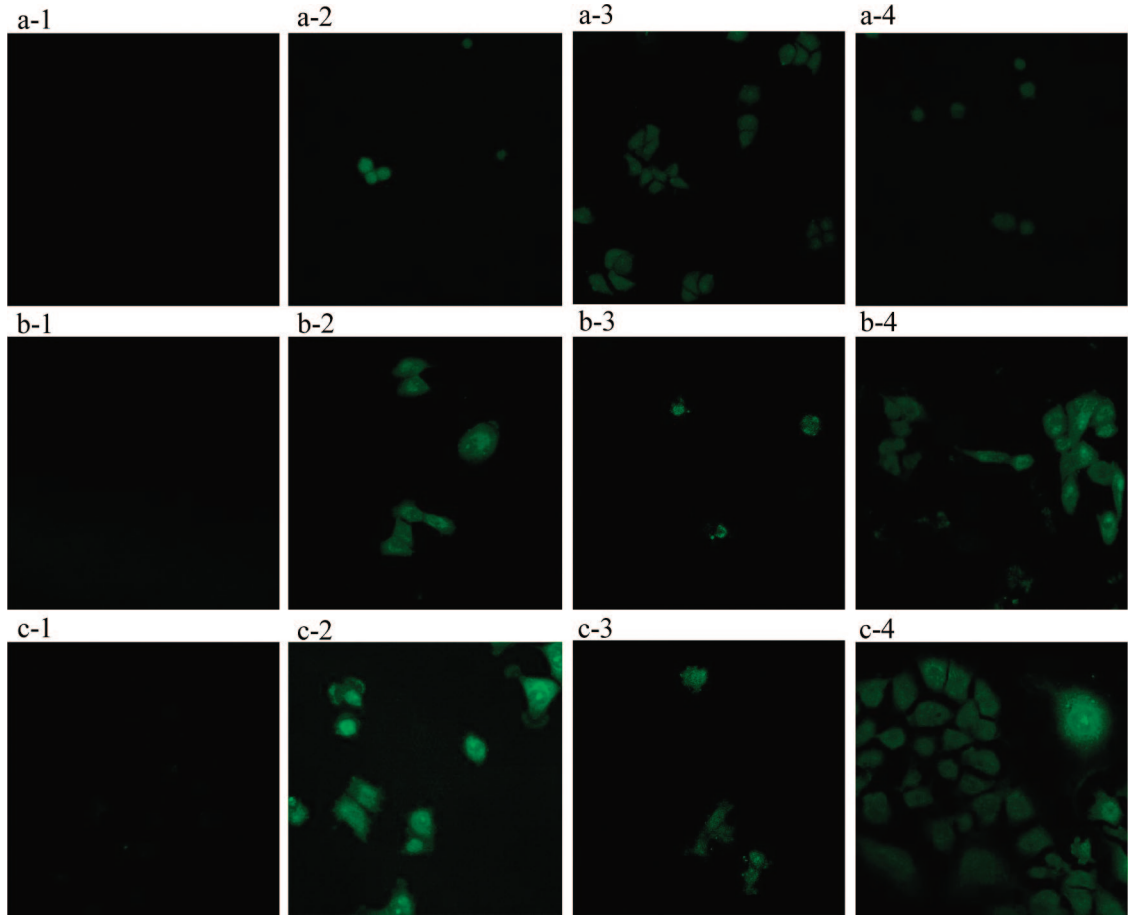


Figure 3. Analysis of apoptotic cell death in cancer cells treated with thiopeptide **1**, **2**, or **3c**: a-1, HT-29 colon cell line negative control; a-2, HT-29 with compound thiopeptide **1**; a-3, HT-29 with compound **2**; a-4, HT-29 with compound **3c**; b-1, A-549 lung cell line negative control; b-2, A-549 with compound thiopeptide **1**; b-3, A-549 with compound **2**; b-4, A-549 with compound **3c**; c-1, MDA-MB-231 breast cell line negative control; c-2, MDA-MB-231 with compound thiopeptide **1**; c-3, MDA-MB-231 with compound **2**; c-4, MDA-MB-231 with compound **3c**.

Peptide-Heterocycle 2. A solution of **9e** (0.85 g, 1.01 mmol) in 98% HCO_2H (25 mL) was refluxed for 1 h. The cooled reaction mixture was poured into a solution of $NaHCO_3$ and extracted with CH_2Cl_2. The organic layer was dried and concentrated in vacuo to give the keto-deprotected product as a yellow oil. A solution of the oil in ethanol (80 mL) was stirred at 85 °C for 48 h. Concentration in vacuo gave a brown residue, which was purified by chromatography on silica gel. Elution with CH_2Cl_2−MeOH (100 to 95:5) and final purification were achieved by Isco Flash (4.3 g reverse phase C18 RediSep column), H_2O (0.04% TFA) and MeCN (0.04% TFA) as eluents (gradient 5−20% in 40 min; flow rate 13 mL/min), to afford **2** (73 mg, 11%) as a yellow solid. Mp ($CHCl_3$) 185−187 °C. $[\alpha]_D$ +40.1 (*c* 3.13, DMSO). ^{1}H NMR (DMSO, 400 MHz) δ 0.85−0.92 (m, 9H); 0.98 (d, J = 6.8 Hz, 3H); 1.03−1.10 (m, 2H); 1.58−1.64 (m, 1H); 2.02−2.15 (m, 1H); 4.19−4.24 (m, 1H); 4.44−4.50 (m, 1H); 4.69−4.74 (m, 1H); 4.98 (dd, J = 9.2 and 16.8 Hz, 1H); 7.51−7.58 (m, 4H); 8.33 (d, J = 7.2 Hz, 2H); 8.40 (d, J = 7.2 Hz, 1H); 8.52 (s, 1H); 8.67 (d, J = 8.8 Hz, 1H); 8.8 (s, 1H); 9.05 (s, 1H); 9.12 (s, 1H). ^{13}C NMR (DMSO, 100 MHz) δ 11.8 (q); 14.5 (q); 17.5 (q); 19.6 (q); 25.4 (t); 31.1 (d); 55.2 (d); 57.0 (d); 57.5 (d); 69.5 (t); 121.2 (d); 126.4 (s); 127.2 (d); 127.5 (s); 128.4 (2d); 129.1 (s); 129.4 (s); 129.9 (2d); 135.4 (s); 139.0 (d); 139.5 (d); 140.1 (s); 141.2 (d); 150.5 (s); 152.0 (s); 154.9 (s); 157.3 (s); 159.8 (s); 162.6 (s); 170.0 (s); 170.2 (s); 170.6 (s). MS (ES) *m/z* 714 (M + NH_4, 100), 316 (5), 288 (18). HRMS *m/z* calcd for $C_{34}H_{33}N_8O_7S$ 697.2187 (M + H), found 697.2187.

Macrocyclization−Acylation for Preparation of 3a−d. A solution of **9** (0.72 mmol) in 98% HCO_2H (20 mL) was stirred under reflux for 1 h. The reaction mixture was cooled, poured into a solution of $NaHCO_3$, and then extracted with CH_2Cl_2. The organic layer was dried, and concentrated to give the keto- and O-deprotected product as a yellow oil. A solution of the oil in EtOH (40 mL) was stirred at 85 °C for 48 h. Concentration in vacuo gave a brown residue, which upon elution with CH_2Cl_2−MeOH (9:1) gave the alcohol **3** as a yellow oil.

Peptide-Heterocycle 3a. The alcohol **3** (105 mg, 0.14 mmol) was dissolved in dry THF (20 mL). Ac_2O (0.45 mL, 4.82 mmol) was then added dropwise. The resulting solution was stirred for 8 h at room temperature, washed with $NaHCO_3$, dried, and concentrated. The final product was purified by preparative HPLC using H_2O (0.04% TFA) and MeCN (0.04% TFA) as eluents (gradient 20−40% in 50 min; flow rate 15 mL/min) to afford **3a** (15 mg, 13%) as a yellow solid. Mp ($CHCl_3$) 171−173 °C. $[\alpha]_D$ +71.8 (*c* 0.47, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.93−1.01 (m, 12H); 1.28−1.35 (m, 2H); 1.63−1.75 (m, 1H); 1.85−1.95 (m, 1H); 2.04 (s, 3H); 3.59−3.68 (m, 1H); 4.32−4.49 (m, 1H); 4.65−4.75 (m, 2H); 5.75−5.84 (m, 1H); 6.15−6.24 (bs, 1H); 7.42−7.53 (m, 4H); 7.95 (s, 1H); 8.19 (s, 1H); 8.26 (s, 2H); 8.36 (d, J = 7.2 Hz, 2H); 8.55 (bs, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.8 (q); 14.5 (q); 18.8 (q); 18.9 (q); 20.4 (q); 25.7 (d); 29.6 (t); 30.1 (d); 39.7 (d); 47.0 (d); 56.5 (d); 63.6 (t); 119.6 (d); 126.5 (s); 127.2 (s); 128.0 (d); 128.5 (2d); 129.4 (s); 130.0 (s); 130.2 (s); 130.3 (2d); 135.7 (d); 137.4 (s); 137.7 (d); 139.8 (d); 141.8 (s); 151.4 (s); 153.0 (s); 156.0 (s); 158.2 (s); 160.4 (s); 162.4 (s); 170.8 (s); 171.7 (s); 172.0 (s). MS (ES) *m/z* 786 (M + NH_4, 100), 744 (12), 316 (12), 288 (38), 241 (12). HRMS *m/z* calcd for $C_{37}H_{37}N_8O_9S$ 769.2399 (M + H), found 769.2409.

Peptide-Heterocycle 3b. The alcohol **3** (87 mg, 0.12 mmol) was dissolved in dry CH_2Cl_2 (10 mL). 2-(2-Methoxyethoxy)acetic acid (0.156 mL, 1.37 mmol), EDC·HCl (263 mg, 1.37 mmol), and DMAP (25 mg, 0.21 mmol) were then added. The resulting solution was stirred for 8 h at room temperature, washed with $NaHCO_3$,

dried, and concentrated. The final product was purified by preparative HPLC using H_2O (0.04% TFA) and MeCN (0.04% TFA) as eluents (gradient 20−38% in 70 min; flow rate 15 mL/min) to afford **3b** (13 mg, 13%) as a yellow solid. Mp ($CHCl_3$) 145−147 °C. $[\alpha]_D$ −30.2 (*c* 0.61, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.84−1.07 (m, 12H); 1.26−1.35 (m, 2H); 1.52−1.75 (m, 1H); 1.81−1.96 (m, 1H); 3.34 (s, 3H); 3.53−3.71 (m, 6H); 4.09−4.22 (m, 2H); 4.46−4.56 (m, 1H); 4.59−4.81 (m, 1H); 5.55 (bs, 1H); 5.73−5.80 (m, 1H); 7.41−7.51 (m, 4H); 7.94 (s, 1H); 8.20 (s, 1H); 8.24 (s, 1H); 8.27 (s, 1H); 8.34 (d, J = 8.0 Hz, 2H); 8.57 (bs, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.8 (q); 14.2 (q); 18.7 (q); 18.8 (q); 25.8 (d); 29.6 (t); 39.6 (d); 46.7 (d); 56.3 (d); 58.8 (q); 59.7 (d); 67.9 (t); 69.9 (t); 70.5 (t); 71.6 (t); 119.6 (d); 126.5 (s); 127.9 (s); 128.4 (d); 128.5 (2d); 129.9 (s); 130.3 (s); 130.3 (s); 130.4 (2d); 135.7 (s); 137.5 (d); 137.7 (d); 139.7 (d); 139.9 (s); 141.8 (s); 151.3 (s); 152.9 (s); 158.0 (s); 158.3 (s); 160.4 (s); 170.3 (s); 171.8 (s); 172.0 (s). MS (ES) *m/z* 860 (M + NH_4, 100), 744 (55), 726 (45), 316 (45), 288 (98), 242 (22). HRMS *m/z* calcd for $C_{40}H_{43}N_8O_{11}S$ 843.2750 (M + H), found 843.6727.

Peptide-Heterocycle 3c. The alcohol **3** (111 mg, 0.15 mmol) was dissolved in dry CH_2Cl_2 (10 mL). 2-[2-(2-Methoxyethoxy)-ethoxy]acetic acid (0.25 mL, 1.65 mmol), EDC·HCl (316 mg, 1.65 mmol), and DMAP (30 mg, 0.25 mmol) were then added dropwise. The resulting solution was stirred for 8 h at room temperature, washed with $NaHCO_3$, dried, and concentrated. The final product was purified by preparative HPLC using H_2O (0.04% TFA) and MeCN (0.04% TFA) as eluents (gradient 20−38% in 70 min; flow rate 15 mL/min) to afford **3c** (16 mg, 12%) as a yellow solid. Mp ($CHCl_3$) 139−141 °C. $[\alpha]_D$ −63.4 (*c* 0.56, $CHCl_3$). ^{1}H NMR (DMF, 400 MHz) δ 0.93−0.98 (m, 6H); 1.01 (d, J = 6.8 Hz, 3H); 1.07 (d, J = 6.8 Hz, 3H); 1.24−1.30 (m, 2H); 1.38 (d, J = 6.4 Hz, 1H); 1.54−1.74 (m, 1H); 2.11−2.28 (m, 1H); 3.28 (s, 3H); 3.55−3.70 (m, 10H); 4.08−4.20 (m, 2H); 4.70−4.79 (m, 2H); 5.82 (s, 1H); 6.17 (s, 1H); 7.49−7.61 (m, 3H); 8.44 (d, J = 8.0 Hz, 2H); 8.59 (s, 1H); 8.98 (s, 1H); 9.14 (s, 1H); 9.17 (s, 1H); 10.03 (bs, 1H). ^{13}C NMR (DMF, 100 MHz) δ 11.8 (q); 14.5 (q); 18.9 (q); 19.2 (q); 26.8 (d); 29.4 (t); 32.4 (d); 39.4 (d); 58.1 (d); 58.2 (q); 60.1 (d); 68.1 (t); 68.3 (t); 70.3 (t); 70.4 (t); 70.5 (t); 71.9 (t); 121.6 (d); 127.3 (s); 127.9 (2d); 128.4 (s); 128.7 (s); 128.8 (2d); 129.4 (s); 130.3 (s); 130.4 (d); 136.5 (s); 139.8 (d); 140.7 (d); 140.8 (d); 142.2 (s); 152.8 (s); 155.8 (s); 158.1 (s); 158.5 (s); 159.6 (s); 160.7 (s); 171.0 (s); 171.6 (s); 171.9 (s). MS (ES) *m/z* 904 (M + NH_4, 40), 726 (100), 316 (8), 288 (38). HRMS *m/z* calcd for $C_{42}H_{47}N_8O_{12}S$ 887.3029 (M + H), found 887.3037.

Peptide-Heterocycle 3d. The alcohol **3** (50 mg, 0.068 mmol) was dissolved in dry CH_2Cl_2 (7 mL). Boc-L-Arg-(Alloc)$_2$-OH (120 mg, 0.27 mmol), EDC·HCl (52 mg, 0.27 mmol), and DMAP (7 mg, 0.57 mmol) were added dropwise. The resulting solution was stirred for 31 h at room temperature, washed with $NaHCO_3$ and NH_4Cl, dried, and concentrated. The final product was purified by flash chromatography on silica Bondesil C8 using H_2O (0.04% TFA) and MeCN (0.04% TFA) as eluents (gradient 0−50%) to afford **3d** (15.9 mg, 16%) as a yellow solid. $[\alpha]_D$ −11.6 (*c* 0.82, DMSO). ^{1}H NMR (DMSO, 400 MHz) δ 0.82−0.94 (m, 9H); 0.99 (d, J = 6.8 Hz, 3H); 1.07−1.17 (m, 2H); 1.25 (s, 9H); 1.35−1.37 (m, 2H); 1.57−1.64 (m, 2H); 1.73−1.78 (m, 1H); 2.01−2.11 (m, 1H); 3.09−3.16 (m, 2H); 3.57−3.65 (m, 1H); 3.79−3.91 (m, 3H); 4.47−4.51 (m, 2H); 4.62−4.70 (m, 3H); 4.75−4.79 (m, 1H); 5.12−5.40 (m, 4H); 5.87−5.99 (m, 2H); 7.08 (bs, 1H); 7.17 (bs, 1H); 7.30 (bs, 1H); 7.48−7.59 (m, 3H); 8.30−8.33 (m, 2H); 8.51 (s, 1H); 8.53 (s, 1H); 8.79 (bs, 1H); 8.98 (s, 1H); 9.11 (s, 1H); 9.12 (s, 1H); 9.98 (bs, 1H). ^{13}C NMR (DMSO, 100 MHz) δ 12.3 (q); 14.2 (q); 17.9 (q); 18.5 (q); 19.0 (q); 22.4 (d); 26.9 (t); 28.1 (t); 28.9 (t); 31.9 (d); 41.7 (t); 53.4 (2d); 56.9 (d); 58.1 (d); 65.1 (t); 67.0 (t); 69.7 (t); 78.1 (s); 117.1 (s); 117.2 (t); 118.3 (t); 121.2 (d); 126.4 (s); 127.2 (s); 127.5 (s); 127.8 (s); 128.6 (2d); 129.1 (s); 129.6 (2d); 129.7 (s); 130.7 (d); 131.8 (d); 133.5 (s); 133.6 (d); 135.6 (s); 139.5 (d); 140.4 (d); 141.4 (d); 150.4 (s); 152.1 (s); 154.7 (s); 157.4 (s); 157.5 (s); 158.9 (s); 160.0 (s); 170.2 (s); 170.7 (s); 170.9 (s); 173.8 (s). MS (MALDI-TOF) *m/z* 1189 (M + K), 1173 (M + Na, 15), 1151 (M, 10). HRMS *m/z* calcd for $C_{54}H_{63}N_{12}O_{15}S$ 1151.4295 (M + H), found 1151.4251.

Peptide-Heterocycle 3e. The O-deprotected product (74 mg, 0.101 mmol) was dissolved in dry CH_2Cl_2 (10 mL). Boc-L-Arg-(Boc)$_2$-OH (193 mg, 0.41 mmol), EDC·HCl (78 mg, 0.41 mmol), and DMAP (12.4 mg, 0.102 mmol) were then added dropwise. The resulting solution was stirred for 15 h at room temperature, washed with $NaHCO_3$ and NH_4Cl, dried, and concentrated. The final product was purified by flash chromatography on silica Bondesil C8 using H_2O (0.04% TFA) and MeCN (0.04% TFA) as eluents (gradient 0−50%) to give **3e** (19 mg, 17%) as a white solid. $[\alpha]_D$ −19.4 (*c* 0.12, DMSO). ^{1}H NMR (DMSO, 400 MHz) δ 0.83−0.95 (m, 12H); 1.09−1.18 (m, 2H); 1.23 (s, 9H); 1.36 (s, 9H); 1.43 (s, 9H); 1.45−1.52 (m, 4H); 1.60−1.68 (m, 1H); 1.96−2.03 (m, 1H); 3.95−4.04 (m, 1H); 4.11−4.18 (m, 2H); 4.39−4.48 (m, 2H); 4.63−4.69 (m, 1H); 5.63−5.70 (m, 1H); 7.14−7.28 (m, 2H); 7.50−7.75 (m, 3H); 8.17 (bs, 1H); 8.24 (bs, 1H); 8.39−8.41 (m, 2H); 8.55 (s, 1H); 8.72 (bs, 1H); 8.96 (s, 1H); 9.09 (s, 1H); 9.14 (s, 1H); 10.7 (bs, 1H). ^{13}C NMR (DMSO, 100 MHz) δ 12.4 (q); 14.5 (q); 19.5 (q); 18.4 (q); 24.6 (d); 28.0 (q); 28.1 (t); 28.3 (t); 28.4 (q); 28.7 (q); 29.4 (t); 31.2 (d); 46.8 (d); 53.7 (d); 56.0 (d); 60.2 (t); 64.6 (t); 122.4 (d); 128.4 (2d); 129.3 (2d); 131.0 (d); 140.2 (d); 141.1 (d); 141.4 (d). MS (ES) *m/z* 1185 (M + 2H, 60), 1184 (M + H, 100). HRMS *m/z* calcd for $C_{51}H_{63}N_{12}O_{13}S$ (M − Boc) 1083.4352, found 1083.4351.

Peptide-Heterocycle 3f. A solution of **3e** (5 mg, 4.2 μmol) in TFA−CH_2Cl_2 (25:75, 1 mL) was stirred at room temperature for 2 h. The TFA was removed, and the product was used without purification. $[\alpha]_D$ −40.1 (*c* 0.23, DMSO). ^{1}H NMR (DMSO, 500 MHz) δ 0.83−0.98 (m, 12H); 1.07−1.18 (m, 2H); 1.41−1.76 (m, 5H); 2.03−2.08 (m, 1H); 3.57−3.64 (m, 1H); 4.0−4.04 (m, 1H); 4.08−4.18 (m, 3H); 4.49−4.56 (m, 2H); 4.65−4.68 (m, 1H); 5.69−5.73 (m, 1H); 7.51−7.62 (m, 7H); 8.20 (d, J = 5.0 Hz, 1H); 8.39−8.41 (m, 2H); 8.56 (s, 1H); 8.71 (d, J = 8.0 Hz, 1H); 8.80 (d, J = 9.0 Hz, 1H); 8.99 (s, 1H); 9.10 (s, 1H); 9.14 (s, 1H). ^{13}C NMR (DMSO, 125 MHz) δ 12.5 (q); 14.4 (q); 19.5 (q); 19.7 (q); 25.0 (t); 25.1 (t); 26.0 (t); 26.8 (d); 30.4 (d); 46.9 (d); 56.2 (d); 58.4 (d); 60.3 (t); 65.2 (t); 70.4 (d); 122.3 (d); 128.4 (2d); 129.3 (2d); 131.0 (d); 139.6 (d); 140.9 (d); 141.0 (d). MS (ES) *m/z* 883 (M, 100). HRMS *m/z* calcd for $C_{41}H_{47}N_{12}O_9S$ 883.3304, found 883.3317.

Peptide-Heterocycle 9b. Reaction (20 h) of the free acid **7a** (816 mg, 1.28 mmol) and **8b** (500 mg, 1.54 mmol) using the general procedure for amide formation gave **9b** (1.14 g, 94%) as a solid. An analytical sample was purified by column chromatography (4:1 to 2:3 hexane−EtOAc) to afford **9b** as a yellow solid. Mp ($CHCl_3$) 143−145 °C. $[\alpha]_D$ +1.4 (*c* 0.42, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.91−1.02 (m, 12H); 1.07 (s, 9H); 1.19−1.29 (m, 1H); 1.48−1.58 (m, 1H); 2.07−2.15 (m, 1H); 2.20−2.27 (m, 1H); 2.58 (s, 3H); 3.37 (s, 6H); 3.67−3.73 (m, 1H); 3.76−3.80 (m, 1H); 3.89 (s, 2H); 4.46−4.50 (m, 1H); 4.58−4.63 (m, 1H); 5.24−5.37 (m, 1H); 6.70 (d, J = 8.4 Hz, 1H); 7.17 (bs, 1H); 7.38−7.46 (m, 4H); 7.80 (d, J = 8.4 Hz, 1H); 8.24−8.30 (m, 2H); 8.36 (s, 1H); 8.37 (s, 1H); 8.47 (bs, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.6 (q); 11.7 (q); 14.8 (q); 17.7 (q); 19.3 (q); 26.3 (t); 27.2 (q); 30.9 (d); 31.7 (t); 37.2 (d); 48.4 (d); 50.2 (2q); 57.4 (d); 58.3 (d); 62.2 (t); 73.9 (s); 99.3 (s); 124.3 (s); 126.5 (s); 128.3 (2d); 128.5 (2d); 129.6 (s); 130.2 (d); 140.0 (d); 141.2 (s); 142.6 (s); 143.0 (d); 151.3 (s); 151.7 (s); 153.0 (s); 155.8 (s); 161.3 (s); 161.4 (s); 161.6 (s); 170.9 (s); 171.2 (s); 188.3 (s). MS (MALDI-TOF) 965 ($M^{81}Br$ + Na, 100), 963 ($M^{79}Br$ + Na, 85). HRMS *m/z* calcd for $C_{42}H_{54}{}^{79}BrN_8O_{10}S$ 941.2861 and to $C_{42}H_{56}{}^{81}BrN_8O_{10}S$ 943.2850, found 941.2867 and 943.2853.

Peptide-Heterocycle 9c. Reaction (20 h) of the free acid **7b** (1 g, 1.54 mmol) and the N-deprotected **8a** (573 mg, 1.84 mmol) using the general procedure for amide formation gave **9c** (1.38 g, 95%) as a yellow solid. An analytical sample was purified by column chromatography (1:1 to 2:3 hexane−EtOAc) to give **9c** as a yellow solid. Mp ($CHCl_3$) 116−118 °C. $[\alpha]_D$ +2.3 (*c* 0.61, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.88−0.98 (m, 9H); 1.01 (d, J = 6.8 Hz, 3H); 1.06 (s, 9H); 1.18−1.31 (m, 1H); 1.46−1.61 (m, 1H);

2.05−2.16 (m, 1H); 2.19−2.27 (m, 1H); 2.78 (s, 3H); 3.36 (s, 6H); 3.67−3.75 (m, 1H); 3.77−3.81 (m, 1H); 3.87 (s, 2H); 4.45−4.66 (m, 2H); 5.32−5.42 (m, 1H); 6.79−6.88 (m, 1H); 7.36−7.46 (m, 3H); 7.78−7.89 (m, 2H); 8.07 (s, 1H); 8.12−8.17 (m, 1H); 8.24−8.29 (m, 2H); 8.36 (s, 1H); 8.51 (bs, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.6 (q); 12.1 (q); 14.7 (q); 17.7 (q); 19.3 (q); 26.4 (t); 27.2 (q); 30.8 (d); 31.8 (t); 37.2 (d); 48.5 (d); 50.2 (2q); 57.4 (d); 58.3 (d); 62.4 (t); 73.9 (s); 99.2 (s); 125.0 (s); 126.7 (s); 128.2 (2d); 128.3 (2d); 129.4 (s); 130.0 (d); 139.4 (d); 141.4 (s); 143.3 (d); 152.0 (s); 152.4 (s); 152.8 (s); 154.6 (s); 158.8 (s); 161.4 (s); 164.1 (s); 171.0 (s); 171.3 (s); 173.6 (s); 188.0 (s). MS (MALDI-TOF) 965 ($M^{81}Br$ + Na, 100), 963 ($M^{79}Br$ + Na, 80). HRMS *m/z* calcd for $C_{42}H_{54}{}^{79}BrN_8O_{10}S$ 941.2861, found 941.2870.

Peptide-Heterocycle 9d. Reaction (20 h) of the free acid **7b** (763 mg, 1.17 mmol) and **8b** (458 mg, 1.41 mmol) using the general procedure for amide formation gave **9d** as a solid (1.05 g, 94%). An analytical sample was purified by column chromatography (4:1 to 2:3 hexane−EtOAc) to give **9d** as a yellow solid. Mp ($CHCl_3$) 147−149 °C. $[\alpha]_D$ +3.5 (*c* 0.76, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.91−1.03 (m, 12H); 1.09 (s, 9H); 1.22−1.31 (m, 1H); 1.47−1.58 (m, 1H); 2.08−2.17 (m, 1H); 2.21−2.30 (m, 1H); 2.58 (s, 3H); 2.79 (s, 3H); 3.37 (s, 6H); 3.68−3.73 (m, 1H); 3.77−3.80 (m, 1H); 3.88 (s, 2H); 4.43−4.54 (m, 1H); 4.57−4.63 (m, 1H); 5.24−5.37 (m, 1H); 6.69 (d, J = 8.4 Hz, 1H); 7.10 (bs, 1H); 7.38−7.46 (m, 3H); 7.78−7.83 (m, 2H); 8.25−8.30 (m, 2H); 8.37 (s, 1H); 8.44 (bs, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.6 (q); 11.7 (q); 12.1 (q); 14.7 (q); 17.7 (q); 19.3 (q); 26.4 (t); 27.3 (q); 30.9 (d); 31.8 (t); 37.1 (d); 48.4 (d); 50.3 (2q); 57.4 (d); 58.3 (d); 62.2 (t); 73.9 (s); 99.2 (s); 124.4 (s); 125.0 (s); 126.7 (s); 128.3 (2d); 128.4 (2d); 129.4 (s); 130.0 (d); 141.2 (s); 142.9 (d); 151.2 (s); 152.0 (s); 152.5 (s); 152.8 (s); 155.8 (s); 158.8 (s); 161.5 (s); 170.8 (s); 171.0 (s); 171.2 (s); 188.4 (s). MS (MALDI-TOF) 979 ($M^{81}Br$ + Na, 100), 977 ($M^{79}Br$ + Na, 85). HRMS *m/z* calcd for $C_{43}H_{56}{}^{79}BrN_8O_{10}S$ 955.3018 and to $C_{43}H_{56}{}^{81}BrN_8O_{10}S$ 957.3007, found 955.3028 and 957.3009.

Peptide-Heterocycle 9e. Reaction (20 h) of the free acid **7a** (1.37 g, 2.15 mmol) and **8c** (580 mg, 2.59 mmol) using the general procedure for amide formation gave **9e** (1.78 g, 98%) as a solid. An analytical sample was purified by column chromatography (1:1 to 2:3 hexane−EtOAc) to give **9e** as a solid. Mp ($CHCl_3$) 132−134 °C. $[\alpha]_D$ +22.4 (*c* 0.49, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.89−1.07 (m, 12H); 1.22−1.31 (m, 1H); 1.51−1.61 (m, 1H); 2.05−2.18 (m, 1H); 2.21−2.45 (m, 1H); 3.37 (s, 6H); 3.79−3.86 (m, 1H); 3.89 (s, 2H); 4.29−4.84 (m, 3H); 6.77−6.95 (m, 1H); 7.35−7.44 (m, 3H); 7.74−7.85 (m, 2H); 8.04 (s, 1H); 8.09−8.12 (m, 1H); 8.17−8.25 (m, 2H); 8.31 (s, 1H); 8.36 (s, 1H); 8.49 (bs, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.3 (q); 15.0 (q); 17.5 (q); 19.5 (q); 26.0 (t); 29.8 (d); 31.6 (t); 36.4 (t); 36.6 (d); 50.3 (2q); 58.4 (d); 58.6 (d); 99.3 (s); 126.5 (s); 128.2 (2d); 128.4 (2d); 129.4 (s); 130.1 (s); 130.3 (d); 139.6 (d); 140.1 (d); 141.4 (s); 143.3 (d); 151.8 (s); 153.0 (s); 154.5 (s); 161.4 (s); 161.7 (s); 161.9 (s); 162.6 (s); 171.5 (s); 171.8 (s); 188.1 (s). MS (MALDI-TOF) 865 ($M^{81}Br$ + Na, 100), 863 ($M^{79}Br$ + Na, 85). HRMS *m/z* calcd for $C_{36}H_{42}{}^{79}BrN_8O_9S$ 841.1973, found 841.1968.

Cell Lines and Culture. Human-derived established cell lines used in this study were purchased from ATCC (American Type Culture Collection): A-549, human lung carcinoma (ATCC no. CCL-185), HT-29, human colorectal adenocarcinoma (ATCC no. HTB-38), and MDA-MB 231, human breast adenocarcinoma (ATCC no. HTB-26).

All cell lines are maintained in DMEM (Dulbecco's modified Eagle's medium) supplemented with 10% FBS (fetal bovine serum) and 100 units/mL penicillin and streptomycin at 37 °C and 5% CO_2. Triplicate cultures were incubated for 72 h in the presence or absence of test compounds (at 10 concentrations typically ranging from 10 to 0.0026 μg/mL).

A colorimetric assay using sulforhodamine B (SRB) has been adapted for a quantitative measurement of cell growth and viability, following a previously described method.[16] Cells are plated in 96-well microtiter plates at a density of 5×10^3/well and incubated for 24 h. One plate from each different cell line is fixed and stained and used for Tz reference (see next paragraph). After that, cells are treated with vehicle alone (control) or compounds at the concentrations indicated. Treated cells are further incubated for 72 h, and cytotoxic evaluation was performed by colorimetric analysis.

In brief, cells are washed twice with phosphate buffered saline (PBS), fixed for 15 min in 1% glutaraldehyde solution, rinsed twice in PBS, and stained in 0.4% SRB solution for 30 min at room temperature. Cells are then rinsed several times in 1% acetic acid solution and air-dried. SRB was then extracted in 10 mM trizma base solution and the absorbance measured at 490 nm. Cell survival is expressed as percentage of control cell growth.

Dose−response curves are obtained by using the NCI algorithm:[17] Tz = number of control cells at time t_0, C = number of control cells at time t, and T = number of treated cells at time t.

If Tz < T < C (growth inhibition), then the result is $100 \times ([T - \mathrm{Tz}]/[C - \mathrm{Tz}])$.

If T < Tz (net cell killing), then the result is $100 \times ([T - \mathrm{Tz}]/\mathrm{Tz})$.

After dose-curve generation, results are experessed as GI_{50}, the compound concentration that causes 50% cell growth inhibition, compared to control cultures.

Cell Cycle Analysis. Cell cycle analysis was performed by propidium iodide staining to determine DNA content. Subconfluent cells treated with 35 nM **1** or 172 nM **2** or 135 nM **3c** for 12 h were trypsinyzed, collected by centrifugation, resuspended in PBS, and then fixed in 70% ethanol. The fixed cells were incubated with 5 mg/mL propidium iodide (Sigma), 0.1 mg/mL RNase A (Sigma), and 0.1% Triton X-100 for 15 min at 37 °C and analyzed with a Beckman Coulter Epics XL using the 488 nm line of argon laser. The cell cycle profile was analyzed using Cell Quest software.

Apoptosis Assay. The methodology used is based on fluorochrome inhibitors of caspases (FLICA; CaspaTag in situ caspase detection kits, Chemicon International, U.S. and Canada). The inhibitors are cell permeable and noncytotoxic. Once inside the cell, the inhibitor covalently binds to the active caspase. This kit uses a carboxyfluorescein-labeled fluoromethyl ketone peptide inhibitor of caspase-3 (FAM-DEVD-FMK), which fluoresces green. When added to a group of cells, the FAM-DEVD-FMK probe enters each cell and covalently binds to a reactive cysteine residue in the large subunit of the active caspase heterodimer, thereby inhibiting further enzymatic activity.[18]

Subconfluent cells cultured on coverslips were treated with 35 nM **1** or 172 nM **2** or 135 nM **3c** for 24 h. After treatment, 30X FLICA reagent was added at 1:15 dilution in culture medium and incubated for 1 h at 37 °C. The cells were washed with PBS and analyzed using a Leica TCS SP2 laser scanning confocal spectral microscope (Leica Microsystems Heidelberg GmbH, Mannheim, Germany) with a band-pass filter excitation at 490 nm and emission at 520 nm to view the green fluorescence of caspase-positive cells. The green fluorescence signal was a direct measure of the amount of active caspase-3/7 present in the cell at the time the reagent was added.

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Supporting Information Available: Experimental details, characterization of synthesized compounds, and a table of HPLC purities of final compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Yeung, K.; Paterson, I. Actin-binding marine macrolides: total synthesis and biological importance. *Angew. Chem., Int. Ed.* **2002**, *41*, 4632–4653.

(2) Recent revisions about the chemistry and properties can be found in the following: (a) Roy, R. S.; Gehring, A. M.; Milne, J. C.; Belshaw, P. J.; Walsh, C. T. Thiazole and oxazole peptides: biosynthesis and molecular machinery. *Nat. Prod. Rep.* **1999**, *16*, 249–263. (b) Yeh, V. S. C. Recent advances in the total syntheses of oxazole-containing natural products. *Tetrahedron* **2004**, *60*, 11995–12042. (c) Riego, E.; Hernández, D.; Albericio, F.; Álvarez, M. Directly linked polyazoles: important moieties in natural products. *Synthesis* **2005**, 1907–1922. (d) Jin, Z. Imidazole, oxazole and thiazole alkaloids. *Nat. Prod. Rep.* **2006**, *23*, 464–496.

(3) Nagatsu, A.; Kajitani, H.; Sakakibara, J. Muscoride A: a new oxazole peptide alkaloid from freshwater cyanobacterium *Nostoc muscorum*. *Tetrahedron Lett.* **1995**, *36*, 4097–4100.

(4) Kehraus, S.; König, G. M.; Wright, A. D. Leucamide A: a new cytotoxic heptapeptide from the australian sponge *Leucetta microraphis*. *J. Org. Chem.* **2002**, *67*, 4989–4992.

(5) (a) Shin-ya, K.; Wierzba, K.; Matsuo, K.; Ohtani, T.; Yamada, Y.; Furihata, K.; Hayakawa, Y.; Seto, H. Telomestatin, a novel telomerase inhibitor from *Streptomyces anulatus*. *J. Am. Chem. Soc.* **2001**, *123*, 1262–1263. (b) Doi, T.; Yoshida, M.; Shin-ya, K.; Takahashi, T. Total synthesis of (*R*)-telomestatin. *Org. Lett.* **2006**, *8*, 4165–4167.

(6) Lindquist, N.; Fenical, W.; Van Duyne, G. D.; Clardy, J. Isolation and structure determination of diazonamides A and B, unusual cytotoxic metabolites from the marine ascidian *Diazona chinensis*. *J. Am. Chem. Soc.* **1991**, *113*, 2303–2304.

(7) (a) IB-01211 was isolated from the marine microorganism strain ES7-008: Romero, F.; Malet, L.; Cañedo, L, M.; Cuevas, C.; Reyes, J. New Cytotoxic Depsipeptides. WO 2005/000880 A2, 2005. (b) The same structure was proposed for mechercharmycin A, isolated from a marine-derived *Thermoactinomices* sp: Kanoh, K.; Matsuo,Y.; Adachi, K.; Imagawa, H.; Nishizawa, M.; Shizuri, Y. Mechercharmycins A and B, cytotoxic substances from marine-derived *Thermoactinomyces* sp. YM3-251. *J. Antibiot.* **2005**, *58*, 289−292.

(8) (a) Hernández, D.; Vilar, G.; Riego, E.; Cañedo, L. M.; Cuevas, C.; Albericio, F.; Álvarez, M. Synthesis of IB-01211, a cyclic peptide containing 2,4-concatenated thia- and oxazoles, via Hantzsch macrocyclization. *Org. Lett.* **2007**, *9*, 809–811. (b) Hernández, D.; Riego, E.; Francesch, A.; Cuevas, C.; Albericio, F.; Álvarez, M. Preparation of penta-azole containing cyclopeptides: challenges in macrocyclization. *Tetrahedron* **2007**, *63*, 9862–9870.

(9) Sohda, K-y.; Nagai, K.; Yamori, T.; Suzuki, K-i.; Tanaka, A. YM-216391, a novel cytotoxic cyclic peptide from Streptomyces nobilis. *J. Antibiot.* **2005**, *58*, 27–31.

(10) Hydrolysis of the methyl ester was performed using LiOH in aqueous THF and MeOH as detailed in the Experimental Section.

(11) These conditions for the thiazole Hantzsch synthesis afforded an easier purification of crude material than conditions obtained before; see ref 8.

(12) Using the standard conditions of $Pd(PPh_3)_4$, $PhSiH_3$, in CH_2Cl_2.

(13) IC_{50} for YM-216391 in HeLa S3 is 14 nM: Sanada, K.; Takeba-yashi, Y.; Nagai, K.; Hiramoto, M. New antitumor compound. *Jpn. Kokai Tokkyo Koho* **1999**, JP11180997-A; *Chem. Abstr.* **1999**, *131*, 101307j.

(14) Hernández, D.; Riego, E.; Albericio, F.; Álvarez, M. Synthesis of natural product derivatives containing 2,4-concatenated oxazoles. *Eur. J. Org. Chem.* **2008**, 3389–3396.

(15) Ekert, P. G.; Silke, J.; Vaux, D. L. Caspase inhibitors synthesis of natural product derivatives containing 2,4-concatenated oxazoles. *Cell Death Differ.* **1999**, *6*, 1081–1086.

(16) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Waren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. New colorimetric cytotoxicity assay for anticancer drug screening. *J. Natl. Cancer Inst.* **1990**, *82*, 1107–1112.

(17) Boyd, M. R.; Paull, K. D. Some practical considerations and applications of the National Cancer Institute in vitro anticancer drug discovery screen. *Drug Dev. Res.* **1995**, *34*, 91–104.

(18) Rotonda, J.; Nicholson, D. W.; Fazil, K. M.; Gallart, Y. G.; Labelle, M.; Peterson, E. P.; Rasper, D. M.; Ruel, R.; Vaillancourt, J. P.; Thornberry, N. A.; Becker, J. W. The three-dimensional structure of apopain/CPP32, a key mediator of apoptosis. *Nat. Struct. Biol.* **1996**, *3*, 619–625.

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