

# Synthesis of Somatostatin Analogues Containing C-Terminal Adamantane and Their Antiproliferative Properties

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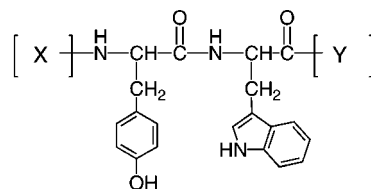
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On the basis of the structure of somatostatin analogue TT-232 (**1**), which exhibited a highly potent antitumor activity, we synthesized small linear peptide derivatives and evaluated their antitumor and apoptotic activity. Of them, Boc-Tyr-D-Trp-1-adamantylamide (**5**) had the most potent cell antiproliferative activity in SW480 and A431 cell lines, which was supported in A431 cell lines by FACS analysis that demonstrated a major increase in DNA fragmentation in the subG1 fraction.

## Introduction

The neuropeptide somatostatin (SRIF) exerts inhibitory effects on the secretory processes in the endocrine and exocrine systems. It also mediates tumor cell growth inhibition<sup>1–5</sup> through a family of seven transmembrane G-protein-coupled receptors (SSTR1–SSTR5).<sup>6,7</sup> SSTRs are distributed throughout human body not only in normal cells but also in tumor cells. Native somatostatin binds with high affinity to all SSTR subtypes, whereas the commercially available somatostatin analogues bind with varying degrees of activity;<sup>8</sup> for example, cyclic and linear octapeptide analogues bind to hSSTR 2, 3, and 5 subtypes with high affinity but with reduced affinity to hSSTR1 and 4 subtypes. Most of the somatostatin analogues developed for clinical use, such as octreotide<sup>9–11</sup> and RC-160,<sup>12</sup> have high affinity to SSTR2 and SSTR5 and act longer than somatostatin with use in the diagnosis and treatment of gastrointestinal disorders, including endocrine tumors.<sup>9,13–16</sup> However, the use of these analogues as antitumor agents has been limited because of their antisecretory effects and poor oral bioavailability. Kéri et al. reported that in vitro and in vivo, a selective somatostatin structural analogue, TT-232 (**1**) [D-Phe-c(Cys-Tyr-D-Trp-Lys-Cys)-Thr-NH<sub>2</sub>], had potent antiproliferative activity without antisecretory action.<sup>17,18</sup> Compound **1**, which binds to SSTR1 and 4 receptors with low affinity but not to SSTR2, 3, and 5 receptors,<sup>19,20</sup> exerts its effects through the tyrosine phosphorylation pathway, and further, **1** is internalized into the cytoplasm and nuclei, where it directly induces apoptosis.<sup>20</sup>

In this paper, we attempt to simplify the structure of **1** to improve biological activity and cellular permeability and to synthesize several new linear analogues (**2–15**, Figure 1). To reduce the size of the active peptide and at the same time improve its antiproliferative activity, we synthesized peptides **2–4**, comprising only the essential biologically relevant amino acid sequence, Tyr-D-Trp-Lys, and studied the resulting structure–activity relationship. The studies on these compounds revealed that placing hydrophobic moieties at the N- and C-termini of the peptide was critical for the manifestation of antiproliferative activity. Therefore, Lys was substituted with



- |                         |                              |
|-------------------------|------------------------------|
| <b>2:</b> X=Boc,        | Y=Lys(Z)-OFm                 |
| <b>3:</b> X=Boc,        | Y=Lys(Z)-OH                  |
| <b>4:</b> X=H,          | Y=Lys(Z)-OH                  |
| <b>5/10:</b> X=Boc-/H-, | Y=1-adamantylamide           |
| <b>6/11:</b> X=Boc-/H-, | Y=2-adamantylamide           |
| <b>7/12:</b> X=Boc-/H-, | Y=1-naphthylamide            |
| <b>8/13:</b> X=Boc-/H-, | Y=t-butylamide               |
| <b>9/14:</b> X=Boc-/H-, | Y=4-methylcoumarin-7-ylamide |
| <b>15:</b> X=H,         | Y=OH                         |

Figure 1. Structures of somatostatin analogues **2–15**.

hydrophobic moieties and N-terminal amino groups were protected by *tert*-butoxycarbonyl (*t*-Boc) groups to give compounds **5–9**. The resulting structures with D-Trp in the middle of the peptide were expected to resist enzymatic degradation. Being small and hydrophobic, these molecules should exhibit potent cell permeability, and in this study, we report the synthesis, physicochemical characterization, and the biological activities of these new analogues.

## Results and Discussion

**Chemistry.** Compounds **3** and **4** were prepared from Boc-Tyr-D-Trp-Lys(Z)-OFm<sup>a</sup> (**2**).<sup>21</sup> The OFm group of **2** was removed by 20% piperidine/DMF to produce **3**, which was converted into **4** by HCl in dioxane. Each compound was purified by semipreparative RP-HPLC. Compounds **5–14** were prepared as follows: Boc-D-Trp-OH was coupled with the corresponding amine by a mixed anhydride method. The Boc group of the resulting compounds was removed by HCl in dioxane to produce their

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<sup>a</sup> Abbreviations: OFm, 9-fluorenyl methyl ester; RP-HPLC, reversed-phase high performance liquid chromatography; TFA, trifluoroacetic acid; AcOEt, ethyl acetate; MeOH, methanol; AcOH, acetic acid; *n*-BuOH, butanol; DMF, *N,N*-dimethylformamide; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-yldiphenyltetrazolium bromide; DIEA, *N,N*-diisopropylethylamine; PyBop, benzotriazol-1-yloxy-tris-pyrrolidinophosphonium hexafluorophosphate.

**Table 1.** Antiproliferative Activities of Somatostatin Analogues 1–15 on A431 Cells by MTT Test

compd	concentration of compound <sup>a</sup>		
	10 $\mu$ M	25 $\mu$ M	50 $\mu$ M
<b>1</b>	50 $\pm$ 9.4	60 $\pm$ 8.5	80 $\pm$ 7.4
cycloheximide	40 $\pm$ 4.6	70 $\pm$ 4.9	80 $\pm$ 3.1
<b>2</b>	2 $\pm$ 6.4	7 $\pm$ 3.9	7 $\pm$ 1.9
<b>3</b>	0 $\pm$ 4.6	0 $\pm$ 7.2	2 $\pm$ 6.2
<b>4</b>	0 $\pm$ 5.2	0 $\pm$ 4.3	0 $\pm$ 6.7
<b>5</b>	72 $\pm$ 1.4	90 $\pm$ 2.6	93 $\pm$ 1.7
<b>6</b>	26 $\pm$ 5.8	43 $\pm$ 5.7	95 $\pm$ 2.3
<b>7</b>	25 $\pm$ 6.1	21 $\pm$ 5.0	19 $\pm$ 3.9
<b>8</b>	3 $\pm$ 7.2	20 $\pm$ 6.0	31 $\pm$ 3.4
<b>9</b>	12 $\pm$ 3.7	18 $\pm$ 1.9	35 $\pm$ 5.7
<b>10</b>	19 $\pm$ 0.8	52 $\pm$ 5.8	91 $\pm$ 1.0
<b>11</b>	30 $\pm$ 5.4	76 $\pm$ 2.0	88 $\pm$ 9.0
<b>12</b>	7 $\pm$ 4.4	18 $\pm$ 7.6	40 $\pm$ 2.7
<b>13</b>	10 $\pm$ 7.2	5 $\pm$ 7.4	16 $\pm$ 1.0
<b>14</b>	0 $\pm$ 6.8	0 $\pm$ 6.5	22 $\pm$ 5.6
<b>15</b>	0 $\pm$ 9.5	8 $\pm$ 5.9	11 $\pm$ 6.8

<sup>a</sup> Antiproliferative activity of compounds is expressed as inhibition of cellular proliferation (%) and listed as the mean  $\pm$  SE.

corresponding amine hydrochlorides, which were coupled with Boc-Tyr-OH by using PyBop as a coupling reagent to give compounds **5**–**9**. Boc groups were removed by HCl in dioxane to produce compounds **10**–**14**, which were purified by semi-preparative RP-HPLC. In the case of the dipeptide (**15**), it was prepared using Boc-Tyr-OH and H-D-Trp-OFm as starting materials. All final compounds exhibited single peaks on analytical HPLC with unique retention times (see Supporting Information Table S3). All compounds were analyzed by MALDI-TOF mass spectrometry, <sup>1</sup>H and <sup>13</sup>C NMR, elemental analyses, and HPLC, in which all compounds (**2**–**15**) exhibited greater than 98% purity (see Supporting Information).

**Antiproliferative Activity.** The growth inhibitory activity of **2**–**15** on A431 human epidermal carcinoma cells was measured by MTT test<sup>22</sup> and summarized in Table 1. The linear peptides (**2**–**4**) containing only the biologically important sequence (-Tyr-D-Trp-Lys-), which is also present in molecule **1**, were examined. Whereas **2** exhibited 10% antiproliferative activity relative to **1**, the inhibitory activity of **3** and **4** was negligible. The HPLC analysis showed that **2** had a higher degree of hydrophobicity than **3** and **4** (retention time: 46.8, 36.6, and 29.4 min, respectively). These data suggested that protected N- and C-termini might contribute to manifestation of the inhibitory activity. With these results in mind, we turned our attention to shortening the peptide with hydrophobic moieties at the N- and C-termini to produce **5**–**9**. Nearly all compounds exhibited antiproliferative activity in a dose-dependent manner as shown in Table 1. Of these compounds, **5** and **6** proved to be the most potent; in fact, **5** exhibited 72% inhibition at a dose of 10  $\mu$ M, which was 1.5 times more potent than **1**. Peptides **5** and **6** have Boc protecting groups at the N-termini and 1- or 2-adamantylamine at the C-termini, with HPLC retention times of 43.0 and 42.0 min, respectively. Peptides **7**, **8**, and **9** have 1-naphthylamine, *tert*-butylamine, and aminomethylcoumarine at their C termini, with their retention times of 39.4, 37.2, and 37.1 min, respectively. Their antiproliferative activity was less than 30% (Table 1) on A431 cells compared to that of **5** and **6**. Removing Boc protecting groups from the N-termini of peptides (**5**–**9**) resulted in compounds (**10**–**14**) in which the peptides containing 1- and 2-adamantylamine (**10** and **11**) exhibited the highest activity on A431 cells, similar to that observed with **1**. Removal of the hydrophobic moieties from N- and C-termini produced H-Tyr-D-Trp-OH (**15**), which exhibited only 10% of the activity compared to **5** and **6**.

**Table 2.** Antiproliferative Activities of Somatostatin Analogues 5, 6, 10, 11 on SW480 cells by MTT Test

compd	concentration of compound <sup>a</sup>		
	10 $\mu$ M	25 $\mu$ M	50 $\mu$ M
<b>1</b>	13 $\pm$ 6.5	47 $\pm$ 0.7	88 $\pm$ 2.6
cycloheximide	68 $\pm$ 1.0	85 $\pm$ 2.6	92 $\pm$ 1.0
<b>5</b>	7 $\pm$ 4.8	53 $\pm$ 1.9	95 $\pm$ 2.4
<b>6</b>	9 $\pm$ 4.4	60 $\pm$ 1.3	74 $\pm$ 3.9
<b>10</b>	0 $\pm$ 3.2	15 $\pm$ 0.2	57 $\pm$ 4.6
<b>11</b>	13 $\pm$ 2.2	15 $\pm$ 1.6	52 $\pm$ 5.5

<sup>a</sup> Antiproliferative activity of compounds is expressed as inhibition of cellular proliferation (%) and listed as the mean  $\pm$  SE.

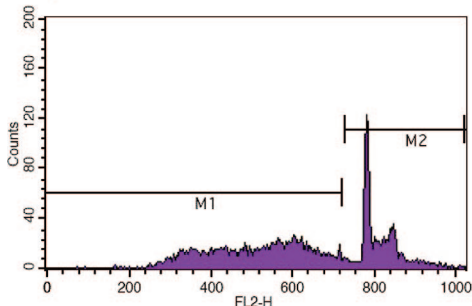
(Table 1). The growth inhibitory activities of **5**, **6**, **10**, and **11** on SW480 human colon carcinoma cells were tested similarly (Table 2). The peptides containing free NH<sub>2</sub> groups (**10** and **11**) lost some of their antiproliferative activity; namely, they exhibited less than 60% of the activity at the highest dose compared to **5** and **6**.

**Apoptosis.** Cell proliferation assay data suggested that there were at least four compounds (**5**, **6**, **10**, and **11**) with very strong effects in reducing the number of tumor cells when quantified by the MTT assay. Another means to decrease the number of cells is to induce apoptosis, which results in a drastic loss. To test this hypothesis, we continued with our biological analysis using flow cytometry (FACS), a method that is very efficient in detecting and measuring DNA fragmentation, a major hallmark of apoptosis. We detected an increase in the subG1 DNA fraction after the treatment with **5**, which antiproliferation studies revealed the greatest potency: **5** exhibited exceptionally potent apoptosis effects (Figure 2), supporting our hypothesis that **5** should have a potent antiproliferative effect through its induction of apoptosis.

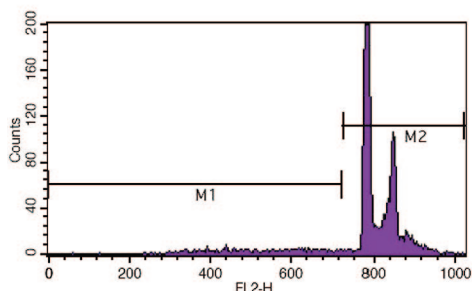
**Microscopic Study.** On the basis of the cell proliferation and FACS analysis data, four of the most active compounds (**5**, **6**, **10**, and **11**) were selected to visualize the morphological changes in tumor cells after the indicated treatment. Not surprisingly, microscopic visualization showed once again that **5** was the peptide that indeed changed cell morphology, thereby indicating directly revealing that apoptosis had been induced (see Supporting Information Figure S1).

## Conclusions

In this study, the new linear somatostatin analogues were prepared containing the biologically essential amino acid sequence (-Tyr-D-Trp-Lys-) present in the tumor-selective heptapeptide **1**. Compounds **5**–**14**, substitutions of the C-terminal Lys of the Tyr-D-Trp-Lys sequence with various hydrophobic and bulky residues (adamantyl-, naphthyl-, *tert*-butyl-, 4-methylcoumarinyl-) with or without Boc group at the N-termini, indicated that the compounds containing adamantylamine (**5**, **6**, **10**, and **11**) showed the most potent antiproliferative activity on A431 cells, even greater than **1**. In addition, **5** with a Boc group was the most active analogue in the induction of apoptosis. These results indicate that the potent activity in the tested cell lines might be attributed to the Tyr-D-Trp sequence coupled with a hydrophobic and bulky residue at the N-terminus and at the C-terminus, which thus increased hydrophobicity in the whole molecule. Our data further suggest that not only hydrophobicity but also aliphatic properties and rigidity are important at the C-terminus. On the basis of these structure–activity studies, we were able to develop and then select a highly promising apoptosis inducing dipeptide amide. Further studies are underway exploring the detailed mechanism

**Compound 5**

Marker	Events	%Gated	%Total
All	7592	100.00	45.11
M1	4715	62.10	28.01
M2	2849	37.53	16.93

**Control**

Marker	Events	%Gated	%Total
All	7129	100.00	73.18
M1	599	8.40	6.15
M2	6521	91.47	66.94

**Figure 2.** FACS analysis data of **5**. Compound **5** induces apoptotic cell death in A431 epidermal carcinoma cells. Cells were treated with **5** (30  $\mu$ M) for 48 h or left untreated and then quantified for their DNA content after propidium iodide (PI) staining. Values shown in boxes are the percent of cells with less DNA content than that of G1 phase cells (sub-G1). The experiment was independently repeated three times. In FACS analysis, FL2-H represents the fluorescence channel 2 and counts represent the counted cells during the analysis. M1 shows the apoptotic fraction of investigated cells (in %), and **5** induced strong apoptosis (~62%) even above the background (spontaneous apoptosis) of 8.4%.

of action and potential applications of our simplified **1** analogues with Tyr-D-Trp as an important biological core template.

## Experimental Section

**Boc-Tyr-D-Trp-Lys(Z)-OFm (2).** Boc-Tyr-OH (1.70 mmol) and H-D-Trp-Lys(Z)-OFm [prepared from Boc-D-Trp-Lys(Z)-OFm (1.2 g, 1.55 mmol) and 4 M HCl/dioxane (1.9 mL, 7.75 mmol)] were coupled with PyBop (1.70 mmol) in DMF (100 mL) containing DIEA (1.1 mL, 6.20 mmol). The mixture was stirred at 0 °C for 10 min and then room temperature overnight. After removal of the solvent, the residue was extracted with AcOEt, which was washed with 10% citric acid, 5% NaHCO<sub>3</sub> solution, and water and dried over Na<sub>2</sub>SO<sub>4</sub>. After removal of Na<sub>2</sub>SO<sub>4</sub> by filtration, the solvent of the filtrate was evaporated and the crude product was purified by open column silica gel chromatography. The compound was crystallized from a suitable solvent and filtered. The crude product was purified by open column silica gel chromatography (AcOEt/hexane = 2:1). The compound was precipitated with hexane. Yield 745 mg (56.1%), mp 97–103 °C,  $[\alpha]_D^{25}$  –10.7°(c 1.0, MeOH),  $R_f$  = 0.64 (CHCl<sub>3</sub>/MeOH/AcOH = 90:8:2).

**Boc-Tyr-D-Trp-Lys(Z)-OH (3).** The title compound was prepared from Boc-Tyr-D-Trp-Lys(Z)-OFm (**2**) (1.2 g, 1.31 mmol) and 20% piperidine/DMF (40 mL), and the crude product was recrystallized from ether. Yield 377 mg (29.0%), mp 108–112 °C,  $[\alpha]_D^{25}$  +9.76°(c 1.0, MeOH),  $R_f$  = 0.47 (CHCl<sub>3</sub>/MeOH/H<sub>2</sub>O = 8:3:1).

**HCl·H-Tyr-D-Trp-Lys(Z)-OH (4).** The title compound was prepared from Boc-Tyr-D-Trp-Lys(Z)-OH (**3**) (795 mg, 1.09 mmol) and 4 M HCl/dioxane (1.4 mL, 5.45 mmol). The crude product was purified with semipreparative RP-HPLC and lyophilized from 1 M HCl. Yield 146 mg (20.1%), amorphous,  $[\alpha]_D^{25}$  –83.5°(c 1.0, H<sub>2</sub>O),  $R_f$  = 0.38 (*n*-BuOH/AcOH/H<sub>2</sub>O = 4:1:5).

**General Procedure for Synthesis of Boc-Tyr-D-Trp-X [X: 1-Adamantylamide (5), 2-Adamantylamide (6), 1-Naphthylamide (7), *tert*-Butylamide (8), and 4-Methylcoumarin-7-ylamide (9)].** Boc-Tyr-OH (576 mg, 2.05 mmol) and H-D-Trp-X [prepared from Boc-D-Trp-X (1.86 mmol) and 4 M HCl/dioxane (2.3 mL, 9.32 mmol)] in DMF (50 mL) were coupled using PyBop reagent (1.1 g, 2.05 mmol) and DIEA (1.9 mL, 11.2 mmol) at room temperature overnight. After removal of the solvent, the residue was extracted with AcOEt, which was washed with 10% citric acid, 5% NaHCO<sub>3</sub>, and water and dried over Na<sub>2</sub>SO<sub>4</sub>. After removal of Na<sub>2</sub>SO<sub>4</sub> by filtration, the solvent of the filtrate was evaporated.

**Boc-Tyr-D-Trp-1-adamantylamide (5).** The crude product was purified by open column silica gel chromatography (AcOEt/hexane = 2:1) and precipitated with petroleum ether. Yield 1.14 g (82.6%), mp 140–143 °C,  $[\alpha]_D^{25}$  +14.7°(c 1.0, MeOH),  $R_f$  = 0.64 (AcOEt/hexane = 1:1).

**Boc-Tyr-D-Trp-2-adamantylamide (6).** The crude product was purified by open column silica gel chromatography (AcOEt/hexane = 2:1) and precipitated with petroleum ether. Yield 1.19 g (86.2%), mp 138–141 °C,  $[\alpha]_D^{25}$  +11.0°(c 1.0, MeOH),  $R_f$  = 0.51 (AcOEt/hexane = 1:1).

**Boc-Tyr-D-Trp-1-naphthylamide (7).** The crude product was recrystallized from MeOH. Yield 825 mg (59.8%), mp 237–239 °C,  $[\alpha]_D^{25}$  +18.0°(c 1.0, MeOH),  $R_f$  = 0.64 (CHCl<sub>3</sub>/MeOH/AcOH = 90:8:2).

**Boc-Tyr-D-Trp-*tert*-butylamide (8).** The crude product was purified by open column silica gel chromatography (AcOEt/hexane = 2:1) and precipitated with petroleum ether. Yield 854 mg (87.6%), mp 113–115 °C,  $[\alpha]_D^{25}$  +15.0°(c 1.0, MeOH),  $R_f$  = 0.57 (AcOEt/hexane = 1:1).

**Boc-Tyr-D-Trp-4-methylcoumarin-7-ylamide (9).** The crude material was purified by open column silica gel chromatography (3–5% MeOH in CHCl<sub>3</sub>) and precipitated with petroleum ether. Yield 286 mg (27.8%), mp 232–235 °C,  $[\alpha]_D^{25}$  +99.6°(c 1.0, MeOH),  $R_f$  = 0.43 (AcOEt/hexane = 1:1).

**General Procedure for Synthesis of HCl·H-Tyr-D-Trp-X [X: 1-Adamantylamide (10), 2-Adamantylamide (11), 1-Naphthylamide (12), *tert*-Butylamide (13), and 4-Methylcoumarin-7-ylamide (14)].** Boc-Tyr-D-Trp-X (0.166 mmol) was treated with 4 M HCl/dioxane (0.4 mL, 1.67 mmol) at room temperature for 1 h. Dry ether was added to a solution to form a precipitate, which was collected by filtration and washed with dry ether. The crude product was purified by semipreparative RP-HPLC and lyophilized from 1 M HCl.

**HCl·H-Tyr-D-Trp-1-adamantylamide (10).** Yield 150 mg (83.8%), amorphous,  $[\alpha]_D^{25}$  +24.6°(c 1.0, MeOH),  $R_f$  = 0.81 (*n*-BuOH/AcOH/pyridine/H<sub>2</sub>O = 4:1:1:2).

**HCl·H-Tyr-D-Trp-2-adamantylamide (11).** Yield 78.5 mg (87.9%), amorphous,  $[\alpha]_D^{25}$  +28.3°(c 1.0, MeOH),  $R_f$  = 0.82 (*n*-BuOH/AcOH/pyridine/H<sub>2</sub>O = 4:1:1:2).

**HCl·H-Tyr-D-Trp-1-naphthylamide (12).** Yield 73.5 mg (82.2%), amorphous,  $[\alpha]_D^{25}$  +28.2°(c 1.0, MeOH),  $R_f$  = 0.72 (*n*-BuOH/AcOH/pyridine/H<sub>2</sub>O = 4:1:1:2).

**HCl·H-Tyr-D-Trp-*tert*-butylamide (13).** Yield 71.7 mg (81.8%), amorphous,  $[\alpha]_D^{25}$  +35.5°(c 1.0, MeOH),  $R_f$  = 0.75 (*n*-BuOH/AcOH/pyridine/H<sub>2</sub>O = 4:1:1:2).

**HCl·H-Tyr-D-Trp-4-methylcoumarin-7-ylamide (14).** Yield 84.0 mg (93.3%), amorphous,  $[\alpha]_D^{25}$  –24.6°(c 1.0, MeOH),  $R_f$  = 0.74 (*n*-BuOH/AcOH/pyridine/H<sub>2</sub>O = 4:1:1:2).



**HCl·H-Tyr-D-Trp-OH (15).** Boc-Tyr-D-Trp-OFm (211 mg, 0.232 mmol) was treated with 20% piperidine/DMF (10 mL) followed by treatment with 4 M HCl/dioxane (0.6 mL, 2.32 mmol) to give the title compound, which was crystallized from ether. The crude product was purified by semipreparative RP-HPLC and lyophilized from 1 M HCl. Yield 65.8 mg (70.2%), amorphous,  $[\alpha]_D^{25} +26.3^\circ$  (c 1.0, MeOH),  $R_f = 0.46$  (n-BuOH/AcOH/pyridine/H<sub>2</sub>O = 4:1:1:2).

**Antiproliferative Activity.** Two cell lines are well-characterized, fast-growing, and represent excellent model systems for antiproliferative studies. As a routine and quantitative test method, the MTT assay was applied for the quantification of the differences in the number of cells after treatment with the compounds in addition to cycloheximide and **1** that served as positive controls, screening the 14 compounds derived from **1**. Each analogue was tested at 10, 25, and 50  $\mu$ M. Cycloheximide was used as an external standard as a well-known and efficient apoptosis inducer and cell proliferation inhibitor. Antiproliferative activity was examined using the MTT.

**MTT Analysis.** MTT assay is based on the mitochondrial dehydrogenase enzyme that cleaves the tetrazolium rings of the pale-yellow MTT in viable cells and that form a dark-blue formazan crystals which is largely impermeable to membranes resulting in its accumulation in healthy cells. Solubilization of the cells by the addition of DMSO results in the liberation of the crystals which are solubilized. The amount of surviving cells is directly proportional to the level of the formazan product created. The color can be quantified using a colorimetric assay using an ELISA reader at 540 nm based on the reduction of the soluble yellow MTT tetrazolium salt to a blue insoluble formazan product using the mitochondrial succinic dehydrogenase in live cells.

**Apoptosis (Flow Cytometric Analysis).** A431 cells were incubated at 37 °C for 24 h in DMEM supplemented with 10% FCS using 24-well plates, seeding 50 000 cells per well. After treatment, the cells were washed, fixed with 70% ethanol, and stored at -20 °C or used for propidium iodide (PI) staining. The stained cells were subjected to flow cytometry (FACS Calibur, BD Biosciences) to detect and quantify apoptosis. Cells with their DNA content less than that of G1 phase cells (sub-G1) were assumed to be apoptotic. The analysis was performed with CellQuest software.

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**Supporting Information Available:** Synthetic procedures, <sup>1</sup>H and <sup>13</sup>C NMR, elemental analysis, and HPLC data for all the new compounds, and biological assay procedures. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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