Journal of Asian Natural Products Research
Vol. 12, No. 1, January 2010, 36–42

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Synthesis and antitumor activity of bisindolylmaleimide and amino acid ester conjugates

Ke Wang, Xiang-Yan Li, Xiao-Guang Chen and Zhan-Zhu Liu*

Key Laboratory of Bioactive Substances and Resources Utilization of Chinese Herbal Medicine (Ministry of Education), Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China

(*Received 9 September 2009; final version received 13 October 2009*)

A series of novel bisindolylmaleimide and natural amino acid ester conjugates were synthesized and evaluated for their inhibitory activity against six tumor cell lines. Some compounds displayed interesting cytotoxic profiles. The most active compound **8e** showed inhibitory activity against several human cancer cell lines.

Keywords: bisindolylmaleimide; amino acid ester; conjugate; antitumor activity

1. Introduction

Staurosporine (Figure 1), a well-known member of the indolocarbazole alkaloid, was first isolated from *Streptomyces staurosporeus* by Omura *et al.* [1]. It is an appealing molecule with interesting biological properties, including antimicrobial [1], hypotensive [2], cell cytotoxic [2], and inhibition of protein kinase C [3]. However, the lack of specificity limits its utilization as a tool for studying protein kinase. In addition, extremely poor water solubility has plagued the development of staurosporine as a therapeutic agent. Staurosporine has become a lead structure for novel antitumor agents. For example, bisindolylmaleimides [4], in which the planarity of the indolocarbazole ring system has been disrupted, have valuable pharmacological properties. Many derivatives of bisindolylmaleimides have been synthesized, and their biological activities were evaluated [5]. Some of the bisindolylmaleimide derivatives are currently in clinical trial [6].

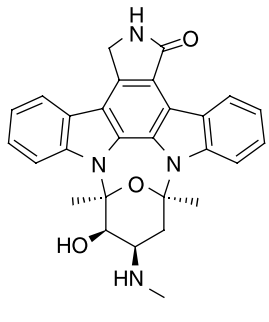
It is well known that amino acids are the fundamental building blocks of biological systems and the biosynthetic precursors of many natural products [7]. With the aim of increasing the water solubility of indolocarbazoles and improving the binding properties to their possible targets, amino acids have already been applied in the modification of indolocarbazoles [8] and bisindolylmaleimide derivatives [9]. In this paper, a series of novel bisindolylmaleimide and natural amino acid ester conjugate derivatives are synthesized, and their antiproliferative activities against six human tumor cell lines are evaluated.

Synthesis of the target compounds basically followed the Suzuki reaction-based synthetic route established previously by our group [10]. Firstly,

*Corresponding author. Email: liuzhanzhu@imm.ac.cn

ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020903413385
http://www.informaworld.com



Staurosporine

Figure 1. Structure of staurosporine.

preparation of the indole boronic acid (**4**) from indole (**1**) was accomplished through the following steps: protection of the indole NH with TsCl and then treatment with $Hg(OAc)_2$ to yield compound **3**; reaction of **3** with BH_3·THF followed by hydrolytic workup gave **4** in a total yield of 82% [11]. Then, bisindolylmaleimide (**5**) was synthesized by the Suzuki cross-coupling of indole boronic acid (**4**) and *N*-methyldibromomaleimide. With compound **5** in hand, cleavage of both indole nitrogen protective groups was readily accomplished [12], and basic treatment of imide **6** led to anhydride **7** [13]. Finally, treatment of **7** with amino acid esters in toluene under refluxing gave the desired product **8** in a reasonable yield (Scheme 1).

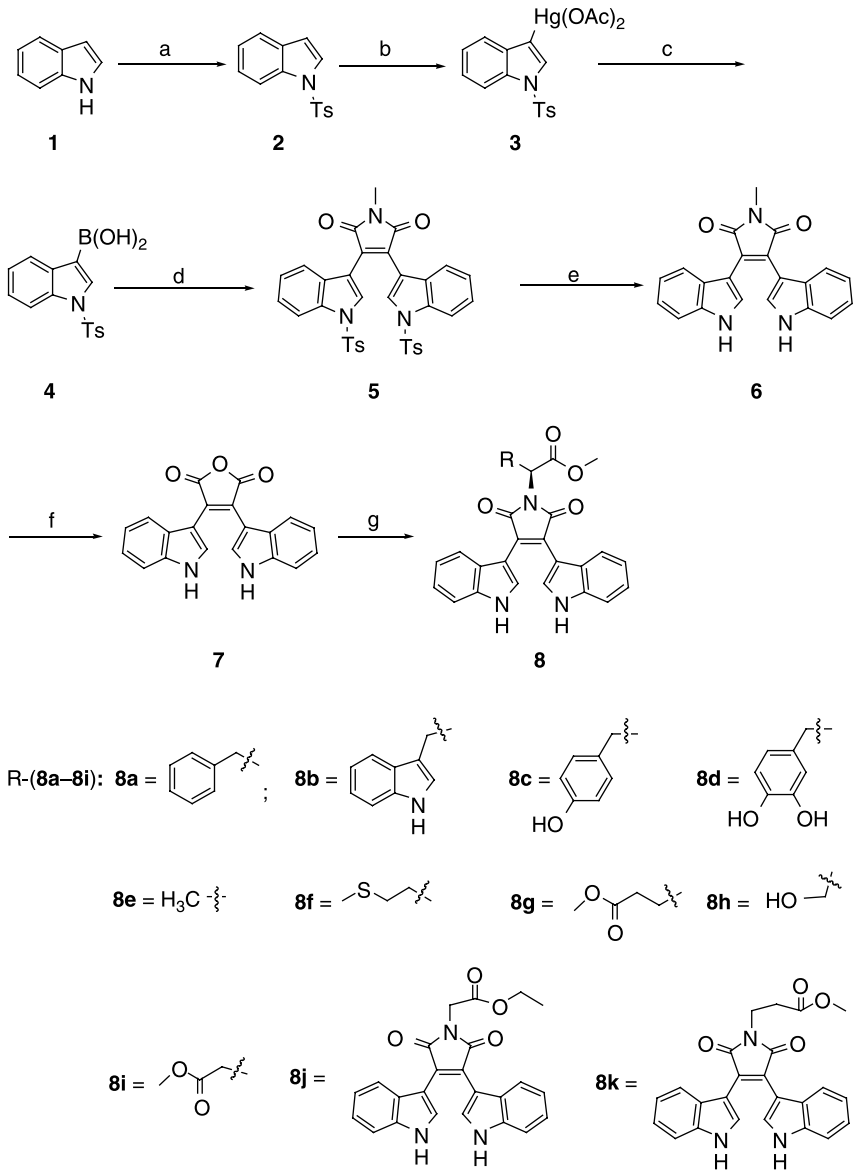
2. Results and discussion

By the MTT assay, cytotoxicity of these derivatives was evaluated against six human cancer cell lines: human hepatocellular liver carcinoma (HepG2), human colon carcinoma (HCT-116), human ileocecal adenocarcinoma (HCT-8), human colon carcinoma (HT-29), human pancreatic adenocarcinoma (Bxpc-3), human erythroleukemia (k562). The results of the cytotoxicity studies are summarized in Table 1.

Among the four derivatives of aromatic amino acids **8a**–**8d**, compound **8a**, which was derived from L-phenylalanine, exhibited inhibitory activity against HepG2, HCT-8, and HT-29 cell lines, with the inhibitory concentration (IC_{50}) values of 14.7, 26.9, and 24.7 μM, respectively. Compound **8c**, which was derived from L-tyrosine, showed cytotoxicity against HCT-116, with the IC_{50} value of 30.1 μM. However, compounds **8b** and **8d**, which were the derivatives of L-tryptophane and L-DOPA, respectively, exhibited no cytotoxicity against all the tumor cell lines tested.

As for the seven derivatives of the aliphatic natural amino acids **8e**–**8k**, compound **8e**, which was derived from L-alanine, exhibited broad-spectrum inhibitory activities against HepG2, HCT-116, HCT-8, and k562 cell lines. However, compounds **8f** and **8h**, which were derived from L-methionine and L-serine, respectively, were inactive to all tumor cell lines screened at a concentration of 100 μM. Compounds **8j** and **8k** were two achiral derivatives which were synthesized from glycine and β-aminopropionic acid, respectively. Compound **8j** exhibited cytotoxicity against Bxpc-3, with an IC_{50} value of 25 μM, while compound **8k** exhibited inhibitory activities against HCT-116 and HT-29 cell lines, with IC_{50} values of 27.7 and 25.4 μM, respectively. Bearing one more carbonyl group on the side chain, compound **8g**, a derivative of L-glutaminic acid, exhibited inhibitory activities against HCT-8 and k562 cell lines, with IC_{50} values of 24.8 and 24.9 μM, respectively, while compound **8i**, a derivative of L-asparagic acid, exhibited inhibitory activities against HCT-116, Bxpc-3, and k562 cell lines, with IC_{50} values of 20.2, 26, and 8.7 μM, respectively.

In summary, we have incorporated a number of natural amino acids into the bisindolylmaleimide skeleton, and the derivatives exhibited antiproliferative activity and selectivity against six human cancer cell lines. Further studies on the mechanism of action and the structure–activity relationship of this type of compounds are in progress and will be reported in due course.



Scheme 1. Reagents and conditions: (a) TsCl, KOH, H_2O, Bu_4NHSO_4, 96%; (b) $Hg(OAc)_2$, AcOH, H_2O, $HClO_4$(cat), 99%; (c) 1. BH_3·THF; 2. H_2O, 85%; (d) *N*-methyldibromomaleimide, $Pd(PPh_3)_4$, MeOH, PhH, 2M Na_2CO_3, 10 h, 46%; (e) K_2CO_3, CH_3OH, H_2O, 84%; (f) KOH, EtOH, 82%; (g) amino acid ester, toluene, reflux.

3. Experimental

3.1 General experimental procedures

Melting points were recorded on a Yanaco melting point apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer Polarimeter 341LC using 10 cm cells and the sodium D line (589 nm) at 20°C and concentration indicated. NMR spectra were recorded on a Varian Oxford 300 (^{1}H: 300 MHz, ^{13}C: 75 MHz) or Varian Oxford 400 (^{1}H: 400 MHz, ^{13}C: 100 MHz), chemical shifts (δ) are expressed in ppm, and the

Table 1. *In vitro* cytotoxicity of compounds **8a**–**8k** (IC_{50}, μM).

Compound	HepG2	HCT-116	HCT-8	HT-29	Bxpc-3	k562
8a	14.7	–	26.9	24.7	–	–
8c	–	30.1	–	–	–	–
8e	14.0	31.1	22.8	–	–	6.1
8g	–	–	24.8	–	–	24.9
8i	–	20.2	–	–	26.0	8.7
8j	–	–	–	–	25.0	–
8k	–	27.7	–	25.4	–	–

Note: The IC_{50} values represent the compound concentration (μM) required to inhibit tumor cell proliferation by 50%.

following abbreviations are used: singlet (s), doublet (d), triplet (t), doubled doublet (dd), multiplet (m). HRMS were carried out by Agilent LC/MSD TOF.

3.2 General procedure for compounds 8a–k

To a solution of anhydride **7** (30 mmol) in toluene (15 ml), amino acid ester (36 mmol) was added. The mixture was refluxed for 12 h. After cooling, the solvent was removed under reduced pressure, and the residue was purified by flash chromatography to give the desired compound.

3.2.1 Compound 8a

Using **7** and (L)-phenylalanine methyl ester as starting materials, the title compound **8a** was obtained as a red solid; mp >250°C; $[\alpha]_D^{20}$: −93.3 (c = 1.0, CH_3OH); ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) 3.45 (m, 2H), 3.72 (s, 3H), 5.18 (dd, 1H, J = 11.4, 4.8 Hz), 6.67 (m, 4H), 6.97 (m, 2H), 7.15 (m, 1H), 7.22 (m, 4H), 7.34 (d, 2H, J = 8.4 Hz), 7.67 (d, 2H, J = 3.0 Hz), 11.69 (d, 2H, J = 2.7 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 34.1, 52.6, 59.7, 105.2, 111.8, 119.5, 120.8, 121.7, 125.1, 126.7, 127.2, 128.3, 128.9, 129.4, 136.0, 137.1, 169.6 (C═O), 170.7 (C═O); HR-ESI-MS: *m/z* 490.1751 $[M+H]^+$ (calcd for $C_{30}H_{24}N_3O_4$, 490.1761).

3.2.2 Compound 8b

Using **7** and (L)-trytophan methyl ester as starting materials, the title compound **8b** was obtained as a red solid; mp >250°C; $[\alpha]_D^{20}$: −123.0 (c = 1.0, CH_3OH); ^{1}H NMR (300 MHz, CD_3OD): δ (ppm) 3.57 (d, J = 3.6 Hz), 3.72 (s, 3H), 5.08 (dd, 1H, J = 6.3, 9.0 Hz), 6.51 (m, 4H), 6.86 (m, 5H), 7.21 (m, 3H), 7.42 (s, 1H), 7.44 (s, 2H); ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) 25.9, 53.2, 54.5, 107.4, 111.3, 112.3, 119.2, 119.9, 120.6, 122.3, 122.6, 123.0, 124.5, 126.9, 128.7, 129.9, 137.7, 172.0 (C═O), 173.1 (C═O); HR-ESI-MS: *m/z* 528.1779 $[M+H]^+$ (calcd for $C_{32}H_{24}N_4O_4$, 528.1792).

3.2.3 Compound 8c

Using **7** and (L)-tyrosine methyl ester as starting materials, the title compound **8c** was obtained as a red solid; mp 217.1–221.3°C; $[\alpha]_D^{20}$: −171.0 (c = 1.0, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 3.49 (m, 2H), 3.81 (s, 3H), 5.07 (dd, 1H, J = 10.2, 6.0 Hz), 6.67 (d, 2H, J = 8.7 Hz), 6.76 (m, 2H), 6.90 (d, 2H, J = 5.2 Hz), 7.08 (m, 4H), 7.31 (d, 2H, J = 8.4 Hz), 7.65 (d, 2H, J = 2.7 Hz), 8.48 (s, 2H); ^{13}C NMR (75 MHz, CD_3OD): δ (ppm) 35.0, 53.2, 54.9, 107.4, 112.4, 116.3, 120.7, 122.5, 123.0, 126.9, 128.6, 129.2, 130.0, 131.3, 137.7, 157.2, 171.7 (C═O), 173.1 (C═O); HR-ESI-MS: *m/z* 506.1708 $[M+H]^+$ (calcd for $C_{30}H_{24}N_3O_5$, 506.1710).

3.2.4 Compound **8d**

Using **7** and (L)-doba methyl ester as starting materials, the title compound **8d** was obtained as a red solid; mp >250°C; $[\alpha]_D^{20}$: −162.4 (c = 1.0, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 3.44 (m, 2H), 3.81 (s, 3H), 5.07 (dd, 1H, J = 10.2, 5.7 Hz), 5.18 (s, 1H), 5.31 (s, 1H), 6.72 (m, 5H), 6.91 (d, 2H, J = 8.1 Hz), 7.07 (t, 2H, J = 8.4 Hz), 7.28 (d, 2H, J = 8.1 Hz), 7.59 (d, 2H, J = 1.5 Hz), 8.49 (s, 2H); ^{13}C NMR (75 MHz, CD_3OD): δ (ppm) 35.2, 53.2, 54.9, 107.4, 112.4, 116.4, 117.3, 120.7, 121.6, 122.6, 123.0, 126.9, 128.6, 130.0, 137.7, 145.1, 146.2, 171.8 (C═O), 173.1 (C═O); HR-ESI-MS: m/z 522.1668 $[M+H]^+$ (calcd for $C_{30}H_{24}N_3O_6$, 522.1660).

3.2.5 Compound **8e**

Using **7** and (L)-alanine methyl ester as starting materials, the title compound **8e** was obtained as a red solid; mp 211.5–214.7°C; $[\alpha]_D^{20}$: + 60.0 (c = 1.0, CH_3OH); ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) 1.56 (d, 3H, J = 6.9 Hz), 3.67 (s, 3H), 4.96 (q, 1H, J = 7.2 Hz), 6.60 (m, 2H), 6.77 (d, 2H, J = 8.1 Hz), 6.97 (m, 2H), 7.36 (d, 2H, J = 8.7 Hz), 7.78 (d, 2H, J = 3.0 Hz), 11.72 (d, 2H, J = 2.4 Hz); ^{13}C NMR (100 MHz, CD_3OD): δ (ppm) 15.7, 24.2, 53.2, 107.6, 112.4, 120.7, 122.6, 123.0, 127.1, 128.7, 130.1, 137.7, 172.6 (C═O), 173.0 (C═O); HR-ESI-MS: m/z 414.1457 $[M+H]^+$ (calcd for $C_{24}H_{20}N_3O_4$, 414.1448).

3.2.6 Compound **8f**

Using **7** and (L)-methionine methyl ester as starting materials, the title compound **8f** was obtained as a red solid; mp 209.0–212.0°C; $[\alpha]_D^{20}$: + 6.0 (c = 1.0, CH_3OH); ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) 2.04 (s, 3H), 2.38 (m, 2H), 2.56 (m, 2H), 3.68 (s, 3H), 5.02 (dd, 1H, J = 8.7, 6.3 Hz), 6.63 (m, 2H), 6.78 (d, 2H, J = 7.8 Hz), 6.98 (m, 2H), 7.36 (d, 2H, J = 8.1 Hz), 7.79 (d, 2H, J = 2.7 Hz), 11.73 (d, 2H, J = 2.4 Hz); ^{13}C NMR (100 MHz, CD_3OD): δ (ppm) 20.9, 24.8, 29.6, 53.1, 53.2, 107.5, 107.6, 112.3, 112.4, 120.6, 120.7, 122.6, 122.7, 122.9, 123.0, 127.0, 127.0, 128.5, 128.6, 130.0, 130.3, 137.7, 137.7, 172.1 (C═O), 173.3 (C═O), 174.1 (C═O); HR-ESI-MS: m/z 474.1577 $[M+H]^+$ (calcd for $C_{26}H_{24}N_3O_4S$, 474.1488).

3.2.7 Compound **8g**

Using **7** and (L)-glutamic dimethyl ester as starting materials, the title compound **8g** was obtained as a red solid; mp >250°C; $[\alpha]_D^{20}$: + 15.0 (c = 1.0, CH_3OH); ^{1}H NMR (300 MHz, CD_3OD): δ (ppm) 2.40 (m, 4H), 3.48 (s, 3 H), 3.66 (s, 3H), 4.85 (dd, 1H, J = 9.0, 5.4 Hz), 6.53 (m, 2H), 6.78 (m, 2H), 6.90 (m, 2H), 7.26 (m, 2H), 7.67 (s, 2H); ^{13}C NMR (75 MHz, CD_3OD): δ (ppm) 25.5, 31.6, 52.2, 52.5, 53.2, 107.5, 112.4, 120.7, 122.6, 123.1, 127.0, 128.6, 130.2, 137.7, 171.6 (C═O), 173.1 (C═O), 174.8 (C═O); HR-ESI-MS: m/z 486.1750 $[M+H]^+$ (calcd for $C_{27}H_{24}N_3O_6$, 486.1660).

3.2.8 Compound **8h**

Using **7** and (L)-serine methyl ester as starting materials, the title compound **8h** was obtained as a red solid; mp 202.4–207.5°C; $[\alpha]_D^{20}$: + 42.0 (c = 1.0, CH_3OH); ^{1}H NMR (300 MHz, CD_3OD): δ (ppm) 3.71 (s, 3H), 4.17 (m, 2H), 4.96 (dd, 1H, J = 8.1, 6.6 Hz), 6.54 (t, 2H, J = 8.1 Hz), 6.77 (d, 2H, J = 8.1 Hz), 6.92 (t, 2H, J = 8.1 Hz), 7.26 (d, 2H, J = 8.1 Hz), 7.71 (s, 2H); ^{13}C NMR (100 MHz, CD_3OD): δ (ppm) 53.1, 55.5, 60.4, 107.6, 112.4, 120.7, 122.6, 123.0, 127.1, 128.8, 130.1, 137.7, 170.3 (C═O), 173.4 (C═O); HR-ESI-MS: m/z 430.1394 $[M+H]^+$ (calcd for $C_{24}H_{20}N_3O_5$, 430.1403).

3.2.9 Compound **8i**

Using **7** and (L)-aspartic acid dimethyl ester as starting materials, the title compound **8i** was obtained as a red solid;

mp >250°C; $[\alpha]_D^{20}$: + 12.0 (c = 1.0, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 3.41 (q, 2H, J = 6.3 Hz), 3.72 (s, 3H), 3.78 (s, 3H), 5.44 (dd, 1H, J = 6.3, 8.4 Hz), 6.74 (m, 2H), 6.94 (d, 2H, J = 8.1 Hz), 7.06 (m, 2H), 7.31 (d, 2H, J = 8.1 Hz), 7.75 (d, 2H, J = 3.0 Hz), 8.62 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 33.7, 47.7, 51.8, 52.9, 105.4, 111.9, 119.5, 121.0, 121.8, 125.2, 126.8, 129.6, 136.0, 169.1 (C═O), 170.4 (C═O), 170.5 (C═O); HR-ESI-MS: m/z 472.1501 $[M+H]^+$ (calcd for $C_{26}H_{22}N_3O_6$, 472.1503).

3.2.10 Compound *8j*

Using **7** and glycine ethyl ester as starting materials, the title compound **8j** was obtained as a red solid; mp 241.3–246.0°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) 1.22 (t, 3H, J = 7.2 Hz), 4.16 (q, 2H, J = 7.2 Hz), 4.39 (s, 2H), 6.63 (m, 2H), 6.77 (d, 2H, J = 8.1 Hz), 6.98 (m, 2H,), 7.36 (d, 2H, J = 8.1 Hz), 7.78 (d, 2H, J = 2.7 Hz), 11.72 (d, 2H, J = 2.4 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 14.0, 30.6, 61.3, 105.4, 111.8, 119.5, 120.9, 121.7, 125.2, 127.0, 129.4, 136.0, 168.0 (C═O), 170.9 (C═O); HR-ESI-MS: m/z 414.1457 $[M+H]^+$ (calcd for $C_{24}H_{20}N_3O_4$, 414.1448).

3.2.11 Compound *8k*

Using **7** and (β)-alanine methyl ester as starting materials, the title compound **8k** was obtained as a red solid; mp 214.3–216.7°C; ^{1}H NMR (300 MHz, CD_3COCD_3): δ (ppm) 2.77 (t, 2H, J = 7.5 Hz), 3.64 (s, 3H), 3.93 (t, 2H, J = 7.5 Hz), 6.60 (m, 2H), 6.94 (m, 4H), 7.39 (m, 2H), 7.85 (d, 2H, J = 2.1 Hz), 10.83 (s, 2H); ^{13}C NMR (100 MHz, CD_3COCD_3): δ (ppm) 33.6, 34.7, 51.9, 107.5, 112.4, 120.4, 122.3, 122.7, 126.8, 128.3, 129.9, 127.3, 171.9 (C═O), 172.4 (C═O); HR-ESI-MS: m/z 414.1451 $[M+H]^+$ (calcd for $C_{24}H_{20}N_3O_4$, 414.1448).

3.3 Cell cultures and proliferation assays

The tumor cell lines panel consisted of HepG2, HCT-8, HCT-116, HT-29, Bxpc-3, and k562. Human cancer cells were cultured in RPMI-1640 or DMEM/F12 supplemented with 10% fetal bovine serum, containing penicillin and streptomycin at 37°C and humidified at 5% CO_2. Briefly, cells were plated in the appropriate media on 96-well plates in a total volume of 100 μl at a density of $1–2.5 \times 10^4$ cells/ml and were allowed to adhere for 24 h before treatment with tested drugs in DMSO solution (10^{-5}, 10^{-6}, 10^{-7} mol/l final concentration). Triplicate wells were treated with media and agents. Cell viability was assayed after 96 h continuous drug exposure with a tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium salt; MTT (0.5 mg/ml, 100 μl); Ameresco Corp., Anchorage, AK, USA] in a fresh medium. After the medium was removed, 150 μl of DMSO was added to each well. The plates were gently agitated until the color reaction was uniform and an OD_{570} was determined using a microplate reader (Wellscan MK3, Labsystems Dragon, Helsinki, Finland). Microsoft_Excel 2003 was used for data analysis. Media-only treated cells served as the indicator of 100% cell viability. The 50% IC_{50} was defined as the concentration that reduced the absorbance of the untreated wells by 50% of the vehicle in the MTT assay. Assays were performed in triplicate on three independent experiments.

References

[1] S. Omura, Y. Iwai, A. Hirano, A. Nakagawa, J. Awaya, H. Tsuchiya, Y. Takahashi, and R. Masumam, *J. Antibiot.* **30**, 275 (1977).

[2] (a) S. Cesar, M. Carmen, and A.S. Jose, *Nat. Prod. Rep.* **23**, 1007 (2006). (b) S. Omura, Y. Iwai, and A. Hirano, *Japan Kokai* 78 73,501, *Chem. Abstr.* **89**,

178086b (1978). (c) S. Moura and A. Iwai, *Ger Offen.* 2,745,326, *Chem. Abstr.* **89**, 58348 (1978).

[3] (a) H. Kase, K. Iwahashi, and Y. Matsuda, *J. Antibiot.* **39**, 1059 (1986). (b) T. Tamaoki, H. Nomoto, I. Takahishi, Y. Kato, M. Morimoto, and F. Tomita, *Biochem. Biophys. Res. Commun.* **135**, 397 (1986).

[4] (a) S. Roy, S. Roy, and G.W. Gribble, *Org. Lett.* **8**, 4975 (2006). (b) S. Bartlett and A. Nelson, *Chem. Commun.* **9**, 1112 (2004).

[5] (a) M.F. Brana, L. Anorbe, G. Tarrason, F. Mitjans, and J. Piulats, *Bioorg. Med. Chem. Lett.* **11**, 2701 (2001). (b) D. Toullec, P. Pianetti, H. Coste, P. Bellevergue, P.T. Grand, M. Ajakane, V. Baudet, P. Boissin, E. Boursier, F. Loriolle, L. Duhamel, D. Charon, and J. Kirilovsky, *J. Biol. Chem.* **266**, 15771 (1991). (c) R.A. Bit, P.D. Davis, L.H. Elliott, W. Harris, C.H. Hill, E. Keech, H. Kumar, G. Lawton, A. Maw, J.S. Nixon, J.D.R. Vesey, J. Wadsworth, and S.E. Wilkinson, *J. Med. Chem.* **36**, 21 (1993).

[6] (a) P.D. Davis, C.H. Hill, and G. Lawton, *Eur. Patent Appl.* EP 328026 (1989). (b) M. Sodeoka, M. Katoh, and M. Fujita, *PCT Int. Appl.* WO 2005068455 (2005). (c) H. Osswald and R. Reck, *Eur. Patent Appl*. EP 397060 (1990). (d) U.H. Dhingra, D.M. Huryn, and D.D. Keith, *PCT Int. Appl.* WO 9804551 (1998).

[7] M. Zhao, L. Bi, W. Wang, C. Wang, J. Ju, and S. Peng, *Bioorg. Med. Chem. Lett.* **14**, 6998 (2006).

[8] (a) P. Moreau, M. Sancelme, C. Bailly, S. Leonce, A. Pierre, J. Hickman, B. Pfeiffer, and M. Prudhomme, *Eur. J. Med. Chem.* **36**, 887 (2001). (b) Z. Li, F. Zhai, L. Zhao, Q. Guo, and Q. You, *Bioorg. Med. Chem. Lett.* **19**, 406 (2009).

[9] G. Xie, J. Zimmermann, T. Meyer, and J.W. Lown, *Bioorg. Med. Chem. Lett.* **5**, 497 (1995).

[10] K. Wang and Z.Z. Liu, *Synth. Commun.* (2009) in press.

[11] N.K. Garg, R. Sarpong, and B.M. Stoltz, *J. Am. Chem. Soc.* **124**, 13179 (2002).

[12] M.M. Faul, K.A. Sullivan, and L.L. Winneroski, *Synthesis* **12**, 1511 (1995).

[13] M. Brenner, H. Rexhausen, B. Steffan, and W. Steglich, *Tetrahedron* **44**, 2887 (1988).