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Chinese Chemical Letters 23 (2012) 462–465

CHINESE CHEMICAL LETTERS

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Synthesis and cytotoxic activities of a series of novel *N*-methylbisindolylmaleimide amino acid ester conjugates

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Received 7 December 2011
Available online 3 March 2012

Abstract

A series of novel *N*-methylbisindolylmaleimides and natural amino acid conjugates were synthesized and evaluated for their inhibitory activity against six tumor cell lines. Most of the compounds exhibited moderate *in vitro* cytotoxic activities in the range of 10–100 μmol/L.

Keywords: Bisindolylmaleimide; Amino acid; Synthesis; Cytotoxicity

Staurosporine (Fig. 1) was isolated from *Streptomyces staurosporeus* in 1977. It has been found to possess a wide range of biological activities, including antifungal, antimicrobial, hypotensive, platelet aggregation activities and cell cytotoxicity. The mechanism of its cytotoxic activity against tumor cells was the inhibition of protein kinase C, which is the most important aspect of its biological profile [1–4]. However, the poor selectivity for kinase family renders it a blunt tool for searching for new drugs. Nonetheless, staurosporine has been a useful lead for the discovery of antitumor drugs. For example, a number of bisindolylmaleimides structurally related to staurosporine has been synthesized, some of which are being in clinical trials [5–7].

As part of our ongoing effort to discover potent anti-tumor compounds through the structural modification of natural products, we recently designed and synthesized a novel series of bisindolylmaleimide amino acid ester conjugates. Amino acids have been introduced into different bioactive molecules to improve their pharmacological profiles including both potency and bioavailability. The rich side chains as well as the carboxylic or amino functionality render amino acids an ideal moiety in the structural modification of bioactive natural products [8]. In our previous paper, a series of *N*-methylbisindolylmaleimide amino acids conjugates featuring an acetyl linker were synthesized and evaluated for their *in vitro* anti-tumor activity [9,10].

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1001-8417/$ – see front matter
doi:10.1016/j.cclet.2012.01.033

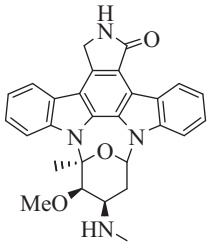
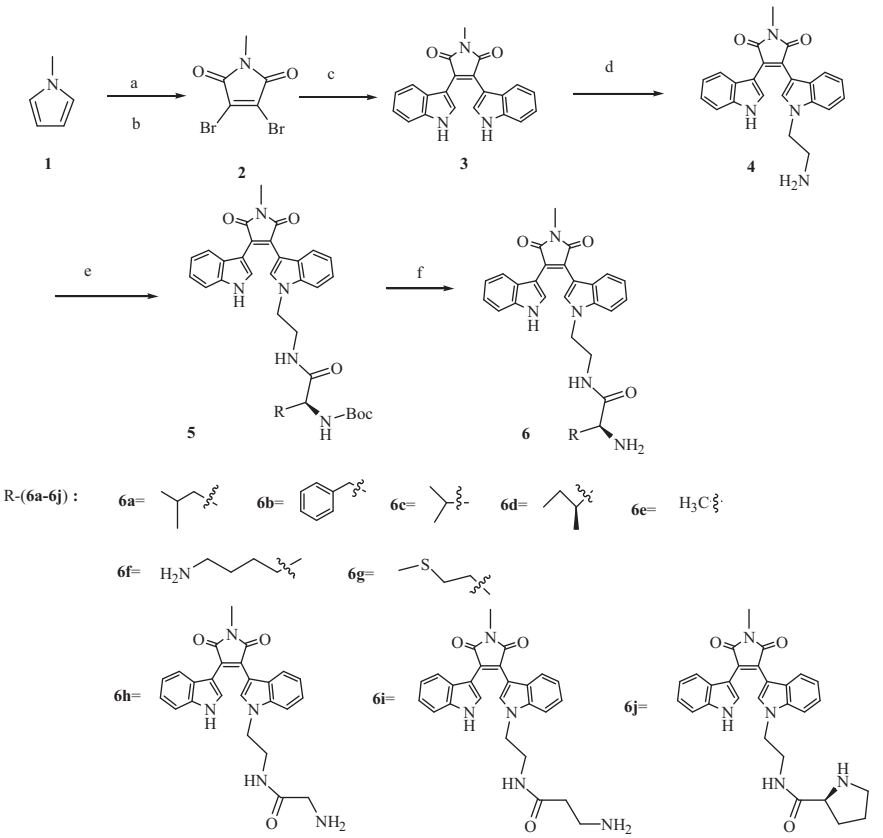


Fig. 1. The structure of Staurosporine.

In this study, a new series of derivatives of *N*-methylmaleimide conjugated with a number of natural amino acids by the linker of ethylamine were designed and synthesized. In addition, the *in vitro* inhibition activities of these derivatives were tested against a panel of human tumor cell lines including human intestinal adenocarcinoma (HCT-8), human hepatoma cell (BEL-7402), human ovarian carcinoma (A2780), human breast carcinoma (MCF-7), non-small cell lung carcinoma (A549), human gastric carcinoma (BGC-823). Furthermore, a preliminary structure–activity relationship was discussed.

Bisindolylmaleimide nucleus was synthesized from *N*-methylpyrrole **1**. Bromination and oxidation of *N*-methylpyrrole gave *N*-methyldibromomaleimide **2** [11]. Indolyllithium was coupled with *N*-methyldibromomaleimide **2** to afford the bisindolylmaleimide **3** [12]. The indole nitrogen was alkylated with 2-bromoethyl amine in the presence of NaH to afford amine **4**. Then **4** was coupled with various *N*-Boc-L-amino acids in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and 4-(dimethylamino)pyridine (DMAP) to give **5**. Finally, deprotection of **5** afforded target compound **6** in a reasonable yield (Scheme 1) [13].



Scheme 1. Reagents and conditions: (a) NBS, THF, (b) HNO_3, 46%; (c) indole, LiHMDS, toluene, 73%; (d) $BrCH_2CH_2NH_2$, NaH, DMF, 80 °C, 48%; (e) EDC, DMAP, CH_2Cl_2, 60–90%; (f) CF_3COOH, CH_2Cl_2, 50–85%.

Table 1
Cytotoxicity of compounds **6a–j**.

Compound	*In vitro* cytotoxicity (IC_{50}, μmol/L)					
	BCT-8	BEL-7402	A2780	MCF-7	A549	BGC-823
6a	20.11	20.78	21.85	20.10	24.77	26.99
6b	15.97	24.35	25.86	19.69	30.38	36.59
6c	19.02	23.63	27.19	22.52	23.72	71.87
6d	26.50	22.62	30.25	18.56	85.17	52.12
6e	27.00	33.79	59.45	40.40	35.55	62.67
6f	40.32	28.97	50.24	76.21	>100	59.29
6g	14.63	18.09	25.96	23.24	26.19	35.02
6h	24.97	24.17	25.23	45.25	78.75	38.02
6i	>100	>100	>100	>100	>100	>100
6j	33.28	36.11	42.71	58.49	59.89	50.24
Cisplatin	3.08	7.39	4.83	3.36	1.75	1.02

The IC_{50} values represent the compound concentration (μmol/L) required to inhibit tumor cell proliferation by 50%.

By the MTT assay, cytotoxicity of these derivatives was evaluated against six human cancer cell lines: human intestinal adenocarcinoma (HCT-8), human hepatoma cell (BEL-7402), human ovarian carcinoma (A2780), human breast carcinoma (MCF-7), non-small cell lung carcinoma (A549), human gastric carcinoma (BGC-823). The results of cytotoxicity studies were summarized in Table 1.

As for the ten derivatives of amino acids, compound **6i**, which was derived from *β*-aminopropionic acid, were the only one that was inactive to all tumor cell lines. Compound **6b**, the only aromatic amino acid derivative, which was derived from L-phenylalanine, showed moderate cytotoxic activities against all tested human tumor cell lines with IC_{50} values of 15–40 μmol/L. Compound **6a**, which was derived from L-leucine, exhibited inhibitory activities against the tested cell lines, with IC_{50} values of 20–30 μmol/L. Interestingly, compound **6d**, which was derived from L-isoleucine, exhibited similar inhibitory potency against the tested cell lines, with IC_{50} values of 20–100 μmol/L. Ala (**6e**) and Val (**6c**) conjugates, which share a similar hydrophobic residue, exhibited similar antiproliferative profile. Lys conjugate **6f**, which has two amino residues, exhibited inhibitory activities against the tested five cell lines with IC_{50} values of 28–80 μmol/L, but was inactive against A549. Compound **6g**, which was derived from L-methionine, exhibited inhibitory activities against the tested cell lines, with IC_{50} values of 14–35 μmol/L. In the cases of the Gly (**6h**) and Pro (**6j**) conjugates, both exhibited inhibitory activities against the tested cell lines, with IC_{50} values of 24–100 μmol/L and 33–60 μmol/L respectively. In addition, it could be seen that all of these derivatives are less active against the human gastric carcinoma (BGC-823).

In conclusion, a series of novel *N*-methylbisindolylmaleimide amino acid conjugates were prepared. All of *α*-amino acid conjugates except the *β*-aminopropionic acid conjugate **6i** showed moderate antiproliferative activity against human cancer cell lines including HCT-8, BEL-7402, A2780, MCF-7, A549 and BGC-823, which means that the *α*-amino acid moiety is essential for the cytotoxicity of this type of *N*-methylbisindolylmaleimide conjugates. This study demonstrated that this type of bisindolylmaleimide derivatives could be promising lead compounds for the discovery of novel antitumor drugs. Further studies of the antiproliferative mechanism of this series of compounds are in progress.

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[13] General procedure and data for the preparation of compounds **6a–j**: To a solution of **5** (0.18 mmol) in anhydrous dichloromethane (2 mL) was added trifluoroacetic acid (2 mL) and the reaction mixture was stirred at 0 °C under argon for 2 h. The reaction mixture was concentrated in vacuo, and then diluted with H_2O (2 mL). Saturated aqueous $NaHCO_3$ was added dropwise until neutralization. After extraction with ethyl acetate, the combined organic phase was dried over Na_2SO_4. The solvent was removed, and the residue was purified on a column of silica gel using MeOH–$CHCl_3$ (1/50) as eluent to give the product **6**. Yield: 50–88%. Compound **6a**: Yield 86.2%, red solid, mp: 142–144 °C; $[\alpha]_{20}^{D}$ +61.3 (*c* 0.006, CH_3OH); HRMS (ESI) calcd. for $C_{29}H_{31}N_5O_3Na$ $[M+Na]^+$: 520.2319, found 520.2318. ^1H NMR (300 MHz, DMSO-d_6): δ 11.73 (s, 1H, N–H), 8.67 (t, 1H, *J* = 4.8 Hz), 8.10 (br, 1H), 7.76 (s, 1H), 7.70 (d, 1H, *J* = 2.7 Hz), 7.52 (d, 1H, *J* = 8.1 Hz), 7.37 (d, 1H, *J* = 8.1 Hz), 7.01 (m, 2H), 6.70 (m, 1H), 6.65 (m, 2H), 4.33 (m, 2H), 3.61 (m, 2H), 3.07 (m, 4H), 1.51 (m, 1H), 1.42 (m, 1H), 0.87 (m, 1H), 0.77 (d, 6H, *J* = 5.4 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.68, 169.44, 136.00, 135.72, 132.44, 129.26, 127.06, 126.23, 125.84, 125.19, 121.84, 121.64, 121.48, 121.11, 119.68, 119.44, 111.79, 110.06, 105.54, 105.15, 50.93, 45.66, 44.78, 38.86, 23.90, 23.44, 22.42, 21.48. Compound **6b**: Yield 83.3%, red solid, mp: 151–153 °C; $[\alpha]_{20}^{D}$ −13.0 (*c* 0.002, CH_2Cl_2); HRMS (ESI) calcd. for $C_{32}H_{30}N_5O_3$ $[M+H]^+$: 532.2348, found 532.2347. ^1H NMR (300 MHz, DMSO-d_6): δ 11.68 (s, 1H), 8.05 (t, 1H), 7.74 (s, 1H), 7.72 (s, 1H), 7.49 (d, 1H, *J* = 8.4 Hz), 7.35 (d, 1H, *J* = 8.1 Hz), 7.21 (m, 4H), 7.00 (m, 3H), 6.80 (t, 2H, *J* = 8.1 Hz), 6.64 (m, 2H), 4.26 (t, 2H, *J* = 6.0 Hz), 3.40 (m, 3H), 3.02 (s, 3H), 2.87 (dd, 1H, *J* = 13.2, 4.5 Hz), 2.55 (m, 1H), 1.98 (br, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 174.49, 171.70, 138.64, 135.97, 135.84, 132.31, 129.23, 128.09, 127.16, 126.35, 126.09, 125.84, 125.26, 121.73, 121.64, 121.29, 121.09, 119.58, 119.44, 111.75, 110.14, 105.59, 105.05, 56.18, 45.14, 40.62, 38.51, 23.92. Compound **6c**: Yield 45%, red solid, mp: 169–171 °C; $[\alpha]_{20}^{D}$ +24.0 (*c* 0.007, CH_3OH); HRMS (ESI) calcd for $C_{28}H_{30}N_5O_3$ $[M+H]^+$: 484.2349, found 484.2325. ^1H NMR (300 MHz, $CDCl_3$): δ 9.08 (s, 1H), 7.70 (d, 1H, *J* = 2.7 Hz), 7.57 (s, 1H), 7.39 (t, 1H, *J* = 6.0 Hz), 7.30 (d, 2H, *J* = 8.4 Hz), 7.01 (m, 4H), 6.75 (t, 2H, *J* = 7.5 Hz), 4.26 (m, 2H), 3.64 (m, 2H), 3.15 (m, 4H), 2.26 (m, 1H), 0.95 (d, 3H, *J* = 6.9 Hz), 0.80 (d, 3H, *J* = 7.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 175.14, 172.45, 136.01, 135.88, 131.68, 128.39, 127.35, 126.34, 125.37, 122.45, 122.30, 121.77, 120.27, 120.08, 111.34, 109.36, 107.03, 106.22, 60.03, 45.91, 38.97, 30.66, 24.16, 19.64, 16.09. Compound **6d**: Yield 34%, red solid, mp: 147–149 °C; $[\alpha]_{20}^{D}$ −42.0 (*c* 0.007, CH_2Cl_2); HRMS (ESI) calcd. for $C_{29}H_{32}N_5O_3$ $[M+H]^+$: 498.2505, found 498.2530. ^1H NMR (300 MHz, $CDCl_3$): δ 9.19 (s, 1H, N–H), 7.69 (d, 1H, *J* = 2.7 Hz), 7.57 (s, 1H), 7.41 (t, 1H, 6.3 Hz), 7.29 (m, 2H), 7.03 (m, 3H), 6.74 (t, 2H, 7.2 Hz), 4.22 (m, 2H), 3.49 (m, 2H), 3.17 (m, 4H), 1.94 (m, 2H), 1.61 (br, 2H), 1.08 (m, 1H), 0.90 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.71, 172.03, 135.60, 135.49, 131.24, 128.03, 126.85, 125.92, 124.94, 121.86, 121.33, 119.84, 119.62, 110.95, 108.93, 106.54, 105.80, 59.37, 45.43, 38.54, 37.37, 23.71, 23.25, 15.62, 11.47. Compound **6e**: Yield 73.2%, red solid, mp: 182–184 °C; $[\alpha]_{20}^{D}$: +10.0 (*c* 0.008, CH_3OH); HRMS (ESI) calcd. for $C_{26}H_{26}N_5O_3$ $[M+H]^+$: 456.2030, found 456.2034. ^1H NMR (300 MHz, $CDCl_3$): δ 9.22 (s, 1H), 7.70 (d, 1H, *J* = 2.4 Hz), 7.56 (s, 1H), 7.29 (m, 3H), 7.01 (m, 4H), 6.74 (t, 2H, *J* = 7.8 Hz), 4.28 (m, 2H), 3.51 (m, 3H), 3.17 (s, 3H), 1.28 (d, 3H, *J* = 7.2 Hz), 0.85 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 176.44, 172.47, 136.09, 135.93, 131.68, 128.47, 127.43, 126.31, 125.37, 122.42, 122.29, 121.75, 120.26, 120.05, 111.38, 109.36, 106.97, 106.25, 50.57, 45.60, 39.14, 24.14, 21.39. Compound **6f**: Yield 63.6%, red solid, mp: 155–157 °C; $[\alpha]_{20}^{D}$ +45.0 (*c* 0.009, CH_3OH); HRMS (ESI) calcd. for $C_{29}H_{33}N_6O_3$ $[M+H]^+$: 513.2614, found 513.2595. ^1H NMR (300 MHz, CD_3OD-d_4): δ 7.69 (s, 1H), 7.60 (s, 1H), 7.37 (d, 1H, *J* = 8.1 Hz), 7.29 (d, 1H, *J* = 8.1 Hz), 6.95 (m, 2H), 6.76 (m, 2H), 6.58 (m, 2H), 4.27 (t, 2H, *J* = 6.0 Hz), 3.52 (m, 2H), 3.11 (t, 1H, *J* = 6.0 Hz), 3.04 (s, 3H), 2.68 (t, 2H, *J* = 7.2 Hz), 1.45 (m, 6H). ^{13}C NMR (125 MHz, CD_3OD-d_4): δ 177.84, 174.01, 137.76, 137.59, 133.39, 133.31, 130.25, 129.27, 129.21, 127.82, 127.79, 127.06, 123.34, 123.10, 123.06, 121.06, 120.74, 112.50, 110.60, 107.62, 58.78, 49.63, 41.92, 40.80, 31.07, 30.07, 24.28, 20.84. Compound **6g**: Yield 71.3%, red solid, mp: 168–171 °C; $[\alpha]_{20}^{D}$ +34.0 (*c* 0.005, CH_2Cl_2); HRMS (ESI) calcd. for $C_{28}H_{30}N_5O_3S$ $[M+H]^+$: 516.2069, found 516.2058. ^1H NMR (300 MHz, $CDCl_3$): δ 9.10 (s, 1H), 7.68 (d, 1H, *J* = 2.7 Hz), 7.57 (s, 1H), 7.38 (t, 1H, *J* = 6.0 Hz), 7.31 (m, 2H), 7.04 (m, 2H), 6.95 (dd, 2H, *J* = 7.8, 3.0 Hz), 6.74 (m, 2H), 4.30 (t, 2H, *J* = 5.1 Hz), 3.55 (m, 2H), 3.41 (dd, 1H, *J* = 8.1, 4.2 Hz), 3.17 (s, 3H), 2.54 (t, 2H, *J* = 6.9 Hz), 2.12 (m, 1H), 2.08 (s, 1H), 1.72 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 175.26, 172.49, 172.42, 136.03, 135.86, 131.75, 128.41, 127.37, 127.28, 126.25, 125.38, 122.45, 122.31, 121.74, 120.26, 120.10, 111.34, 109.33, 106.98, 106.18, 54.06, 45.61, 39.14, 33.65, 30.59, 24.16, 15.23. Compound **6h**: Yield 73.5%, red solid, mp: 123–126 °C; HRMS (ESI) calcd. for $C_{25}H_{24}N_5O_3$ $[M+H]^+$: 442.1879, found 442.1874. ^1H NMR (300 MHz, $CDCl_3$): δ 9.47 (s, 1H), 7.69 (d, 1H, *J* = 2.1 Hz), 7.54 (s, 1H), 7.28 (m, 3H), 7.05 (m, 3H), 6.90 (d, 1H, *J* = 8.1 Hz), 6.73 (m, 2H), 4.28 (t, 2H, *J* = 5.4 Hz), 3.53 (q, 2H, *J* = 5.7 Hz), 3.25 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.16, 172.02, 135.52, 131.26, 128.16, 127.10, 126.69, 125.88, 124.86, 121.84, 121.26, 119.81, 119.54, 111.03, 108.83, 106.36, 105.75, 45.32, 44.09, 38.56, 23.71. Compound **6i**: Yield 73.5%, red solid, mp: 152–154 °C; HRMS (ESI) calcd. for $C_{26}H_{26}N_5O_3$ $[M+H]^+$: 456.2035, found 456.2031. ^1H NMR (300 MHz, CD_3OD-d_4): δ 7.71 (s, 1H), 7.63 (s, 1H), 7.35 (d, 1H, *J* = 8.4 Hz), 7.27 (d, 1H, *J* = 7.8 Hz), 6.99 (t, 1H, *J* = 7.5 Hz), 6.92 (t, 1H, *J* = 7.8 Hz), 6.77 (t, 2H, *J* = 8.7 Hz,), 6.55 (m, 2H), 4.31 (t, 2H, *J* = 6.3 Hz), 3.51 (t, 2H), 3.25 (d, 3H, *J* = 0.9 Hz), 2.95 (t, 2H, *J* = 6.9 Hz), 2.40 (t, 2H, *J* = 6.3 Hz). ^{13}C NMR (125 MHz, CD_3OD-d_4): δ 172.84, 172.62, 136.40, 136.28, 131.74, 128.84, 127.89, 126.58, 126.52, 125.62, 121.82, 121.67, 121.64, 121.15, 119.60, 119.30, 111.10, 109.22, 106.20, 105.98, 44.92, 38.95, 36.06, 32.70, 22.85. Compound **6j**: Yield 72.5%, red solid, mp: 171–174 °C; $[\alpha]_{20}^{D}$ +75.0 (*c* 0.008, CH_2Cl_2); HRMS (ESI) calcd. for $C_{28}H_{28}N_5O_3$ $[M+H]^+$: 482.2192, found 482.2178. ^1H NMR (300 MHz, $CDCl_3$): δ 9.38 (s, 1H), 7.71 (s, 2H), 7.55 (s, 1H), 7.32 (t, 2H, *J* = 8.1 Hz), 7.04 (m, 3H), 6.92 (d, 1H, *J* = 8.1 Hz), 6.75 (t, 2H, *J* = 7.2 Hz), 4.27 (m, 2H), 3.72 (q, 1H), 3.57 (m, 1H), 3.17 (s, 3H), 2.91 (m, 1H), 2.72 (m, 1H), 2.09 (m, 1H), 1.84 (m, 1H), 1.62 (m, 2H), 0.86 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 174.49, 172.47, 136.28, 136.04, 131.64, 128.60, 127.60, 127.43, 126.31, 125.40, 122.46, 122.36, 122.34, 121.86, 120.32, 120.11, 111.50, 109.43, 107.00, 106.36, 60.30, 46.97, 45.68, 39.33, 30.45, 24.16, 22.68.