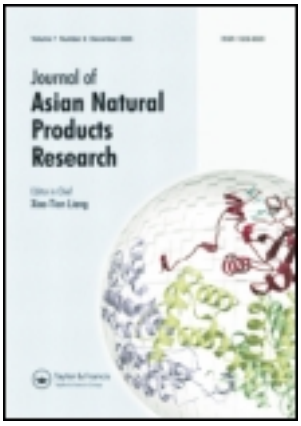

Journal of Asian Natural Products Research

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/ganp20

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Available online: 13 Jul 2011

To cite this article: Xiao-Bin Li, Jin-Shan Tang, Hao Gao, Rong Ding, Jia Li, Kui Hong & Xin-Sheng Yao (2011): A new staurosporine analog from Actinomycetes *Streptomyces* sp. (172614), Journal of Asian Natural Products Research, 13:8, 765-769

To link to this article: http://dx.doi.org/10.1080/10286020.2011.586342

Journal of Asian Natural Products Research
Vol. 13, No. 8, August 2011, 765–769

A new staurosporine analog from Actinomycetes *Streptomyces* sp. (172614)

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(*Received 14 March 2011; final version received 4 May 2011*)

A new staurosporine analog, 10′-{5″-[(methoxycarbonyl)amino]-2″-methyl}-phenylaminocarbonylstaurosporine (**1**), together with staurosporine (**2**), was obtained from the culture broth of Actinomycetes *Streptomyces* sp. (172614). Their structures were elucidated by comprehensive spectroscopic analysis including UV, MS, NMR, and CD spectra. Pharmacological experiments revealed that **1** and **2** showed significant cytotoxicity against human colon tumor cell HCT-116.

Keywords: *Streptomyces* sp.; staurosporine analog; cytotoxicity; HCT 116

1. Introduction

The Actinomycetes are a prolific source of structurally interesting and biologically active metabolites responsible for over 45% of all microbial natural products [1], including the clinically important antibiotics and antineoplastic. Indolocarbazole alkaloid is one kind of main components and much attention has been paid to it due to its chemodiversity and, especially, interesting biological activities [2,3]. Recently, during our search for new anticancer agents from microbial resources, a strain of Actinomycetes *Streptomyces* sp. (172614) has been obtained from the mangrove soil, which exhibited strong cytotoxicity against HCT-116. By bioassay-guided separation, a new staurosporine analog (**1**), together with a known compound staurosporine (**2**) [4], was isolated from *Streptomyces* sp. (172614) (Figure 1). **1** and **2** showed significant cytotoxicity against HCT-116 colon cancer cell lines *in vitro* by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay method with IC_{50} values of 0.37 and 0.04 μM, respectively. In this paper, we report the isolation and structural determination of compound **1**.

2. Results and discussion

Compound **1** was obtained as a yellow powder with $[\alpha]_D^{24}$ +90.2 ($c = 0.30$, MeOH). HR-ESI-MS showed a pseudomolecular ion peak at *m/z* 695.2569 $[M + Na]^+$, indicating the molecular formula of $C_{38}H_{36}N_6O_6$. The UV spectrum showed the absorption maxima at 205 (4.7), 292 (4.7), 319 (4.1), 334 (4.1), 355 (3.9), and 372 (3.9) nm, suggesting the

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ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020.2011.586342
http://www.informaworld.com

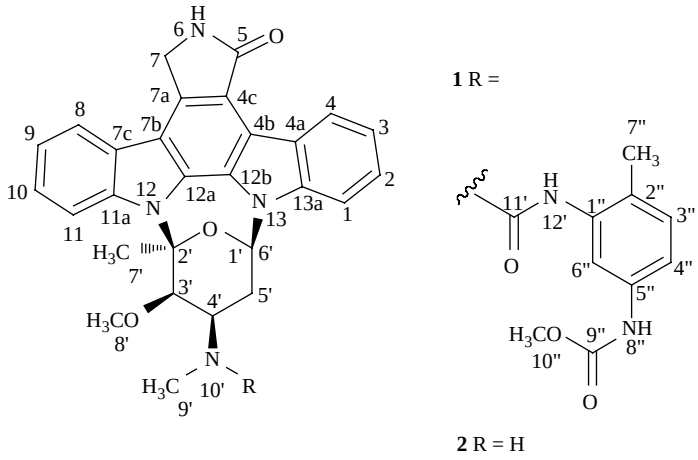

Figure 1. The structures of **1** and **2**.

presence of an indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5(6*H*)-one skeleton as chromophore in **1** [5].

The ^{1}H NMR spectrum of **1** showed downfield signals of aromatic protons at δ 9.28 (1H, d, $J = 8.0$ Hz), 8.05 (1H, d, $J = 8.0$ Hz), 8.00 (1H, d, $J = 8.0$ Hz), 7.70 (1H, d, $J = 8.0$ Hz), 7.49 (2H, t, $J = 8.0$ Hz), 7.36 (1H, t, $J = 8.0$ Hz), and 7.30 (1H, t, $J = 8.0$ Hz), forming two ABCD coupling systems and three aromatic protons at δ 7.10 (1H, d, $J = 8.3$ Hz), 7.18 (1H, dd, $J = 8.3$, 2.0 Hz), and 7.43 (1H, d, $J = 2.0$ Hz) forming an ABX coupling system. There were five methyl protons at δ 2.14 (3H, s), 2.33 (3H, s), 2.82 (3H, s), 2.87 (3H, s), and 3.67 (3H, s) in the ^{1}H NMR spectrum of **1**. The ^{13}C NMR signals also exhibited the presence of the methyl groups and aromatic rings. With the aid of the 2D NMR (^{1}H–^{1}H COSY, HSQC, and HMBC) spectra, all the ^{1}H and ^{13}C NMR signals of **1** were assigned and shown in Table 1.

The sugar moiety was elucidated on the basis of 2D NMR correlations (Figure 2). The ^{1}H–^{1}H COSY spectrum showed the following key correlations of H-3′/H-4′/H-5′/H-6′. The HMBC spectrum showed key correlations from H-3′ to C-8′, from H-6′ to C-2′, from H-7′ to C-2′, C-3′, from H-8′ to C-3′, and from H-9′ to C-4′.

Detailed analysis of the HMBC correlations of the methyl at δ_H 2.14 with C-1″ at δ_C 138.0/C-3″ at δ_C 129.9, NH$^-$ at δ_H 9.54 with C-4″ at δ_C 115.2/C-6″ at δ_C 116.8, OMe$^-$ at δ_H 3.67 with C-9″ at δ_C 154.0, NH$^-$ at δ_H 7.99 with C-11′ at δ_C 156.0/C-2″ at δ_C 127.6, and the methyl at δ_H 2.82 with C-11′ at δ_C 156.0 revealed the presence of *N*-carbonyl-*N*′-methoxycarbonyl-2,4-diaminotoluene moiety, which was further confirmed by the ESI-MS^2 spectroscopic data of **1**. The ESI-MS^2 of the precursor ion at *m/z* 695 $[M + Na]^+$ gave a strong fragment ion at *m/z* 489, which was attributed to the product ion of staurosporine moiety. The stereochemistry of **1** was determined on the basis of ROESY and CD spectra. In the ROESY spectrum of **1**, correlations of H-7′ and H-4′, H-3′ and H-4′, and H-4′ and H-6′ were observed (Figure 3). The CD spectrum of **1** was consistent with that of **2** (Figure 4). Thus, the structure of **1** was elucidated as 10′-{5″-[(methoxycarbonyl)amino]-2″-methyl}-phenylaminocarbonylstaurosporine (Figure 1).

Table 1. ^{1}H and ^{13}C NMR spectral data of **1** and **2** (DMSO-d_6, δ in ppm).

	1		**2**			**1**		**2**	
No.	δ_H (mult, J in Hz)	δ_C	δ_H (mult, J in Hz)	δ_C	No.	δ_H (mult, J in Hz)	δ_C	δ_H (mult, J in Hz)	δ_C
1	7.70 (d, 8.0)	109.0	7.56 (d, 8.2)	108.3	2′	–	94.8	–	91.1
2	7.49 (t, 8.0)	125.3	7.45 (td, 7.6, 0.9)	124.8	3′	4.31 (m)	83.9	4.04 (d, 3.5)	82.8
3	7.30 (t, 8.0)	119.4	7.28 (t, 7.6)	119.0	4′	4.87 (m)	49.0	3.25 (m)	50.1
4	9.28 (d, 8.0)	125.6	9.30 (d, 7.8)	125.6	5′	2.67 (m), 2.25 (m)	27.3	Overlapped	29.4
4a	–	122.6	–	122.5	6′	7.05 (t, 7.6)	82.4	6.68 (dd, 4.0, 2.9)	79.9
4b	–	114.6	–	113.5	7′	2.33 (s)	29.5	2.29 (s)	29.7
4c	–	119.3	–	118.8	8′	2.87 (s)	60.4	3.32 (s)	57.2
5	–	171.9	–	172.3	9′	2.82 (s)	30.3	1.45 (s)	33.3
6	8.58 (s)	–	8.50 (s)	–	11′	–	156.0		
7	5.00 (s)	45.4	4.96 (s)	45.4	12′	7.99 (s)	–		
7a	–	132.7	–	132.0	1″	–	138.0		
7b	–	114.1	–	114.1	2″	–	127.6		
7c	–	123.7	–	123.9	3″	7.10 (d, 8.3)	129.9		
8	8.05 (d, 8.0)	121.4	7.96 (d, 6.5)	120.8	4″	7.18 (dd, 8.3, 2.0)	115.2		
9	7.36 (t, 8.0)	120.2	7.28 (t, 7.3)	119.7	5″	–	135.8		
10	7.49 (t, 8.0)	125.0	7.41 (td, 7.8, 1.0)	124.3	6″	7.43 (d, 2.0)	116.8		
11	8.00 (d, 8.0)	113.8	7.98 (d, 7.8)	115.2	7″	2.14 (s)	17.3		
11a	–	139.0	–	139.4	8″	9.54 (s)	–		
12a	–	129.1	–	130.0	9″	–	154.0		
12b	–	124.6	–	126.7	10″	3.67 (s)	51.5		
13a	–	136.3	–	136.3					

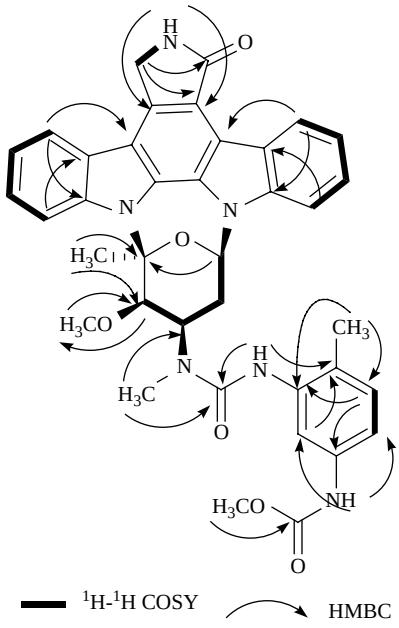

Figure 2. Key HMBC and $^{1}H-^{1}H$ COSY correlations of **1**.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured using a JASCO P-1020 polarimeter. CD spectra were recorded on a JASCO J-810 CD spectrometer. IR spectra (KBr) were recorded on a JASCO FT/IR-480 Plus Fourier Transform Infrared Spectrometer. The UV spectra were obtained on a JASCO V-550 UV–VIS Spectrophotometer. ^{1}H (400 MHz), ^{13}C (100 MHz), and 2D NMR spectra were recorded in DMSO-d_6 on a Bruker AV-400 spectrometer. HR-ESI-MS spectra were measured using an Agilent 6210 LC/MSD TOF mass spectrometer. HPLC was recorded on a Gilson system with a 306 pump and a UV–vis-152 detector, using a reversed-phase column (5 μm, 21.2 × 250 mm, Welch XB-C18) at 8 ml/min and monitor at 290 nm. Column chromatographies (CCs) were carried out using silica gel (200–300 mesh, Qingdao Marine Chemical Group Corporation, Qingdao, China) and Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden). Silica gel GF_{254} (Qingdao Marine Chemical Group Corporation) was used for analytical TLC.

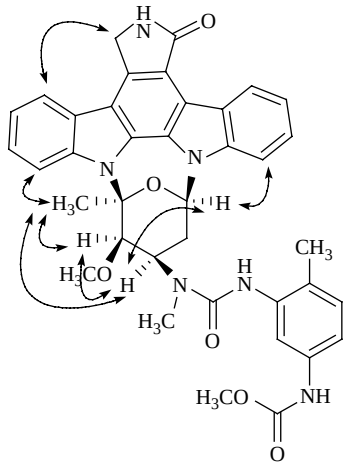

Figure 3. Key ROESY correlations of **1**.

3.2 Bacterial material

Streptomyces sp. was isolated from soil collected from the Jiulongjiangkou Mangrove, Fujian, China. A voucher specimen has been deposited in the Institute of Tropical Biosciences and Biotechnology, Haikou, China (No. 172614).

3.3 Extraction and isolation

The fermentation broth (50 l) was lyophilized and macerated with acetone overnight four times and then filtered. The filtrate was concentrated to dryness under vacuum which yielded 2000 g crude extract. The crude extract was suspended with water and extracted with EtOAc for three times. The organic layer (8.9 g) was directly subjected to a silica gel open column, eluted with $CHCl_3$–MeOH in gradient (100:0 to 0:100) to yield 11 fractions. The water layer was chromatographed over a macroporous resin Diaion HP20 column, eluted with a gradient MeOH–H_2O (0, 10, 30, 50, and 100%)

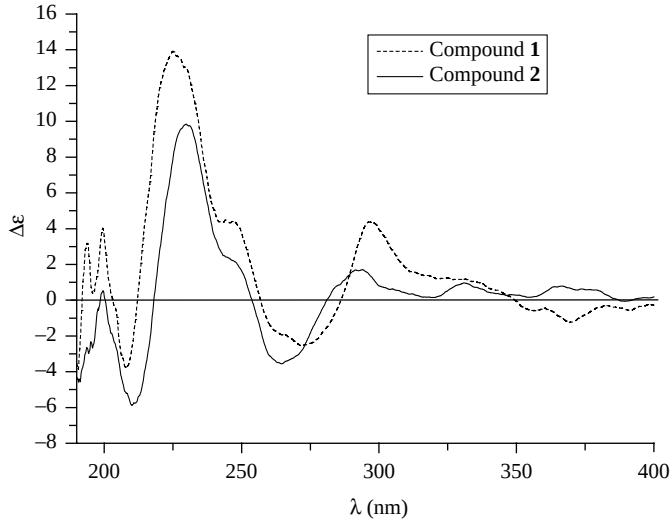

Figure 4. CD spectra of compounds **1** and **2** (in MeOH).

to yield five fractions [W1 (1357.7 g), W2 (17.2 g), W3 (28.3 g), W4 (17.6 g), and W5 (2.9 g)]. The results of MTT experiment revealed that fraction W5 exhibited strong cytotoxic activity. The active fraction W5 (2.9 g, eluted with MeOH) was subjected to Sephadex LH-20 CC, eluted with 70% MeOH–H_2O to give seven fractions (fractions W5-1–W5-7). Fraction W5-7 (111.6 mg) was separated by silica gel CC and eluted with gradient $CHCl_3$–MeOH (100:0, 99:1, 97:3, 0:100, v/v) to yield six subfractions (fractions W5-7-1–W5-7-6). Fraction W5-7-5 [26.4 mg, eluted with $CHCl_3$–MeOH (97:3)] was subjected to a preparative HPLC [70% MeOH–H_2O (0.05% aqueous ammonia), 8.0 ml/min] to obtain compounds **1** (2.3 mg) and **2** (3.8 mg).

*3.3.1 10′-{5″-[(methoxycarbonyl)amino]-2″-methyl}-phenylaminocarbonylstaurosporine (**1**)*

A yellow powder; $[\alpha]_D^{24}$ +90.2 ($c = 0.30$, MeOH). UV (MeOH) λ_{max} (log ε): 205 (4.7), 292 (4.7), 319 (4.1), 334 (4.1), 355 (3.9), and 372 (3.9) nm. IR (KBr) ν_{max}: 3422, 2921, 1665, 1456, 1317, 1233, 1073, and 749 cm^{-1}. ^1H and ^{13}C NMR spectral data, see Table 1. HR-ESI-MS: *m/z* 695.2569 $[M + Na]^+$ (calcd for $C_{38}H_{36}N_6O_6Na$, 695.2589).

Acknowledgements

The authors are grateful to Mr Zhen-Qiang Mu and Miss Yuan-Fan Huang for NMR measurements, Ms Ming-Yan Liu for MS measurements, Mrs Hui-Nan Zhao for HR-ESI-MS measurements. This work was financially supported by grants from the Ministry of Science and Technology of China (2009CB522300, and 2009ZX09302-004), and the Ministry of Education of China/Fok Ying Tung Education Foundation (121039).

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