

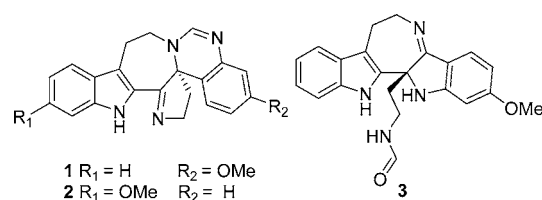
Three New Indole Alkaloids from
*Trigonostemon lii*Cheng-Jian Tan,^{†,‡,§} Ying-Tong Di,[†] Yue-Hu Wang,[†] Yu Zhang,[†] Yi-Kang Si,[†]
Qiang Zhang,[†] Suo Gao,[†] Xu-Jia Hu,[†] Xin Fang,[†] Shi-Fei Li,[†] and
Xiao-Jiang Hao^{†,*}

State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, Yunnan, PRC, Graduate School of Chinese Academy of Sciences, Beijing 100039, PRC, The Key Laboratory of Chemistry for Natural Products of Guizhou Province, Chinese Academy of Sciences, Guiyang 550002, PRC, and Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing 100050, PRC

haoxj@mail.kib.ac.cn

Received March 26, 2010

ABSTRACT



Three unprecedented indole alkaloids, trigonoliimines A–C (1–3) with a unique polycyclic system, were isolated from the leaves of *Trigonostemon lii* Y. T. Chang. The structures of 1–3 were determined by the spectroscopic, computational, and CD exciton chirality approaches. Trigonoliimine A showed modest anti-HIV-1 activity (EC₅₀ = 0.95 μg/mL, TI = 7.9).

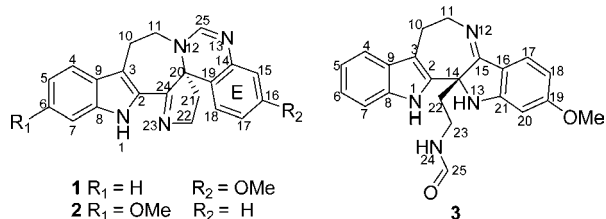
The genus *Trigonostemon* (Euphorbiaceae) comprising ca. 50 species is widely distributed in India, Malaysia, and middle Asia.¹ Previous chemical investigations on this genus have involved an array of structurally interesting compounds such as modified daphnane-type diterpenoids,² alkaloids,³

diterpenoids, and phenanthrenes.⁴ In this study, three novel indole alkaloids, trigonoliimines A–C (1–3), with unprecedented polycyclic skeletons, were isolated from the extract of the leaves of *Trigonostemon lii* Y. T. Chang collected in Yunnan Province of China. Here, we report the isolation, structural elucidation, and anti-HIV-1 activity of compounds 1–3.

The powdered leaves (12.0 kg) of *T. lii* were percolated three times with 95% EtOH to give a crude extract (1.0 kg). The extract was suspended in 1.5 L of water and then partitioned with petroleum ether, EtOAc, and *n*-BuOH

[†] Kunming Institute of Botany.[‡] Graduate School of the Chinese Academy of Sciences.[§] The Key Laboratory of Chemistry for Natural Product of Guizhou Province.^{*} Institute of Materia Medica, Chinese Academy of Medical Sciences.(1) Chen, S. K.; Chen, B. Y.; Li, H. *Flora of China* (Zhongguo Zhiwu Zhi); Science Press: Beijing, 1997; Vol. 44 (2), p 162.(2) (a) Jayasuriya, H.; Zink, D. L.; Singh, S. B.; Borris, R. P.; Nanakorn, W.; Beck, H. T.; Balick, M. J.; Goetz, M. A.; Slayton, L.; Gregory, L.; Zakson-Aiken, M.; Shoop, W.; Singh, S. B. *J. Am. Chem. Soc.* **2000**, *122*, 4998–4999. (b) Jayasuriya, H.; Zink, D. L. *Prod.* **2004**, *67*, 228–231. (c) Soonthornchareonnon, N.; Sakayarojkul, M.; Isaka, M.; Mahakittikun, V.; Chuakul, W.; Wongsinkongman, P. *Chem. Pharm. Bull.* **2005**, *53*, 241–243. (d) Tempeam, A.; Thasana, N.; Pavaro, C.; Chuakul, W.; Siripong, P.; Ruchirawat, S. *Chem. Pharm. Bull.* **2005**, *53*, 1321–1323. (e) Chen, H. D.; He, X. F.; Ai, J.; Geng, M. Y.; Yue, J. M. *Org. Lett.* **2009**, *11*, 4080–4083. (f) Zhang, L.; Luo, R. H.; Wang, F.; Jiang, M. Y.; Dong, Z. J.; Yang, L. M.; Zheng, Y. T.; Liu, J. K. *Org. Lett.* **2010**, *12*, 152–155.(3) (a) Kanchanapoom, T.; Kasai, R.; Chumsri, P.; Kraissintu, K.; Yamasaki, K. *Tetrahedron Lett.* **2002**, *43*, 2941–2943. (b) Hu, X. J.; Di, Y. T.; Wang, Y. H.; Kong, L. Y.; Gao, S.; Li, C. S.; Liu, H. Y.; He, H. P.; Ding, J.; Xie, H.; Hao, X. J. *Planta Med.* **2009**, *75*, 1157–1161.(4) (a) Kokpol, U.; Thebpatiphat, S.; Boonyaratavej, S.; Chedchusulchai, V.; Ni, C. Z.; Clardy, J.; Chaichantipyuth, C.; Chittawong, V.; Miles, D. H. *J. Nat. Prod.* **1990**, *53*, 1148–1151. (b) Yin, S.; Su, Z. S.; Zhou, Z. W.; Dong, L.; Yue, J. M. *J. Nat. Prod.* **2008**, *71*, 1414–1417. (c) Hu, X. J.; Wang, Y. H.; Kong, L. Y.; He, H. P.; Gao, S.; Liu, H. Y.; Ding, J.; Xie, H.; Di, Y. T.; Hao, X. J. *Tetrahedron Lett.* **2009**, *50*, 2917–2919.

successively. The *n*-BuOH-soluble fraction (250 g) was subjected to RP-18 column eluted with 10%–100% MeOH. The 70% MeOH fraction was further chromatographed over Sephadex LH-20 gel, followed by semipreparative HPLC [MeOH/H₂O (containing 0.1% Et₂NH), 70/30] to give trigonoliimines A (**1**, 5.3 mg), B (**2**, 3.1 mg), and C (**3**, 15.7 mg).



Trigonoliimine A (**1**)⁵ was obtained as a light yellow gum. The molecular formula of **1** was established as C₂₂H₂₀N₄O by positive HRESIMS data (*m/z* 357.1709 [M + H]⁺, calcd 357.1715), with 15 degrees of unsaturation. The UV absorptions at 205 and 306 nm suggested the indole moiety, whereas the IR spectrum showed bands at 3425 and 1627 cm⁻¹, suggesting the presence of NH and imine functionalities, respectively. The ¹H NMR spectrum of **1** (Table 1)

Table 1. ¹H and ¹³C NMR Data of Trigonoliimine A (**1**) and ¹³C NMR Data of Trigonoliimine B (**2**)

no.	1		2	
	δ _C ^a	δ _H ^b	δ _C ^c	δ _C ^c
1 (NH)	-	11.50 (s)	-	-
2	127.9	-	126.5	125.1
3	115.6	-	117.4	117.7
4	119.1	7.44 (d, 7.5)	119.0	119.3
5	119.1	6.99 (t, 7.5)	119.6	110.0
6	123.4	7.15 (t, 7.5)	124.3	157.6
7	111.7	7.32 (d, 7.5)	111.4	93.4
8	136.5	-	136.8	137.4
9	127.1	-	127.2	121.0
10α	29.1	3.06 (m)	29.4	29.0
10β		2.95 (m)		
11α	46.6	4.00 (br d, 14.5)	47.9	47.5
11β		3.74 (t, 12.5)		
14	143.0	-	141.0	139.6
15	109.2	6.55 (overlapped)	108.6	123.4
16	159.6	-	160.1	128.2
17	110.3	6.54 (overlapped)	111.4	124.8
18	123.2	6.53 (overlapped)	123.2	121.8
19	115.0	-	113.7	121.3
20	76.5	-	77.2	76.5
21α	40.6	2.05 (m)	40.4	39.8
21β		2.14 (m)		
22α	56.2	3.55 (m)	56.0	55.2
22β		4.10 (dd, 8.5, 16.0)		
24	166.4	-	167.4	166.6
25	150.2	7.48 (s)	149.9	149.0
OMe	55.1	3.65 (3H, s)	55.0	54.6

^a Measured in DMSO-*d*₆ at 125 MHz. ^b Measured in DMSO-*d*₆ at 500 MHz. ^c Measured in CDCl₃/CD₃OD (3:1) at 125 MHz.

indicated the presence of a methoxyl group (δ_H 3.65) and one 1,2-disubstituted aromatic ring [δ_H 7.44 (d, *J* = 7.5 Hz, H-4), 6.99 (t, *J* = 7.5 Hz, H-5), 7.15 (t, *J* = 7.5 Hz, H-6) and 7.32 (d, *J* = 7.5 Hz, H-7)]. Proton resonance at δ_H 11.50 was attributable to an indolic nitrogen proton. Two sets of units NCH₂CH₂ in the high field of ¹H NMR were deduced from the ¹H–¹H COSY and HSQC spectra. These deductions were further confirmed by the ¹³C NMR, which also revealed an additional one benzene moiety, two imine groups, a fully substituted double bond, as well as one sp³ quaternary carbon. The aforementioned groups represented nine degrees of unsaturation. Thus, there must be six rings in the structure.

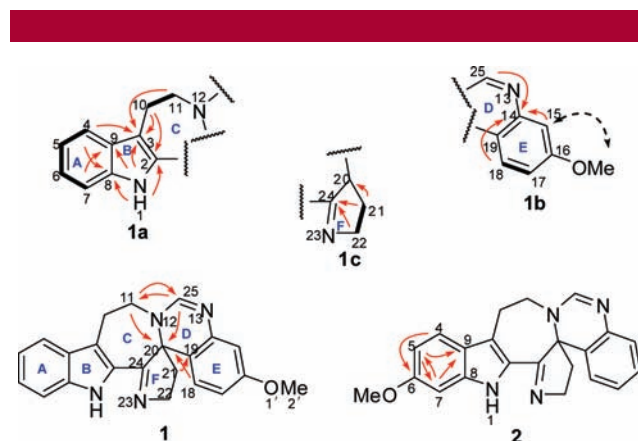


Figure 1. Partial fragments (**1a–1c**), ¹H–¹H COSY (–), key HMBC (→) correlations of **1** and **2**, and key ROESY (←–→) correlation of **1**.

Comprehensive analysis of the 1D and 2D NMR spectra, especially HMBC (Figure 1), elucidated that compound **1** featured a unique ring system. A detailed account of the structural elucidation of **1** is presented below. The observed HMBC correlations of the indolic nitrogen proton (NH-1) to C-2, C-3, C-8, and C-9, H-4 to C-3 and C-8, and H-7 to C-9 were consistent with a 2,3-disubstituted indole moiety. In addition, one of the ethanamine fragments attached to C-3 was confirmed by the HMBC correlations from H₂-10 to C-3 and C-2 and H-11β to C-3. The above data established the partial structure (**1a**, Figure 1).

Moiety **1b** contained the 1,2,4-trisubstituted benzene ring fragment, the imine group, and the methoxyl group. HMBC correlations (in CDCl₃/CD₃OD (3:1), Supporting Information) of H-15 and H-18 to C-14 (δ_C 143.0) and of H-17 to C-19 indicated that the imine group attached to C-14 by the C–N bond. The ROESY correlations (in CDCl₃/CD₃OD (3:1), Supporting Information) of H-15 with the OCH₃ indicated that the methoxy group was linked at C-16.

The ring F (fragment **1c**, Figure 1) consisting of the NCH₂CH₂ moiety (C-21–C-22), C-20, C-24, and N-23 was

(5) Trigonoliimine A (**1**): a light yellow gum; [α]_D¹⁰ = +13.3 (*c* 0.3, CHCl₃); CD (CHCl₃) 222 nm (Δε +9.7), 225 nm (Δε –2.5); UV (MeOH) λ_{max} (log ε) 205 nm (4.5), 306 nm (4.1); IR (KBr) ν_{max} 3425, 2960, 2925, 1627, and 1452 cm⁻¹; ¹H and ¹³C NMR data (Table 1); ESIMS *m/z* 357 [M + H]⁺; HRESIMS *m/z* 357.1709 [M + H]⁺ (calcd for C₂₂H₂₀N₄O, 357.1715).

really established by the HMBC correlations of H₂-21 to C-20 and H-21 α and H₂-22 to C-24, respectively. Furthermore, the key HMBC correlations of H-25 and H-11 α to C-20, H-25 to C-11, and H-11 β to C-25 supported the connectivity of C-20 to the two moieties **1a** and **1b** through heteroatom N-12. HMBC crosspeaks of H₂-21 to C-19 and H-18 to C-20 further connected C-19 to C-20, and the remaining C-2 therefore must be attached to C-24 forming an azepane agreement with the molecular weight. Thus, the gross structure of trigonoliimine A was unambiguously assigned as **1** with an unusual fused-hexacyclic ring system (two five-, three six-, and one seven-membered rings).

To determine its absolute configuration, chemical computation was employed. First, A SPARTAN 08 search of conformations of compound **1** was performed, which identified two stable conformations, I and II, only with different rotated angle of the OCH₃ around the C-16–O-1' single bond. Reoptimization of the structure of the stable conformations was carried out at HF/6-31G* and then B3LYP/6-31G(2d, p) levels in Gaussian 03. Harmonic vibrational frequencies of each conformation were then calculated using B3LYP/6-31G (2d, p) to confirm their stability. Then, the potential energy surface (PES) was scanned, which started from the optimized geometry of conformation I and varied the dihedral angle C15C16O1'C2'. The results exhibited only two minima and suggested that the occurrence of the comparatively rapid interconversion of I and II was a logical process in solution at room temperature. Relative and free energy and equilibrium populations at room temperature of the two conformations were further calculated and listed in Table 2. Lastly, the “self-consistent reaction field” method (SCRF) was employed to perform the OR calculation of the two major conformers of compound **1** in chloroform at the B3LYP-SCRF/6-311++G (d, 2p) level.⁶ As shown in Table 2, the computed optical rotation value (+18.1) of **1** is matched up to the experimental value (+13.3) for the S enantiomer, thus tentatively assigning the 20S configuration.

Table 2. Computed Energy, Populations, and Optical Rotation for Trigonoliimine A (**1**)

conformation	Trigonoliimine A		exptl
	I (1 S)	II (2 S)	
relative energy [kcal mol ⁻¹]	0.00	0.12	
equilibrium populations (%)	53.4	46.6	
[α] _D	-23.88	+ 62.24	
sum of [α] _D		+ 18.11	+ 13.3

The molecular formula of **2**⁷ was indicated to be C₂₂H₂₀N₄O by positive HRESIMS (*m/z* 357.1718 [M + H]⁺, (calcd: 357.1715)), which was the same as that of **1**. Comparison of the 1D NMR spectroscopic data of **2** with that

(6) (a) Cheeseman, J. R.; Frisch, M. J.; Devlin, F. J.; Stephens, P. J. *J. Phys. Chem. A* **2000**, *104*, 1039–1046. (b) Stephens, P. J.; Devlin, F. J.; Cheeseman, J. R.; Frisch, M. J. *J. Phys. Chem. A* **2001**, *105*, 5356–5371. (c) Stephens, P. J.; Pan, J. J.; Devlin, F. J.; Cheeseman, J. R. *J. Nat. Prod.* **2008**, *71*, 285–288.

Table 3. ¹H (500 MHz) and ¹³C NMR (100 MHz) Data of Trigonoliimine C (**3**)

no.	δ_C^a	δ_H^a	δ_C^b
1 (NH)	-	10.64 (s)	-
2	131.8	-	131.4
3	108.8	-	110.3
4	117.8	7.41 (d, 7.5)	118.5
5	118.6	6.98 (t, 7.5)	119.7
6	121.6	7.08 (t, 7.5)	122.7
7	110.9	7.36 (d, 7.5)	111.5
8	134.8	-	136.3
9	127.9	-	129.2
10 α	23.3	3.05 (br d, 11.0)	24.3
10 β		2.80 (t, 11.0)	
11 α	46.5	4.24 (t, 12.0)	47.5
11 β		3.99 (br d, 12.0)	
13 (NH)	-	6.83 (br s)	-
14	66.4	-	68.1
15	170.3	-	174.9
16	116.4	-	116.5
17	123.6	7.34 (d, 8.0)	125.2
18	105.7	6.26 (dd, 2.5, 8.0)	108.4
19	164.3	-	166.8
20	94.0	6.27 (d, 2.5)	95.3
21	156.8	-	159.0
22 α	39.5	2.29 (m)	40.2
22 β		2.51 (m)	
23	33.6	3.14 (2H, m)	34.9
24 (NH)	-	7.99 (br s)	-
25	161.1	7.93 (s)	163.4
OMe	55.3	3.77 (s)	55.8

^a Measured in DMSO-*d*₆. ^b Measured in CDCl₃/CD₃OD (3:1).

of **1** showed a resemblance. All these suggested that **2** should be an isomer of **1**, which was further confirmed by 2D NMR experiments. Specifically, the HMBC correlations of H-7 to C-5 and C-9, H-5 to C-7 and C-9, and H-4 to C-6 indicated that the OMe group was linked to C-6, as shown in Figure 1.

Trigonoliimine C (**3**)⁸ was isolated as a light yellow gum. The positive HRESIMS at *m/z* 375.1813 [M + H]⁺ gave molecular formula C₂₂H₂₂N₄O₂ (calcd: 375.1821), indicating 14 degrees of unsaturation. The ¹³C NMR spectrum of compound **3** (Table 3) showed 22 well-resolved resonances, including sp³ carbons (1 quaternary carbon, 4 methylenes, and 1 methoxyl) and sp² carbons (8 methines and 8 quaternary carbons). Among them, the carbon resonances at δ_C 170.3 and 161.1 were assigned to imine and formamide functionalities, respectively. In its ¹H NMR spectrum, only 19 protons showed HSQC correlations to these carbons.

(7) Trigonoliimine B (**2**): a light yellow gum; [α]_D¹⁰ = + 5.0 (c 0.5, CHCl₃); UV (MeOH) λ_{max} (log ϵ) 192 nm (4.2), 207 nm (4.4), 220 nm (4.4), 321 nm (4.0); IR (KBr) ν_{max} 3439, 2959, 2924, 1619, and 1452 cm⁻¹; ¹³C NMR data (Table 1); ESIMS *m/z* 357 [M + H]⁺; HRESIMS *m/z* 357.1718 [M + H]⁺ (calcd for C₂₂H₂₀N₄O, 357.1715).

(8) Trigonoliimine C (**3**): a light yellow gum; [α]_D¹⁰ = - 4.8 (c 0.45, CHCl₃); CD (CHCl₃) 224 nm ($\Delta\epsilon$ - 4.5), 228 nm ($\Delta\epsilon$ + 8.4); UV (MeOH) λ_{max} (log ϵ) 224 nm (4.4), 253 nm (3.8); IR (KBr) ν_{max} 3440, 2961, 2926, 1622, and 1453 cm⁻¹; ¹H and ¹³C NMR data (Table 2); ESIMS *m/z* 375 [M + H]⁺; HRESIMS *m/z* 375.1813 [M + H]⁺ (calcd for C₂₂H₂₂N₄O₂, 375.1821).

Besides, the remaining three [δ_{H} 10.64 (s, NH-1); 6.83 (br s, NH-13); and 7.99 (br s, NH-24)] were assigned to exchangeable protons. 1D NMR and $^1\text{H}-^1\text{H}$ COSY further revealed signals for one 1,2-disubstituted aromatic ring, one 1,2,4-trisubstituted benzene ring, and two NCH_2CH_2 moieties. Apart from nine degrees of unsaturation occupied by sp^2 carbons, the remaining five degrees of unsaturation required **3** to have a pentacyclic core ring system.

Interpretation of two-dimensional (2D) NMR data led to three partial moieties **a**–**c**. Unit **a** contained a 2,3-disubstituted 1*H*-indole ring on the basis of HMBC cross peaks of the exchangeable proton at δ_{H} 10.64 (NH-1) to C-2, C-3, C-8, and C-9, H-4 to C-3 and C-8, and H-7 to C-9 (**3a**, Figure 2). In addition, the NCH_2CH_2 fragment attached to C-3 was confirmed by the HMBC correlations of H₂-10 to C-3 and C-2. The HMBC correlations of NH-13 to C-14, C-15 and C-16, H-20 to C-16 and C-21, and H-17 to C-15 confirmed the unit **b** (**3b**, Figure 2). The unit **c**, consisting of another NCH_2CH_2 moiety and the formamide group, was assigned as the *N*-ethylformamide fragment (**3c**, Figure 2) by the observed HMBC correlations of NH-24 to C-25 and H-25 to C-23.

The connectivity of these units was further indicated by HMBC experiments. The three-bond correlations from H-11 α and H-17 to C-15 indicated the connection between fragments **3a** and **3b**. The connectivity of fragments **3a** and **3b** with **3c** was confirmed by the HMBC correlations of H-22 β to C-2, H₂-22 and NH-13 to C-14, which also permit the seven-membered imine-containing rings. Thus, the gross structure of **3** was established as shown.

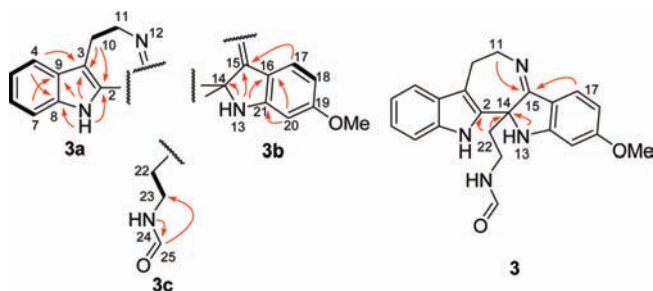


Figure 2. Partial fragments (**3a**–**3c**), $^1\text{H}-^1\text{H}$ COSY (–), and key HMBC (→) correlations of **3**.

The CD spectrum of **3** exhibited significantly positive cotton effects in 220–230 nm resulting from the exciton

coupling between the two different chromophores of the indole moiety and the 6-methoxyindolin-3-imine moiety,⁹ indicating that the transition dipole moments of the two chromophores were oriented in a clockwise manner (Figure 3). So, the absolute configuration of **3** was assigned as 14*R*.

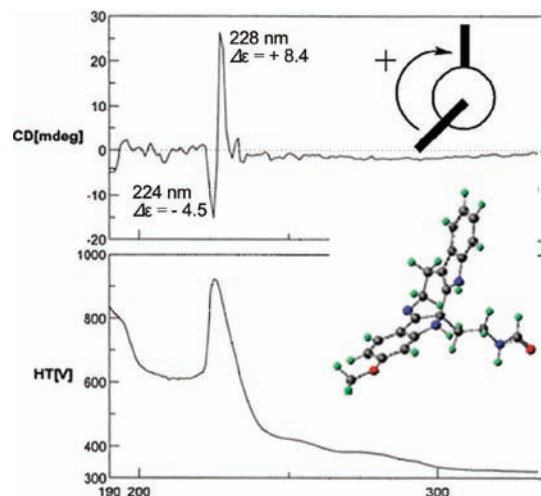


Figure 3. CD spectra of **3**. Bold lines denote the electric transition dipole of the chromophores for **3**.

The anti-HIV-1 activity of **1** and **3** was tested by a microtiter syncytium formation infectivity assay, with AZT (EC_{50} = 0.02 $\mu\text{g}/\text{mL}$, TI = 59924) as a positive control.¹⁰ Compound **1** showed modest anti-HIV-1 activity (EC_{50} = 0.95 $\mu\text{g}/\text{mL}$, TI = 7.9).

Acknowledgment. The work was financially supported by NSFC (No. 1130830114, 30801434), the Ministry of Science and Technology (2009CB940900 and 2009CB52230), and the young Academic and technical leader Raising Foundation of Yunnan Province (3930830114, 2009CI072).

Supporting Information Available: General experimental procedures, optical rotation calculation, and 1D and 2D NMR spectra of **1**–**3**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

OL100715X

(9) Dong, J. G.; Bornmann, W.; Nakanishi, K.; Berova, N. *Phytochemistry* **1995**, *40*, 1821–1824.

(10) Wang, J. H.; Tam, S. C.; Huang, H.; Ouyang, D. Y.; Wang, Y. Y.; Zheng, Y. T. *Biochem. Biophys. Res. Commun.* **2004**, *317*, 965–971.