Trigoflavidols A–C, Degraded Diterpenoids with Antimicrobial Activity, from Trigonostemon flavidus

11 AUTHORS, INCLUDING:

Yu-Cheng Gu
Syngenta
274 PUBLICATIONS **2,246** CITATIONS

SEE PROFILE

Shun-Lin Li
Chinese Academy of Sciences
82 PUBLICATIONS **573** CITATIONS

SEE PROFILE

Hong-Ping He
Chinese Academy of Sciences
177 PUBLICATIONS **1,843** CITATIONS

SEE PROFILE

Xiao-Jiang Hao
Chinese Academy of Sciences
191 PUBLICATIONS **1,835** CITATIONS

SEE PROFILE

Trigoflavidols A−C, Degraded Diterpenoids with Antimicrobial Activity, from *Trigonostemon flavidus*

Gui-Hua Tang,[†,‡] Yu Zhang,[†] Yu-Cheng Gu,[§] Shi-Fei Li,[†,‡] Ying-Tong Di,[†] Yue-Hu Wang,[⊥] Cui-Xian Yang,[‖] Guo-Ying Zuo,[‖] Shun-Lin Li,[†] Hong-Ping He,*[,†] and Xiao-Jiang Hao*[,†]

[†]State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, People's Republic of China

[‡]Graduate University of Chinese Academy of Sciences, Beijing 100049, People's Republic of China

[§]Syngenta, Jealott's Hill International Research Centre, Bracknell, Berkshire, RG42 6EY, U.K.

[⊥]Key Laboratory of Economic Plants and Biotechnology, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, People's Republic of China

[‖]Research Center of Natural Medicine, Clinical School of Kunming General Hospital of Chengdu Military Command, Kunming, 650032, People's Republic of China

S *Supporting Information*

ABSTRACT: Trigoflavidols A (**1**) and B (**2**), tetranorditerpenoid dimers possessing a rearrangement skeleton with a spiroketal core moiety, and trigoflavidol C (**3**), a hexanorditerpenoid, have been isolated from *Trigonostemon flavidus* along with two known compounds. Compounds **1** and **2** showed moderate antimicrobial activities (MIC values: 3.12−6.25 μg/mL) against *Staphylococcus aureus*, 8[#]MRSA, and 82[#]MRSA, and **1**, **2**, and **5** showed weak activities (IC$_{50}$ values: 3.75−28.99 μM) against various human tumor cell lines.

1 and **2** with MIC values of 3.12−6.25 μg/mL against *Staphylococcus aureus*, 8[#]MRSA, and 82[#]MRSA
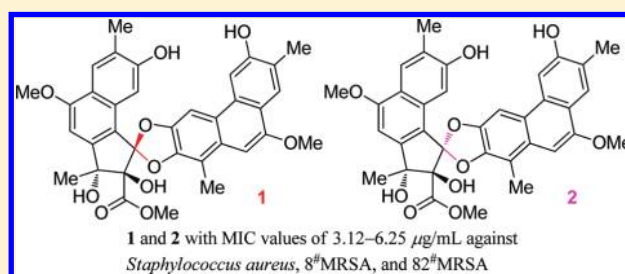
Plants of the genus *Trigonostemon* (about 50 species) belonging to the Euphorbiaceae family are trees or shrubs that are distributed mainly in tropical and subtropical Asia, extending from Sri Lanka and India to New Guinea.[1] Phytochemical investigations of *Trigonostemon* species have revealed the occurrence of indole alkaloids,[2−6] modified daphnane diterpenoids,[7−12] 3,4-*seco*-cleistanthanic diterpenoids,[13] and degraded diterpenoids.[13−16] Some degraded diterpenoids also exist in plants of other genera of the Euphorbiaceae such as *Actephila*,[17] *Domohinea*,[18] and *Neoboutonia*.[19] Previously we reported the isolation of degraded diterpenoids together with daphnane diterpenoids and indole alkaloids from *Trigonostemon*.[3,4,6,16,20]

Trigonostemon flavidus Gagnepain, also known as *Trigonostemon heterophyllus* Merrill, is an evergreen shrub that grows in dense forests, and the chemical constituents of this plant have not been extensively researched previously.[21,22] In the course of our ongoing work, we have carried out a phytochemical investigation on the stems of *T. flavidus* collected in Hainan Province, China. Herein, we report the identification of five degraded diterpenoids, including the tetranorditerpenoid dimers trigoflavidols A (**1**) and B (**2**) and the hexanorditerpenoid trigoflavidol C (**3**), as well as their biological activities.
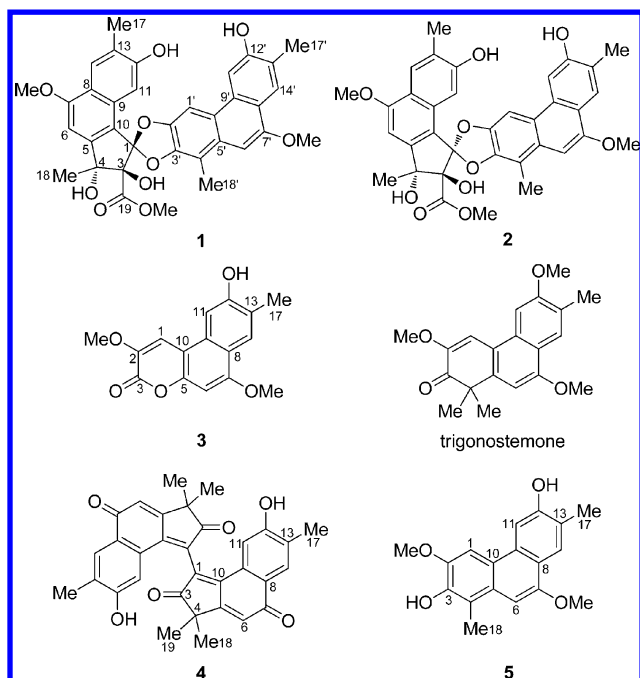
Trigoflavidol A (**1**) was determined to have the molecular formula $C_{35}H_{32}O_{10}$ with 20 degrees of unsaturation based on the [M + Na]$^+$ ion at *m/z* 635.1898 (calcd 635.1893) in its positive HRESIMS. The IR absorptions at 3432, 1631, 1588,

and 1469 cm^{-1} indicated the presence of hydroxy and phenyl functionalities. The 35 carbon signals observed in the ^{13}C NMR and DEPT spectra (Table 1) were classified as seven methyls including three *O*-methyl groups, seven methines, and 21 quaternary carbons including one ester carbonyl, six oxygenated aromatic carbons, and three other oxygenated carbons. In addition, one tertiary methyl at δ_H 1.61 (3H, s), three aromatic methyls at δ_H 2.33, 2.42, and 2.45 (each 3H, s), seven uncoupled aromatic protons at δ_H 6.90, 7.00, 7.20, 7.62, 7.90 (each 1H, s), and 8.05 (2H, s), and four hydroxy protons at δ_H 4.71, 4.77 (each 1H, s) and 8.89 (2H, s) were distinguished through further analysis of the NMR spectra. It was obvious from the comparison of the aforementioned values with the data of actephilol A[17] and from analysis of the 2D NMR data of **1** that trigoflavidol A was a heterodimer comprising two different highly aromatized tetranorditerpenoids, A and B, shown in Figure 1.

The constituent units were defined by analysis of the HMBC and ROESY correlations. The structure of part A, as shown in Figure 1, was determined by a set of HMBC correlations involving 3-O*H* to C-1, C-3, C-4, and C-19, 4-O*H* to C-3, C-4, C-5, and C-18, H$_3$-18 to C-3, C-4, and C-5, H-6 to C-4, C-8, and C-10, H-11 to C-8, C-10, and C-13, H-14 to C-7, C-9, C-12, and C-17, H$_3$-17 to C-12, C-13, and C-14, and 12-O*H* to C-

Table 1. ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) Data of 1 and 2 in Acetone-d_6 (δ in ppm)

position	1		2	
	δ_C, type	δ_H, multi	δ_C, type	δ_H, multi
1	121.9, C		121.8, C	
3	92.9, C		92.6, C	
4	80.9, C		80.9, C	
5	149.8, C		149.6, C	
6	96.4, CH	6.90, s	96.5, CH	6.90, s
7	159.6, C		159.5, C	
8	121.5, C		121.4, C	
9	131.4, C		131.3, C	
10	119.6, C		119.9, C	
11	106.26, CH	7.20, s	106.30, CH	7.22, s
12	156.8, C		156.8, C	
13	127.1, C		127.1, C	
14	125.2, CH	8.05, s	125.1, CH	8.05, s
17	17.1, CH$_3$	2.33, s	17.1, CH$_3$	2.33, s
18	27.8, CH$_3$	1.61, s	27.7, CH$_3$	1.64, s
19	173.3, C		173.3, C	
1′	98.4, CH	7.62, s	98.8, CH	7.65, s
2′	147.09, C		146.6, C	
3′	147.12, C		147.7, C	
4′	112.3, C		111.5, C	
5′	128.9, C		129.4, C	
6′	96.8, CH	7.00, s	96.7, CH	6.99, s
7′	153.5, C		153.6, C	
8′	119.9, C		119.9, C	
9′	132.6, C		132.6, C	
10′	121.9, C		121.5, C	
11′	106.33, CH	7.90, s	106.34, CH	7.91, s
12′	156.4, C		156.4, C	
13′	126.1, C		126.0, C	
14′	125.0, CH	8.05, s	125.0, CH	8.05, s
17′	16.8, CH$_3$	2.42, s	16.8, CH$_3$	2.42, s
18′	11.7, CH$_3$	2.45, s	11.9, CH$_3$	2.45, s
7-OMe	56.2, CH$_3$	4.09, s	56.2, CH$_3$	4.09, s
19-OMe	53.1, CH$_3$	3.62, s	53.1, CH$_3$	3.59, s
7′-OMe	55.7, CH$_3$	4.10, s	55.7, CH$_3$	4.10, s
3-OH		4.77, s		4.73, s
4-OH		4.71, s		4.75, s
12-OH		8.89, s		8.91, s
12′-OH		8.89, s		8.91, s

11, C-12, and C-13. The connection of C-1 with C-10 to form the five-membered ring was deduced on the basis of the chemical shifts of these carbons at δ_C 121.9 (C-1) and 119.6 (C-10) and on the structure of part B (see Figure 1). Two methoxy groups at δ_H 3.62 and 4.09 were deduced to be located at C-19 and C-7 by the HMBC correlations from 19-OCH_3 to C-19 and from 7-OCH_3 to C-7, respectively. The proposed structure was further confirmed by the ROESY cross-peaks of H$_3$-18/H-6, H-6/4-OH, H-6/7-OCH_3, H-11/12-OH, H$_3$-17/H-14, and H-14/7-OCH_3. Thus, part A of **1** comprised a degraded diterpenoid with a rearranged skeleton.

The structure of part B was deduced by the interpretation of HMBC and ROESY spectra (see Figure 1), which were similar to those of trigonochinene E (**5**).[13] Although there were no direct HMBC correlations available to link parts A and B, the two parts accounted for 19 degrees of unsaturation, and the remaining degree of unsaturation required that parts A and B be connected to form a ring. The presence of two oxygenated aromatic carbon signals at δ_C 147.09 (C-2′) and 147.12 (C-3′) and of a typical ketal carbon at δ_C 121.9 (C-1) suggested that compound **1** was produced by the condensation reaction of parts A and B. Finally, a structure containing a unique spiroketal moiety was deduced for **1**; this structure is fully consistent with the molecular composition of this compound.

The relative configuration of compound **1** was determined via a ROESY experiment. The ROESY correlations of 3-OH/H$_3$-18 and 19-OCH_3/H$_3$-18′ indicated that H$_3$-18, 3-OH, and the oxygen bridge between C-1 and C-2′ were cofacial and were arbitrarily assigned a β-orientation, as shown in Figure 1.

The molecular formula of trigoflavidol B (**2**) was established as $C_{35}H_{32}O_{10}$, identical to that of **1**, on the basis of the positive HRESIMS ion at m/z 635.1896 [M + Na]$^+$ (calcd 635.1893). Comparison of the NMR data demonstrated that **2** showed remarkable similarity with **1** (Table 1). Only the chemical shifts of H-18, H-1′, 19-OCH_3, 3-OH, and 4-OH in the ^{1}H NMR spectrum and the chemical shifts of C-2′, C-3′, C-4′, and C-5′ in the ^{13}C NMR spectrum displayed certain differences. Analysis of the 2D NMR data confirmed that **2** had the same gross structure as **1**. However, the key ROESY cross-peaks of 3-OH/

H$_3$-18 and 19-OCH_3/H-1′ indicated that **2** was the C-1 epimer of **1**.

Positive HRESIMS analysis of compound **3** exhibited a quasimolecular ion peak at m/z 287.0924 [M + H]$^+$ (calcd 287.0919), corresponding to the molecular formula $C_{16}H_{14}O_5$ with 10 degrees of unsaturation. The ^{1}H NMR spectrum of **3** displayed signals for four aromatic protons (δ_H 6.80, 7.53, 7.56, and 7.90, each 1H, s), one hydroxy proton (δ_H 10.10, 1H, s), two methoxy groups (δ_H 3.92 and 3.97, each 3H, s), and a tertiary methyl group (δ_H 2.31, 3H, s), which showed correlations in the HSQC and HMBC spectra with the aromatic carbon signals at δ_C 93.0, 110.9, 104.6, 123.7, 156.7, 141.5, and 155.9, the methoxy carbon signals at δ_C 56.2 and 56.0, and the aromatic methyl carbon at δ_C 16.7, respectively. Six low-field quaternary carbons were additionally observed in the ^{13}C NMR and DEPT spectra of **3**. Comparing the MS and NMR data **3** to those of trigonostemone[14] revealed that both had the same degrees of unsaturation and similar structure with
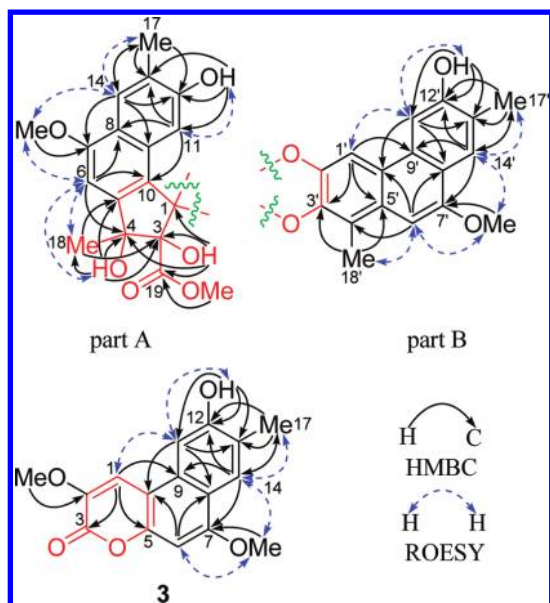
Figure 1. HMBC and ROESY correlations for parts A and B and compound **3**.

the presence of a naphthalene moiety bearing an aromatic methyl and two oxygenated substituents; one difference was that the moiety of the quaternary carbon bearing a *gem*-dimethyl group was missing in **3**. It was deduced that **3** may be a degradation product that is homologous to trigonostemone. The structure of **3** was further established on the basis of the HMBC and ROESY correlations (shown in Figure 1). Thus, compound **3** is a new hexanorditerpenoid, trivially named trigoflavidol C.

A known compound, neoboutomannin (**4**),[15] was identified by comparison of its spectroscopic data with literature data. Moreover, the structure of compound **5** was determined by analysis of its 2D NMR spectrum, which was identical to that of trigonochinene E.[13] However, the ^{13}C NMR assignment for C-8 in **5** should be corrected (Supporting Information).

The isolated degraded diterpenoids were screened for their antimicrobial activity against six microorganisms, *Monilia albicans, Pseudomonas aeruginosa, Escherichia coli, Staphyloccocus aureus*, 8[#]MRSA (MRSA, methicillin-resistant *Staph. aureus*), and 82[#]MRSA, using the agar plate punch assay. The minimum inhibitory concentrations (MICs) of compounds **1**–**3** and **5** against *Staph. aureus*, 8[#]MRSA, and 82[#]MRSA were determined by the 2-fold dilution method. Moreover, the cytotoxicities of compounds **1**–**5** against five human tumor cell lines, HL-60 (premyelocytic leukemia), SMMC-7721 (hepatocellular carci-

noma), A-549 (lung adenocarcinoma), MCF-7 (breast cancer), and SW480 (colon adenocarcinoma), were tested using the MTT method. The results (Table 2) revealed that compounds **1** and **2** exhibited moderate activities against *Staph. aureus*, 8[#]MRSA, and 82[#]MRSA, and compounds **1**, **2**, and **5** showed weak cytotoxicities against the test human tumor cell lines.

■ EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were detected on a Shimadzu UV 2401 spectrometer. IR spectra were determined on a Bruker Tensor-27 infrared spectrophotometer with KBr disks. 1D and 2D NMR spectra were recorded on Bruker AM-400, Bruker DRX-500, and Bruker Avance III 600 spectrometers using TMS as an internal standard. MS and HRESIMS analyses were carried out on an API Qstar Pulsar 1 instrument. Semipreparative HPLC was performed using an Agilent 1200 series pump equipped with a diode array detector and a Zorbax SB-C$_{18}$ column (5.0 μm, 9.4 × 250 mm). Silica gel G (80−100 and 300−400 mesh, Qingdao Makall Group Co., Ltd.), MCI gel CHP 20P (75−150 μm, Mitsubishi Chemical Corporation, Tokyo), C$_{18}$ silica gel (40−75 μm, Fuji Silysia Chemical Ltd.), silica gel H (10−40 μm), and Sephadex LH-20 (GE Healthcare Bio-Xciences AB) were used for column chromatography, and silica gel GF254 (Qingdao) was used for preparative TLC in the form of precoated plates. TLC spots were visualized under UV light and by dipping into 5% H$_2$SO$_4$ in EtOH followed by heating.

Plant Material. The stems of *T. flavidus* were collected from Sanya, Hainan Province, People's Republic of China, in October 2010. The plant was identified by one of the authors (G.-H.T.), and a voucher specimen (H20101011) was deposited at State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany.

Extraction and Isolation. The air-dried and powdered stems (16 kg) of *T. flavidus* were extracted with MeOH (3 × 25 L) three times (4, 3, and 3 h, respectively) under reflux. The crude residue (570 g) was suspended in H$_2$O (4 L) and then partitioned successively with EtOAc and n-BuOH to give two corresponding portions (110 and 200 g). The EtOAc portion (110 g) was subjected to repeated column chromatography over silica gel, C$_{18}$ silica gel, and Sephadex LH-20 and then further purified by semipreparative HPLC to obtain **1** (4.2 mg), **2** (5.6 mg), **3** (4.7 mg), **4** (4.7 mg), and **5** (159.4 mg).

*Trigoflavidol A (**1**):* white, amorphous powder; $[\alpha]^{22}_D$ −4.6 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 363 (3.11), 346 (3.20), 292 (4.43), 250 (4.70), 197 (4.50) nm; IR ν_{max} (KBr) 3432, 1729, 1631, 1604, 1588, 1469, 1442, 1267, 1240, 1198, 1169, 1143, 1078 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; ESIMS *m/z* 635 [M + Na]$^+$; HRESIMS *m/z* 635.1898 [M + Na]$^+$ (calcd for C$_{35}$H$_{32}$O$_{10}$Na, 635.1893).

*Trigoflavidol B (**2**):* white, amorphous powder; $[\alpha]^{22}_D$ −3.7 (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ε) 363 (3.25), 345 (3.33), 292 (4.48), 249 (4.74), 196 (4.54) nm; IR ν_{max} (KBr) 3432, 1728, 1631, 1603, 1588, 1470, 1441, 1266, 1240, 1198, 1169, 1144, 1078 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; ESIMS *m/z* 635 [M + Na]$^+$;

Table 2. Antimicrobial Activities and Cytotoxicities of 1−5^a

compound[b]	antimicrobial activities (MIC in μg/mL)			cytotoxicities (IC$_{50}$ in μM)				
	Staph. aureus	8[#]MRSA	82[#]MRSA	HL-60	SMMC-7721	A-549	MCF-7	SW480
1	1.56	3.12	6.25	21.05	21.58	19.76	14.63	15.86
2	1.56	3.12	3.12	>40	19.49	17.05	14.64	15.42
5	>50	>50	>50	3.75	28.99	21.01	18.4	20.48
positive control	1.56[c]	0.78[c]	0.78[c]	1.14[d]	14.51[d]	12.76[d]	15.85[d]	15.11[d]

^{a}*Staph. aureus* (*Staphylococcus aureus*), MRSA (methicillin-resistant *Staphylococcus aureus*), HL-60 (human premyelocytic leukemia cell line), SMMC-7721 (human hepatocellular carcinoma cell line), A-549 (human lung adenocarcinoma cell line), MCF-7 (human breast cancer cell line), SW480 (human colon adenocarcinoma cell line). ^{b}Results of compounds **3** and **4** against the bacteria (MIC > 50 μg/mL) and the five human tumor cell lines (IC$_{50}$ > 40 μM) were not listed. ^{c}Vancomycin hydrochloride as positive control. ^{d}*cis*-Platin as positive control.

HRESIMS m/z 635.1896 $[M + Na]^+$ (calcd for $C_{35}H_{32}O_{10}Na$, 635.1893).

Trigoflavidol C (3): white, amorphous powder; UV (MeOH) λ_{max} (log ε) 356 (2.90), 254 (3.34), 217 (3.37), 202 (3.27) nm; IR ν_{max} (KBr) 3426, 1710, 1637, 1594, 1451, 1249, 1031, 1003 cm^{-1}; ^{1}H NMR (DMSO-d_6, 600 MHz) δ 10.10 (1H, s, 12-OH), 7.90 (1H, s, H-14), 7.56 (1H, s, H-11), 7.53 (1H, s, H-1), 6.80 (1H, s, H-6), 3.97 (3H, s, 7-OCH_3), 3.92 (3H, s, 2-OCH_3), 2.31 (3H, s, H-17); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 157.2 (C, C-3), 156.7 (C, C-12), 155.9 (C, C-7), 149.0 (C, C-5), 141.5 (C, C-2), 129.2 (C, C-9), 125.9 (C, C-13), 123.7 (CH, C-14), 116.5 (C, C-8), 110.9 (CH, C-1), 105.4 (C, C-10), 104.6 (CH, C-11), 93.0 (CH, C-6), 56.2 (CH$_3$, 3-OCH$_3$), 56.0 (CH$_3$, 7-OCH$_3$), 16.7 (CH$_3$, C-17); ESIMS m/z 309 $[M + Na]^+$; HRESIMS m/z 287.0924 $[M + H]^+$ (calcd for $C_{16}H_{15}O_5$, 287.0919).

Trigonochinene E (5): ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.60 (1H, s, 12-OH), 8.03 (1H, s, H-14), 7.94 (1H, s, H-11), 7.74 (1H, s, H-1), 7.70 (1H, s, 3-OH), 6.95 (1H, s, H-6), 4.07 (3H, s, 7-OCH_3), 4.04 (3H, s, 2-OCH_3), 2.54 (3H, s, H-18), 2.44 (3H, s, H-17); ^{13}C NMR (acetone-d_6, 100 MHz) δ 156.0 (C, C-12), 153.2 (C, C-7), 146.3 (C, C-2), 144.9 (C, C-3), 132.3 (C, C-9), 128.4 (C, C-5), 125.2 (C, C-13), 124.8 (CH, C-14), 119.9 (C, C-8), 119.7 (C, C-10), 117.2 (C, C-4), 106.5 (CH, C-11), 101.4 (CH, C-1), 96.5 (CH, C-5), 56.1 (CH$_3$, 2-OCH$_3$), 55.4 (CH$_3$, 7-OCH$_3$), 16.7 (CH$_3$, C-17), 11.4 (CH$_3$, C-18).

Antimicrobial Assays. The strains used in antimicrobial tests were obtained from the Research Center of Natural Medicine, Clinical School of Kunming General Hospital of Chengdu Military Command. For the agar plate punch assay,[23] all compounds were dissolved in DMSO at a concentration of 500 μg/mL. Then, 50 μL of the solution was added onto a well (6 mm in diameter) that had been punched in the appropriate agar growth medium smeared with a suspension of the test organism (1.5 × 10^9 cfu/mL; cfu, colony forming unit). The test organisms in this bioassay were the bacteria *P. aeruginosa*, *E. coli*, *Staph. aureus*, 8$^\#$MRSA, and 82$^\#$MRSA (all grown on MH medium) and the fungus *M. albicans* (grown on Sabauraud's medium). All active compounds with a diameter of inhibition greater than 10 mm were submitted to minimum inhibitory concentration testing. The MICs of compounds 1−3 and 5 against *Staph. aureus*, 8$^\#$MRSA, and 82$^\#$MRSA were determined using a 2-fold dilution method.[23] The 2-fold serially diluted compounds in MH broth were dispensed into 96-well microtiter plates (100 μL/well), and then an aliquot of 5 × 10^5 cfu/mL of bacterial culture was added to each well (100 μL/well) to final concentrations in a range of 0.39−50 μg/mL. After incubating at 37 °C for 18 h, the lowest concentration without any colony growth was recorded as the MIC value. The resulting values were compared with the value for a positive control (vancomycin hydrochloride, range 0.01−25 μg/mL) under the same conditions.

Cytotoxicity Assays. Cytotoxicity evaluations were performed according to the previously described protocol.[24,25]

■ ASSOCIATED CONTENT

ⓢ Supporting Information
1D and 2D NMR, IR, UV, and HRESIMS spectra of 1−3 and 5; a flowchart for the isolation of chemical constituents from *T. flavidus*. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author
*Tel/Fax: +86-871-5223070. E-mail: haoxj@mail.kib.ac.cn; hehongping@mail.kib.ac.cn.

Notes
The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

■ REFERENCES

(1) Li, B. T.; Gilbert, M. G. *Flora of China*; Science Press: Beijing, 2008; Vol. *11*, pp 272−274
(2) Kanchanapoom, T.; Kasai, R.; Chumsri, P.; Kraisintu, K.; Yamasaki, K. *Tetrahedron Lett.* **2002**, *43*, 2941−2943.
(3) 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) Tan, C. J.; Di, Y. T.; Wang, Y. H.; Zhang, Y.; Si, Y. K.; Zhang, Q.; Gao, S.; Hu, X. J.; Fang, X.; Li, S. F.; Hao, X. J. *Org. Lett.* **2010**, *12*, 2370−2373.
(5) Zhu, Q.; Tang, C. P.; Ke, C. Q.; Li, X. Q.; Liu, J.; Gan, L. S.; Weiss, H. C.; Gesing, E. R.; Ye, Y. *J. Nat. Prod.* **2010**, *73*, 40−44.
(6) Li, S. F.; Di, Y. T.; He, H. P.; Zhang, Y.; Wang, Y. H.; Yin, J. L.; Tan, C. J.; Li, S. L.; Hao, X. J. *Tetrahedron Lett.* **2011**, *52*, 3186−3188.
(7) 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.
(8) Jayasuriya, H.; Zink, D. L.; Borris, R. P.; Nanakorn, W.; Beck, H. T.; Balick, M. J.; Goetz, M. A.; Gregory, L.; Shoop, W. L.; Singh, S. B. *J. Nat. Prod.* **2004**, *67*, 228−231.
(9) Chen, H. D.; He, X. F.; Ai, J.; Geng, M. Y.; Yue, J. M. *Org. Lett.* **2009**, *11*, 4080−4083.
(10) Chen, H. D.; Yang, S. P.; He, X. F.; Ai, J.; Liu, Z. K.; Liu, H. B.; Geng, M. Y.; Yue, J. M. *Org. Lett.* **2010**, *12*, 1168−1171.
(11) Chen, H. D.; Yang, S. P.; He, X. F.; Liu, H. B.; Ding, J.; Yue, J. M. *Tetrahedron* **2010**, *66*, 5065−5070.
(12) 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.
(13) Yin, S.; Su, Z. S.; Zhou, Z. W.; Dong, L.; Yue, J. M. *J. Nat. Prod.* **2008**, *71*, 1414−1417.
(14) Kokpol, U.; Thebpatiphat, S.; Boonyaratavej, S.; Chedchuskulcai, V.; Ni, C. Z.; Clardy, J.; Chaichantipyuth, C.; Chittawong, V.; Miles, D. H. *J. Nat. Prod.* **1990**, *53*, 1148−1151.
(15) Tene, M.; Tane, P.; Tamokou, J. D.; Kuiate, J. R.; Connolly, J. D. *Phytochem. Lett.* **2008**, *1*, 120−124.
(16) 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.
(17) Ovenden, S. P. B.; Yew, A. L. S.; Glover, R. P.; Ng, S.; Rossant, C. J.; Regalado, J. C.; Soejarto, D. D.; Buss, A. D.; Butler, M. S. *Tetrahedron Lett.* **2001**, *42*, 7695−7697.
(18) Long, L.; Lee, S. K.; Chai, H. B.; Rasoanaivo, P.; Gao, Q.; Navarro, H.; Wall, M. E.; Wani, M. C.; Farnsworth, N. R.; Cordell, G. A.; Pezzuto, J. M.; Kinghorn, A. D. *Tetrahedron* **1997**, *53*, 15663−15670.
(19) Tchinda, A. T.; Tsopmo, A.; Tene, M.; Kamnaing, P.; Ngnokam, D.; Tane, P.; Ayafor, J. F.; Connolly, J. D.; Farrugia, L. J. *Phytochemistry* **2003**, *64*, 575−581.
(20) Li, S. F.; Di, Y. T.; Li, S. L.; Zhang, Y.; Yang, F. M.; Sun, Q. Y.; Simo, J. M.; He, H. P.; Hao, X. J. *J. Nat. Prod.* **2011**, *74*, 464−469.
(21) Dong, S. H.; Liu, H. B.; Xu, C. H.; Ding, J.; Yue, J. M. *J. Nat. Prod.* **2011**, *74*, 2576−2581.
(22) Li, Y. X.; Mei, W. L.; Zuo, W. J.; Zhao, Y. X.; Dong, W. H.; Dai, H. F. *Phytochem. Lett.* **2011**, *5*, 41−44.

(23) Xu, S. Y.; Bian, R. L.; Chen, X. *Pharmacological Experiment Methodology*, 3rd ed.; People's Medical Publishing House: Beijing, 2002; pp 1647−1719.

(24) Fang, X.; Di, Y. T.; Geng, Z. L.; Tan, C. J.; Guo, J.; Ning, J.; Hao, X. J. *Eur. J. Org. Chem.* **2010**, *2010*, 1381−1387.

(25) Zhang, Y.; Di, Y. T.; He, H. P.; Li, S. F.; Lu, Y.; Gong, N. B.; Hao, X. J. *Eur. J. Org. Chem.* **2011**, *2011*, 4103−4107.