Two New Cytotoxic Alkaloids from *Mappianthus iodoides* HAND.-MAZZ.

by **Xi-Bin Xiao***[a]), **Yue-Xing Lin**[b]), **Geng-Bo Xu**[a]), **Xu-Bo Gong**[a]), **Ying Gu**[a]), **Jie-Feng Tong**[a]), and **Jing Yang**[a])

[a]) Department of Hematology, Second Affiliated Hospital, Zhejiang University, No. 88 Jiefang Road, Hangzhou 310009, P. R. China (phone: +86-571-87783652; e-mail: xiaoxibincsu@163.com)

[b]) Blood Bank, Second Affiliated Hospital, Zhejiang University, Hangzhou 310009, P. R. China

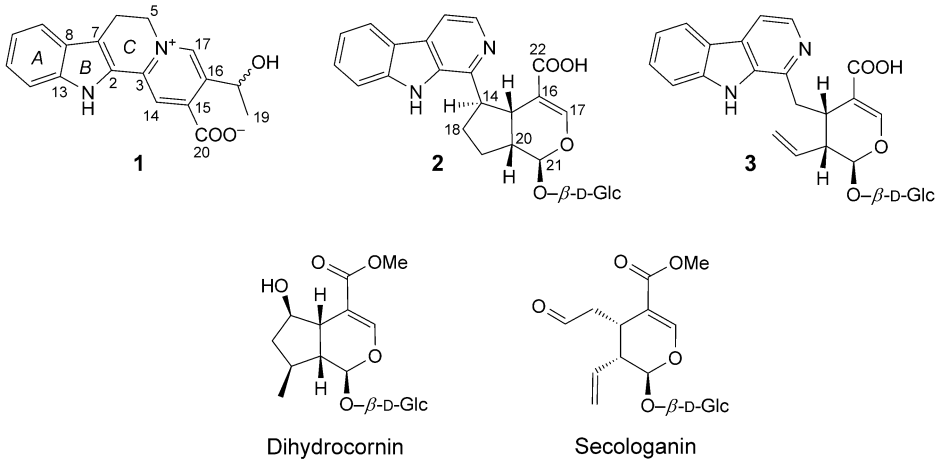
Two new alkaloids, mappine A (**1**) and mapposidic acid (**2**), together with eleven known compounds, were isolated from the stems of *Mappianthus iodoides* HAND.-MAZZ. Their structures were elucidated by means of spectroscopic and mass-spectrometric analyses, particularly 1D- and 2D-NMR spectroscopy. The cytotoxic activities of the two new alkaloids were also evaluated.

Introduction. – The genus *Mappianthus* (Icacinaceae family) consists of three species which are distributed in subtropical and tropical zone. *Mappianthus iodoides* HAND.-MAZZ., one species occurring in China, is used by local people for the treatment of traumatic injury, rheumatalgia, arthralgia, *etc.* [1].

There were no phytochemical studies on this genus except that a sesquiterpene named (−)-cedrol had been reported from *Mappianthus iodoides* HAND.-MAZZ. [2]. Our phytochemical investigation on the 95% EtOH extract of the title plant led to the isolation of 13 compounds, comprising seven alkaloids, *i.e.*, mappine A (**1**), mapposidic acid (**2**), 1-methyl-β-carboline [3], strictosidine [4], strictosidine acid [4], 5-carboxy-strictosidine [5], and lyalosidic acid (**3**) [6], three lignans, *i.e.*, pinoresinol [7], (+)-(1*R*,2*S*,5*R*,6*S*)-2,6-bis(4-hydroxyphenyl)-3,7-dioxabicyclo[3.3.0]octane [8], and pinoresinol dimethyl ether [9], two iridoid glycosides, *i.e.*, dihydrocornin [10] and secologanin [11], and one phenolic glycoside, *i.e.*, 3,4,5-trimethoxyphenol-1-*O*-[β-D-apiofuranosyl-(1 → 6)]-β-D-glucopyranoside [12]. The above compounds were reported for the first time from this genus. Here, we report on the isolation and structure elucidation of the two new alkaloids **1** and **2**, and the evaluation of their cytotoxic activities.

Results and Discussion. – *Structure Elucidation.* Compound **1**, isolated as an amorphous powder, was assigned the molecular formula $C_{18}H_{16}N_2O_3$, on the basis of the $[M+H]^+$ ion peak at *m*/*z* 309.1234 (calc. 309.1239) in HR-ESI-MS (positive-ion mode). The ^{1}H-NMR spectrum (DMSO) showed signals for four aromatic H-atoms due to the *A* ring of the indole system at δ(H) 7.70 (*d*, *J* = 8.2, H–C(9))[1]), 7.15 (*dd*, *J* = 8.2, 8.2, H–C(10)), 7.33 (*dd*, *J* = 8.2, 8.2, H–C(11)), and 7.52 (*d*, *J* = 8.2, H–C(12)), and for two CH_2 groups at δ(H) 4.87–4.91 (*m*, CH_2(5)) and 3.32–3.36 (*m*, CH_2(6)), suggesting that the skeleton of **1** resembled tryptamine (*Table 1*). A methine C-atom

[1]) Arbitrary C-atom numbering as indicated in the formula. For systematic names, *cf.* the *Exper. Part.*



bearing a OH group, as indicated by the chemical shift of the corresponding H-atom at δ(H) 5.18–5.20 (*q*, $J = 6.5$, H–C(18)), coupled with a Me group δ(H) 1.43 (*d*, $J = 6.5$, H–C(19)). HMBC Correlations of H–C(19) with C(16) and C(18), and of H–C(18) with C(15), C(16), and C(19) indicated that the 1-hydroxyethyl fragment was located at C(16).

The signals in the ^{13}C-NMR spectrum were attributed to one COO^- C-atom (δ(C) 165.9 (C(20))) and five olefinic C-atoms, and the corresponding downfield signals at δ(H) 8.23 (*s*, H–C(14)) and 8.78 (*s*, H–C(17)) in the ^{1}H-NMR spectrum of **1**, together with a strong absorption band (1606 cm^{-1}) in the IR spectrum indicated the presence of a pyridine ring in compound **1**. A characteristically highly deshielded signal at δ(H) 8.78 (*s*, H–C(17)) indicated the presence of a H-atom at a C-atom adjacent to a positively charged N-atom [13], which was verified by the HMBC experiment. HMBC Correlations (*Table 1*) of H–C(17) with C(3), C(5), C(15), C(16), and C(18), of H–C(14) with C(2), C(3) and C(20) established the structure of **1**, with the chemical shift at δ(C) 165.9 indicating the C(20)OO^- group [13]. Accordingly, **1** was assigned an inner salt form and named mappine.

Compound **2** was isolated as an amorphous powder, and its molecular formula was established as $C_{26}H_{28}N_2O_9$, based on the HR-ESI-MS (*m*/*z* 513.1868 ($[M+H]^+$; calc. 513.1873)). The ^{1}H-NMR spectrum (CD_3OD) showed signals of two H-atoms of *C* ring of a β-carboline moiety at δ(H) 8.11 (*d*, $J = 5.7$, H–C(5))[1]) and 7.90 (*d*, $J = 5.7$, H–C(6)), along those of four aromatic H-atoms on the *A* ring (δ(H) 8.12 (*d*, $J = 8.4$, H–C(9)), 7.22 (*dd*, $J = 8.4$, 8.4, H–C(10)), 7.52 (*dd*, $J = 8.4$, 8.4, H–C(11)), and 7.70 (*d*, $J = 8.4$, H–C(12))), one acetal H-atom (δ(H) 5.57 (*d*, $J = 6.9$, H–C(21))), one anomeric H-atom of a β-linked glucose (δ(H) 4.73 (*d*, $J = 7.8$, H–C(1′))), and one olefinic H-atom of the acrylic part moiety (δ(H) 7.30 (*s*, H–C(17))) (*Table 2*). The ^{13}C-NMR spectrum (CD_3OD) showed signals for 26 C-atoms including 13 olefinic atoms, one COOH (δ(C) 176.2 (C(22))), one acetal (δ(C) 97.4 (C(21))), and one anomeric C-atom (δ(C) 100.7 (C(1′))), and one glucose unit. The hydrolysis in acidic medium provided the free sugar, which could be identified as D-glucose by the sign of its optical rotation and comparison

Table 1. *^{1}H- and ^{13}C-NMR Data and HMBC Correlations for Compound* **1**. Recorded in (D_6)DMSO at 400 and 100 MHz, respectively; δ in ppm, J in Hz. Atom numbering as indicated in the formula.

	δ(H)	δ(C)	HMBC (H → C)
C(2)		138.8	
C(3)		141.3	
CH_2(5)	4.87–4.91 (*m*)	55.0	C(3), C(6), C(7), C(17)
CH_2(6)	3.32–3.36 (*m*)	18.9	C(2), C(5), C(7), C(8)
C(7)		116.0	
C(8)		124.7	
H–C(9)	7.70 (*d*, *J* = 8.2)	120.3	C(7), C(11), C(13)
H–C(10)	7.15 (*dd*, *J* = 8.2, 8.2)	120.4	C(8), C(12)
H–C(11)	7.33 (*dd*, *J* = 8.2, 8.2)	125.6	C(9), C(13)
H–C(12)	7.52 (*d*, *J* = 8.2)	112.6	C(8), C(10)
C(13)		139.1	
H–C(14)	8.23 (*s*)	118.7	C(2), C(3), C(20)
C(15)		156.5	
C(16)		138.8	
H–C(17)	8.78 (*s*)	142.8	C(3), C(5), C(15), C(16), C(18)
H–C(18)	5.19 (*q*, *J* = 6.5)	64.6	C(15), C(16), C(19)
Me(19)	1.43 (*d*, *J* = 6.5)	24.1	C(16), C(18)
C(20)		165.9	

of retention times (t_R) of its Me_3Si (TMS) ethers with the t_R value of a standard sample in gas chromatography.

The ^{1}H- and ^{13}C-NMR spectra of **2** were very similar to those of lyalosidic acid (**3**; *Table 2*), except for two CH_2 C-atoms in **2** instead of two olefinic C-atoms in **3**. Due to the same molecular weight of the two compounds, it may be deduced that **2** was derived from lyalosidic acid by cyclization between C(14) and C(18), was also supported by the HMBC correlations of H–C(18) with C(3) and C(20), and of H–C(14) with C(2), C(16), and C(20). In the ROESY spectrum of **2**, correlation peaks H–C(15)/H–C(20) and H–C(14)/H–C(21) were observed. Thus, the iridoid part of **2** bears a loganin skeleton, on the other hand, dihydrocornin and secologanin were also obtained in this phytochemical study which shared the same relative configuration. Dihydrocornin [10] and secologanin [11] were identified by comparison of their NMR data and the physical properties with those reported in the literature. From a biogenetic view, H–C(15) and H–C(20) of **2** should be β-oriented, while H–C(14) and H–C(21) have α-orientation. All the above information strongly indicated that **2** was a cyclized product of lyalosidic acid, named mapposidic acid.

The cytotoxicities of compounds **1** and **2** were evaluated. The results were listed in *Table 3*. Compound **1** demonstrated inhibitory activity against K562 and Lovo cell lines with IC_{50} values of less than 10 μM, and compound **2** showed cytotoxicity against the K562, HepG2, KB, and LoVo cell lines with IC_{50} values of 15.3, 6.9, 2.2 and 4.2 μM, respectively.

Table 2. *^{1}H- and ^{13}C-NMR Spectral Data and HMBC Correlations for Compounds* **2** *and* **3**. Recorded in CD_3OD at 400 and 100 MHz, respectively; δ in ppm, J in Hz. Atom numbering as indicated in the formula of **2**.

	2			**3**	
	δ(H)	δ(C)	HMBC (H → C)	δ(H)	δ(C)
C(2)		137.2			138.2
C(3)		147.1			144.8
H–C(5)	8.11 (*d*, *J* = 5.7)	137.5	C(3), C(6), C(7)	8.05 (*d*, *J* = 5.6)	136.7
H–C(6)	7.90 (*d*, *J* = 5.7)	114.6	C(2), C(5), C(8)	7.91 (*d*, *J* = 5.6)	114.9
C(7)		129.9			130.8
C(8)		122.8			122.1
H–C(9)	8.12 (*d*, *J* = 8.4)	118.4	C(7), C(11), C(13)	8.14 (*d*, *J* = 8.4)	122.7
H–C(10)	7.22 (*dd*, *J* = 8.4, 8.4)	120.7	C(8), C(12)	7.19 (*dd*, *J* = 8.4, 8.4)	121.2
H–C(11)	7.52 (*dd*, *J* = 8.4, 8.4)	129.4	C(9), C(13)	7.54 (*dd*, *J* = 8.4, 8.4)	130.3
H–C(12)	7.70 (*d*, *J* = 8.4)	113.5	C(8), C(10)	7.73 (*d*, *J* = 8.4)	113.4
C(13)		142.6			135.8
H–C(14) or CH_2(14)	2.47 – 2.51 (*m*)	36.7	C(2), C(16), C(20)	2.14 – 2.17 (*m*), 2.35 – 2.37 (*m*)	36.6
H–C(15)	3.66 – 3.69 (*m*)	36.1	C(3), C(17)	2.98 – 3.01 (*m*)	36.0
C(16)		117.8			114.8
H–C(17)	7.30 (*s*)	149.3	C(15), C(16), C(21)	7.71 (*s*)	151.2
CH_2(18)	2.68 – 2.72 (*m*), 3.10 – 3.14 (*m*)	28.7	C(3), C(20)	5.18 (*d*, *J* = 11.0), 5.28 (*d*, *J* = 17.6)	118.8
CH_2(19) or H–C(19)	2.47 – 2.51 (*m*), 2.67 – 2.71 (*m*)	29.5	C(15), C(21)	5.75 (*ddd*, *J* = 17.6, 11.0, 7.5)	134.6
H–C(20)	2.51 – 2.53 (*m*)	47.1	C(14), C(16), C(21)	2.66 – 2.68 (*m*)	46.4
H–C(21)	5.57 (*d*, *J* = 6.9)	97.4	C(17), C(1′)	5.78 (*d*, *J* = 7.2)	97.6
C(22)		176.2			174.7
H–C(1′)	4.73 (*d*, *J* = 7.8)	100.7	C(21), C(3′), C(5′)	4.72 (*d*, *J* = 7.6)	100.4
H–C(2′)	3.21 (*dd*, *J* = 7.8, 9.3)	74.9	C(3′), C(4′)	3.17 (*dd*, *J* = 7.6, 9.0)	74.6
H–C(3′)	3.17 – 3.35 (*m*)	78.2	C(1′), C(5′)	3.32 (*dd*, *J* = 9.0, 9.0)	78.4
H–C(4′)	3.26 (*dd*, *J* = 9.3, 9.3)	71.9	C(2′), C(5′)	3.26 – 3.29 (*m*)	71.7
H–C(5′)	3.27 – 3.35 (*m*)	78.5	C(1′), C(3′), C(6′)	3.27 – 3.32 (*m*)	78.1
CH_2(6′)	3.66 (*dd*, *J* = 6.6, 11.9), 3.93 (*dd*, *J* = 2.1, 11.9)	63.1	C(4′), C(5′)	3.58 (*dd*, *J* = 6.9, 11.7), 3.93 (*dd*, *J* = 2.1, 11.7)	63.2

Table 3. *Cytotoxic Activities (IC_{50} [μM] ± SD) of Compounds* **1** *and* **2** *against Four Tumor Cell Lines*

Compound	K562	HepG2	KB	LoVo
1	4.6 ± 0.4	> 100	> 100	9.8 ± 0.8
2	15.3 ± 1.5	6.9 ± 0.6	2.2 ± 0.2	4.2 ± 0.3
Taxotere[a])	> 400[b])	> 400[b])	0.0011 ± 0.00006	0.0021 ± 0.00004
Adriamycin[a])	0.09 ± 0.01	0.07 ± 0.0067	> 400[b])	> 400[b])

[c]) Positive control substances. [b]) No cytotoxic activity.

Experimental Part

General. Column chromatography (CC): silica gel (200 – 300 and 100 – 200 mesh, *Qingdao Marine Chemical*, China); C_{18} reversed-phase (RP) silica gel (20 – 45 μm, *Fuji Silica Chemical*, Japan) and

Sephadex LH-20 (*Pharmacia*). TLC: GF_{254} silica-gel plates (*Yantai Marine Chemical Co., Ltd.*, China). UV Spectra: *Hitachi UV-3200* spectrophotometer; λ_{max} (log ε) in nm. Optical rotations: *Perkin-Elmer 341* polarimeter. IR Spectra: *Perkin-Elmer 577-IR* spectrometer; in KBr; $\tilde{\nu}$ in cm^{-1}. ^{1}H- and ^{13}C-NMR spectra: *Bruker AM-400* spectrometer; δ in ppm rel. to Me_4Si as internal standard, J in Hz. ESI-MS: *Finnigan LCQ-DECA* instrument; HR-ESI-MS: *Waters Q-TOF* spectrometer; in m/z.

Plant Material. Samples of *Mappianthus iodoides* HAND.-MAZZ. were collected in Jinxiu, Guangxi Province, P. R. China, in July 2007. The specimen was identified by Prof. *Yu Zhao*, Department of Pharmacy, Zhejiang University. A voucher specimen (No. 20070703) was deposited with the Herbarium of the College of Agriculture and Biotechnology.

Extraction and Isolation. Powdered and air-dried stems (1.0 kg) of *Mappianthus iodoides* HAND.-MAZZ. were extracted exhaustively with cold 95% EtOH (3 × 4.0 l, 1.5 h each). Evaporation of EtOH gave a residue (120 g), which was then suspended in 15% EtOH (0.8 l) and filtered. The filtrate was concentrated (1.0 l) and partitioned sequentially with petroleum ether (3 × 1.0 l), AcOEt (3 × 1.0 l), and BuOH (4 × 1.0 l). The AcOEt-soluble fraction (20 g) was concentrated and subjected to CC (silica gel; $CHCl_3$/MeOH 20:1–2:1) to yield eight fractions. *Fr. 1* was purified by repeated CC (silica gel; petroleum ether (PE)/acetone 8:1–2:1; and *Sephadex LH-20*; MeOH as eluent) to afford 1-methyl-β-carboline (14 mg), pinoresinol (18 mg), (+)-(1*R*,2*S*,5*R*,6*S*)-2,6-bis(4′-hydroxyphenyl)-3,7-dioxabicyclo[3.3.0]octane (14 mg), and pinoresinol dimethyl ether (9 mg). *Fr. 2* was subjected to CC (silica gel; PE/acetone 3:1) to give dihydrocornin (18 mg). *Frs. 3–8* were further purified similarly by CC (*ODS*; MeOH/H_2O with gradient increased MeOH; and *Sephadex LH-20*; MeOH as eluent) to give secologanin (8 mg), strictosidine (19 mg), strictosidine acid (10 mg), **1** (10 mg), **2** (15 mg), 5-carboxystrictosidine (19 mg), and lyalosidic acid (20 mg). The BuOH-soluble fraction (36 g) submitted to CC (first silica gel; $CHCl_3$/MeOH 9:1; and then *Sephadex LH-20*; MeOH/H_2O 1:1) to yield 3,4,5-trimethoxyphenol-1-*O*-[β-D-apiofuranosyl-(1 → 6)]-β-D-glucopyranoside (9 mg). The structures of the eleven known isolates were identified by a combination of spectroscopic methods (MS, and ^{1}H- and ^{13}C-NMR) and comparisons with data in the literature or co-TLC with authentic samples.

Mappine A (=*7,12-Dihydro-3-(1-hydroxyethyl)-6*H-*indolo[2,3-*a*]quinolizin-5-ium-2-carboxylate*; **1**). Amorphous powder. UV (DMSO): 254 (3.30), 310 (3.50). $[\alpha]_D^{23} = +16$ ($c = 0.12$, DMSO). IR (KBr): 3384, 1656, 1606, 1573, 1439, 1298, 1194, 1082. ^{1}H- and ^{13}C-NMR: *Table 1*. ESI-MS: 309.3 ($[M+H]^+$). HR-ESI-MS: 309.1234 ($[M+H]^+$; calc. 309.1239).

Mapposidic Acid (=(*1S,4aS,5R,7aR*)*-1-(β-*D*-Glucopyranosyloxy)-1,4a,5,6,7,7a-hexahydro-5-(9*H*-pyrido[3,4-*b*]indol-1-yl)cyclopenta[*c*]pyran-4-carboxylic acid 1*; **2**). Amorphous powder. UV (MeOH): 231 (3.30), 298 (3.41), 311 (3.24). $[\alpha]_D^{23} = -47$ ($c = 0.17$, MeOH). IR (KBr): 3390, 1624, 1444, 1389, 1311, 1066. ^{1}H- and ^{13}C-NMR: *Table 2*. ESI-MS: 513.2 ($[M+H]^+$). HR-ESI-MS: 513.1868 ($[M+H]^+$; calc. 513.1873).

Acid Hydrolysis of **2**. A soln. of **2** (4 mg) in MeOH (5 ml) containing 1N HCl (2 ml) was refluxed for 4 h, concentrated under reduced pressure, diluted with H_2O, and extracted with AcOEt. The aq. phase was concentrated to obtain the sugar moiety which was identified as D-glucose by the sign of its optical rotation ($[\alpha]_D^{23} = +51.8$ ($c = 0.02$, MeOH)). It was further confirmed by comparing retention times (t_R) of its Me_3Si (TMS) ethers (α-anomer, 3.7 min; β-anomer, 5.1 min) with t_R of a standard sample in GC. Preparation of TMS ether and its subsequent GC was carried out according to the protocol described in [14].

Cell Cultures. Human HepG2 (hepatocellular carcinoma), K562 (human leukemia), KB (cervix carcinoma), and LoVo (colon adenocarcinoma) cell lines were obtained from the Shanghai Cell Bank, Chinese Academy of Sciences. The cells were maintained in RPMI1640 medium with 10% FBS (fetal bovine serum). In each case, 100 U/ml of penicillin and 100 U/ml of streptomycin were added.

Cytotoxicity Assay. Cells were cultured in 96-well microtiter plates for the assay. After incubation for 24 h and treatment with 10^{-2} to 10^2 μM of the test compounds for 72 h, growth inhibition of the cancer cells was evaluated by the SRB method (adherent cells: HepG2, KB, and LoVo) or WST-1 method (suspended cell: K562) as described in [15][16]. The activities are given as IC_{50} values. Results are expressed as the mean values of triplicate data points. Adriamycin and taxotere were used as positive controls.

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REFERENCES

[1] D. Fang, D. H. Qin, *Acta Bot. Yunn.* **2002**, *24*, 709.
[2] C. S. Chen, Q. G. Chen, L. M. Zeng, *Acta Sci. Nat. Univ. Sunyatsen* **2000**, *39*, 120.
[3] H. Tsuchiya, M. Sato, I. Watanabe, *J. Agric. Food Chem.* **1999**, *47*, 4167.
[4] Y. Yamazaki, A. Urano, H. Sudo, M. Kitajima, H. Takayama, M. Yamazaki, N. Aimi, K. Saito, *Phytochemistry* **2003**, *62*, 461.
[5] N. Aimi, H. Seki, S. Sakai, *Chem. Pharm. Bull.* **1992**, *40*, 2588.
[6] N. Aimi, H. Murakami, T. Tsuyuki, T. Nishiyama, S. Sakai, J. Haginiwa, *Chem. Pharm. Bull.* **1986**, *34*, 3064.
[7] E. Okuyama, K. Suzumura, M. Yamazaki, *Chem. Pharm. Bull.* **1995**, *43*, 2200.
[8] M. Kobayashi, Y. Ohta, *Phytochemistry* **1983**, *22*, 1257.
[9] T. Iida, M. Nakano, T. Ito, *Phytochemistry* **1982**, *21*, 673.
[10] N. Tanaka, T. Tanaka, T. Fujioka, H. Fujii, K. Mihashi, K. Shimomura, K. Ishimaru, *Phytochemistry* **2001**, *57*, 1287.
[11] I. Calis, O. Sticher, *Phytochemistry* **1984**, *23*, 2539.
[12] I. Kitagawa, H. Wei, S. Nagao, T. Mahmud, K. Hori, M. Kobayashi, T. Uji, H. Shibuya, *Chem. Pharm. Bull.* **1996**, *44*, 1162.
[13] W. Song, S. Li, S. Wang, Y. Wu, J. Zi, M. Gan, Y. Zhang, M. Liu, S. Lin, Y. Yang, J. Shi, *J. Nat. Prod*. **2008**, *71*, 922.
[14] S. Hara, H. Okabe, K. Mihashi, *Chem. Pharm. Bull.* **1987**, *35*, 501.
[15] P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J. T. Warren, H. Bokesch, S. Kenney, M. R. Boyd, *J. Natl. Cancer Inst*. **1990**, *82*, 1107.
[16] Y. Zhou, W. Zhu, Q. X. Zhang, *J. Trop. Med.* **2005**, *5*, 580.

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