Two New Indole Alkaloids from *Emmenopterys henryi*

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Two new indole alkaloids, 5-oxodolichantoside (**1**) and deglycocadambine (**2**), were isolated from the twigs and leaves of *Emmenopterys henryi*, together with four known indole alkaloids and five known iridoids. The structures of the new compounds were elucidated on the basis of extensive spectroscopic analyses, including 1D- and 2D-NMR experiments, and confirmed by single-crystal X-ray diffraction studies. This is the first report on the isolation of indole alkaloids from this species. The indole alkaloids were evaluated for their cytotoxic activities against five human cancer lines.

Introduction. – Plants of the Rubiaceae family are rich resources of indole alkaloids [1], some of which exhibited significant cytotoxic [2], antimalarial [1a], and antihypertensive [3] activities. *Emmenopterys henryi* OLIV., a monotypic plant belonging to the Rubiaceae family, is an endemic species growing in western and southwestern parts of China [4]. Its roots and barks have long been used in traditional Chinese medicine for the treatment of nausea, vomiting, bruises, and injures from falls [5]. The chemistry of *E. henryi* has previously been studied, and a few coumarins, triterpenoids, and steroids have been identified [6]. However, so far, no alkaloids have been reported from this species. During our continuing search for bioactive metabolites from the monotypic species endemic to China [7], an 95% EtOH extract of *E. henryi* was investigated, resulting in the isolation of two new indole alkaloids, 5-oxodolichantoside (**1**) and deglycocadambine (**2**), together with four known indole alkaloids, cadambine (**3**) [8], 3α-dihydrocadambine (**4**) [9], strictosidine (**5**) [10], and 3,4,5,6-tetradehydrodolichantoside (**6**) [11], and five known iridoids, loganetin (**7**) [12], cachineside I (**8**) [13], loganic acid (**9**) [14], loganin (**10**) [12], 6-[(*E*)-caffeoyl]loganic acid (**11**) [15] (*Fig. 1*). To the best of our knowledge, this is the first report on the isolation of indole alkaloids from this species. Herein, we report the isolation and structural elucidation of the new compounds, as well as their cytotoxic activities of the indole alkaloids **1**–**6**.

Results and Discussion. – 5-Oxodolichantoside (**1**) was isolated as optically active, corless crystals. Its molecular formula was established as $C_{28}H_{34}N_2O_{10}$ on the basis of HR-ESI-MS (*m/z* 593.1919 ([M+Cl]$^-$; calc. 593.1901), requiring 13 degrees of

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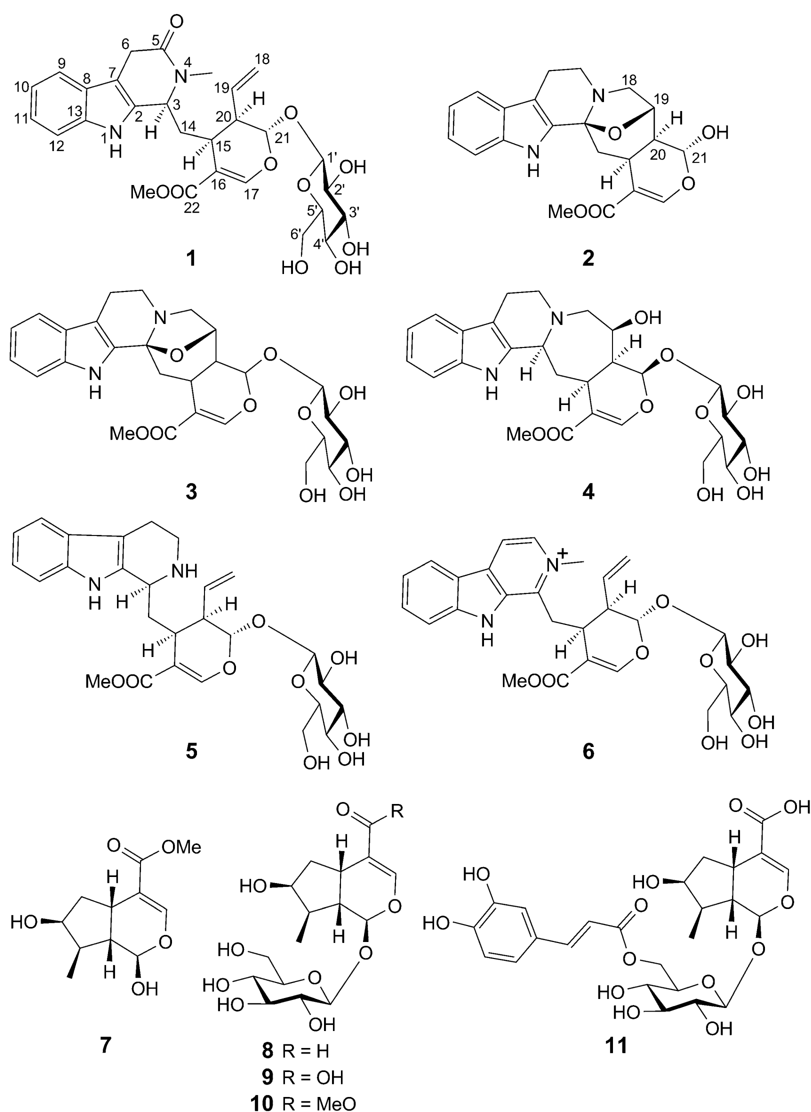

Fig. 1. *Structures of compounds* **1**–**11** *isolated from* E. henryi

unsaturation. The IR absorption bands at 3425, 1622, 1704 cm^{-1} indicated the presence of OH and C=O groups. The ^{1}H-NMR spectrum (*Table*) displayed signals for an unsubstituted indole nucleus at δ(H) 7.45 (*d*, *J* = 7.5, H–C(9)), 7.06 (*t*, *J* = 7.5, H–C(10)), 7.16 (*t*, *J* = 7.5, H–C(11)), and 7.36 (*d*, *J* = 7.5, H–C(12)), a monosubstituted C=C bond at δ(H) 5.13 (*dd*, *J* = 10.7, 10.7, H_a–C(18)), 4.69 (*dd*, *J* = 17.3, 1.5, H_b–C(18)), and 5.53 (*dt*, *J* = 17.3, 10.7, H–C(19)), two Me groups at δ(H) 3.21 (*s*), 3.66 (*s*), as well as for an anomeric H-atom at 4.58 (*d*, *J* = 7.9, H–C(1′)). The ^{13}C-NMR and DEPT spectra (*Table*) exhibited 28 C-atom resonances due to two Me groups (δ(C) 34.6

Table 1. *^1H- and ^{13}C-NMR Data* (400 and 100 MHz, resp.; CD_3OD) *of Compounds* **1** *and* **2** (δ in ppm, J in Hz). Atom numbering as indicator in *Fig. 1.*

Position	**1**		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
2		134.7 (*s*)		133.9 (*s*)
3	4.85 (*dd*, *J* = 5.6, 2.3)	58.8 (*d*)		93.1 (*s*)
5		172.9 (*s*)	3.14 – 3.19 (*m*), 2.80 – 2.84 (*m*)	54.0 (*t*)
6	3.68 – 3.74 (*m*)	30.6 (*t*)	2.79 – 2.85 (*m*)	23.1 (*t*)
7		105.7 (*s*)		111.6 (*s*)
8		126.9 (*s*)		127.3 (*s*)
9	7.45 (*d*, *J* = 7.5)	119.0 (*d*)	7.49 (*d*, *J* = 7.6)	120.1 (*d*)
10	7.06 (*t*, *J* = 7.5)	120.4 (*d*)	7.02 (*t*, *J* = 7.6)	120.4 (*d*)
11	7.16 (*t*, *J* = 7.5)	123.0 (*d*)	7.13 (*t*, *J* = 7.6)	123.6 (*d*)
12	7.36 (*d*, *J* = 7.5)	112.3 (*d*)	7.34 (*d*, *J* = 7.6)	112.8 (*d*)
13		138.4 (*s*)		138.9 (*s*)
14	2.75 – 2.82 (*m*), 1.98 – 2.06 (*m*)	32.2 (*t*)	2.02 – 2.07 (*m*)	43.0 (*t*)
15	2.78 – 2.84 (*m*)	26.7 (*d*)	3.26 (*t*, *J* = 5.8)	27.4 (*d*)
16		111.0 (*s*)		111.2 (*s*)
17		153.5 (*d*)		155.6 (*d*)
18	5.13 (*dd*, *J* = 10.7, 10.7), 4.69 (*dd*, *J* = 17.3, 1.5)	120.9 (*t*)	3.52 (*d*, *J* = 10.7), 3.03 (*dd*, *J* = 10.7, 7.3)	59.9 (*t*)
19	5.53 (*dt*, *J* = 17.3, 10.7)	134.7 (*d*)	4.90 – 4.95 (*m*)	75.2 (*d*)
20	1.89 – 1.96 (*m*)	45.1 (*d*)	1.63 – 1.69 (*m*)	43.0 (*d*)
21	5.22 (*d*, *J* = 9.1)	95.6 (*d*)	5.67 (*d*, *J* = 9.1)	95.7 (*d*)
22		168.7 (*s*)		169.5 (*s*)
1′	4.58 (*d*, *J* = 7.9)	99.7 (*d*)		
2′	3.17 – 3.24 (*m*)	74.7 (*d*)		
3′	3.32 – 3.38 (*m*)	78.1 (*d*)		
4′	3.26 – 3.33 (*m*)	71.6 (*d*)		
5′	3.24 – 3.32 (*m*)	78.4 (*d*)		
6′	3.84 – 3.90 (*m*), 3.59 – 3.66 (*m*)	62.8 (*t*)		
Me–N(4)	3.21 (*s*)	34.6 (*q*)		
*Me*O–C(22)	3.66 (*s*)	51.7 (*q*)	3.66 (*s*)	52.0 (*q*)

(Me–N(4)) and 51.7 (MeO–C(22))), three CH_2 (one olefinic) and ten CH (one acetal, two olefinic, and four aromatic) groups, five quaternary C-atoms (one olefinic and four aromatic), one amide C=O C-atom (δ(C) 172.9 (C(5)), one ester C=O C-atom (δ(C) 168.7 (C(22)), and a glucosyl moiety (δ(C) 99.7 (C(1′)), 74.7 (C(2′)), 78.1 (C(3′)), 71.6 (C(4′)), 78.4 (C(5′)), and 62.8 (C(6′))). These data suggested that **1** was an indole alkaloid glucoside. The ^{1}H- and ^{13}C-NMR data of **1** (*Table*) indicated they were similar to those of dolichantoside [16], except for the replacement of the CH_2 group at C(5) in dolichantoside by an amide C=O group in **1**, as deduced from the HMBCs (*Fig. 2*) of H–C(3), CH_2(6), and Me–N(4) with the amide C=O C-atom.

The relative configuration of **1** was deduced from ROESY and X-ray diffraction experiments. The ROESY correlations between H_b–C(14) and H–C(19) suggested that H–C(15) and H–C(20) were both α-oriented. The H–C(21) was established as β-oriented based on the ROESY correlation H–C(19)/H–C(21). However, the ROESY

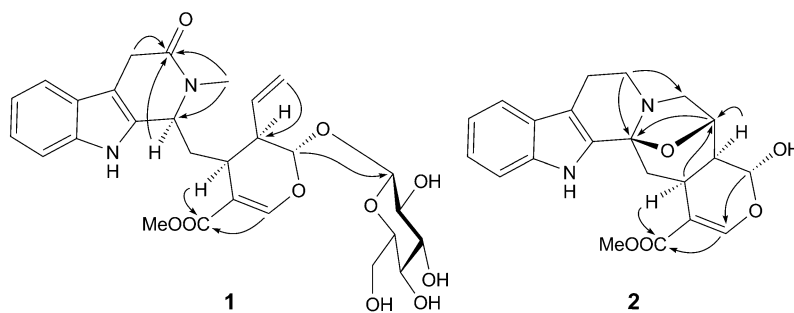

Fig. 2. *Key HMBCs of compounds* **1** *and* **2**

spectrum could not provide sufficient evidence to establish the orientation of H–C(3). Fortunately, a single crystal of **1** was obtained from MeOH and X-ray crystallographic analysis was carried out (*Fig. 3*), unambiguously providing the relative configuration and structure of **1**.

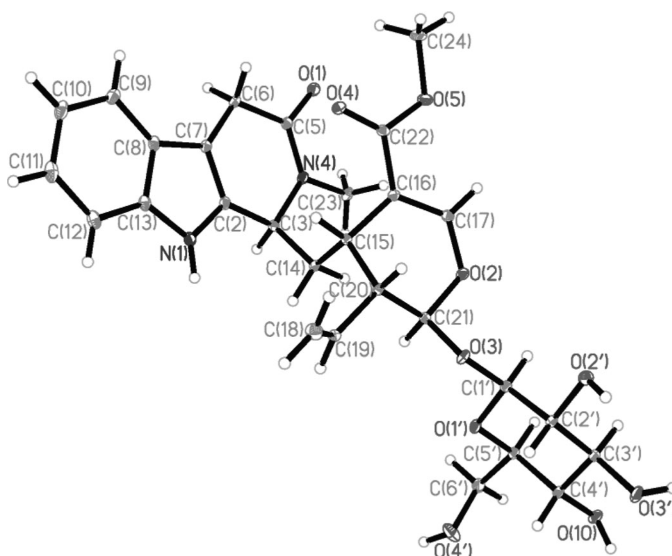

Fig. 3. *X-Ray crystal structure of compound* **1**

Deglycocadambine (**2**), colorless crystals, has the molecular formula $C_{21}H_{22}N_2O_5$ as deduced from HR-ESI-MS (*m*/*z* 5381.1456 ([*M*–H]$^-$; calc. 381.1450), corresponding to twelve degrees of unsaturation. The ^{1}H- and ^{13}C-NMR spectra (*Table*) revealed the presence of a 1,2-disubstituted phenyl ring (δ(H) 7.49 (*d*, *J* = 7.6, H–C(9)), 7.02 (*t*, *J* = 7.6, H–C(10)), 7.13 (*t*, *J* = 7.6, H–C(11)), and 7.34 (*d*, *J* = 7.6, H–C(12)); δ(C) 127.3 (C(8)), 120.1 (C(9)), 120.4 (C(10)), 123.6 (C(11)), 112.8 (C(12)), and 138.9 (C(13))). The ^{13}C-NMR spectrum (*Table*) exhibited 15 additional C-atom signals, including those

of one Me (δ(C) 52.0 (MeO–C(22))) group, four CH_2 and four CH (one olefinic, and one O-bearing ones) groups, four quaternary C-atoms (one O-bearing, one olefinic and two aromatic ones), one hemiacetal C-atom (δ(C) 95.7 (C(21))), and one ester C=O C-atom (δ(C) 169.5 (C(22))). These data were similar to those of cadambine (**3**), except that the glucosyl at C(21) was missing in **2**, which suggested that **2** was the aglycone of compound **3**. Detailed analysis of the 1D- and 2D-NMR data (*Fig. 2*) allowed us to assign the planar structure. In the ROESY spetrum, the correlations H–C(15)/H–C(20)/H_α–C(18) suggested that these H-atoms were α-oriented. In addition, ROESY correlations H_β–C(18)/H–C(19)/H–C(21) indicated that the O-bridge between C(3) and C(19), and H–C(21) were β-oriented. Finally, an X-ray diffraction analysis of **2**, (*Fig. 4*), confirmed its structure and relative configuration.

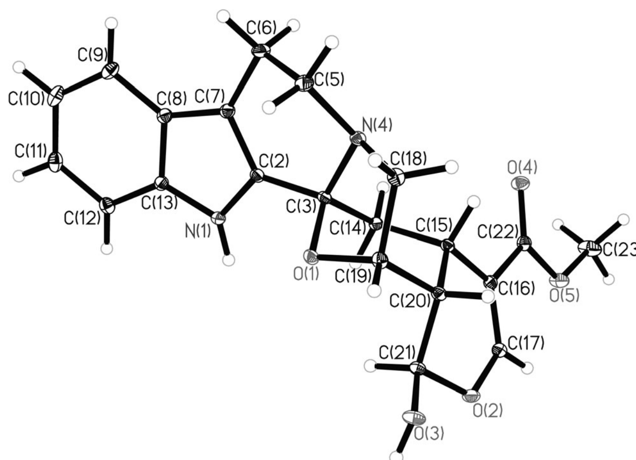

Fig. 4. *X-Ray crystal structure of compound* **2**

The indole alkaloids **1**–**6** were evaluated for cytotoxic activities against human myeloid leukemia (HL-60), hepatocellular carcinoma (SMMC-7721), lung cancer (A-549), breast cancer (MCF-7), and colon cancer (SW-480) cell lines *in vitro*. However, none of them exhibited activity with IC_{50} values >40 μM.

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Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 200–300 mesh; *Qingdao Marine Chemical*, Qingdao, P. R. China), *Lichroprep RP-18* gel (40–63 μm, *Merck*, DE-Darmstadt), and *Sephadex LH-20* (*Amersham Pharmacia Biotech*; Sweden). TLC: SiO_2 Plates; detection by spraying with 10% H_2SO_4 in EtOH, followed by heating. Semi-prep. HPLC: *Agilent 1200* apparatus; Z*orbax SB-C-18* column (9.4 mm × 25 cm; *Agilent*). MPLC: *Lisui EZ Purify III* System. M.p.: *X-4* microscope melting-point apparatus. Optical rotations: *Horiba SEPA–300* polarimeter. UV Spectra: *Shimadzu UV-2401A* spectrophotometer. IR Spectra: *Tensor 27* spectrophotometer; KBr pellets. 1D- and 2D-NMR spectra:

Bruker AM-400 or *Avance III 600* spectrometer with TMS as the internal standard, chemical shifts (δ) in ppm with reference to the solvent signals. ESI-MS and HR-ESI-MS: *API QSTAR time-of-flight* spectrometer. EI-MS and HR-EI-MS: *Waters Autospec Premier P776* spectrometer.

Plant Material. The twigs and leaves of *E. henryi* were collected in Kunming Botany Garden, Yunnan province, P. R. China, in December 2010, and identified by one of the authors (Prof. *X. Gong*), Kunming Institute of Botany. A voucher specimen (KIB20090911e) was deposited with the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. The air-dried and powdered twigs and leaves of *E. henryi* (15.0 kg) were extracted three times with 95% EtOH at r.t. The filtrate was concentrated under reduced pressure to yield a residue, which was suspended in H_2O and then partitioned with AcOEt to afford AcOEt (750 g) and H_2O layer (180 g). The AcOEt fraction was subjected to CC (SiO_2; petroleum ether (PE)/Me_2CO 1:0 → 0:1): *Frs. A – F*. *Fr. B* was further subjected to CC (SiO_2; $CHCl_3$/Me_2CO 8:2 → 7:3, and, then *Sephadex LH-20*; MeOH): **7** (54 mg). *Fr. E* was fractionated by MPLC (*RP-18*; MeOH/H_2O, 20:80 → 100:0): *Frs. E1 – E5*. *Fr. E2* was separated by CC (SiO_2; $CHCl_3$/Me_2CO 8:2 → 6:4; then *RP-18*; MeOH/H_2O 25:75 → 65:35): **8** (18 mg), **9** (34 mg), and **10** (15 mg). Compound **2** (10 mg) was obtained from *Fr. E3* by CC (SiO_2; $CHCl_3$/MeOH 9:1). *Fr. E4* was repeatedly purified by CC (SiO_2; $CHCl_3$/Me_2CO 7:3 and then $CHCl_3$/MeOH 8.5:1.5): **11** (36 mg). The H_2O layer was chromatographed on CC (SiO_2, $CHCl_3$/MeOH 9:1 → 1:1): *Frs. 1 – 5*. *Fr. 2* was further separated by CC (SiO_2; AcOEt/MeOH 8:2 → 6:4): *Frs. 2.1 – 2.6*. *Fr. 2.2* was subjected to CC(*Sephadex LH-20*; $CHCl_3$/MeOH 9:1) and then purified by semi-prep. HPLC (MeOH/H_2O 45:55): **1** (35 mg). *Fr. 2.3* was repeatedly submitted to CC (SiO_2; $CHCl_3$/MeOH 9:1 → 7:3 and then AcOEt/MeOH 8:2 → 6:4) and further purified by CC(*Sephadex LH-20*; MeOH): **4** (5 mg), **5** (9 mg), and **6** (8 mg). *Fr. 2.4* was subjected to CC (SiO_2; $CHCl_3$/MeOH 8:2) and then purified by semi-prep. HPLC (MeOH/H_2O 20:80): **3** (12 mg).

5-Oxodolichantoside (= *Methyl (2*R**,3*S**,4*R**)-3-Ethenyl-2-(β-*D*-glucopyranosyloxy)-3,4-dihydro-4-{[(1*R**)-2,3,4,9-tetrahydro-2-methyl-3-oxo-1*H*-β-carbolin-1-yl]methyl}-2*H*-pyran-5-carboxylate*; **1**). Colorless crystals. M.p. 150 – 153°. $[\alpha]_D^{18.2} = -95.83$ ($c = 0.36$, MeOH). UV (MeOH): 273 (3.98), 222 (4.71), 200 (4.63). IR (KBr): 3425, 2924, 1704, 1622, 1459, 1438, 1405, 1282, 1186, 1075, 933, 746. ^1H- and ^{13}C-NMR: see the *Table*. ESI-MS (neg.): 593 ($[M+Cl]^-$). HR-ESI-MS (neg.): 593.1919 ($[M+Cl]^-$, $C_{28}H_{34}ClN_2O_{10}^-$; calc. 593.1901).

Deglycocadambine (= *Methyl (4*R**,4a*S**,5*S**,14b*S**,15a*S**)-4a,5,6,8,9,14,15,15a-Octahydro-4-hydroxy-8*H*-5,14b-epoxypyrano[4'',3'':4',5']azepino[1',2':1,2]pyrido[3,4-*b*]indole-1-carboxylate*; **2**). Colorless crystals. M.p. 135 – 137°. $[\alpha]_D^{17.8} = -142.57$ ($c = 0.10$, MeOH). UV (MeOH): 273 (4.15), 222 (4.75), 200 (4.65). IR (KBr): 3425, 2926, 1691, 1629, 1550, 1438, 1384, 1323, 1277, 1206, 1100, 985. ^1H- and ^{13}C-NMR: see the *Table*. ESI-MS (neg.): 381 ($[M-H]^-$). HR-ESI-MS (neg.): 381.1456 ($[M-H]^-$ $C_{21}H_{21}N_2O_5^-$; calc. 381.1450).

X-Ray Crystallography of **1** *and* **2**. Colorless crystals of **1** and **2** were obtained from MeOH. The intensity data were collected on a *Bruker-APEX-DUO* diffractometer with MoK^{α} radiation. The crystal structures of **1** and **2** were solved by direct methods (SHLXS-97), expanded using difference *Fourier* technique, and refined by the program and the full-matrix least-squares calculations. The non-H-atoms were refined anisotropically, H-atoms were fixed at calculated positions.

Crystallographic Data of **1**[1]). $C_{28}H_{34}N_2O_{10} \cdot 3\,H_2O$; M_r 612.62; crystal size, 0.06 × 0.28 × 0.43 mm; space group, triclinic, P_1; T 100(2) K; a = 6.8950(6) Å, b = 8.9636(8) Å, c = 12.1832(11) Å; α = 87.0970(10)°, β = 87.8420(10)° γ = 79.4280(10)°, V = 738.9 (11) Å^3; $F(000) = 326$, $Z = 1$, $D_x = 1.377$ Mg/m^3; 10467 reflections collected with 7608 independent $R_{int} = 0.0178$, data, restraints, and parameters 7608, 3, and 400; goodness-of-fit on $F^2 = 1.043$, final indices $R_1 = 0.0299$, $wR_2 = 0.0772$; largest differences peak and hole 0.278 and −0.204 e Å^{-3}, resp.

Crystallographic Data of **2**[1]). $C_{21}H_{22}N_2O_5 \cdot H_2O$; M_r 400.42; crystal size, 0.23 × 0.26 × 0.80 mm; space group, orthorhombic, $P2_12_12_1$; T 100(2) K; a = 9.1220(9) Å, b = 12.1164(12) Å, c = 17.2633(17) Å; $\alpha = \beta = \gamma$ = 90°, V = 1908.0 (3) Å^3; $F(000) = 848$, $Z = 4$, $D_x = 1.394$ Mg/m^3; 20312 reflections collected with

[1]) CCDC-919885 (**1**) and -919886 (**2**) contain the supplementary crystallographic data for this article. These data can be obtained free of charge *via* www.ccdc.cam.ac.uk/data_request/cif.

5419 independent $R_{int} = 0.0241$, data, restraints, and parameters 5419, 0, and 266; goodness-of-fit on $F^2 = 1.073$, final indices $R_1 = 0.0306$, $wR_2 = 0.0834$; largest differences peak and hole 0.324 and -0.242 e · Å^{-3}, resp.

Cytotoxicity Assay. The cytotoxicities of compounds **1**–**6** against HL-60, SMMC-7721, A-549, MCF-7, and SW480 cell lines was assessed using the MTT method [17]. Cells were plated in 96-well plates 12 h before treatment and continuously exposed to different concentrations of compounds. After 48 h, 20 μl of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) soln. were added to each well, which were incubated for another 4 h. Then, 20% SDS (= sodium dodecyl sulfate; 100 μl) were added to each well. After 12 h at r.t., the OD value of each well was recorded at 595 nm. The IC_{50} value of each compound was calculated by the *Reed* and *Muench* method [18].

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