FULL PAPER

Four New Pregnane Steroids from *Aglaia abbreviata* and Their Cytotoxic Activities

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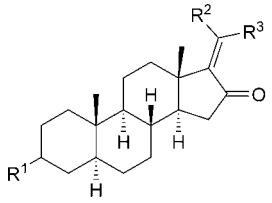
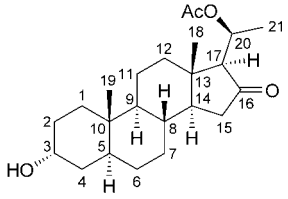
Four new pregnane steroids, aglaiasterols A – D (**1** – **4**), have been isolated from the EtOH extract of stems of *Aglaia abbreviata*. They were identified as (3*α*,5*α*,17*Z*)-3-hydroxypregn-17-en-16-one (**1**), (3*β*,5*α*,17*E*)-3-hydroxypregn-17-en-16-one (**2**), (3*β*,5*α*,17*Z*)-3-hydroxypregn-17-en-16-one (**3**), and (3*α*,5*α*,20*S**)-3-hydroxy-16-oxopregnan-20-yl acetate (**4**) on the basis of spectroscopic methods, including 1D- and 2D-NMR techniques. Compounds **1** – **4** were evaluated for their cytotoxic activities against K562 (human leukemia), MCF-7 (human breast cancer), and KB (human oral epithelium cancer) cells, and drug-resistant cells of K562/A02, MCF-7/ADM, and KB/VCR. These isolates showed weak to moderate inhibitory effects on the growth of the tested cell lines.

Introduction. – The genus *Aglaia* (Meliaceae) consists of *ca.* 130 species of dioecious trees and shrubs mainly distributed in tropical and subtropical regions [1]. Several species of this genus were reported to show antifungal [2], antineoplastic [3], antiviral [4], and insecticidal [5] activities. Previous chemical investigations of plants of this genus have led to the identification of several classes of compounds including flavaglines, bisamides [6], triterpenoids [7], and pregnane steroids [8]. Some pregnane steroids and their glycosides were found to show significant cytotoxic effects [9][10]. *Aglaia abbreviata* is a wild shrub indigenous to the Yunnan Province of China [11][12]. In our phytochemical research on this plant, four new pregnane steroids, aglaiasterols A – D (**1** – **4**), were isolated from the more polar fractions. Their structures were determined by detailed analysis of their spectroscopic data. In this article, we report the isolation, structure elucidation, and antitumor activities of these new pregnane steroids. Given that multidrug resistance is one of the most serious obstacles in cancer chemotherapy, the evaluation of antitumor activity involves not only sensitive tumor cells, but also multidrug-resistant (MDR) tumor cells.



1 R^1 = *α*-OH, R^2 = H, R^3 = Me
2 R^1 = *β*-OH, R^2 = Me, R^3 = H
3 R^1 = *β*-OH, R^2 = H, R^3 = Me



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Results and Discussion. – *Structure Elucidation.* Compound **1** was obtained as colorless needles. The HR-ESI-MS result at *m*/*z* 339.2287 ([*M* + Na]$^+$; calc. 339.2295) indicated that it has a molecular formula of $C_{21}H_{32}O_2$ with six degrees of unsaturation. The IR spectrum showed strong absorption bands at 3502, 1712, and 1644 cm^{-1}, ascribable to a OH group and an *α*,*β*-unsaturated cyclopentanone moiety. The ^{13}C-NMR spectrum showed 21 signals (*Table 1*), which could be classified with the help of HSQC data as three Me, eight CH_2, and six CH groups (one O-bearing and one olefinic), and four C_q-atoms (one C=O and one olefinic), suggesting that **1** possesses a tetracyclic ring system. In the ^{1}H-NMR spectrum (*Table 1*), two upfield-shifted tertiary Me groups at *δ*(H) 0.83 and 0.90 and one olefinic H-atom at 5.70 (*q*, *J* = 7.5) representing an ethenyl moiety were also observed.

The aforementioned data indicated that **1** is most likely a C_{21} pregnane steroid with a vinyl side chain. The constitutional formula was deduced from HSQC and HMBC spectra (*Fig. 1, a*). In the HMBC spectrum, the correlations of the H-atom at *δ*(H) 0.83 (Me(19)) with the C-atoms C(1), C(5), C(9), and C(10), and of the H-atom at 0.90 (Me(18)) with C(12), C(13), C(14), and C(17) enabled the assignments of the two *singlet* Me groups and their neighboring C-atoms. The HMBC cross-peaks of H–C(3) at *δ*(H) 4.06 (*t*, *J* = 2.6) with C(2) and C(4) indicated that the OH group is located at C(3); those of the CH_2 H-atoms at 2.23 (*dd*, *J* = 17.0, 7.5, H_α) and 1.93 (*dd*, *J* = 17.0, 14.6, H_β), attached to the C-atom at *δ*(C) 39.2, with C(13) and C(14) and the further HMB correlation of these CH_2 H-atoms with the C=O C-atom at *δ*(C) 208.0, assigned the CH_2 group at C(15) and the C=O group at C(16). The configuration of the OH group could be assigned as *α* on the basis of the multiplicity of H–C(3) (*t*, *J* = 2.6). In addition, a *quadruplet* corresponding to an olefinic H-atom and a *doublet* assigned to a vinylic Me group indicated that the vinylic Me group and the olefinic H-atom are attached to C(20), which was also confirmed by the HMB correlation of H–C(20) at *δ*(H) 5.70 with C(17).

DOI: 10.1002/hlca.201500228

Table 1. *^{1}H- and ^{13}C-NMR Data* (500 and 125 MHz, resp.; in $CDCl_3$) *of* **1** *and* **2**

Position	**1**		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
1	1.39 – 1.42 (*m*, H_α), 0.90 – 0.92 (*m*, H_β)	32.0	1.70 – 1.73 (*m*, H_α), 0.97 – 0.99 (*m*, H_β)	36.7
2	1.30 – 1.34 (*m*, H_α), 1.07 – 1.11 (*m*, H_β)	29.0	1.80 – 1.84 (*m*, H_α), 1.39 – 1.41 (*m*, H_β)	31.5
3	4.06 (*t*, *J* = 2.6)	66.5	3.58 – 3.62 (*m*)	71.5
4	1.20 – 1.23 (*m*, H_α), 1.01 – 1.04 (*m*, H_β)	35.8	1.67 – 1.69 (*m*, H_α), 1.27 – 1.31 (*m*, H_β)	38.1
5	1.65 – 1.68 (*m*)	39.4	1.25 – 1.27 (*m*)	44.8
6	1.20 – 1.24 (*m*, H_α), 1.01 – 1.05 (*m*, H_β)	28.3	1.11 – 1.13 (*m*, H_α), 1.30 – 1.34 (*m*, H_β)	28.5
7	1.44 – 1.49 (*m*, H_α), 1.01 – 1.05 (*m*, H_β)	31.9	1.63 – 1.67 (*m*, H_α), 0.95 – 0.98 (*m*, H_β)	32.0
8	1.55 – 1.59 (*m*)	34.6	1.66 – 1.69 (*m*)	34.3
9	0.90 – 0.92 (*m*)	54.3	0.84 – 0.87 (*m*)	54.2
10		35.7		35.8
11	1.71 – 1.73 (*m*, H_α), 1.39 – 1.44 (*m*, H_β)	20.5	1.67 – 1.70 (*m*, H_α), 1.46 – 1.49 (*m*, H_β)	21.0
12	1.35 – 1.40 (*m*, H_α), 1.25 – 1.28 (*m*, H_β)	35.9	2.26 – 2.29 (*m*, H_α), 1.65 – 1.68 (*m*, H_β)	36.8
13		42.6		43.4
14	1.42 – 1.44 (*m*)	49.6	1.47 – 1.49 (*m*)	50.1
15	2.23 (*dd*, *J* = 17.0, 7.5, H_α), 1.93 (*dd*, 17.0, 14.6, H_β)	39.2	2.19 (*dd*, *J* = 17.0, 7.6, H_α), 1.98 (*dd*, *J* = 17.0, 14.6, H_β)	38.0
16		208.0		206.8
17		148.0		148.1
18	0.90 (*s*)	19.6	1.01 (*s*)	17.7
19	0.83 (*s*)	11.2	0.86 (*s*)	12.3
20	5.70 (*q*, *J* = 7.5)	129.8	6.48 (*q*, *J* = 7.5)	128.9
21	2.07 (*d*, *J* = 7.5)	14.0	1.84 (*d*, *J* = 7.5)	13.1

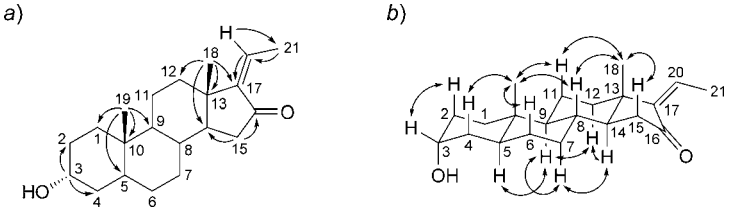

Fig. 1. *a*) *Key HMB* (H → C) *correlations of* **1**. *b*) *Key ROESY* (H ↔ H) *correlations of* **1**.

The relative configuration of **1** was determined from the ROESY spectrum (*Fig. 1*,*b*). The cross-peaks of Me(19) with H_β–C(4) and H_β–C(6), but not with H–C(5), indicated a *trans* fusion between rings *A* and *B* (*i.e.*, 5α-pregnane series) that was also supported by the chemical shift of Me(19) at δ(C) 11.2 according to the following empirical rule. Thus, the chemical shift of this Me group in an *A*/*B trans* junction would be in an upfield range of δ(C) 11 – 12; whereas that in the corresponding *A*/*B cis* junction would be in a range of 22 – 24 ppm [13]. ROESY correlations of H–C(8) with both Me(18) and Me(19) indicated that the H-atom and the Me groups are β-oriented. The correlations of H–C(9) with H–C(5) and H–C(14) demonstrated an α-orientation of these H-atoms. The (*Z*) geometry of the vinyl group of **1** was assigned by comparison of the NMR data with those of (*E*)- and (*Z*)-volkendousin [14]. The (*Z*) geometry of the C=C bond of **1** was supported by the chemical shift at a higher field (δ(H) 5.70), compared to the (*E*) isomer [14] at *ca.* 6.40, due to the deshielding effect of the C=O group at C(16). Thus, the structure of **1** was elucidated as (3α,5α,17*Z*)-3-hydroxypregn-17-en-16-one.

Compounds **2** and **3** showed OH and α,β-unsaturated ketone absorptions in their IR spectra, and their HR-ESI-MS spectra showed the same molecular formula ($C_{21}H_{32}O_2$). Their ^{1}H- and ^{13}C-NMR spectra were closely related to those of **1** (*Tables 1* and *2*). Both compounds showed signals of three Me groups, two of them tertiary (δ(H) 1.01 and 0.86 for **2**, 0.91 and 0.85 for **3**) and the other a vinylic Me group (1.84 (*d*, *J* = 7.5) for **2** and 2.08 (*d*, *J* = 7.3) for **3**). In addition, there was one olefinic H-atom appearing as *quadruplet* (δ(H) 6.48 (*q*, *J* = 7.5) for **2** and 5.68 (*q*, *J* = 7.3) for **3**), indicating that the vinylic Me group and the olefinic H-atom are attached to the same C-atom (C(20)). The (*Z*)/(*E*) configuration of the side chain moiety and the position of the C=O group in **2** and **3** were deduced as follows. In **2**, the olefinic H-atom is deshielded due to the proximity of the C=O group and appeared at δ(H) 6.48. In **3**, this H-atom is farther away from the C=O group and appeared at higher field (δ(H) 5.68) compared with **2**, and, inversely, the vinylic Me group was shifted downfield by *ca.* 0.2 in **3** compared with that of **2**. The chemical shifts and coupling constants of the side chain moiety in both compounds were very similar to those of **1** or (*E*)-pregnenes [15]. The CH–O H-atoms (δ(H) 3.58 – 3.62 (*m*) for **2** and **3** each) and the CH–O C-atoms (δ(C) 71.5 for **2** and 71.2 for **3**) were assigned to C(3) by HMBC spectra. The relative configurations of **2** and **3** were

Table 2. *^{1}H- and ^{13}C-NMR Data* (500 and 125 MHz, resp.; in $CDCl_3$) *of* **3** *and* **4**

Position	**3**		**4**	
	δ(H)	δ(C)	δ(H)	δ(C)
1	1.70 – 1.73 (*m*, H_α), 0.96 – 0.98 (*m*, H_β)	36.8	0.94 – 0.99 (*m*, H_α), 1.47 – 1.49 (*m*, H_β)	31.9
2	1.80 – 1.84 (*m*, H_α), 1.40 – 1.42 (*m*, H_β)	31.5	1.53 – 1.56 (*m*, H_α), 1.64 – 1.68 (*m*, H_β)	29.0
3	3.58 – 3.62 (*m*)	71.2	4.06 (*t*, *J* = 2.6)	66.5
4	1.67 – 1.69 (*m*, H_α), 1.27 – 1.31 (*m*, H_β)	38.1	1.39 – 1.42 (*m*, H_α), 1.53 – 1.55(*m*, H_β)	35.8
5	1.25 – 1.27 (*m*)	45.0	1.56 – 1.57 (*m*)	39.1
6	1.11 – 1.13 (*m*, H_α), 1.30 – 1.34 (*m*, H_β)	28.3	1.23 – 1.25 (*m*, H_α), 1.14 – 1.20 (*m*, H_β)	28.3
7	1.63 – 1.67 (*m*, H_α), 0.95 – 0.98 (*m*, H_β)	32.0	1.60 – 1.63 (*m*, H_α), 1.03 – 1.06 (*m*, H_β)	32.1
8	1.65 – 1.69 (*m*)	34.2	1.51 – 1.54 (*m*)	34.6
9	0.83 – 0.88 (*m*)	54.4	0.94 – 0.97 (*m*)	54.1
10		35.7		36.2
11	1.67 – 1.69 (*m*, H_α), 1.44 – 1.47 (*m*, H_β)	20.6	1.62 – 1.66 (*m*, H_α), 1.33 – 1.35 (*m*, H_β)	20.4
12	2.27 – 2.30 (*m*, H_α), 1.63 – 1.66 (*m*, H_β)	35.9	1.96 – 2.01 (*m*, H_α), 1.44 – 1.46 (*m*, H_β)	39.2
13		42.6		42.9
14	1.41 – 1.44 (*m*)	50.0	1.47 – 1.49 (*m*)	50.3
15	2.13 (*dd*, *J* = 17.0, 7.5, H_α), 1.93 (*dd*, *J* = 17.0, 14.6, H_β)	39.5	2.25 (*dd*, *J* = 17.0, 7.5, H_α), 1.85 (*dd*, *J* = 17.0, 14.5, H_β)	39.2
16		206.7		215.7
17		148.1	2.05 (*d*, *J* = 9.5)	67.2
18	0.91 (*s*)	19.7	0.77 (*s*)	13.6
19	0.85 (*s*)	12.3	0.81 (*s*)	11.2
20	5.68 (*q*, *J* = 7.3)	129.9	5.06 (*qd*, *J* = 9.5, 7.0)	69.3
21	2.08 (*d*, 7.3)	13.1	1.43 (*d*, *J* = 7.0)	20.3
AcO			2.02 (*s*)	21.4
				170.0

established to be identical to that of **1**, apart from C(3), on the basis of a ROESY experiment. Finally, a detailed comparison of the ^{13}C-NMR data of both compounds with those of **1** established **2** and **3** to be (3*β*,5*α*,17*E*)-3-hydroxypregn-17-en-16-one and (3*β*,5*α*,17*Z*)-3-hydroxypregn-17-en-16-one, respectively.

Compound **4** was obtained as colorless needles. The molecular formula, $C_{23}H_{36}O_4$, was determined by HR-ESI-MS at *m*/*z* 399.2512 ($[M+Na]^+$; calc. 399.2506), indicating that there are six degrees of unsaturation in **4**. The IR spectrum showed strong absorption bands at 3440 and 1731 cm^{-1}, ascribable to OH and C=O groups, respectively. The ^{13}C-NMR spectrum showed 23 C-atom signals (*Table 2*), which were classified by ^{1}H-NMR and HSQC spectra as four Me, eight CH_2, and seven CH groups (two O-bearing), and four C_q-atoms (two C=O groups), suggesting that **4** possesses a tetracyclic ring system. In the ^{1}H-NMR spectrum (*Table 2*), two upfield-shifted tertiary Me groups at δ(H) 0.77 and 0.81, one Me group of AcO at 2.02, a secondary Me group at 1.43 (*d*, *J* = 7.0), and two CH–O groups at 4.06 (*t*, *J* = 2.6) and 5.06 (*qd*, *J* = 9.5, 7.0) were also observed.

Inspection of the ^{1}H- and ^{13}C-NMR data showed that **4** has the same pregnane skeleton as **1** with a C=O group at C(16), Me groups at C(10) and C(13), but with an additional AcO group and without the C(17)=C(20) bond. The chemical shifts, multiplicities, and coupling constants in conjunction with the presence of the vicinal coupling between H–C(20) and H–C(17), suggested that there is an O-bearing substituent at C(20), which is absent in **1**. The position of the AcO group was determined as C(20) by HMB correlations of H–C(20) with C(17), C(21), and the C=O C-atom (*Fig. 2*,*a*). The relative configuration of **4** was established to be identical to that of **1** on the basis of a ROESY experiment (*Fig. 2*,*b*). The (20*S**) configuration of **4** was established by comparing the ^{13}C-NMR shift values of C(20) (δ(C) 69.3), C(21) (20.3), and C(17) (67.2) with the corresponding signals of (3*β*,5*α*,20*S*)-16-oxopregnane-3,20-diyl diacetate [16]. Thus, the structure of **4** was finally elucidated as (3*α*,5*α*,20*S**)-3-hydroxy-16-oxopregnan-20-yl acetate. The assignments of the NMR signals (*Table 2*) of **4** were made unambiguously on the basis of HSQC and HMBC experiments.

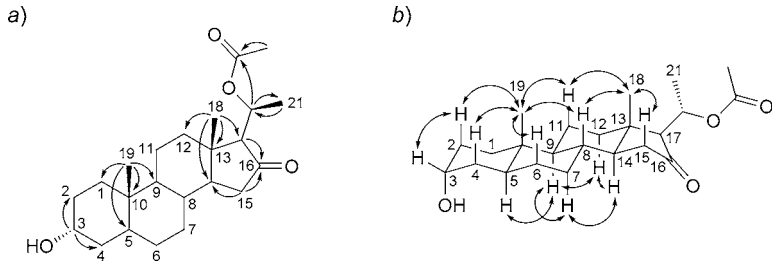

Fig. 2. *a*) *Key HMB* (H → C) *correlations of* **4**. *b*) *Key ROESY* (H ↔ H) *correlations of* **4**.

Table 3. *Cytotoxic Activities of* **1** – **4**[a])

Compound	Cell line					
	MCF-7	MCF-7/ADM	KB	KB/VCR	K562	K562/A02
1	> 50	> 50	49.1 ± 3.8	> 50	14.4 ± 2.0	> 50
2	> 50	> 50	40.2 ± 3.3	> 50	23.5 ± 2.3	> 50
3	> 50	> 50	> 50	> 50	17.2 ± 2.2	> 50
4	> 50	15.6 ± 1.9	12.1 ± 2.5	3.9 ± 0.8	2.8 ± 0.5	10.2 ± 2.43
5-Fluorouracil	> 50	> 50	6.8 ± 1.2	33.4 ± 2.5	2.1 ± 0.3	3.3 ± 0.44
Doxorubicin	0.54 ± 0.07	> 50	0.012 ± 0.005	0.45 ± 0.03	0.17 ± 0.02	19.2 ± 1.6

[a]) Results are expressed as IC_{50} values in μM.

Cytotoxic Activity. Compounds **1** – **4** were evaluated for their cytotoxic activities against MCF-7 (human breast cancer), KB (human oral epithelium cancer), and K562 (human leukemia) cells, and their MDR counterparts MCF-7/ADM, KB/VCR, and K562/A02. It is worth noting that **4** exhibited the strongest cytotoxic activity on various tumor cells (*Table 3*). Especially on MCF-7/ADM and KB/VCR cells, which all belong to a solid tumor, the inhibition effect was even better than that on their sensitive parental ones. Although, as to leukemia cells, the cytotoxic activity was weaker on K562/A02 cells than that on K562, its extraordinary antitumor effects on both sensitive and MDR cells was also worth further investigation. As we know, cytotoxic activities of compounds in the genus *Aglaia* were reported widely [17][18]. However, there were few studies developing the antitumor effect on MDR cells. In fact, multidrug resistance is one of the major obstacles to cancer chemotherapy, which makes cancer cells respond insufficiently to a spectrum of plural structurally and functionally unrelated anticancer agents. Since the mechanism involves drug efflux increase, DNA repair, survival/apoptotic signaling pathways alteration, *etc.* [19], the extraordinary cytotoxic activity of **4** on MDR cells may attribute to such specific mechanisms. In addition, **1** – **3** showed moderate to weak cytotoxic activities against K562, KB, and MCF-7 cells, and their MDR counterparts, reflecting variations among tumor species. Besides, the cytotoxic activities of these four compounds on SMMC-7721 cells (human hepatocellular carcinoma) was also evaluated, but the antitumor effect was weak (data not shown).

Overall, **4** was sensitive against MDR cancer cell lines. Therefore, it may be a new potential lead for chemotherapeutic agents directly against MDR tumor. Further research will try to reveal the structure–activity relationship and provide more potent derivatives with suitable modification.

Experimental Part

General. All reagents and solvents were of anal. grade (*Jiangsu Hanbang Sci. & Tech. Co., Ltd.*). Na_2SO_4 was the drying agent used in all work-up procedures. Thin layer chromatography (TLC): silica gel plates (SiO_2); visualized by heating and immersing in vanillin/H_2SO_4 in EtOH. Column chromatography (CC): commercial SiO_2 (100 – 200 and 200 – 300 mesh; *Qingdao Marine Chemical Industrial*), *Sephadex LH-20* (*Pharmacia*), and reversed-phase C_{18} (*RP-C*$_{18}$; 40 – 63 μm; *YMC*); fractions were monitored by TLC. Anal. HPLC: *Agilent 1100* instrument with multiple wavelength diode array detector. Prep. HPLC: *SHIMADZU* apparatus with *Shimpak RP-C*$_{18}$ column (20 × 200 mm i.d., 5 μm). Optical rotations: *Jasco P-1020* polarimeter (Na filter, λ 589 nm); in $CHCl_3$ soln. UV Spectra: *SHIMADZU UV-2450* spectropolarimeter; λ_{max} (log ε) in nm. IR Spectra: *Bruker Tensor 27* spectrometer; KBr disks; $\tilde{\nu}$ in cm^{-1}. ^1H- and ^{13}C-NMR spectra: *Bruker ACF-500* NMR instrument (500 and 125 MHz, resp.); in $CDCl_3$; at r.t.; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. ESI-MS: *Agilent 1100* series LC/MSD Trap mass spectrometer; in *m*/*z*. HR-ESI-MS: *Mariner* time-of-flight mass spectrometer with an electrospray interface; in *m*/*z*.

Plant Material. Air-dried stems of *Aglaia abbreviata* were collected from Xishuangbanna, Yunnan Province, P. R. China, in May 2013, and were identified by Prof. *Jingyuan Cui*, Xishuangbanna Botanical Garden, Chinese Academy of Sciences, P. R. China. A voucher specimen has been deposited in the College of Pharmacy, Henan University (Accession number AP201305).

Extraction and Isolation. Air-dried stems (10 kg) were extracted three times with 95% EtOH (3 × 40 l; 3 h, 3 h, and 2 h, resp.) under reflux. After removal of EtOH under reduced pressure with a rotary evaporator, the viscous residue was suspended in H_2O (1 l) and partitioned successively with $CHCl_3$ (20 l) and AcOEt (10 l). The $CHCl_3$-soluble extract (130 g) was fractionated by CC (*D101* porous resin; aq. EtOH, gradient) to give six fractions, *Frs. 1 – 6*, combined according to the TLC results. *Fr. 2* (8 g) was further subjected to CC (SiO_2; petrol ether/AcOEt 20 : 1 to 1 : 2, gradient) to give four subfractions, *Frs. 2.1 – 2.4*. *Fr. 2.2* was subjected to CC (*RP-C*$_{18}$; MeOH/H_2O 5 : 5 to 9 : 1) to give three subfractions, *Frs. 2.2.1 – 2.2.3*. *Fr. 2.2.2* (1 g) was separated by CC (*RP-C*$_{18}$; MeOH/H_2O 75 : 25) to yield **1** (7 mg). *Fr. 2.3* (2 g) was subjected to CC (*RP-C*$_{18}$; MeOH/H_2O 5 : 5 to 9 : 1) to give four subfractions, *Frs. 2.3.1 – 2.3.4*. *Fr. 2.3.1* (110 mg) was separated by prep. HPLC (MeOH/H_2O 68 : 32, 10 ml min^{-1}) to give **4** (8 mg). *Fr. 2.3.4* (80 mg) was separated by prep. HPLC (MeOH/H_2O 70 : 30, 10 ml min^{-1}) to give **2** (8 mg) and **3** (8 mg).

Aglaiasterol A (=*(3α,5α,17*Z*)-3-Hydroxypregn-17-en-16-one*; **1**). Colorless needles (MeOH). M.p. 125 – 127°. $[\alpha]_D^{26} = -110.8$ ($c = 0.220$, $CHCl_3$). UV (MeCN): 235 (1.73). IR: 3502, 2915, 1712, 1644, 1451, 1379, 1065, 1004. ^1H- and ^{13}C-NMR: see *Table 1*. ESI-MS: 655.6 ($[2M + Na]^+$). HR-ESI-MS: 339.2287 ($[M + Na]^+$, $C_{21}H_{32}NaO_2^+$; calc. 339.2295).

Aglaiasterol B (=*(3β,5α,17*E*)-3-Hydroxypregn-17-en-16-one*; **2**). Colorless needles (MeOH). M.p. 115 – 128°. $[\alpha]_D^{26} = +30.6$ ($c = 0.210$, $CHCl_3$). UV (MeCN): 240 (1.82). IR: 3501, 2915, 1712, 1644, 1453, 1370, 1060, 1000. ^1H- and ^{13}C-NMR: see *Table 1*. ESI-MS: 317 ($[M + H]^+$). HR-ESI-MS: 339.2304 ($[M + Na]^+$, $C_{21}H_{32}NaO_2^+$; calc. 339.2295).

Aglaiasterol C (=*(3β,5α,17*Z*)-3-Hydroxypregn-17-en-16-one*; **3**). Colorless needles (MeOH). M.p. 105 – 120°. $[\alpha]_D^{26} = +60.8$ ($c = 0.250$, $CHCl_3$). UV (MeCN): 238 (1.63). IR: 3502, 2915, 1712, 1645, 1453,

1378, 1065, 1004. ^{1}H- and ^{13}C-NMR: see *Table 2*. ESI-MS: 317 ($[M+H]^+$). HR-ESI-MS: 339.2296 ($[M+Na]^+$, $C_{21}H_{32}NaO_2^+$; calc. 339.2295).

Aglaiasterol D (=*(3α,5α,20S*)-3-Hydroxy-16-oxopregnane-20-yl acetate*; **4**). Colorless needles (MeOH). M.p. 170 – 172°. $[\alpha]_D^{26} = -100.7$ ($c = 0.262$, $CHCl_3$). IR: 3440, 2938, 1731, 1640, 1449, 1379, 1243, 1070, 1029. ^{1}H- and ^{13}C-NMR: see *Table 2*. ESI-MS: 399.3 ($[M+Na]^+$). HR-ESI-MS: 399.2512 ($[M+Na]^+$, $C_{23}H_{36}NaO_4^+$; calc. 399.2506).

Cell Culture and Cytotoxicity Assay. The following human tumor cell lines were used: K562 (leukemia), MCF-7 (breast cancer), KB (oral epithelial cancer), SMMC-7721 (hepatocellular carcinoma), and MDR cells of K562/A02, MCF-7/ADM, and KB/VCR. All cells were cultured in *RPMI-1640* or *Dulbecco*'s Modified *Eagle*'s Medium (*HyClone*, Logan, UT), supplemented with 10% fetal bovine serum (*HyClone*) in 5% CO_2 at 37°. The cytotoxicity assay was performed according to the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) method in 96-well microplates [20]. Briefly, 180 µl of the cell suspension was seeded into each well of 96-well cell culture plates and allowed to adhere for 12 h before test compound addition, while suspended cells were seeded just before test compound addition with an initial density of $1 \cdot 10^5$ cells/ml. Each tumor cell line was exposed to each test compound at concentrations of 0.1, 1, 10, 100, and 500 µM in triplicate for 48 h, with 5-fluorouracil and doxorubicin (*Sigma*, St. Louis, MO), used as positive controls. After treatment, cell viability was detected and IC_{50} values were calculated by the *Reed* and *Muench* method [21].

This work was supported by the *National Natural Science Foundation of China* (Grant No. U1204830) and the *Science and Technology Key Project* of *Department of Education of Henan Province* (Grant No. 13A310064).

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Received January 25, 2015
Accepted April 17, 2015

