Phenolic Compounds from the Whole Plants of *Gentiana rhodantha* (Gentianaceae)

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Gentiana rhodantha FRANCH. ex HEMSL. (Gentianaceae), an annual herb widely distributed in the southwest of China, has been medicinally used for the treatment of inflammation, cholecystitis, and tuberculosis by the local people of its growing areas. Chemical investigation on the whole plants led to the identification of eight new phenolic compounds, rhodanthenones A–D (**1**–**4**, resp.), apigenin 7-*O*-glucopyranosyl-(1→3)-glucopyranosyl-(1→3)-glucopyranoside (**5**), 1,2-dihydroxy-4-methoxybenzene 1-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (**6**), 1,2-dihydroxy-4,6-dimethoxybenzene 1-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (**7**), and methyl 2-*O*-β-D-glucopyranosyl-2,4,6-trihydroxybenzoate (**8**), together with eleven known compounds, **9**–**19**. Their structures were determined on the basis of detailed spectroscopic analyses and chemical methods. Acetylcholinesterase (AChE) inhibition and cytotoxicity tests against five human cancer cell lines showed that only rhodanthenone D (**4**) and mangiferin (**12**) exhibited 18.4 and 13.4% of AChE inhibitory effects at a concentration of 10^{-4} M, respectively, while compounds **1**–**5** and the known xanthones lancerin (**11**), mangiferin (**12**), and neomangiferin (**13**) displayed no cytotoxicity at a concentration of 40 μM.

Introduction. – *Gentiana rhodantha* FRANCH. ex HEMSL. (Gentianaceae) is an annual herb widely distributed in the southwest of China. The whole plants have been medicinally used for the treatment of inflammation, cholecystitis, and tuberculosis by the local people of its growing areas. Previous investigations led to a series of secoiridoid glucosides as the main secondary metabolites from this herb [1–3]. Several xanthones from *G. campestris* and *G. amarella* ssp. *acuta* showed potent inhibitory activities on acetylcholinesterase (AChE) [4][5]. The close taxonomic links between these two species and *G. rhodantha* prompted us to investigate the title plant for new anti-AChE compounds. As a result, eight new compounds, including three benzophenone glucosides, rhodanthenones A–C (**1**–**3**, resp.), one xanthone, rhodanthenone D (**4**), one apigenin 7-*O*-glycoside, **5**, and three simple aromatic glycosides, 1,2-dihydroxy-4-methoxybenzene 1-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (**6**), 1,2-dihydroxy-4,6-dimethoxybenzene 1-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (**7**), and methyl 2-*O*-β-D-glucopyranosyl-2,4,6-trihydroxybenzoate (**8**), were obtained from the whole plants of *G. rhodantha*, together with eleven known compounds. The isolates, including **1**–**5**, and three known xanthones, lancerin (**11**), mangiferin (**12**), and neomangiferin (**13**), were tested for their inhibitory activities on AChE and cytotoxicities against five human cancer cell lines. The results are presented in this article.

Results and Discussion. – The MeOH extract of the whole plants of *G. rhodantha* was defatted with petroleum ether and then applied to repeated column chromatography (CC) on *Diaion HP-20SS*, *Sephadex LH-20*, silica gel, *MCI-gel CHP20P*, and *Chromatorex ODS*, to afford eight new phenolic compounds, **1**–**8** (*Fig. 1*). In addition, eleven known compounds were identified as rhyncoside D (**9**) [6], foliachinenoside C (**10**) [7], lancerin (**11**) [8], mangiferin (**12**) [9], neomangiferin (**13**) [10], vanilloloside (**14**) [11], 2-(*β*-D-glucopyranosyloxy)-3-hydroxybenzoic acid (**15**) [12], glucosyringic acid (**16**) [13], alangionoside O (**17**) [14], 1-*O*-*β*-D-glucopyranosyl-4-epiamplexine (**18**) [15], and (−)-syringaresinol *O*-*β*-D-glucopyranoside (**19**) [16], by direct comparison of their spectral and physical data with those of authentic samples and literature.

Rhodanthenone A (**1**) was obtained as a yellow amorphous powder. HR-FAB-MS (*m*/*z* 407.0982 ($[M-H]^-$)), in combination with ^{13}C-NMR (DEPT) spectroscopic data (*Table 1*), provided the molecular formula $C_{19}H_{20}O_{10}$ for **1**. The IR (KBr) spectrum displayed the characteristic absorptions for OH (3385 cm^{-1}) and C=O (1626 cm^{-1}) groups. Ninteen C-atom signals were displayed in the ^{13}C-NMR spectrum of **1**, twelve of which were between *δ*(C) 95 and 163 arising from two benzene rings. In addition, signals of one C=O C-atom at *δ*(C) 199.3, and of a hexosyl unit at *δ*(C) 101.6, 74.6, 78.1, 70.9, 77.7, and 62.4 were observed. The ^{1}H-NMR spectrum showed an anomeric H-atom signal at *δ*(H) 4.83 (*d*, *J* = 7.7) and six aromatic H-atom signals at *δ*(H) 6.20, 6.05, 7.12

Table 1. *^{13}C-* (100 MHz) *and ^{1}H-NMR* (400 MHz) *Data of Compounds* **1**–**3**[a]). In CD_3OD, *δ* in ppm, *J* in Hz.

Position	**1**		**2**		**3**	
	δ(C)	*δ*(H)	*δ*(C)	*δ*(H)	*δ*(C)	*δ*(H)
1	109.5 (*s*)		110.4 (*s*)		107.9 (*s*)	
2	163.6 (*s*)		158.3 (*s*)		160.7 (*s*)	
3	95.6 (*d*)	6.20 (*s*)	95.7 (*d*)	6.10 (*s*)	103.4 (*s*)	
4	159.4 (*s*)		161.9 (*s*)		164.9 (*s*)	
5	98.1 (*d*)	6.05 (*s*)	98.0 (*d*)	6.04 (*s*)	92.3 (*d*)	6.08 (*s*)
6	161.7 (*s*)		159.2 (*s*)		161.8 (*s*)	
1′	142.8 (*s*)		132.3 (*s*)		143.2 (*s*)	
2′	116.8 (*d*)	7.12 (*s*)	117.7 (*d*)	7.16 (*s*)	116.1 (*d*)	7.08 (*s*)
3′	158.2 (*s*)		145.6 (*s*)		158.2 (*s*)	
4′	120.3 (*d*)	6.94 (*d*, *J* = 7.3)	151.8 (*s*)		119.9 (*d*)	6.94 (*d*, *J* = 7.3)
5′	130.1 (*d*)	7.24 (*dd*, *J* = 7.3, 7.5)	115.5 (*d*)	6.74 (*d*, *J* = 7.6)	130.3 (*d*)	7.21 (*dd*, *J* = 7.6, 7.3)
6′	121.4 (*d*)	7.20 (*d*, *J* = 7.5)	124.8 (*d*)	7.06 (*d*, *J* = 7.6)	121.1 (*d*)	7.12 (*d*, *J* = 7.6)
C=O	199.3 (*s*)		197.5 (*s*)		200.3 (*s*)	
MeO					56.9 (*q*)	3.72 (*s*)
Glc						
1″	101.6 (*d*)	4.83 (*d*, *J* = 7.7)	101.0 (*d*)	4.70 (*d*, *J* = 7.6)	76.0 (*d*)	4.88 (*d*, *J* = 7.6)
2″	74.6 (*d*)	2.90 (*dd*, *J* = 8.4, 8.3)	73.3 (*d*)	2.90 (*dd*, *J* = 8.1, 7.6)	71.5 (*d*)	3.28–3.32 (*m*)
3″	78.1 (*d*)	3.32–3.35 (*m*)	77.1 (*d*)	3.17–3.19 (*m*)	79.9 (*d*)	3.41–3.43 (*m*)
4″	70.9 (*d*)	3.26 (*t*, *J* = 9.2)	69.5 (*d*)	4.07 (*t*, *J* = 9.0)	73.0 (*d*)	3.93 (*t*, *J* = 9.1)
5″	77.7 (*d*)	3.19–3.23 (*m*)	76.4 (*d*)	3.21–3.24 (*m*)	82.5 (*d*)	3.28–3.32 (*m*)
6″	62.4 (*t*)	3.85 (*dd*, *J* = 11.9, 1.3), 3.70 (*dd*, *J* = 11.9, 4.8)	60.7 (*t*)	3.65 (*dd*, *J* = 11.4, 1.3), 3.43 (*dd*, *J* = 11.4, 6.7)	62.5 (*t*)	3.83 (*dd*, *J* = 11.4, 1.5), 3.70 (*dd*, *J* = 11.4, 6.7)

[a]) The assignments were based on DEPT, HMQC, HMBC, and ROESY experiments.

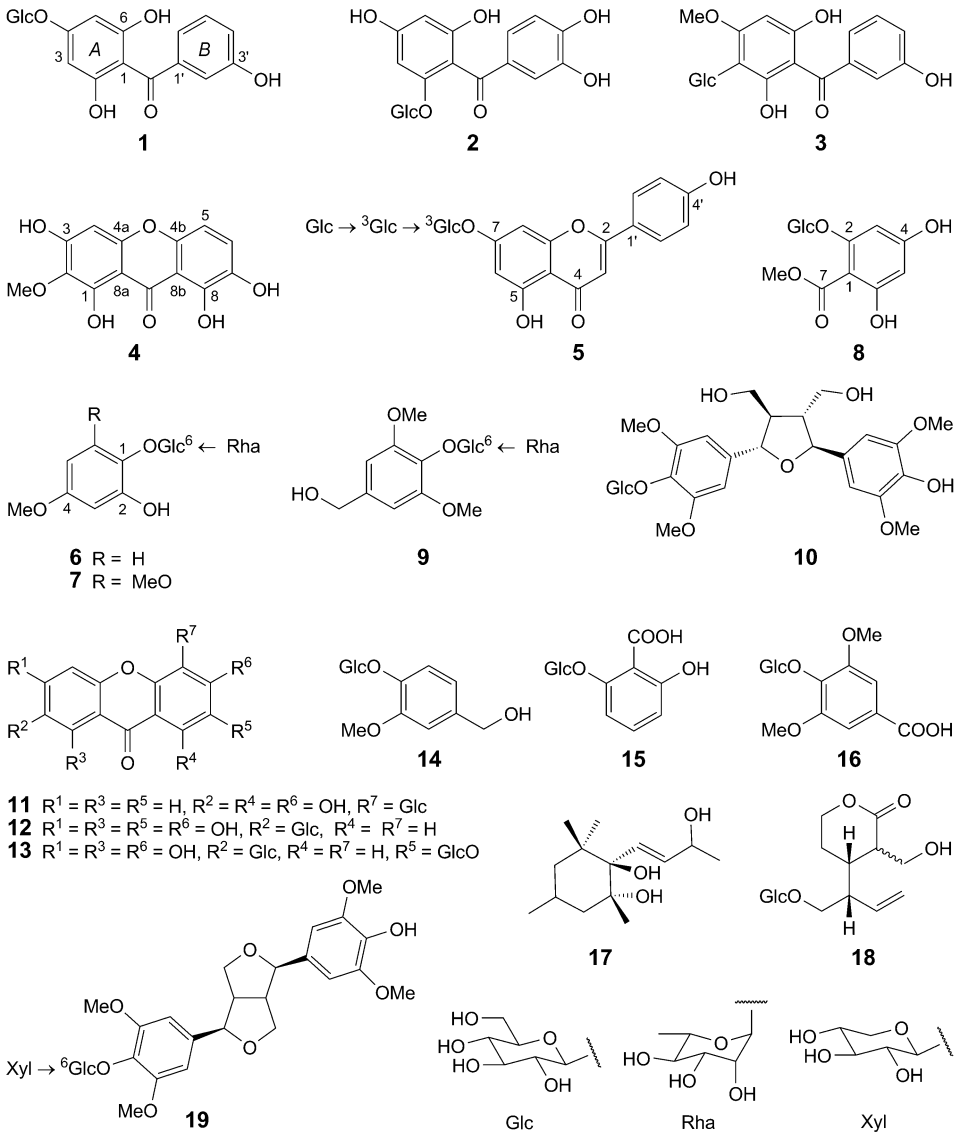

Fig. 1. *Compounds* **1**–**19** *isolated from the whole plants of* Gentiana rhodantha

(*s*, 1 H each), 6.94 (*d*, $J = 7.3$), 7.24 (*dd*, $J = 7.3, 7.5$), and 7.20 (*d*, $J = 7.5$), corresponding to a 1,2,4,6-tetrasubstituted and a 1,3-disubstituted benzene ring, respectively. The above data suggested that compound **1** could be a benzophenone glycoside [17][18]. Acidic hydrolysis of **1** with 1M HCl in dioxane afforded D-glucose as sugar residue, which was determined by GC analysis of its corresponding trimethylsilylated L-cysteine adduct [19]. The benzophenone skeleton was confirmed by the HMBC correlations (*Fig. 2*) from both H–C(2′) (δ(H) 7.12) and H–C(6′) (δ(H) 7.20) to the C=O C-atom

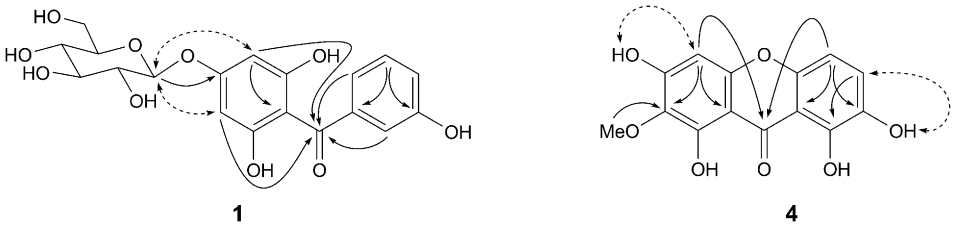

Fig. 2. *Key HMBC* (H→C) *and ROESY* (H↔H) *correlations of* **1** *and* **4**

(δ(C) 199.3), and the ^4J(C,H) HMBC correlations from both H–C(3) (δ(H) 6.20) and H–C(5) (δ(H) 6.05) to the same C=O C-atom. The HMBC correlations (*Fig. 2*) from the glucosyl anomeric H-atom (δ(H) 4.83) to C(4) (δ(C) 159.4), and the ROESY correlation of the glucosyl anomeric H-atom (δ(H) 4.83) with both H–C(3) and H–C(5) determined the location of the glucosyl unit on the benzophenone as C(4). Therefore, the structure of rhodanthenone A (**1**) was determined to be 2,4,6,3′-tetrahydroxybenzophenone 4-*O*-β-D-glucopyranoside.

Rhodanthenone B (**2**) was obtained as a yellow amorphous powder. Its molecular formula was determined as $C_{19}H_{20}O_{11}$, based on HR-FAB-MS (*m/z* 423.0906 ([*M* – H]$^-$)), and ^{13}C-NMR and DEPT spectra, which indicated one more O-atom than in **1** (*Table 1*). The ^{13}C-NMR spectrum of **2** indicated the presence of one hexosyl moiety, along with 13 signals due to a benzophenone aglycone, which contains two benzene rings and one C=O C-atom (δ(C) 197.5), as in **1**. The ^{1}H-NMR spectrum of **2** showed signals for one 1,3,4-trisubstituted (δ(H) 7.16 (*s*, H–C(2′)), 7.06 (*dd*, *J* = 7.6, H–C(6′)), 6.74 (*d*, *J* = 7.6, H–C(5′))) and one 1,2,4,6-tetrasubstituted (δ(H) 6.04, 6.10 (2*s*, H–C(3,5))) benzene rings, as well as an anomeric H-atom signal at δ(H) 4.70 (*d*, *J* = 7.6). Acidic hydrolysis of **2** with 1M HCl in dioxane afforded D-glucose as the carbohydrate moiety. The ^3J(C,H) HMBC correlations from both H–C(2′) (δ(H) 7.16) and H–C(6′) (δ(H) 7.06) to the C=O C-atom (δ(C) 197.5), and the ^4J(C,H) HMBC correlations from H–C(5) (δ(H) 6.04) and H–C(3) (δ(H) 6.10) to the same C=O C-atom led to the linkage position of the two benzene rings as shown in *Fig. 1*. The glucosyl unit was determined to be at C(2) by the HMBC correlation between the anomeric H-atom (δ(H) 4.70) and C(2) (δ(C) 158.3). This was further confirmed by the ROESY correlation of the anomeric H-atom with H–C(3) (δ(H) 6.10). Thus, rhodanthenone B (**2**) was elucidated as 2,4,6,3′,4′-pentahydroxybenzophenone 2-*O*-β-D-glucopyranoside.

Rhodanthenone C (**3**) was isolated as a yellow amorphous powder. Its molecular formula, $C_{20}H_{22}O_{10}$, was established on the basis of HR-FAB-MS (*m/z* 421.0906 ([*M* – H]$^-$)) and NMR data. The ^{1}H- and ^{13}C-NMR spectra of **3** were closely related to those of **1**, revealing the presence of two benzene rings, and one C=O and one hexosyl moieties. Compared with compound **1**, in addition to the same 1,3-disubstituted benzene ring and a C=O C-atom (δ(C) 200.3), the NMR spectra of **3** indicated the presence of a pentasubstituted benzene ring (δ(H) 6.08 (*s*, H–C(5)) and an additional MeO group (δ(C) 56.9) in the benzophenone skeleton, as well as, with a set of signals, one β-glucopyranosyl moiety (δ(H) 4.88 (*d*, *J* = 7.6, H–C(1) of Glc); δ(C) 76.0, 71.5,

79.9, 73.0, 82.5, and 62.5). In HMBC spectrum, both H–C(2′) (δ(H) 7.08) and H–C(6′) (δ(H) 7.12) correlated with the C=O C-atom (δ(C) 200.3), and the ^4J(C,H) HMBC correlation from the H–C(5) at δ(H) 6.08 to the same C=O C-atom was observed. Moreover, the HMBC correlations from the anomeric H-atom (δ(H) 4.88) to C(3) (δ(C) 103.4), C(4) (δ(C) 164.5), and C(2) (δ(C) 160.7), from the MeO H-atoms (δ(H) 3.72) to C(4) (δ(C) 164.9), and the ROESY correlation between the MeO group (δ(H) 3.72) and H–C(5) (δ(H) 6.08) confirmed the C,C connections of glucosyl unit with C(3) and the MeO group with C(4). Consequently, rhodanthenone C (**3**) was characterized as 2,6,3′-trihydroxy-4-methoxybenzophenone 3-β-D-glucosylpyranoside.

Rhodanthenone D (**4**) was obtained as a yellow amorphous powder with the molecular formula $C_{14}H_{10}O_7$, established by HR-FAB-MS (*m*/*z* 289.2056 ($[M-H]^-$)), ^{13}C-NMR, and DEPT data. On the basis of its 1D- and 2D-NMR data, the structure of **4** was determined to be 1,3,7,8-tetrahydroxy-2-methoxyxanthone. In the ^{13}C-NMR spectrum of **4**, signals of twelve C-atoms between δ(C) 98 and 160 due to two benzene rings, of one C=O C-atom at δ(C) 184.1, and of one MeO group at δ(C) 60.8 were observed. The ^{1}H-NMR spectrum displayed signals for four exchangeable H-atoms at δ(H) 11.70 (br. *s*, OH–C(8)), 11.60 (br. *s*, OH–C(1,3)), 9.37 (br. *s*, OH–C(7)), for one aromatic H-atom at δ(H) 6.26 (*s*, H–C(4)), and two *ortho*-coupled aromatic *doublet*s at δ(H) 6.94, 7.27 (*d*, J = 7.9, H–C(5,6)), in addition to a MeO signal at δ(H) 3.77. These above data supported that compound **4** was a polyhydroxylated xanthone. In the HMBC spectrum of **4** (*Fig. 2*), H–C(4) (δ(H) 6.26) correlated with C(8a) (δ(C) 100.7), C(2) (δ(C) 127.5), and the C=O C-atom (δ(C) 184.1), and H–C(5) (δ(H) 6.94 correlated with C(8b) (δ(C) 108.0) and the same C=O C-atom. Moreover, the ROESY correlations of two exchangeable H-atoms with signals at δ(H) 11.60 (*s*, OH–C(3)) and 9.37 (*s*, OH–C(7)) with those of aromatic H-atoms at δ(H) 6.26 (H–C(4)) and δ(H) 7.27 (H–C(6)), respectively, were observed. These observations confirmed the locations of the OH and MeO groups in **4** as shown in *Fig. 2*. Thus, the structure of rhodanthenone D (**4**) was determined as 1,3,7,8-tetrahydroxy-2-methoxyxanthone.

Compound **5**, yellow amorphous powder, had the molecular formula $C_{33}H_{40}O_{20}$, as deduced from the HR-FAB-MS (*m*/*z* 755.2056 ($[M-H]^-$)) and NMR data. The UV spectrum showed the absorption at 327, 271, and 206 nm. In the ^{13}C-NMR spectrum of **5**, signals corresponding to 33 C-atoms were observed, including 14 aromatic signals and one C=O C-atom signal at δ(C) 182.0 due to a flavone aglycone, as well as those of three hexosyl moieties. The ^{1}H-NMR spectrum displayed signals of two exchangeable H-atoms (δ(H) 12.92 (*s*, OH–C(5)), 10.45 (*s*, OH–C(4′)), two aromatic *singlet*s at δ(H) 6.20, 6.87 (*s*, H–C(6,8)) arising from a 1,2,3,5-tetrasubstituted aromatic ring, two aromatic *doublet*s (δ(H) 7.93 (*d*, J = 8.6, H–C(2′,6′)), 6.93 (*d*, J = 8.6, H–C(3′,5′))) due to a symmetrical 1,4-bisubstituted aromatic ring, one aromatic signals at δ(H) 6.85 (*s*, H–C(3)) and three anomeric H-atom signals (δ(H) 5.02 (*d*, J = 7.0, Glc H–C(1″)), 4.60 (*d*, J = 7.4, Glc H–C(1‴), and 4.51 (*d*, J = 7.6, Glc H–C(1⁗)). Acidic hydrolysis of **5** afforded D-glucose as the carbohydrate moiety. The above data supported that **5** was an apigenin triglucoside [20]. The connections and location of the sugar moieties were determined by the HMBC experiment, in which correlations from the terminal glucosyl anomeric H-atom at δ(H) 4.51 (Glc H–C(1⁗)) to the middle glucosyl C(3‴) (δ(C) 83.2), from the middle glucosyl anomeric H-atom at δ(H) 4.60 (Glc H–C(1‴)) to the inner glucosyl C(3″) (δ(C) 83.0), and from the inner glucosyl anomeric H-atom at

(δ(H) 5.02 Glc H–C(1″)) to the aglycone C(7) (δ(C) 162.7) were observed. Therefore, the structure of **5** was elucidated as apigenin 7-*O*-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranoside.

Compound **6** was obtained as a brown amorphous powder and possessed the molecular formula $C_{19}H_{28}O_{12}$, as determined from its HR-FAB-MS (*m/z* 447.1507 ($[M-H]^-$)) and ^{13}C-NMR data. The ^{13}C-NMR spectrum of **6** (*Table 2*) exhibited 19 C-atom signals, including those of one benzene ring, a MeO C-atom (δ(C) 56.7), and those of two hexosyl moieties. The *ABX* spin system at δ(H) 6.39 (*d*, *J* = 2.0), 6.66 (*dd*, *J* = 2.0, 8.9), and 7.18 (*dd*, *J* = 8.9, 2.0), one MeO signal at δ(H) 3.84, two anomeric H-atom signals at δ(H) 4.72 (*d*, *J* = 7.3, Glc H–C(1)) and 4.71 (br. *s*, Rha H–C(1)), and one *doublet* at δ(H) 1.23 (*d*, *J* = 6.2, Rha H–C(6)) arising from a 6-deoxy sugar were displayed in ^{1}H-NMR spectrum (*Table 2*). Acidic hydrolysis of **6** produced D-glucose and L-rhamnose as the sugar moieties. The HMBC correlations from the glucosyl anomeric H-atom (δ(H) 4.72) to C(1) (δ(C) 143.7), and the rhamnosyl anomeric H-atom at δ(H) 4.71 to the glucosyl C(6′) (δ(C) 67.9) were observed. Moreover, the ROSEY correlations of the MeO H-atoms (δ(H) 3.84) with both H–C(3) (δ(H) 6.39) and H–C(5) (δ(H) 6.66), and of glucosyl H–C(1) (δ(H) 4.72) with H–C(6) (δ(H) 7.18)

Table 2. *^{13}C-* (100 MHz) *and ^{1}H-NMR* (400 MHz) *Data of Compounds* **6**–**8**[a]). In CD_3OD, δ in ppm, *J* in Hz.

Position	**6**		**7**		**8**	
	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)
1	143.7 (*s*)		132.5 (*s*)		96.6 (*s*)	
2	147.5 (*s*)		155.4 (*s*)		161.2 (*s*)	
3	108.5 (*d*)	6.39 (*d*, *J* = 2.0)	97.0 (*d*)	6.38 (*d*, *J* = 2.6)	96.6 (*d*)	6.23 (*d*, *J* = 2.1)
4	157.4 (*s*)		158.0 (*s*)		164.8 (*s*)	
5	110.6 (*d*)	6.66 (*dd*, *J* = 2.0, 8.9)	98.9 (*d*)	6.23 (*d*, *J* = 2.6)	98.3 (*s*)	
6	120.6 (*d*)	7.18 (*d*, *J* = 8.9)	150.7 (*s*)		164.7 (*d*)	6.00 (*d*, *J* = 2.1)
COO					172.0 (*s*)	
MeO	56.7 (*q*)	3.84 (*s*)	56.9 (*q*), 56.0 (*q*)	3.84 (*s*), 3.92 (*s*)	52.4 (*q*)	3.85 (*s*)
Glc						
1′	102.2 (*d*)	4.72 (*d*, *J* = 7.3)	105.6 (*d*)	4.86 (*d*, *J* = 7.3)	102.5 (*d*)	4.87 (*d*, *J* = 7.3)
2′	74.8 (*d*)	3.38–3.40 (*m*)	74.6 (*d*)	3.28–3.32 (*m*)	74.9 (*d*)	3.27–3.31 (*m*)
3′	77.7 (*d*)	3.46–3.52 (*m*)	77.8 (*d*)	3.36–3.39 (*m*)	78.4 (*d*)	3.48–3.51 (*m*)
4′	71.5 (*d*)	3.40–3.45 (*m*)	71.6 (*d*)	3.32–3.35 (*m*)	71.1 (*d*)	3.41–3.44 (*m*)
5′	76.9 (*d*)	3.49 (*t*, *J* = 10.9)	77.3 (*d*)	3.40–3.42 (*m*)	78.3 (*d*)	3.46–3.49 (*m*)
6′	67.9 (*t*)	4.00 (*dd*, *J* = 12.8, 1.2), 3.69 (*dd*, *J* = 12.8, 5.1)	68.1 (*t*)	3.92 (*dd*, *J* = 11.6, 1.1), 3.55 (*dd*, *J* = 11.6, 4.6)	62.4 (*t*)	3.91 (*dd*, *J* = 11.9, 1.2), 3.72 (*dd*, *J* = 11.9, 4.8)
Rha						
1″	104.0 (*d*)	4.71 (br. *s*)	102.3 (*d*)	4.70 (*d*, *J* = 1.2)		
2″	72.4 (*d*)	3.57–3.60 (*m*)	72.1 (*d*)	3.65–3.67 (*m*)		
3″	72.2 (*d*)	3.60–3.62 (*m*)	74.2 (*d*)	3.63–3.65 (*m*)		
4″	74.0 (*d*)	3.40–3.43 (*m*)	71.7 (*d*)	3.33–3.36 (*m*)		
5″	69.8 (*d*)	3.58–3.61 (*m*)	69.7 (*d*)	3.56–3.60 (*m*)		
6″	18.0 (*q*)	1.23 (*d*, *J* = 6.2)	17.9 (*q*)	1.21 (*d*, *J* = 6.2)		

[a]) The assignments were based on DEPT, HMQC, HMBC, and ROESY or NOESY experiments.

confirmed the linkage positions of the two sugar moieties and the MeO group as shown in *Fig. 1*. Therefore, the structure of compound **6** was assigned to be 1,2-dihydroxy-4-methoxybenzene 1-*O*-*α*-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside.

Compound **7**, with the molecular formula $C_{20}H_{30}O_{13}$, showed IR absorptions of OH group (3425 cm^{-1}). The ^{1}H- and ^{13}C-NMR data (*Table 2*) of **7** were very similar to those of **6**, except for the appearance of an additional MeO group for **7**. This led to the change of the *ABX* spin system in **6** to an *AM* spin system (δ(H) 6.38, 6.23 (*d*, *J* = 2.6, H–C(3,5))), indicating that **7** possessed a 1,2,4,6-tetrasubstituted aromatic ring. In the HMBC spectrum, the two MeO H-atoms at δ(H) 3.84 and 3.92 correlated with C(6) and C(4), respectively. Moreover, the NOESY correlations from one MeO (δ(H) 3.92) to H–C(5) (δ(H) 6.23) and H–C(3) (δ(H) 6.38), and of another MeO (δ(H) 3.84) to H–C(5) (δ(H) 6.23) further confirmed the connection of the additional MeO group at C(6). Accordingly, the structure of **7** was elucidated as 1,2-dihydroxy-4,6-dimethoxybenzene 1-*O*-*α*-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside.

Compound **8** had the molecular formula $C_{14}H_{18}O_{10}$, as deduced from the negative-ion-mode HR-FAB-MS (*m*/*z* 345.0837 ($[M-H]^-$)). Signals of six aromatic C-atoms, one MeO (δ(C) 52.4), one C=O C-atom (δ(C) 172.0), and one hexosyl moiety were observed in the ^{13}C-NMR spectrum (*Table 2*). The ^{1}H-NMR spectrum (*Table 2*) exhibited two *meta*-coupled aromatic *doublet*s at δ(C) 6.23, 6.00 (*d*, *J* = 2.1, each 1 H), one MeO signal at δ(H) 3.85, as well as an anomeric H-atom signal at δ(H) 4.87 (*d*, *J* = 7.3). Acidic hydrolysis of **8** afforded D-glucose as the sugar moiety. The skeleton of **8**, and the locations of the MeO group and glucosyl linkage were determined by the HMBC correlation of MeO H-atoms (δ(H) 3.85) with the C=O C-atom (δ(C) 172.0), and the ROESY correlation of anomeric H-atom (δ(H) 4.87) with H–C(3) (δ(H) 6.23). Accordingly, the structure of **8** was elucidated as methyl 2-*O*-*β*-D-glucopyranosyl-2,4,6-trihydroxybenzoate.

Rhodanthenones A–E (**1**–**5**, resp.) and three known xanthones (lancerin (**11**), mangiferin (**12**), and neomangiferin (**13**)) were tested *in vitro* for their inhibitory activity against acetylcholinesterase (AChE), an enzyme implicated in aging-related neurodegenerative diseases such as *Alzheimer*'s disease [21]. When compared to the positive control (tacrine) with 49.6% inhibitory activity at a concentration of 3.3×10^{-7} M, only rhodanthenone D (**4**) and mangiferin (**12**) exhibited 18.4 and 13.4% inhibitory effects at a concentration of 10^{-4} M, respectively. All of the tested compounds showed no cytotoxicity at a concentration of 40 μM, against five human cancer cell lines, using the MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) method [22][23].

In conclusion, 19 compounds have been isolated and identified from the title plant, including eight new and eleven known compounds found for the first time in this species. Based on the structures, these compounds could be classified into benzophenone glucosides, **1**–**3**, flavonoids and xanthones, **4**–**5** and **11**–**13**, lignan glycosides, **10** and **19**, and simple phenolic glycosides, **6**–**9** and **14**–**18**. The new compounds **1**–**3** are the first glucosides of benzophenones isolated from the genus *Gentiana*. Thus, the secondary metabolites of *G. rhodantha*, which is classified in the section Stenogyne, are totally different from those found in species from the other sections of the genus *Gentiana*. *Ho et al.* suggested that section *Stenogyne* FRANCH should be separated from the genus *Gentiana* as a newly erected genus *Metagentiana* T. N. HO & S. W. LIU in the

family Gentianaceae on the basis of morphology [24]. *Yuan et al.*, and *Chen et al.* supported this claim on the basis of nuclear ribosomal ITS sequences [25][26]. Our results could be a supportive evidence for the above taxonomic system, in view of phytochemistry. However, further systematic phytochemical research on other species of section *Stenogyne* FRANCH in genus *Gentiana* is necessary.

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Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 200–300 mesh; *Qingdao Marine Chemical Factory*), *Diaion HP20SS* (*Mitsubishi Chemical Industry, Ltd.*), *MCI-gel CHP20P* (75–150 μm; *Mitsubishi Chemical Industry, Ltd.*), or *Chromatorex ODS* (100–200 mesh; *Fuji Silysia Chemical Co., Ltd.*). TLC: SiO_2 *G* precoated plates (*Qingdao Haiyang Chemical Co.*) with $CHCl_3$/MeOH/H_2O 7:3:0.5; spots were detected by spraying with 10% of H_2SO_4, followed by heating. GC: *Agilent Technologies HP5890* gas chromatograph, equipped with a H_2 flame ionization detector; *30QC2/AC-5* quartz cap. column (30 m × 0.32 mm); conditions: column temp., 180°/280°; programmed increase, 3°/min; carrier gas, N_2 (1 ml/min); injection and detector temp., 250°; injection volume, 4 ml, split ratio, 1:50. Optical rotations: *SEPA-3000* automatic digital polarimeter. UV Spectra: *Shimadzu UV-2401A* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Bio-Rad FTS-135* spectrometer; $\tilde{\nu}$ in cm^{-1}. 1D- and 2D-NMR spectra: *Bruker DRX-500* MHz instrument at 400 and 100 MHz for ^1H and ^{13}C, resp.; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. MS: *VG Autospect 3000* spectrometer; in *m*/*z*.

Plant Material. The air-dried whole plants of *G. rhodantha* FRANCH. ex HEMSL. were collected from Wenshan, Yunnan Province, P. R. China, on July 2004, and identified by *C.-R. Y.* The voucher specimen (KUN 0552165) was deposited with the Herbarium of Kunming Institute of Botany (KIB), Chinese Academy of Sciences (ACS).

Extraction and Isolation. The powdered air-dried whole plant of *G. rhodantha* (2.0 kg) was extracted with MeOH (3 × 2000 ml) at r.t. After removal of the solvent, the resulting residue (100 g) was suspended in H_2O (500 ml) and defatted with petroleum ether (3 × 500 ml). The aq. layer was subjected to CC (*Diaion HP20SS*; H_2O/MeOH 1:0–0:1) to give four fractions, *Frs. A1–A4*. *Fr. A1* (19.3 g) was subjected to repeated CC (*Sephadex LH-20*; H_2O/MeOH 1:0–0:1; SiO_2; $CHCl_3$/MeOH/H_2O 9:1:0:1–7:3:0.5; *MCI-gel CHP20P*; H_2O/MeOH 9:1–6:4; and *Chromatorex ODS*; H_2O/MeOH 7:3) to afford **6** (298 mg), **9** (93 mg), **15** (73 mg), **16** (14 mg). In the same manner, **8** (23 mg), **10** (27 mg), **14** (12 mg), **17** (13 mg), and **18** (4 mg) were obtained from *Fr. A2* (26.0 g). *Fr. A3* (14.6 g) was subjected to CC (*MCI-gel CHP20P*; H_2O/MeOH 7:3–4:6; *Chromatorex ODS*; H_2O/MeOH 6:4; and SiO_2; $CHCl_3$/MeOH/H_2O 8:2:0.2) to yield **1** (41 mg), **2** (303 mg), **3** (53 mg), **7** (23 mg), **12** (271 mg), **13** (2.1 g), and **19** (58 mg). *Fr. A4* (24.7 g) was separated by CC (*Sephadex LH-20*; H_2O/MeOH 3:2–1:4; *Chromatorex ODS*; H_2O/MeOH 1:1; and SiO_2; $CHCl_3$/MeOH/H_2O 8:2:0.2–7:3:0.5) to give **1** (721 mg), **5** (97 mg), **4** (18 mg), and **11** (32 mg).

Rhodanthenone A (*= 3,5-Dihydroxy-4-(3-hydroxybenzoyl)phenyl β-*D*-Glucopyranoside*; **1**)*:* Yellow amorphous powder. $[\alpha]_D^{18} = -8.1$ (*c* = 0.35, MeOH). UV (MeOH): 307 (3.91), 258 (3.84), 207 (4.52). IR (KBr): 3385, 1626, 1452, 1291, 1074. ^1H- and ^{13}C-NMR (CD_3OD): *Table 1*. FAB-MS (neg.): 407 ($[M-H]^-$), 245 ($[M-Glc-H]^-$). HR-FAB-MS (neg.): 407.0982 ($[M-H]^-$, $C_{19}H_{19}O_{10}^-$; calc. 407.0978).

Rhodanthenone B (*= 2-(3,4-Dihydroxybenzoyl)-3,5-dihydroxyphenyl β-*D*-Glucopyranoside*; **2**)*:* Yellow amorphous powder. $[\alpha]_D^{18} = +0.46$ (*c* = 0.36, MeOH). UV (MeOH): 361 (3.87), 256 (3.93), 207 (4.49). IR (KBr): 3419, 1620, 1459, 1168, 1073. ^1H- and ^{13}C-NMR (CD_3OD): *Table 1*. FAB-MS (neg.):

423 ([$M-H$]$^-$), 261 ([$M-$Glc$-H$]$^-$). HR-FAB-MS (neg.): 423.0906 ([$M-H$]$^-$, $C_{19}H_{19}O_{11}^-$; calc. 423.0927).

Rhodanthenone C (=*(1S)-1,5-Anhydro-1-[2,4-dihydroxy-3-(3-hydroxybenzoyl)-6-methoxyphenyl]-*D-*glucitol*; **3**)*:* Yellow amorphous powder. $[\alpha]_D^{18} = +0.46$ ($c = 0.36$, MeOH). UV (MeOH): 306 (3.99), 211 (4.53). IR (KBr): 3419, 1620, 1459, 1073. ^{1}H- and ^{13}C-NMR (CD_3OD): *Table 1*. FAB-MS (neg.): 421 ([$M-H$]$^-$). HR-FAB-MS (neg.): 421.0906 ([$M-H$]$^-$, $C_{20}H_{21}O_{10}^-$; calc. 421.0927).

Rhodanthenone D (=*1,3,7,8-Tetrahydroxy-2-methoxy-9*H*-xanthen-9-one*; **4**)*:* Yellow amorphous powder. $[\alpha]_D^{18} = +26$ ($c = 0.42$, MeOH). UV (MeOH): 327 (4.00), 271 (4.24), 206 (4.31). IR (KBr): 3421, 1607, 1027. ^{1}H-NMR ((D$_6$)DMSO): 6.26 (*s*, H–C(4)); 6.94 (*d*, $J = 7.9$, H–C(5)); 7.27 (*d*, $J = 7.9$, H–C(6)); 3.77 (*s*, MeO–C(7)); 11.70 (*s*, OH–C(8)); 11.60 (*s*, OH–C(1,3)); 9.37 (*s*, OH–C(7)). ^{13}C-NMR ((D$_6$)DMSO): 157.1 (C(1)); 127.5 (C(2)); 159.4 (C(3)); 98.1 (C(4)); 106.2 (C(5)); 124.0 (C(6)); 140.5 (C(7)); 147.0 (C(8)); 149.8 (C(4a)); 147.9 (C(4b)); 100.7 (C(8a)); 108.0 (C(8b)); 184.1 (C=O); 60.8 (*Me*O–C(7)). FAB-MS (neg.): 289 ([$M-H$]$^-$). HR-FAB-MS (neg.): 289.2056 ([$M-H$]$^-$, $C_{14}H_9O_7^-$; calc. 289.2034).

*Apigenin 7-*O*-β-*D*-Glucopyranosyl-(1→3)-β-*D*-glucopyranosyl-(1→3)-β-*D*-glucopyranoside* (=*7-{[*O*-β-*D*-Glucopyranosyl-(1→3)-*O*-β-*D*-glucopyranosyl-(1→3)-β-*D*-glucopyranosyl]oxy}-5-hydroxy-2-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one*; **5**)*:* Yellow amorphous powder. $[\alpha]_D^{18} = +26$ ($c = 0.42$, MeOH). UV (MeOH): 327 (4.15), 271 (4.18), 206 (4.44). IR (KBr): 3421, 1607, 1027. ^{1}H-NMR ((D$_6$)DMSO): 12.92 (*s*, OH–C(5)); 10.45 (*s*, OH–C(4′)); 6.85 (*s*, H–C(3)); 6.20 (*s*, H–C(6)); 6.87 (*s*, H–C(8)); 7.93 (*d*, $J = 8.6$, H–C(2′,6′)); 6.93 (*d*, $J = 8.6$, H–C(3′,5′)); 5.02 (*d*, $J = 7.0$, H–C(1″)); 3.57 (*m*, H–C(2″)); 3.56 (*m*, H–C(3″)); 3.23 (*m*, H–C(4″)); 3.26 (*m*, H–C(5″)); 3.43 (*dd*, $J = 10.3, 2.3$, H_a–C(6″)); 3.25 (*dd*, $J = 10.3, 5.0$, H_b–C(6″)); 4.60 (*d*, $J = 7.43$, H–C(1‴)); 3.19–3.01 (*m*, H–C(2‴)); 3.32–3.36 (*m*, H–C(3‴)); 3.04–3.06 (*m*, H–C(4‴)); 3.21–3.24 (*m*, H–C(5‴)); 3.72 (*dd*, $J = 11.0, 1.3$, H_a–C(6‴)); 3.57 (*dd*, $J = 11.0, 4.2$, H_b–C(6‴)); 4.51 (*d*, $J = 7.6$, H–C(1⁗)); 3.02–3.04 (*m*, H–C(2⁗)); 3.31–3.33 (*m*, H–C(3⁗)); 3.04–3.06 (*m*, H–C(4⁗)); 3.22–3.24 (*m*, H–C(5⁗)); 3.54 (*dd*, $J = 11.3, 2.1$, H_a–C(6⁗)); 3.11 (*dd*, $J = 11.3, 4.6$, H_b–C(6⁗)). ^{13}C-NMR ((D$_6$)DMSO): 164.3 (C(2)); 103.1 (C(3)); 182.0 (C(4)); 156.9 (C(5)); 98.3 (C(6)); 162.7 (C(7)); 94.9 (C(8)); 161.1 (C(9)); 105.3 (C(10)); 121.6 (C(1′)); 128.6 (C(2′,6′)); 116.1 (C(3′,5′)); 161.4 (C(4′)); 99.6 (Glc C(1″)); 75.5 (C(2″)); 83.0 (C(3″)); 68.7 (C(4″)); 76.1 (C(5″)); 60.5 (C(6″)); 102.3 (Glc C(1‴)); 76.1 (C(2‴)); 83.2 (C(3‴)); 69.2 (C(4‴)); 77.0 (C(5‴)); 61.0 (C(6‴)); 104.1 (Glc C(1⁗)); 74.6 (C(2⁗)); 77.4 (C(3⁗)); 69.7 (C(4⁗)); 77.0 (C(5⁗)); 60.3 (C(6⁗)). FAB-MS (neg.): 755 ([$M-H$]$^-$). HR-FAB-MS (neg.): 755.2056 ([$M-H$]$^-$, $C_{33}H_{39}O_{20}^-$; calc. 755.2034).

*1,2-Dihydroxy-4-methoxybenzene 1-*O*-α-*L*-rhamnopyranosyl-(1→6)-β-*D*-glucopyranoside* (=*2-Hydroxy-4-methoxyphenyl 6-*O*-(6-Deoxy-α-*L*-mannopyranosyl)-β-*D*-glucopyranoside*; **6**)*:* Brown amorphous powder. $[\alpha]_D^{18} = -57$ ($c = 0.16$, MeOH). UV (MeOH): 222 (4.35), 281 (4.11). IR (KBr): 3424, 1593, 1214, 1048. ^{1}H- and ^{13}C-NMR (CD_3OD): *Table 2*. FAB-MS (neg.): 447 ([$M-H$]$^-$), 311 ([$M-H-$Rha]$^-$). HR-FAB-MS (neg.): 447.1507 ([$M-H$]$^-$, $C_{19}H_{27}O_{12}^-$; calc. 447.1502).

*1,2-Dihydroxy-4,6-dimethoxybenzene 1-*O*-α-*L*-rhamnopyranosyl-(1→6)-β-*D*-glucopyranoside* (=*2-Hydroxy-4,6-dimethoxyphenyl 6-*O*-(6-Deoxy-α-*L*-mannopyranosyl)-β-*D*-glucopyranoside*; **7**)*:* Yellow amorphous powder. $[\alpha]_D^{18} = -41$ ($c = 0.38$, MeOH). UV (MeOH): 223 (4.26), 282 (3.88). IR (KBr): 3425, 1598, 1097, 1069. ^{1}H- and ^{13}C-NMR (CD_3OD): *Table 2*. FAB-MS (neg.): 477 ([$M-H$]$^-$). HR-FAB-MS (neg.): 477.1601 ([$M-H$]$^-$, $C_{20}H_{29}O_{13}^-$; calc. 477.1608).

*Methyl 2-(β-*D*-Glucopyranosyloxy)-4,6-dihydroxybenzoate* (**8**)*:* Yellow amorphous powder. $[\alpha]_D^{18} = -65$ ($c = 0.38$, MeOH). UV (MeOH): 221 (4.28), 283 (3.61). IR (KBr): 3424, 1680. ^{1}H- and ^{13}C-NMR (CD_3OD): *Table 2*. FAB-MS (neg.): 345 ([$M-H$]$^-$). HR-FAB-MS (neg.): 345.0837 ([$M-H$]$^-$, $C_{14}H_{17}O_{10}^-$; calc. 345.0821).

Acidic Hydrolysis of Compounds **1**–**2** *and* **5**–**8***:* Compounds **1**–**2** and **5**–**8** (*ca.* 5 mg each) in 1M HCl/dioxane 1:1 (5 ml) were heated at 85° on water-bath for 6 h. The mixture was partitioned between $CHCl_3$ and H_2O four times. The aq. layer was passed through an *Amberlite IRA-401* (OH^- form), and the eluate was concentrated to dryness to give a saccharide mixture. Glucose and rhamnose were identified as being present in the mixture by direct TLC analysis compared with authentic samples: R_f (glucose) 0.65; R_f (rhamnose) 0.69 (i-PrOH/MeOH/H_2O 25:1:2). The solns. of the sugar residues of the compounds **1**–**2** and **5**–**8** in 1.5 ml of pyridine were added to L-cysteine methyl ester hydrochloride (1.0 mg) and kept at 60° for 1 h, resp. 1-(Trimethylsilyl)-1*H*-imidazole (1.5 ml) was added to the mixture and kept again at 60°

for 30 min. The supernatants (4 µl) were analyzed by GC, resp., and the retention times of L-rhamnose and D-glucose were 15.85 and 19.37 min, resp.

Acetylcholinesterase (AChE) *Inhibitory Assay.* Acetylcholinesterase (AChE) inhibitory activity was determined using the colorimetric technique described by *Guimaraes et al.* [21] with minor modification. Phosphate buffer soln. (PBS, pH 8.0), 5,5-dithiobis(2-nitrobenzoic acid) (DTNB; 6.25 mM; color reagent) and acetylthiocholine iodide (6.25 mM) in PBS (pH 8.0) were used as the substrate. Tacrine was used as pos. control. A mixture of 10 µl of testing substance dissolved in 2% DMSO in H_2O (or without testing sample as neg. control), 110 µl of PBS (pH 8.0), and 40 µl of AChE (1000 unit/10 ml) in PBS (pH 8.0) was incubated at 30° for 20 min. Then, 20 µl of DTNB and 20 µl of acetylthiocholine iodide were added and incubated at 30° for 30 min. The absorption at 405 nm was measured using a *Molecular Devices E-max* plate reader. Inhibitory activity was determined by the following equation:

$$\text{Inhibition } \% = [A_{control} - A_{sample}]/A_{control} \times 100\%.$$

Cytotoxicity Assay. Five human cancer cell lines, *i.e.*, breast cancer MCF-7, hepatocellular carcinoma SMMC-7721, human myeloid leukemia HL-60, colon cancer SW480, and lung cancer A-549 cells, were used in the cytotoxicity assay. The detailed procedure has been described in [22][23].

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