Five New Nortriterpenoids from the Stems of *Schisandra neglecta*

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Five new nortriterpenoids, schicagenins D–F (**1**–**3**, resp.) and negleschidilactones A and B (**4** and **5**, resp.), together with eleven known ones, were isolated from the stems of *Schisandra neglecta*. Their structures were established on the basis of extensive spectroscopic analyses. All the compounds were evaluated for their activities regarding insulin sensitivity in 3T3-L1 differentiated adipocytes. None of them showed a significant bioactivity at 10 μM concentration.

Introduction. – The plants from the genus *Schisandra* of the family Schisandraceae has been widely used in Traditional Chinese Medicine in the treatment of cough, premature ejaculation, chronic dysentery, and insomnia for thousands of years [1][2]. The previous phytochemical investigations have revealed that this genus is rich in lignans and triterpenoids, which possess various pharmaceutical effects, such as antihepatitis [3][4], anti-HIV [5], antitumor activities [6], and other functions [7][8]. Over the past ten years, our group has conducted phytochemical investigations on more than ten *Schisandra* species from mainland of China, which led to the isolation and characterization of a series of highly oxygenated, polycylic nortriterpenoids endowed with various skeletons, and some of them showed promising bioactivities [9–14].

In the course of our continuing investigation on this genus, the chemical constituents of *Schisandra neglecta* A. C. SMITH, which was collected in the Linzhi region of Tibet in China, has been phytochemically studied. As a result, five new nortriterpenoids, schicagenins D–F (**1**–**3**, resp.) and negleschidilactones A and B (**4** and **5**, resp.), together with eleven known compounds, **6**–**16**, were isolated. This was the second discovery of schisandra triterpenoids characterized with a tetracyclic oxa-cage moiety and a C_9 side chain as in compounds **1**–**3** [12]. Here, we describe the isolation and structure elucidation of the compounds.

Results and Discussion. – The stems of *S. neglecta* (2.2 kg) were extracted with aqueous acetone at room temperature, and then the extract was partitioned between AcOEt and H_2O. Repeated chromatography of the AcOEt-soluble portions (108 g) yielded five new nortriterpenoids, **1**–**5**, together with eleven known ones, **6**–**16** (*Fig. 1*). The known compounds were identified as schicagenin C (**6**) [12], micrandi-

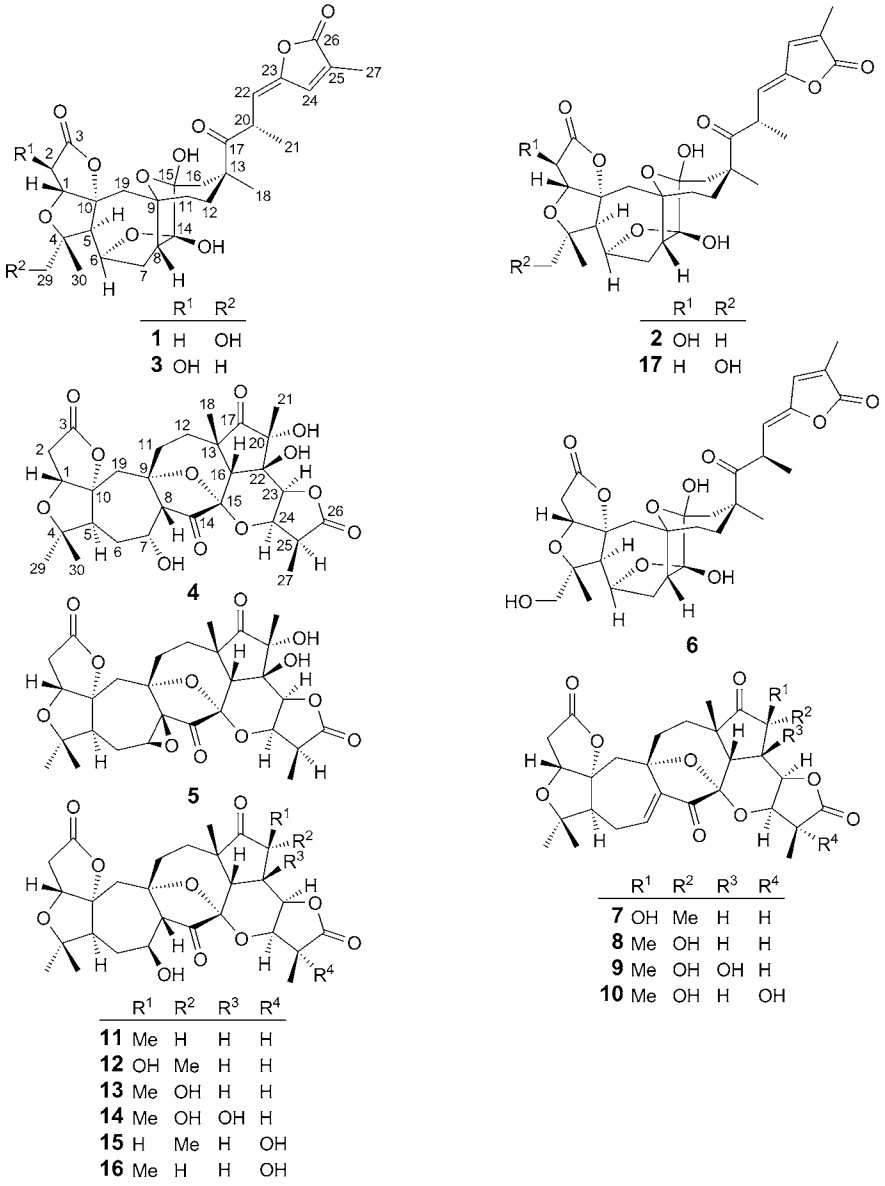

Fig. 1. *Structures of compounds* **1**–**16**

lactone F (**7**) [15], henridilactones A (**8**) [16], henridilactone B (**9**) [16], schirubridilactone E (**10**) [17], lancifodilactone C (**11**) [18], micrandilactone E (**12**) [15], micrandilactone D (**13**) [15], micrandilactone A (**14**) [19], lancifodilactone E (**15**) [18], and lancifodilactone L (**16**) [13], respectively, by comparing their spectroscopic data with those in the literature.

Table 1. *^{1}H-NMR Data of Compounds* **1**–**5**. Recorded in C_5D_5N, δ in ppm, J in Hz.

H-Atom	**1**[a])	**2**[b])	**3**[b])	**4**[c])	**5**[a])
H–C(1)	4.16 (*d*, *J* = 5.5)	4.43 (*s*)	4.35 (*s*)	4.18 (*d*, *J* = 5.9)	4.14 (*d*, (5.9)
CH_2(2) or H–C(2)	2.34 (*d*, *J* = 18.0), 2.54 (*dd*, *J* = 18.0, 5.5)	4.61 (*s*)	4.48 (*s*)	2.76 (*d*, *J* = 18.3), 2.90 (*dd*, *J* = 18.3, 5.9)	2.76 (*d*, *J* = 18.5), 2.90 (*dd*, *J* = 18.5, 5.9)
H–C(5)	3.32 (*s*)	2.50–2.60 (*m*)	2.44–2.55 (*m*)[d])	2.91–2.95 (*m*)	2.39 (*dd*, *J* = 14.5, 2.6)
H–C(6) or CH_2(6)	4.70 (*dd*, *J* = 8.5, 1.3)	4.44–4.51 (*m*)	4.42–4.50 (*m*)	1.79–1.85 (*m*)[d]), 1.63–1.68 (*m*)	2.16–2.22 (*m*)[d]), 1.38–1.48 (*m*)
CH_2(7) or H–C(7)	2.62–2.69 (*m*), 2.23 (*d*, *J* = 13.4)	2.60–2.72 (*m*), 2.29 (*d*, *J* = 13.6)	2.61–2.69 (*m*), 2.25 (*d*, *J* = 13.6)	4.57–4.60 (*m*)	3.87–3.92 (*m*)
H–C(8)	2.84 (*d*, *J* = 5.9)	2.81 (*d*, *J* = 5.9)	2.83 (*d*, *J* = 5.9)	4.20 (*d*, *J* = 7.5)	
CH_2(11)	1.83–1.87 (*m*), 1.29–1.34[d])	2.15–2.25 (*m*)[d]), 1.35–1.44 (*m*)	1.75–1.84(*m*), 1.26–1.33 (*m*)[d])	2.18–2.23 (*m*), 1.86–1.90 (*m*)	1.98–2.07 (*m*), 1.86–1.93 (*m*)
CH_2(12)	2.26–2.32 (*m*), 1.50–1.58 (*m*)	2.15–2.25 (*m*)[d]), 1.51–1.62 (*m*)	2.19–2.29 (*m*)[d]), 1.47–1.57 (*m*)	2.02–2.10 (*m*), 1.68–1.75 (*m*)	2.12–2.20 (*m*)[d]), 1.80–1.89 (*m*)
CH_2(16) or H–C(16)	3.21 (*d*, *J* = 16.1), 3.04 (*d*, *J* = 16.1)	3.09 (*d*, *J* = 15.9), 3.01 (*d*, *J* = 15.9)	3.20 (*d*, *J* = 16.1), 3.05 (*d*, *J* = 16.1)	3.35 (*s*)	3.19 (*s*)
Me(18)	1.19 (*s*)	1.09 (*s*)	1.17 (*s*)	1.62 (*s*)	1.59 (*s*)
CH_2(19)	1.80–1.84 (*m*), 2.13 (*d*, *J* = 15.7)	2.58–2.65 (*m*), 2.47–2.57 (*m*)	2.44–2.55 (*m*)[d]), 2.40 (*d*, *J* = 16.1)	2.02 (*d*, *J* = 16.3), 2.50 (*d*, *J* = 16.3)	2.10–2.16 (*m*)[d]), 2.17–2.25 (*m*)[d])
H–C(20) or C(20)	5.11–5.22 (*m*)	4.95–5.03 (*m*)	5.09–5.18 (*m*)		
Me(21)	1.32 (*d*, *J* = 7.1)	1.48 (*d*, *J* = 7.0)	1.32 (*d*, *J* = 7.1)	1.83 (*s*)	1.79 (*s*)
H–C(22) or C(22)	6.30 (*d*, *J* = 10.7)	5.94 (*d*, *J* = 7.0)	6.29 (*d*, *J* = 10.5)		6.29 (*d*, *J* = 10.5)
H–C(23)				4.96–5.02 (*m*)	5.01 (*s*)
H–C(24)	8.00 (*s*)	7.68 (*s*)	7.95 (*s*)	5.00–5.06 (*m*)	5.30 (*d*, *J* = 2.5)
H–C(25)				3.16–3.20 (*m*)	3.28–3.35 (*m*)
Me(27)	1.88 (*s*)	1.73 (*s*)	1.98 (*s*)	1.39 (*d*, *J* = 7.2)	1.70 (*d*, *J* = 7.2)
CH_2(29) or Me(29)	3.73 (*d*, *J* = 12.0), 3.57 (*d*, *J* = 12.0)	1.12 (*s*)[d])	1.12 (*s*)	1.26 (*s*)	1.21 (*s*)
Me(30)	1.18 (*s*)	1.12 (*s*)[d])	1.10 (*s*)	1.04 (*s*)	0.93 (*s*)

[a]) Recorded at 500 MHz. [b]) Recorded at 400 MHz. [c]) Recorded at 600 MHz. [d]) Overlapping signals.

Compound **1** was isolated as a white amorphous solid, and has a molecular formula $C_{29}H_{36}O_{11}$, as determined by HR-ESI-MS (*m/z* 583.2140 ($[M+Na]^+$)), requiring twelve degrees of unsaturation. The IR absorptions of **1** indicated the presence of OH (3443 cm^{-1}) and γ-lactone (1764 cm^{-1}) functional groups. The ^{1}H-NMR spectrum showed signals for two olefinic H-atoms (δ(H) 8.00 (*s*) and δ(H) 6.30 (*d*, $J = 10.7$)), a secondary Me, and three tertiary Me groups (*Table 1*). The ^{13}C-NMR and DEPT spectra exhibited 29 signals, accounting for four Me, seven CH_2, and seven CH groups (including two olefinic and two oxidized ones), eleven quaternary C-atoms (including five oxidized and two olefinic ones), and three CO groups. These data were consistent with the above elemental formula deduced from the HR-ESI-MS and suggested that **1** was a highly oxygenated nortriterpenoid, which is structurally similar to schicagenin B (**17**) [12] (*Fig. 1*).

The main differences of ^{13}C-NMR (*Table 2*) data of **1** and schicagenin B (**17**) were observed in the chemical shifts of C(18), C(21), C(22), C(24), and C(25). The

Table 2. *^{13}C-NMR Data of Compounds* **1**–**5**. Recorded in C_5D_5N, δ in ppm.

C-Atom	**1**[a])	**2**[b])	**3**[a])	**4**[b])	**5**[a])
C(1)	82.9 (*d*)	88.7 (*d*)	88.6 (*d*)	80.3 (*d*)	80.7 (*d*)
C(2)	35.7 (*t*)	72.8 (*d*)	72.9 (*d*)	35.6 (*t*)	35.2 (*t*)
C(3)	174.6 (*s*)	177.1 (*s*)	176.3 (*s*)	174.6 (*s*)	175.0 (*s*)
C(4)	86.1 (*s*)	83.5 (*s*)	83.3 (*s*)	84.2 (*s*)	83.6 (*s*)
C(5)	57.8 (*d*)	63.0 (*d*)	63.3 (*d*)	53.8 (*d*)	54.0 (*d*)
C(6)	78.6 (*d*)	78.1 (*d*)	78.2 (*d*)	33.7 (*t*)	28.1 (*t*)
C(7)	31.5 (*t*)	31.4 (*t*)	31.4 (*t*)	63.3 (*d*)	63.6 (*d*)
C(8)	50.5 (*d*)	50.9 (*d*)	50.5 (*d*)	55.2 (*d*)	61.2 (*s*)
C(9)	80.0 (*s*)	80.5 (*s*)	80.2 (*s*)	81.4 (*s*)	80.3 (*s*)
C(10)	96.5 (*s*)	97.0 (*s*)	96.3 (*s*)	96.8 (*s*)	95.5 (*s*)
C(11)	37.9 (*t*)	37.8 (*t*)	38.2 (*t*)	37.1 (*t*)	36.6 (*t*)
C(12)	36.2 (*t*)	35.3 (*t*)	36.1 (*t*)	33.3 (*t*)	33.6 (*t*)
C(13)	49.3 (*s*)	49.8 (*s*)	49.3 (*s*)	49.1 (*s*)	49.8 (*s*)
C(14)	112.7 (*s*)	112.9 (*s*)	113.0 (*s*)	207.5 (*s*)	207.2 (*s*)
C(15)	104.0 (*s*)	103.8 (*s*)	104.0 (*s*)	100.1 (*s*)	99.2 (*s*)
C(16)	46.3 (*t*)	47.7 (*t*)	46.2 (*t*)	53.6 (*d*)	56.1 (*d*)
C(17)	216.3 (*s*)	216.6 (*s*)	216.1 (*s*)	220.3 (*s*)	220.2 (*s*)
C(18)	31.4 (*q*)	30.5 (*q*)	31.4 (*q*)	30.8 (*q*)	31.6 (*q*)
C(19)	45.4 (*t*)	46.6 (*t*)	46.3 (*t*)	44.6 (*t*)	38.7 (*t*)
C(20)	41.3 (*d*)	40.6 (*d*)	41.2 (*d*)	80.2 (*s*)	75.8 (*s*)
C(21)	21.1 (*q*)	19.7 (*q*)	21.4 (*q*)	19.8 (*q*)	19.0 (*q*)
C(22)	113.7 (*d*)	116.3 (*d*)	114.1 (*d*)	75.3 (*s*)	80.0 (*s*)
C(23)	148.9 (*s*)	149.4 (*s*)	148.9 (*s*)	76.7 (*s*)	76.7 (*s*)
C(24)	135.7 (*d*)	140.7 (*d*)	135.9 (*d*)	75.0 (*d*)	74.7 (*d*)
C(25)	131.6 (*s*)	127.3 (*s*)	131.3 (*s*)	42.5 (*s*)	43.0 (*s*)
C(26)	171.9 (*s*)	171.8 (*s*)	171.9 (*s*)	177.6 (*s*)	177.9 (*s*)
C(27)	11.1 (*q*)	10.3 (*q*)	11.3 (*q*)	8.3 (*q*)	8.4 (*q*)
C(29)	68.0 (*t*)	28.4 (*q*)	28.6 (*q*)	27.7 (*q*)	27.5 (*q*)
C(30)	18.1 (*q*)	22.2 (*q*)	22.3 (*q*)	20.9 (*q*)	20.5 (*q*)

[a]) Recorded at 125 MHz. [b]) Recorded at 100 MHz.

constitutional formula of the side chain in **1** was deduced to be the same as that of **17** by the obvious HMBCs of Me(27) (δ(H) 1.88 (*s*)) with δ(C) 135.7 (C(24)), 131.6 (C(25)), and 171.9 (C(26)); of H–C(22) (δ(H) 6.30 (*d*, $J = 10.7$)) with δ(C) 216.3 (C(17)), 21.1 (C(21)), 148.9 (C(23)), and 135.7 (C(24)); and of Me(21) (δ(H) 1.32 (*d*, $J = 7.1$)) with δ(C) 216.3 (C(17)), 41.3 (C(20)) and 113.7 (C(22)); along with a H-atom spin system deduced from ^{1}H,^{1}H-COSY correlations Me(21)/H–C(20) (δ(H) 5.11 – 5.22 (*m*))/H–C(22) (*Fig. 2*). There evidences suggested that **1** and **17** might be *C(20)*-epimers or they had a different geometry of the C(22)=C(23) bond. The CD spectrum of **1** and **17** exhibited similar *Cotton* effects. Compound **1** showed a positive *Cotton* effect at 307 nm ($\Delta\varepsilon = +18.66$) and a negative *Cotton* effect at 272 nm ($\Delta\varepsilon = -21.88$), which assigned the (*S*)-configuration to C(20) of **1**, the same as that in schicagenin B (**17**) [12]. Thus, the difference may result from the change in the geometry of the C(22)=C(23) bond of **1**, which was deduced as (*E*) by the ROESY correlation of H–C(24) (δ(H) 8.00 (*s*))/H–C(20) and supported by the disappearance of the ROESY correlation H–C(24)/H–C(22) (*Fig. 3*). Therefore, the structure of **1** was determined as depicted in *Fig. 1* and named schicagenin D.

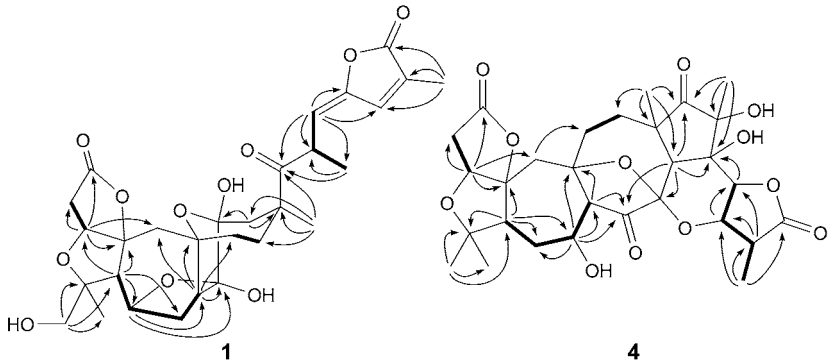

Fig. 2. *Key HMBCs of* **1** *and* **4**

Compound **2**, an amorphous powder, has the molecular formula $C_{29}H_{36}O_{11}$, as determined by HR-ESI-MS (*m*/*z* 583.2155 ($[M + Na]^+$)). Comparison of the NMR data (*Tables 1* and *2*) of **2** with those of **6** indicated that most of them were closely similar, except that two CH_2 signals at δ(C) 35.7 and 68.0 in **6** were replaced by one Me signal (δ(C) 22.2) and one O-bearing CH signal (δ(C) 72.8) in **2**. CH_2(29) in **6** was replaced by the Me group Me(30) (δ(C) 22.2) in **2** through the HMBCs of the H-atom signal of this Me group (δ(H) 1.12 (*s*)) with those at δ(C) 83.5 (C(4)), 63.0 (C(5)), and 22.2 (C(30)) in **1**. The position of the O-bearing CH groups (δ(C) 72.8) was determined as C(2) in **2**, deduced by the HMBCs of the H-atom signal (δ(H) 4.61 (*s*)) to those at δ(C) 88.7 (C(1)) and 97.0 (C(10)), from H–C(1) (δ(H) 4.43 (*s*)) to δ(C) 72.8 (C(2)), 177.1 (C(3)), 97.0 (C(10)), and 46.6 (C(19)), and the ^{1}H,^{1}H-COSY correlation of H–C(1) with the H-atom signal (δ(H) 4.61 (H–C(2))). In addition, both ^{1}H-NMR signals of H–C(1) and H–C(2) were *singlet*s, suggesting that the dihedral angle between

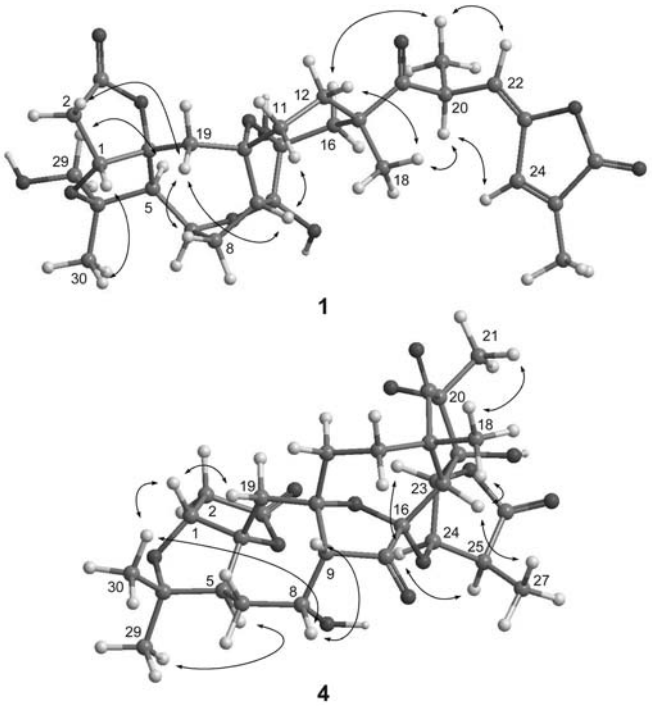

Fig. 3. *Selected ROESY correlations of* **1** *and* **4**

H–C(1) and H–C(2) was near 90°, which further indicated that HO–C(2) should be β-oriented, since H–C(1) was biogenetically assigned as β [12][20]. The absence of the ROESY correlation between H–C(1) and H–C(2) also supported this deduction. In addition, the absolute configuration at C(20) (δ(C) 40.6) in compound **2** was determined as (*S*), deduced from the *Cotton* effects in the CD spectrum similar to those of **6**, which showed a positive *Cotton* effect at 312 nm ($\Delta\varepsilon = +12.40$) and a negative *Cotton* effect at 272 nm ($\Delta\varepsilon = -17.3$). Furthermore, the ROESY correlation of H–C(22) (δ(H) 5.94 (*d*, $J = 7.0$)) with H–C(24) (δ(H) 7.68 (*s*)) indicated that the geometry of the C(22)=C(23) bond (δ(C) 116.3 (C(22)) and 149.3 (C(23))) of **2** is (*Z*). Therefore, compound **2** was elucidated as shown in *Fig. 1* and named schicagenin E.

Compound **3**, obtained as an amorphous powder, possesses the molecular formula $C_{29}H_{36}O_{11}$, as derived from the HR-ESI-MS (*m*/*z* 559.2176 ($[M-H]^-$)). Comparison of the ^{1}H- and ^{13}C-NMR data of **3** with those of **2** (*Tables 1* and *2*, resp.) revealed that most signals of **3** were very similar to those of **2**, except for the chemical shifts of C(21) to C(25). The observed ROESY correlation of H–C(24) (δ(H) 7.95 (*s*)) with H–C(20) (δ(H) 5.09–5.18 (*m*)) showed that the C(22)=C(23) bond (δ(C) 114.1 (C(22)) and 148.9 (C(23))) of **3** is (*E*)-configured. The CD spectrum of **3** showed a positive *Cotton* effect at 308 nm ($\Delta\varepsilon = +5.41$) and a negative *Cotton* effect at 273 nm ($\Delta\varepsilon = -6.93$), which were similar to the *Cotton* effects detected for **2**, indicating (*S*)-configuration at

C(20). Thus, the structure of **3** was deduced as depicted in *Fig. 1* and named schicagenin F.

Compound **4** was isolated as an amorphous powder. The molecular formula was deduced as $C_{29}H_{36}O_{12}$ based on HR-ESI-MS (*m/z* 575.2133 ($[M-H]^-$)). In the IR spectrum, the characteristic absorptions suggested the presence of OH (3441 cm^{-1}) and CO (1783 and 1746 cm^{-1}) groups. The ^{13}C-NMR and DEPT spectra (*Table 2*) displayed signals of five Me, five CH_2, and eight CH groups (four O-bearing) and eleven quaternary C-atoms (four CO groups and six O-bearing), which were closely resembling the corresponding signals of the known compound micrandilactone A (**14**) [19]. Analysis of 1D- and 2D-NMR data showed that the two compounds have the same constitutional formula. Comparison of 1D-NMR data of **4** with those of **14** revealed that the difference is due to the different relative configuration at C(7). The coupling constant between H–C(7) and H–C(8) in compound **4** ($J = 7.5$ Hz) was smaller than that in **14** ($J = 10.1$ Hz), indicating that HO–C(7) is α-oriented in **4**, in contrast to that found in compound **14**. This deduction was supported by the downfield chemical shift of H–C(5) from δ(H) 2.47 in **14** to δ(H) 2.91–2.95 in **4**, due to the deshielding effect by the α-positioned HO–C(7) in **4**, and by the ROESY correlation of Me(30) (δ(H) 1.04 (*s*)) with H–C(7) (δ(H) 4.57–4.60 (*m*)) (*Fig. 3*). Hence, the structure of **4** was determined as shown in *Fig. 1* and named negleschidilactone A.

Compound **5** was isolated as an amorphous powder. The HR-ESI-MS (*m/z* 573.1957 ($[M-H]^-$)) indicated its molecular formula to be $C_{29}H_{34}O_{12}$, requiring thirteen degrees of unsaturation. The IR spectrum showed the presence of OH groups (3425 cm^{-1}) and CO groups (1775 and 1704 cm^{-1}). Analysis of the ^{1}H-, ^{13}C-NMR, and DEPT data (*Tables 1* and *2*) revealed that this compound is structurally similar to **14**. Comparison of the spectroscopic data of **5** with those of **14** indicated that they are quite similar, except that the molecular weight of **5** is two mass units lower than that of **14**, implying that **5** has one degree of unsaturation more than **14**. In addition, the C-atom signals at δ(C) 63.6 and 61.2, and a H-atom signal at δ(H) 3.87–3.92 (*m*) indicated an additional epoxy ring in **5** [21][18]. HMBCs from H–C(5) (δ(H) 2.39 (*dd*, $J = 14.5$, 2.6)) to δ(C) 63.6 (C(7)), from H–C(7) (δ(H) 3.87–3.92 (*m*)) to δ(C) 28.1 (C(6)) and 61.2 (C(8)), and from CH_2(19) (δ(H) 2.10–2.16 (*m*), 2.17–2.25 (*m*)) to C(8) in **5** suggested that **5** was the 7,8-epoxy derivative of **14**. Moreover, the ROESY correlation of H–C(7) with H–C(5) in **5** indicated that the epoxy ring is β-oriented. Thus, the structure of **5** was determined as depicted in *Fig. 1* and named negleschidilactone B.

Biological Studies. Insulin regulates GLUT4 translocation to the plasma membrane in adipocytes and skeleton muscles to downregulate the glucose level in blood. Dysfunction of GLUT4 translocation to the plasma membrane accounts for insulin resistance and high glucose in Type 2 diabetes. Quantification of GLUT4 translocation in these tissues allows us to measure insulin sensitivity and to screen compounds which can increase insulin sensitivity and eventually may be applied in drug development for type 2 diabetes. In our present study, some compounds have been screened for their acute activities in cell-based insulin sensitivity assay as described in [22]. Adipocytes were incubated with 10 μM compound for 30 min and underwent insulin stimulation. No significant activities were observed of these compounds when applied at a relatively high concentration (10 μM) (*Fig. 4*).

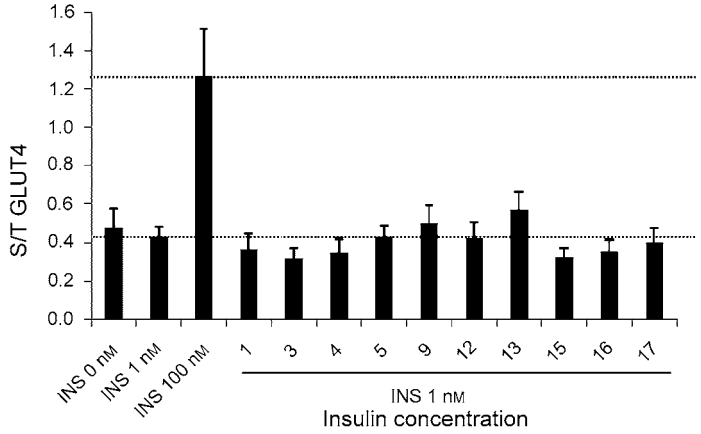

Fig. 4. *Acute activities of the tested compounds on insulin sensitivity in 3T3-L1-differentiated adipocytes*

Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; *Qingdao Marine Chemical Inc.*, Qingdao, P. R. China), *Lichroprep RP-18* gel (40–63 μm, *Merck*, D-Darmstadt), and *MCI-gel CHP 20P* (75–150 μm, *Mitsubishi Chemical Corp.*, Tokyo, Japan). Semi-prep. HPLC: *Agilent 1100* and *1200* liquid chromatographs with a *Zorbax SB-C_{18}* column. TLC: Silica gel *60 F_{254}* on glass plates (*Qingdao Marine Chemical Inc.*) using various solvent systems, and spots visualized by heating the silica gel plates sprayed with 95–98% H_2SO_4/EtOH (*v*/*v* 10:90). Optical rotations: *JASCO P-1020* digital polarimeter. UV Spectra: *Shimadzu UV-2401A* spectrometer. CD Spectra: *Applied Photophysics Chirascan* spectrometer. IR Spectra: *Tenor 27* spectrophotometer with KBr pellets. 1D- and 2D-NMR spectra: *Bruker AM-400*, *DRX-500*, and *AVANCE III-600* MHz spectrometers, with Me_4Si as an internal standard. ESI-MS: *Xevo TQ-S* mass spectrometer. HR-ESI-MS: *API QSTAR* Time-of flight spectrometer.

Plant Material. The aerial part of *S. neglecta* were collected in the Linzhi region of Tibet, P. R. China, in October 2010. The plant was identified by Prof. *Xi-Wen Li* at the Kunming Institute of Botany. A voucher specimen (No. KIB 10172011) 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 aerial parts (2.2 kg) of *S. neglecta* were extracted with 70% aq. acetone (3 × 20 l, each 3 d) at r.t. and filtered. Then, the filtrate was concentrated and partitioned between AcOEt and H_2O. The AcOEt-soluble portion (108 g) was decolorized on *MCI* gel column (7 × 60 cm; MeOH/H_2O 9:1; 5000 ml), and, after concentration, the residue (85 g) was subjected to CC (silica gel (100–200 mesh, 10 × 100 cm, 700 g); $CHCl_3$/acetone 1:0, 9:1, 8:1, 2:1, 1:1, and 1:0; each 5000 ml) to afford *Fractions 1–6*. *Fr. 3* (5.0 g) was subjected to CC (*RP-18* (5 × 30 cm); MeOH/H_2O 2:8, 3:7, 4:6, 5:5, and 10:0; each 2000 ml) to afford subfractions *3A–3G*, *Subfr. 3B* (330 mg) was then subjected to semiprep. HPLC (*Zorbax SB-C_{18}* (9.4 × 250 mm); MeCN/H_2O 3:7; flow rate, 3 ml/min) to afford compounds **2** (11.0 mg), **3** (2.0 mg), **4** (1.2 mg), and **5** (3.5 mg). *Subfr. 3C* (685 mg) was subjected to semiprep. HPLC (*Zorbax SB-C_{18}* (9.4 × 250 mm); MeOH/H_2O 4:6; flow rate, 3 ml/min) to yield compounds **7** (3.8 mg), **8** (3.2 mg), **9** (5.5 mg), and **10** (3.0 mg). *Subfr. 3D* (596 mg) was subjected to semiprep. HPLC (*Zorbax SB-C_{18}* (9.4 × 250 mm); MeOH/H_2O 3:7; flow rate, 3 ml/min) to give **11** (1.2 mg), **12** (5.0 mg), and **13** (190.0 mg). *Subfr. 3E* (110 mg) was subjected to semiprep. HPLC (*Zorbax SB-C_{18}* (9.4 × 250 mm); MeCN/H_2O 4:6; flow rate, 3 ml/min) to afford **14** (9.3 mg), **15** (27.5 mg), and **16** (5.0 mg). *Fr. 4* (3.9 g) was submitted to CC (SiO_2 (200–300 mesh, 4 × 40 cm, 90 g); $CHCl_3$/MeOH, 20:1, 15:1, 10:1, 5:1; each 1000 ml) to yield five subfractions *4A–4E*, *Subfr. 4C* (1.2 g) was purified by CC (*RP-18* (2 × 30 cm); MeOH/H_2O 3:7, 4:6, 5:5 and 10:0; each 500 ml) to afford four subfractions *4C1–*

4C4, and *Subfr. 4C3* (60 mg) was separated by semiprep. HPLC (*Zorbax SB-C_{18}* (9.4 × 250 mm); MeOH/H_2O, 4:6, flow rate, 3 ml/min) successively to afford **1** (165.0 mg) and **6** (4.0 mg).

Schicagenin D (=*(3a*R,*5*R,*5a*S,*6*S,*7a*R,*8*S,*9*S,*11*S,*13a*S,*14a*R*)-Dodecahydro-8,9-dihydroxy-5-(hydroxymethyl)-5,11-dimethyl-11-[(2*S*,*3*E*)-2-methyl-3-(4-methyl-5-oxofuran-2(5*H*)-ylidene)propanoyl]-2*H,*5*H-*6,8:9,13a-diepoxycycloocta[5,6]cyclohepta[1,2-*c*]furo[3,2-*b*]furan-2(14*H*)-one*; **1**). White amorphous solid. $[\alpha]_D^{14} = +54.7$ ($c = 0.28$, MeOH). UV (MeOH): 275 (3.44), 221 (3.81). CD ($c = 0.28$, MeOH): 307 (+18.66). IR (KBr): 3443, 2971, 2934, 1764, 1696, 1625, 1462, 1383, 1297, 1221, 1073. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. HR-ESI-MS: 583.2140 ($[M+Na]^+$, $C_{29}H_{36}NaO_{11}^+$; calc. 583.2155).

Schicagenin E (=*(3*R,*3a*R,*5a*S,*6*S,*7a*R,*8*S,*9*S,*11*S,*13a*S,*14a*R*)-Dodecahydro-3,8,9-trihydroxy-5,5,11-trimethyl-11-[(2*S*,*3*Z*)-2-methyl-3-(4-methyl-5-oxofuran-2(5*H*)-ylidene)propanoyl]-2*H,*5*H-*6,8:9,13a-diepoxycycloocta[5,6]cyclohepta[1,2-*c*]furo[3,2-*b*]furan-2(14*H*)-one*; **2**). White amorphous solid. $[\alpha]_D^{17} = +36.4$ ($c = 0.25$, MeOH). UV (MeOH): 275 (3.46). CD ($c = 0.32$, MeOH): 312 (+12.40). IR (KBr): 3438, 2974, 2932, 1767, 1698, 1462, 1223, 1168, 1073. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. HR-ESI-MS: 583.2155 ($[M+Na]^+$, $C_{29}H_{36}NaO_{11}^+$; calc. 583.2155).

Schicagenin F (=*(3*R,*3a*R,*5a*S,*6*S,*7a*R,*8*S,*9*S,*11*S,*13a*S,*14a*R*)-Dodecahydro-3,8,9-trihydroxy-5,5,11-trimethyl-11-[(2*S*,*3*E*)-2-methyl-3-(4-methyl-5-oxofuran-2(5*H*)-ylidene)propanoyl]-2*H,*5*H-*6,8:9,13a-diepoxycycloocta[5,6]cyclohepta[1,2-*c*]furo[3,2-*b*]furan-2(14*H*)-one*; **3**). White amorphous solid. $[\alpha]_D^{17} = +45.1$ ($c = 0.17$, MeOH). UV (MeOH): 236 (3.00). CD ($c = 0.32$, MeOH): 308 (+5.41). IR (KBr): 3431, 2974, 2933, 1768, 1694, 1628, 1376, 1222, 1168, 1074. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. HR-ESI-MS: 559.2176 ($[M-H]^-$, $C_{29}H_{35}O_{11}^-$; calc. 559.2179).

Negleschidilactone A (=*(1S,3a*S,*3b*R,*4*R,*5a*S,*7a*S,*8a*R,*11a*R,*13a*S,*15*R,*15a*R,*16a*S,*16b*R,*17a*R*)-Tetradecahydro-3b,4,15-trihydroxy-1,4,5a,13,13-pentamethyl-2*H,*10*H-*7a,16a-epoxy-3,9,12,17-tetraoxacyclopenta[3′,3a′]azuleno[6′,5′:5,6]cycloocta[1,2,3-*cd*]-*as-*indacene-2,5,10,16(1*H,*8*H,*13*H*)-tetrone*; **4**), White amorphous solid. $[\alpha]_D^{18} = +164.0$ ($c = 0.04$, MeOH). UV (MeOH): 310 (2.31), 244 (2.98). IR (KBr): 3441, 2933, 1783, 1746, 1631, 1384, 1212, 1166, 1104. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. HR-ESI-MS: 575.2133 ($[M-H]^-$, $C_{29}H_{35}O_{12}^-$; calc. 575.2128).

Negleschidilactone B (=*(1S,3a*S,*3b*R,*4*R,*5a*S,*7a*S,*8a*R,*11a*R,*13a*S,*14a*S,*15a*S,*16a*S,*16b*R,*17a*R*)-Tetradecahydro-3b,4-dihydroxy-1,4,5a,13,13-pentamethyl-2*H,*10*H-*7a,16a-epoxy-3,9,12,15,17-pentaoxacyclopenta[3′,3a′]cyclopropa[6′,7′]azuleno[6′,5′:5,6]cycloocta[1,2,3-*cd*]-*as-*indacene-2,5,10,16(1*H,*8*H*)-tetrone*; **5**). White amorphous solid. $[\alpha]_D^{18} = +65.4$ ($c = 0.19$, MeOH). IR (KBr): 3425, 2913, 1775, 1704, 1625, 1433, 1377, 1213, 1168, 1040. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. HR-ESI-MS: 573.1957 ($[M-H]^-$, $C_{29}H_{33}O_{12}^-$; calc. 573.1972).

Insulin-Sensitivity Assay. 3T3-L1 Fibroblasts (ATCC, USA) were cultured and differentiated into adipocytes as described in [22]. Differentiated adipocytes were then transformed with HA-GLUT4-GFP cDNA by electroporation (*Eppendorf Multiporator*, Germany). After 24 h, the cells were starved for 2 h in serum-free medium, and incubated with 10 μM compounds for 30 min. The cells were stimulated with/without 1 or 100 nM insulin (*Sigma*, *I5500*, bovine pancreas, ≥27 USP units/mg in HPLC) for 1/2 h, and then were fixed with 3.7% formaldehyde . For this acute insulin-sensitivity assay, 100 nM insulin was a positive control as described in [23][24]. To measure insulin sensitivity, cells were immune-stained for HA and Cy3 antibodies without permeablization, and images were taken by fluorescence microscope (*Nikon Ti-E*, Japan). The ratio (intensity in Cy3 channel)/(intensity in GFP channel) represents surface GLUT4 distribution related to the total GLUT4 expression at various insulin stimulations.

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