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New cytotoxic *neo*-clerodane diterpenoids from *Scutellaria strigillosa*

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ABSTRACT

Three new *neo*-clerodane diterpenoids, named scutestrigillosins A–C (**1**–**3**), were isolated from the whole plant of *Scutellaria strigillosa*. Their chemical structures including absolute configurations were established on the basis of detailed physical data analyses. In vitro, the isolated three new compounds exhibited significant cytotoxic activities against four tumor cell lines (HONE-1, P-388, MCF7 and HT29), and gave IC_{50} values in the range 3.5–7.7 μM.

Scutellaria is a unique cosmopolitan genus of the subfamily Scutellarioideae belonging to Lamiaceae (Labiatae) family. About 360 species are found to spread throughout the world and in different climatic areas. Plants of this genus have been widely used in local medicine of many countries of the world for thousands of years, and modern pharmacology research has confirmed that their extracts or monomeric compounds possess antitumor effects.[1,2] On the basis of cDNA microarray analysis, the mechanism underlying the antitumor activity appears to involve DNA damage, cell cycle control, nucleic acid binding, protein phosphorylation and dephosphorylation, and dendritic cell functions. There is evidence that these actions are triggered by *neo*-clerodane diterpenoids.[3–6]

In the course of ongoing search for more new *neo*-clerodane diterpenoids, we investigated the whole plants of *Scutellaria strigillosa*, which is a perennial herb and mainly distributed in Hebei, Shandong, Zhejiang and Jilin provinces in China. In traditional Chinese medicine, the whole plants have been used to clear away heat-evil, expel superficial evils, eliminate stasis and reduce edema.[7] This phytochemical investigation led to the isolation of three new *neo*-clerodane diterpenoids, named scutestrigillosins A–C (Fig. 1, **1**–**3**), the structures of which were elucidated by means of extensive spectroscopic analyses. Furthermore, three new compounds were screened for cytotoxicity against selected cancer cell lines, including HONE-1, P-388, MCF7 and HT29. Herein, we report on the structural elucidation and cytotoxicity of three new *neo*-clerodane diterpenoids.

The air-dried whole plant of *Scutellaria strigillosa* (45.0 kg) was extracted with refluxing EtOH and then partitioned with $CHCl_3$. The $CHCl_3$ fraction (199.3 g) was chromatographed over various columns and preparative HPTLC to obtain compounds **1** (206 mg), **2** (114 mg), and **3** (116 mg) (see detailed experimental procedures in the Supporting information).

Scutestrigillosin A (**1**)[8] was obtained as white needles with optical rotation $[\alpha]_D^{29}$ −24.7° (*c* 0.79, MeOH), and exhibited a positive response to Dragendoff reagent. In the HR-ESI mass spectrum, **1** gave a positive quasi-molecular ion peak at *m*/*z* 681.2799 $[M+H]^+$, corresponding to a molecular formula $C_{39}H_{40}N_2O_9$. The IR spectrum showed absorption bands at 1753, 1638, 1599, 1560, 1489, 1423, 1390, 1285, 1221 and 1105 cm^{-1}, which were in agreement with carbonyl, conjugated carbonyl, aromatic and γ-lactone groups. In the ^1H NMR spectrum, the following signals were observed: four tertiary methyl groups [δ_H 1.43 (3H, s, H-17); 1.60 (3H, s, H-18); 1.50 (3H, s, H-19); 1.28 (3H, s, H-20)], two nicotinic acid ester moieties [δ_H 9.29 (1H, br s, H-3′), 8.89 (1H, br s, H-5′), 7.55 (1H, dd, *J* = 4.8, 7.8 Hz, H-6′), 8.37 (1H, br d, *J* = 8.0 Hz, H-7′); 8.99 (1H, br s, H-3″), 8.71 (1H, br s, H-5″), 7.31 (1H, overlap, H-6″), 8.06 (1H, br d, *J* = 8.0 Hz, H-7″)], a benzoyloxy moiety [δ_H 7.77 (2H, d, *J* = 7.3 Hz, H-3‴ and H-7‴), 7.30 (2H, overlap, H-4‴ and H-6‴), 7.47 (t, *J* = 7.5 Hz, H-5‴)], a tri-substituted double bond unit (δ_H 5.36, 1H, br s, H-3) and an oxygenated methylene group [δ_H 4.35 (1H, d, *J* = 9.5 Hz, Ha-16), 4.50 (1H, d, *J* = 9.5 Hz, Hb-16)]. The ^{13}C NMR displayed 39 carbon resonances and the DEPT spectrum was consistent with the presence of four methyls, five methylenes (sp^3 hybridized), eighteen methines (fourteen sp^2 hybridized and four sp^3 hybridized), and twelve quaternary carbons (eight sp^2 hybridized and four sp^3 hybridized). Detailed analysis of above 39 carbon signals displayed that 20 carbons were due to one *neo*-clerodane diterpenoid skeleton with a 13-spiro-15,16-γ-lactone moiety,[1,9,10] 12 carbons to two nicotinoyloxy moieties,

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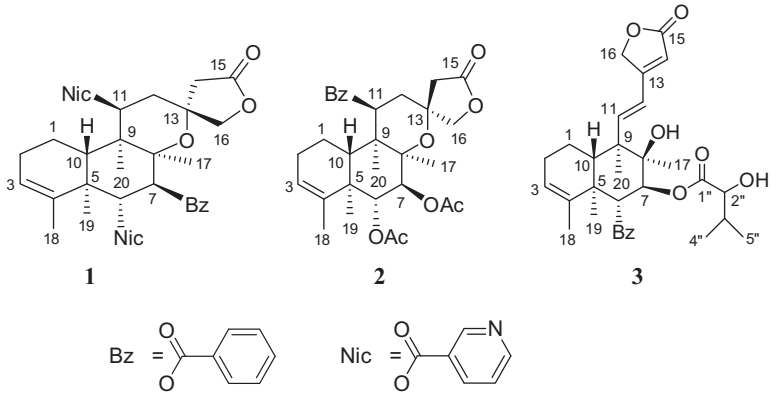

Figure 1. The structures of new *neo*-clerodane diterpenoids from *Scutellaria strigillosa*.

and 7 carbons to a benzoyloxy moiety, respectively. The cross peaks in the HMBC spectrum of H-6/C-1″, H-7/C-1‴ and H-11/C-1′ confirmed the benzoyloxy group was attached to C-7 and two nicotinoyloxy moieties were attached to C-6 and C-11 (Fig. 2), respectively. The relative configuration of **1** was elucidated by the ^{1}H–^{1}H coupling constant and ROESY spectrum. The large coupling constant (10.2 Hz) of H-6 with H-7 indicated two protons must be in the *trans*-diaxially oriented. In the ROESY spectrum (Fig. 3), cross peaks from H_3-20 to H-7, H-11, H_3-17 and H_3-19, from H-6 to H-10, from H_3-17 to H-7, H-11, H_a-14, H_b-14 and H_3-20, as well as from H-11 to H_a-14, H_b-14, H_3-17 and H_3-20 indicated that H_3-17, H_3-19, H_3-20, H-7, H-11 and H_2-14 were co-facial and α-oriented, while H-6 and H-10 were on the opposite side of the molecular plane and thus β-oriented. Furthermore, the absolute configuration of **1** was determined by the ECD exciton chirality method. The ECD spectrum of **1** showed the negative first Cotton effect at 240 nm ($\Delta\varepsilon$ −5.0) and positive second Cotton effect at 223 nm ($\Delta\varepsilon$ +5.6), which were consistent with the absolute configuration 5*R*,6*R*,7*S*,8*R*,9*R*,10*R*,11*S*,13*R*, as was determined for barbatine B.[11]

Scutestrigillosin B (**2**)[12] was isolated as white needles with optical rotation $[\alpha]_D^{29}$ −68.3° (*c* 0.14, MeOH), and the molecular formula was established as $C_{31}H_{38}O_9$ by HR-ESI mass spectrum, which showed a positive quasi-molecular ion at *m*/*z* 555.2589 $[M+H]^+$. The IR spectrum exhibited absorption bands at 1770, 1731, 1642, 1595, 1466, 1385, 1229 and 1020 cm^{-1}, which were indicative of carbonyl, conjugated carbonyl, aromatic and γ-lactone groups. In the ^{1}H and ^{13}C NMR spectra (Table 1), it showed the signals of four tertiary methyl groups and characteristic of one *neo*-clerodane diterpenoid skeleton as in **1**. In addition, a benzoyloxy moiety

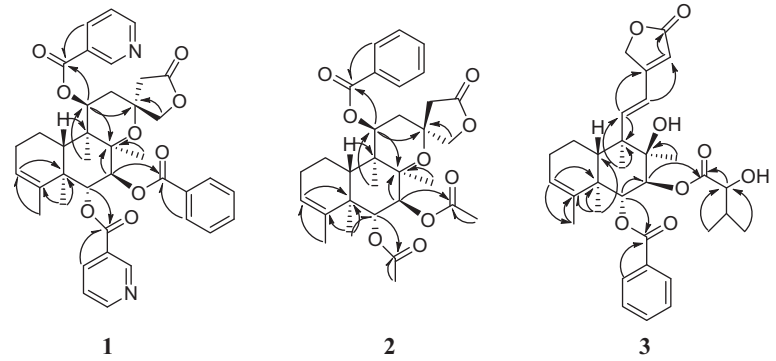

Figure 2. Key HMBC correlations for compounds **1**–**3**.

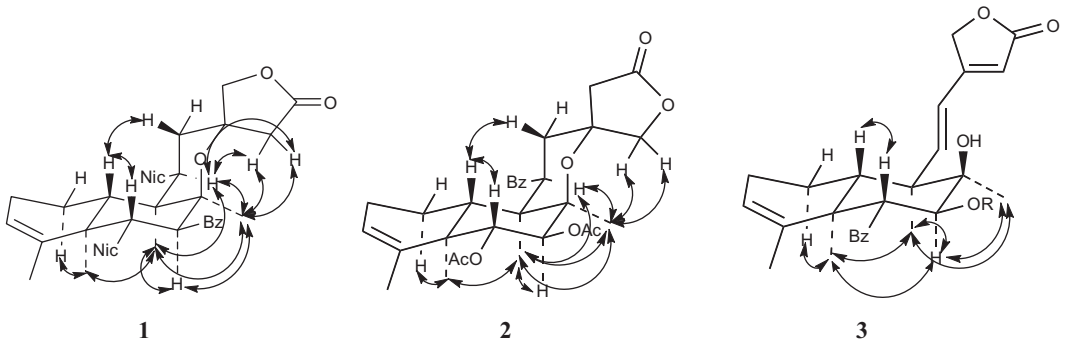

Figure 3. Selected NOE correlations for compounds **1**–**3**.