

## Novel Bioactive Dibenzocyclooctadiene Lignans from *Schisandra neglecta*

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Quatro novas dibenzociclooctadienolignanas, neglectalignanas A-D (**1-4**), juntamente com dezenove compostos conhecidos (**5-23**), foram isolados das hastes de *Schisandra neglecta*. Suas estruturas e esteroquímicas foram elucidadas por métodos espectroscópicos, incluindo as técnicas de ressonância magnética nuclear (RMN) de 1D e 2D, e espectrometria de massas de alta resolução com ionização por electrospray (HR-ESI-MS). Avaliou-se as atividades anti-HIV e a citotoxicidade dos compostos **1-4**, e os resultados mostraram que os compostos **1-4** apresentam atividade anti-HIV moderadas com valores de índice terapêutico (TI) acima de 61,7, 22,6, 57,7 e 27,9, respectivamente, e atividades citotóxicas fracas contra algumas das linhas celulares selecionadas.

Four new dibenzocyclooctadiene lignans, neglectalignans A-D (**1-4**), together with nineteen known compounds (**5-23**) were isolated from the stems of *Schisandra neglecta*. Their structures and stereochemistries were elucidated by spectroscopic methods, including 1D-, 2D-nuclear magnetic resonance (NMR) and high-resolution electrospray ionization mass spectrometry (HR-ESI-MS) techniques. Compounds **1-4** were evaluated for their anti-HIV activities and cytotoxicities. The results revealed that compounds **1-4** showed moderate anti-HIV-1 activities with therapeutic index (TI) values above 61.7, 22.6, 57.7, and 27.9, respectively, and weak cytotoxic activities for some selected cell lines.

**Keywords:** *Schisandra neglecta*, dibenzocyclooctadiene lignans, anti-HIV-1 activities, cytotoxicities

## Introduction

The family *Schisandraceae*, consisting of *Schisandra* and *Kadsura* genera, is medicinally important. The stems and fruits of *Schisandraceae* plant are commonly used in traditional Chinese medicine for their diverse beneficial bioactivities.<sup>1,2</sup> Previous studies showed that plant of the *Schisandraceae* family are rich in lignans, especially dibenzocyclooctadienes, which have been found to possess some beneficial pharmacological effects, including anti-HIV, antitumor, cytotoxic, antioxidant and antihepatotoxic effects.<sup>3-5</sup>

*Schisandra neglecta* A. C. Smith is a climbing plant mainly distributed in southwest China. In previous studies, some new dibenzocyclooctadiene lignans were isolated from the fruits of *S. neglecta* from Dali Prefecture, Yunnan Province,<sup>6</sup> the stems of *S. neglecta* from Xizang Autonomous Region,<sup>7,8</sup> and the stems of *S. neglecta* from the Xichang Prefecture, Sichuan Province.<sup>9</sup> In our continuing efforts to identify bioactive natural products from the *Schisandraceae* medicinal plants, a chemical investigation on the stem of *S. neglecta*, indigenous to the Dali Prefecture of Yunnan Province, was carried out, leading to the characterization of four new dibenzocyclooctadiene lignans, together with nineteen known compounds. In addition, the anti-HIV-1 activities and cytotoxicities of compounds **1-4** were evaluated.

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## Results and Discussion

The stems of *S. neglecta* were extracted with 70% acetone. The extract was subjected repeatedly to column chromatography on silica gel, Sephadex LH-20, RP-18, and semi-preparative reverse phase high performance liquid chromatography (RP-HPLC) to afford four new dibenzocyclooctadiene lignans, named as neglectalignans A-D (**1-4**), together with nineteen known compounds (**5-23**), which were identified by comparison with literature data as rubrisandrin A (**5**),<sup>10</sup> schinegllignan A (**6**),<sup>6</sup> schinegllignan B (**7**),<sup>6</sup> marlignan B (**8**),<sup>11</sup> rubrilignan B (**9**),<sup>12</sup> marlignan G (**10**),<sup>11</sup> epigomisin O (**11**),<sup>13</sup> gomisin D (**12**),<sup>13</sup> wilsonilignan C (**13**),<sup>14</sup> rubschizantherin (**14**),<sup>13</sup> isogomisin O (**15**),<sup>15</sup> gomisin T (**16**),<sup>16</sup> schizandrin (**17**),<sup>13</sup> (+)-gomisin K (**18**),<sup>13</sup> angeloygomisin Q (**19**),<sup>13</sup> tigloygomisin Q (**20**),<sup>13</sup> benzoylgomisin Q (**21**),<sup>17</sup> gomisin D (**22**),<sup>18</sup> and gomisin E (**23**)<sup>15</sup> (Figure 1).

Compound **1** was obtained as a yellow gum, and the molecular formula was determined as  $C_{23}H_{28}O_7$  by HRESIMS at  $m/z$  439.1725  $[M+Na]^+$  (calcd  $m/z$  439.1733). Its  $^1H$  and  $^{13}C$  NMR spectra (Table 1) showed signals for 28 hydrogens and 23 carbons, respectively, corresponding to two aromatic rings with two aromatic protons ( $\delta_H$  7.02 and 6.54), two methylene carbons ( $\delta_C$  36.0 and 39.8), two methine carbons ( $\delta_C$  34.1 and 41.8), two methyl groups ( $\delta_C$  12.8 and 21.8), three methoxy groups ( $\delta_C$  60.1, 60.6, and

60.5), two phenolic hydroxy groups ( $\delta_H$  10.61 and 11.16), and an acetoxy group ( $\delta_C$  169.9 and 21.0). UV spectrum displayed absorption bands at 210 and 245 nm. The IR spectrum showed the presence of hydroxy group ( $3452\text{ cm}^{-1}$ ) and ester group ( $1748\text{ cm}^{-1}$ ). In addition,  $^1H$ - $^1H$  COSY correlations of H-6/H-7/H-8/H-9, H-7/H-17, and H-8/H-18 (Figure 2), together with the HMBC correlations (Figure 2) of H-11 ( $\delta_H$  6.54) with C-9 ( $\delta_C$  36.0), C-10 ( $\delta_C$  134.9) and C-15 ( $\delta_C$  119.8), and of H-4 ( $\delta_H$  7.02) with C-5 ( $\delta_C$  135.2), C-6 ( $\delta_C$  39.8), and C-16 ( $\delta_C$  122.0) implied that **1** is a dibenzocyclooctadiene lignan possessing three methoxy groups, two phenolic hydroxy groups, and an acetoxy group. The  $^1H$  and  $^{13}C$  NMR spectra of **1** were found to be similar to those of marlignan A.<sup>11</sup> Analysis of the  $^1H$  and  $^{13}C$  NMR data of **1** suggested that the only difference was due to a hydroxy group in marlignan A on an aromatic ring being replaced by an acetoxy group in **1**. A hydroxy group located at C-3 was supported by HMBC correlations of the hydroxy proton signal at  $\delta_H$  10.61 with C-2 ( $\delta_C$  140.5), C-3 ( $\delta_C$  150.3) and C-4 ( $\delta_C$  107.0), and another hydroxy group located at C-14 was supported by HMBC correlations of proton signal at  $\delta_H$  11.16 with C-13 ( $\delta_C$  140.0), C-14 ( $\delta_C$  140.0), and C-15 ( $\delta_C$  140.0). The HMBC correlations of three methoxy protons ( $\delta_H$  3.84, 3.90, 3.89) with C-1, C-2, and C-13, suggested that these methoxy groups could be positioned at C-1 ( $\delta_C$  151.6), C-2 ( $\delta_C$  140.5), and C-13 ( $\delta_C$  140.0), respectively. Since the positions of hydroxy

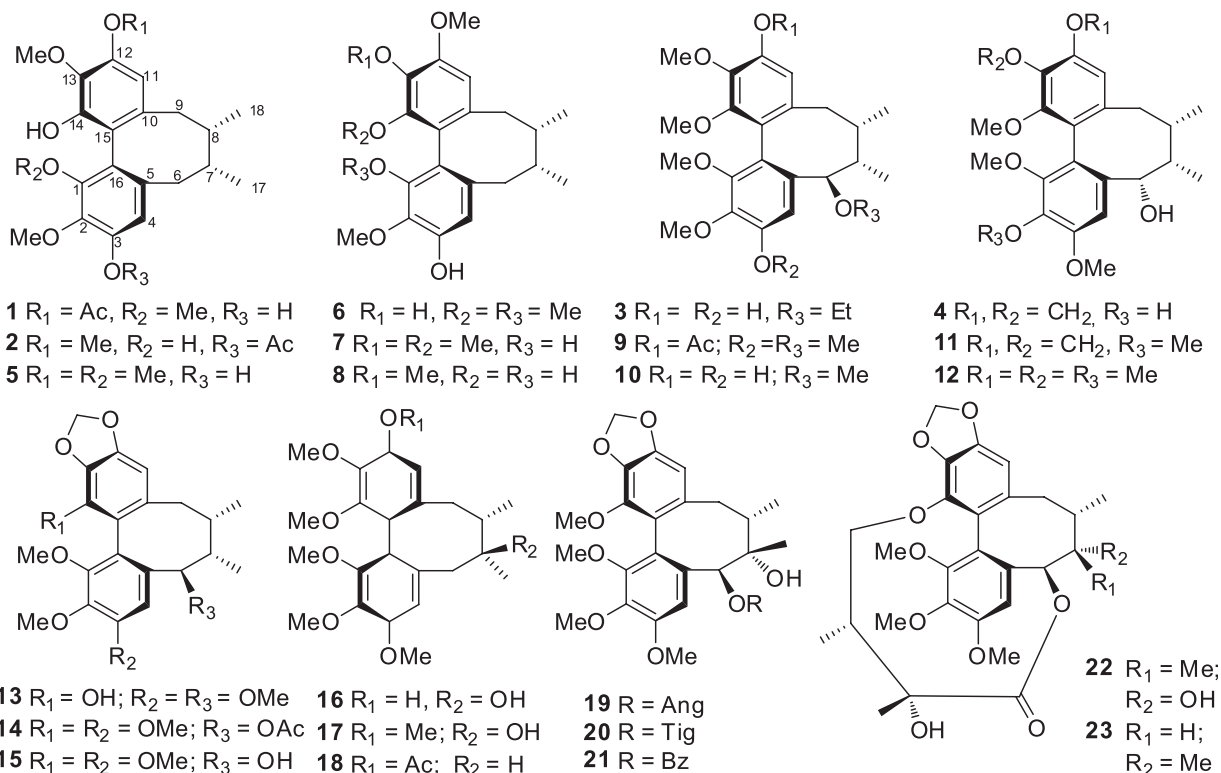
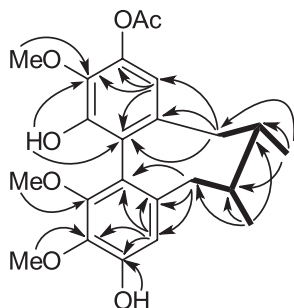


Figure 1. The structure of dibenzocyclooctadiene lignans from *S. neglecta*.

and methoxyl groups were determined, the acetoxy group should be located at C-12. In the cyclooctadiene ring, the signals for two methines were assigned to C-7 and C-8, two benzylic methylenes were attributed to C-6 and C-9, and two methyl groups located at C-17 and C-18, respectively,



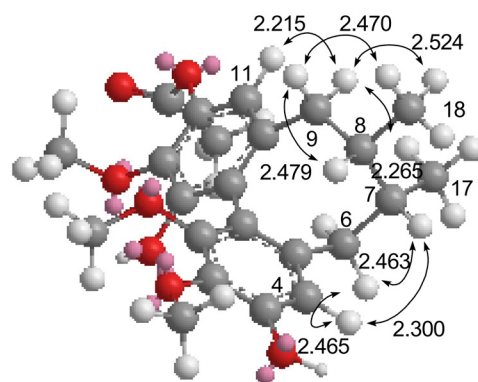
**Figure 2.** Selected HMBC (↷) and  $^1\text{H}$ - $^1\text{H}$  COSY (→) correlations of **1**.

**Table 1.**  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR Data of compounds **1** and **2** (obtained in  $\text{C}_5\text{D}_5\text{N}$ , 500 and 125 MHz)

| No.      | Compound <b>1</b>       |                                   | Compound <b>2</b>       |                                   |
|----------|-------------------------|-----------------------------------|-------------------------|-----------------------------------|
|          | $\delta_{\text{C}}$ (m) | $\delta_{\text{H}}$ (m, $J$ / Hz) | $\delta_{\text{C}}$ (m) | $\delta_{\text{H}}$ (m, $J$ / Hz) |
| 1        | 151.6 (s)               |                                   | 145.9 (s)               |                                   |
| 2        | 140.5 (s)               |                                   | 141.8 (s)               |                                   |
| 3        | 150.3 (s)               |                                   | 144.5 (s)               |                                   |
| 4        | 107.0 (d)               | 7.02 (s)                          | 114.3 (d)               | 7.02 (s)                          |
| 5        | 135.2 (s)               |                                   | 135.1 (s)               |                                   |
| 6        | 39.8 (t)                | 2.63 (dd, $J$ 13.5, 7.0)          | 39.3 (t)                | 2.50 (overlap)                    |
|          |                         | 2.67 (d, $J$ 13.3)                |                         | 2.63 (d, $J$ 13.3)                |
| 7        | 34.1 (d)                | 1.85 (overlap)                    | 34.2 (d)                | 1.80 (m)                          |
| 8        | 41.8 (d)                | 1.85 (overlap)                    | 41.7 (d)                | 1.85 (m)                          |
| 9        | 36.0 (t)                | 2.07 (d, $J$ 13.0)                | 36.2 (t)                | 2.12 (d, $J$ 12.9)                |
|          |                         | 2.48 (dd, $J$ 9.2, 12.9)          |                         | 2.52 (overlap)                    |
| 10       | 134.9 (s)               |                                   | 134.2 (s)               |                                   |
| 11       | 108.5 (d)               | 6.54 (s)                          | 106.1 (d)               | 6.62 (s)                          |
| 12       | 141.4 (s)               |                                   | 151.0 (s)               |                                   |
| 13       | 140.0 (s)               |                                   | 138.7 (s)               |                                   |
| 14       | 148.7 (s)               |                                   | 142.3 (s)               |                                   |
| 15       | 119.8 (s)               |                                   | 118.4 (s)               |                                   |
| 16       | 122.0 (s)               |                                   | 122.4 (s)               |                                   |
| 17       | 12.8 (q)                | 0.77 (d, $J$ 6.7)                 | 13.1 (q)                | 0.71 (d, $J$ 7.0)                 |
| 18       | 21.8 (q)                | 0.89 (d, $J$ 6.7)                 | 22.0 (q)                | 0.91 (d, $J$ 7.0)                 |
| OMe-1    | 60.1 (q)                | 3.84 (s)                          |                         |                                   |
| OMe-2    | 60.5 (q)                | 3.90 (s)                          | 60.9 (q)                | 3.84 (s)                          |
| OMe-12   |                         |                                   | 55.9 (q)                | 3.81 (s)                          |
| OMe-13   | 60.6 (q)                | 3.89 (s)                          | 60.9 (q)                | 3.92 (s)                          |
| Ar-OH-1  |                         |                                   |                         | 10.42 (brs)                       |
| Ar-OH-3  |                         | 10.61 (brs)                       |                         |                                   |
| Ar-OH-14 |                         | 11.16 (brs)                       |                         | 11.09 (brs)                       |
| -OAc     | 169.9 (s)               |                                   | 169.7 (s)               |                                   |
|          | 21.0 (q)                | 1.95 (s)                          | 21.0 (q)                | 1.94 (s)                          |

based on the analysis of its  $^1\text{H}$ - $^1\text{H}$  COSY and HMBC spectra. Thus, the planar structure of **1** was established.

The CD spectrum of **1** gave a negative Cotton effect at 252 nm and a positive Cotton effect at 225 nm, indicating that **1** has a *S*-biphenyl configuration.<sup>19,20</sup> The ROESY correlations between H-4/H-7, H-4/H-6 $\beta$ , H-9 $\beta$ /H-11, H-9 $\beta$ /CH<sub>3</sub>-17, H-9 $\alpha$ /CH<sub>3</sub>-18, H-9 $\alpha$ /H-8, and CH<sub>3</sub>-17/CH<sub>3</sub>-18 in **1** suggested a twist-boat-chair (TBC) conformation for the cyclooctadiene ring.<sup>19,20</sup> The substituent positions and stereochemical assignments in the cyclooctadiene ring of **1** were supported by computer generated molecular model using MM2 force field in CS Chem 3D (Figure 3). Thus, the structure of **1** was determined as shown, and this compound has been given the trivial name as neglectaphenol A.



**Figure 3.** Computer generated molecular model showing key ROESY correlations and corresponding interatomic distance (Å) of compound **1**.

Compound **2** was obtained as a yellow gum, and showed sodiated molecular ions at  $m/z$  439.1736 in the HRESIMS (calcd  $m/z$  439.1733), indicating the same molecular formula with that of compound **1**. The  $^1\text{H}$ - and  $^{13}\text{C}$  NMR spectra of **2** were similar to those of **1** (Table 1). The IR spectrum also showed the presence of hydroxy group (3458  $\text{cm}^{-1}$ ) and ester group (1746  $\text{cm}^{-1}$ ). The obvious chemical shift differences resulted from the substituent group variations in the aromatic rings. Analysis of the HSQC, HMBC, and ROESY spectra of **2** showed that three methoxy groups are located at C-2, C-12, and C-13, two phenolic hydroxy groups at C-1 and C-14, and one acetoxy group located at C-3. Thus, the structure of **2** was established, and it has been accorded the trivial name neglectaphenol B.

Compound **3**, obtained as a yellow gum, was assigned the molecular formula,  $\text{C}_{24}\text{H}_{32}\text{O}_7$ , from its HRESIMS at  $m/z$  455.2034 [ $\text{M}+\text{Na}$ ]<sup>+</sup> (calcd  $m/z$  455.2046). Its  $^1\text{H}$ ,  $^{13}\text{C}$  and DEPT NMR spectra showed signals for 24 carbons and 32 hydrogens (Table 2), corresponding to two aromatic rings with two aromatic protons ( $\delta_{\text{H}}$  6.98 and 6.69), one methylene

( $\delta_C$  37.6), two methines ( $\delta_C$  37.8 and 36.5), one oxygenated methine ( $\delta_C$  88.2), two methyl ( $\delta_C$  17.1 and 17.3), four methoxy ( $\delta_C$  60.5, 60.9, 60.3 and 60.7), two phenolic hydroxy groups ( $\delta_H$  9.72 and 10.00), and an ethoxy group ( $\delta_C$  63.3, 15.5). The HMBC correlations of H-11 ( $\delta_H$  6.69) with C-9 ( $\delta_C$  37.6), C-10 ( $\delta_C$  137.2), and C-15 ( $\delta_C$  121.2), and of H-4 ( $\delta_H$  6.98) with C-5 ( $\delta_C$  136.0), C-6 ( $\delta_C$  88.2), and C-16 ( $\delta_C$  123.5), together with  $^1\text{H}$ - $^1\text{H}$  COSY correlations of H-6/H-7/H-8/H-9, H-7/H-17, and H-8/H-18, as well as UV absorption bands at 205 and 240 nm, implied that **3** was also a dibenzocyclooctadiene lignan.<sup>19,20</sup> However, the IR spectrum was different from that of **2** with the absence of an ester group ( $1746\text{ cm}^{-1}$ ). The ethoxy group located at C-6 was supported by the HMBC correlations of the oxygenated methylene proton ( $\delta_H$  3.22) with C-6 ( $\delta_C$  88.2), and of H-6 ( $\delta_H$  4.06) with oxygenated methylene carbon ( $\delta_C$  63.3). In dibenzocyclooctadiene lignans, the chemical shifts of methoxy groups at C-3 and C-12 occur at  $\delta_C$  55-56, whereas those of methoxy groups at C-1, C-2, C-13, C-14 are found to be  $\delta_C$  60-61.<sup>3,21</sup> Four methoxy groups located at C-1, C-2, C-13, and C-14 were confirmed by the analysis of its HMBC spectrum in **3**. According to the molecular formula, the quaternary carbon at C-3 and C-12 should both be substituted by a hydroxy group. In the cyclooctadiene ring, the oxygenated methine carbon was assigned to C-6 on the basis of the HMBC correlation from H-4 ( $\delta_H$  6.98) to C-6 ( $\delta_C$  88.2). The CD spectrum of **3** (negative Cotton effect at 249 nm and a positive Cotton effect at 220 nm) indicated that **3** has a *S*-biphenyl configuration.<sup>19,20</sup> The ROESY correlations between H-4/ $\text{CH}_3$ -17 and H-11/H-9 $\beta$  in **3** suggested a twist-boat-chair (TBC) conformation for the cyclooctadiene ring.<sup>19,20</sup> The configuration of the ethoxy group attached to C-6 was deduced as being  $\beta$ -oriented by the chemical shift ( $\delta_C$  88.2), which was similar to  $\beta$ -oriented derivatives of the marlignan J,<sup>11</sup> and distinct from that of 6- $\alpha$ -oriented components in dibenzocyclooctadiene lignan family.<sup>22</sup> This was confirmed by the ROESY correlations between H-4/H-6 $\alpha$  and H-4/ $\text{CH}_3$ -17. Thus, the structure of **3** (neglectaphenol C) was established, as shown.

Compound **4** was obtained as a yellow gum. Its molecular formula was determined as  $\text{C}_{22}\text{H}_{26}\text{O}_7$  by its HRESIMS  $m/z$  425.1577 [ $\text{M}+\text{Na}$ ] $^+$ . It showed absorption maxima in the UV spectrum at 210, 243 nm, and a strong negative Cotton effect at 250 nm in the CD spectrum, indicating that **4** is a  $\text{C}_{18}$  dibenzocyclooctadiene lignan with an *S*-biphenyl configuration.<sup>18,19</sup> The  $^{13}\text{C}$  NMR spectrum showed the signals of 12 carbons belonging to a biphenyl at  $\delta_C$  103.9-153.4 (Table 2). Besides the aromatic protons of biphenyl that appeared at  $\delta_H$  6.89 and 6.62 (1H each, s), the  $^1\text{H}$  NMR spectrum of **4** also indicated the presence of one methylenedioxy unit at  $\delta_H$  5.92, 5.99 (1H, each

**Table 2.**  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR Data of compounds **3** and **4** (500 and 125 MHz)

| No.      | Compound <b>3</b> <sup>a</sup> |                               | Compound <b>4</b> <sup>b</sup> |                               |
|----------|--------------------------------|-------------------------------|--------------------------------|-------------------------------|
|          | $\delta_C$ (m)                 | $\delta_H$ (m, <i>J</i> / Hz) | $\delta_C$ (m)                 | $\delta_H$ (m, <i>J</i> / Hz) |
| 1        | 151.9 (s)                      |                               | 153.4 (s)                      |                               |
| 2        | 141.1 (s)                      |                               | 139.5 (s)                      |                               |
| 3        | 150.3 (s)                      |                               | 151.5 (s)                      |                               |
| 4        | 116.1 (d)                      | 6.98 (s)                      | 110.0 (d)                      | 6.89 (s)                      |
| 5        | 136.0 (s)                      |                               | 137.4 (s)                      |                               |
| 6        | 88.2 (d)                       | 4.06 (d, <i>J</i> 8.2)        | 73.2 (d)                       | 4.93 (brs)                    |
| 7        | 38.7 (d)                       | 1.80 (m)                      | 44.5 (d)                       | 2.05 (m)                      |
| 8        | 36.5 (d)                       | 2.05 (m)                      | 40.2 (d)                       | 2.44 (overlap)                |
| 9        | 37.6 (t)                       | 2.19 (m)                      | 35.7 (t)                       | 2.15 (d, <i>J</i> 16.4)       |
|          |                                | 2.48 (m)                      |                                | 2.44 (overlap)                |
| 10       | 137.2 (s)                      |                               | 139.0 (s)                      |                               |
| 11       | 111.0 (d)                      | 6.69 (s)                      | 103.9 (d)                      | 6.62 (s)                      |
| 12       | 149.3 (s)                      |                               | 149.2 (s)                      |                               |
| 13       | 139.7 (s)                      |                               | 136.3 (s)                      |                               |
| 14       | 151.0 (s)                      |                               | 143.3 (s)                      |                               |
| 15       | 121.2 (s)                      |                               | 121.5 (s)                      |                               |
| 16       | 123.5 (s)                      |                               | 122.8 (s)                      |                               |
| 17       | 17.1 (q)                       | 0.85 (overlap)                | 8.7 (q)                        | 0.96 (d, <i>J</i> 7.2)        |
| 18       | 17.3 (q)                       | 0.85 (overlap)                | 22.1 (q)                       | 1.03 (d, <i>J</i> 7.2)        |
| OMe-1    | 60.5 (q)                       | 3.72 (s)                      | 60.8 (q)                       | 3.93 (s)                      |
| OMe-2    | 60.9 (q)                       | 3.85 (s)                      |                                |                               |
| OMe-3    |                                |                               | 55.9 (q)                       | 3.98 (s)                      |
| OMe-13   | 60.3 (q)                       | 3.81 (s)                      |                                |                               |
| OMe-14   | 60.6 (q)                       | 3.75 (s)                      | 60.9 (q)                       | 3.96 (s)                      |
| Ar-OH-2  |                                |                               |                                | 10.81 (brs)                   |
| Ar-OH-3  |                                | 9.72 (brs)                    |                                |                               |
| Ar-OH-12 |                                | 10.00 (brs)                   |                                |                               |
| 1'       | 63.3 (t)                       | 3.22 (m)                      | 101.4 (t)                      | 5.92, 5.99 (s)                |
| 2'       | 15.5 (q)                       | 0.96 (t, <i>J</i> 7.0)        |                                |                               |

<sup>a</sup>obtained in  $\text{CDCl}_3$ ; <sup>b</sup>obtained in  $\text{C}_5\text{D}_5\text{N}$ .

s), three methoxy groups at  $\delta_H$  3.93, 3.98, and 3.96 (3H each, s), one phenolic hydroxy group ( $\delta_H$  10.81, brs), and two secondary methyls at  $\delta_H$  0.96 (3H each, d, *J* 7.2 Hz) and 1.03 (3H, d, *J* 7.2 Hz). From the HMBC spectrum of **4**, it was found that the single  $\text{sp}^3$  oxymethine carbon resonating at C-6 ( $\delta_C$  73.2, d) correlated with a proton at H-6 ( $\delta_H$  4.93, 1H, brs), and correlations observed from H-6 ( $\delta_H$  4.93, brs) to the aromatic C-4 ( $\delta_C$  110.0 d) and C-16 ( $\delta_C$  122.8 s) were used to assign the oxymethine group at C-6. Further analysis of the HMBC spectrum showed that the methylenedioxy unit was attached to C-12 and C-13, the three methoxy groups were located at C-1, C-3, and C-14, and the phenolic hydroxy group located at C-2 respectively.

The *a*-orientation of the hydroxy group at C-6 was confirmed by its chemical shift of  $^{13}\text{C}$  ( $\delta_{\text{C}}$  73.2 d), and  $^1\text{H}$  ( $\delta_{\text{H}}$  4.93, brs), which was similar to that of the *a*-oriented derivatives of gomisins.<sup>15,23</sup> This was further confirmed by the ROESY correlation for one of the C-9 protons ( $\delta_{\text{H}}$  2.15) with the aromatic H-11 ( $\delta_{\text{H}}$  6.62), which allowed the assignment of  $\beta$  H-9 orientation. The ROESY correlations found between H-9 $\beta$  ( $\delta_{\text{H}}$  2.15) and H-8 ( $\delta_{\text{H}}$  2.44) and between H-7 ( $\delta_{\text{H}}$  2.05) and H-6 ( $\delta_{\text{H}}$  4.93) confirmed the  $\alpha$ -orientation of 6-OH.<sup>15,23</sup> The above observations were used to establish the structure of neglectaphenol D (**4**) as shown.

Since some dibenzocyclooctadiene lignans from *Schisandra* species are reported to possess anti-HIV activities and cytotoxicities,<sup>11,24,25</sup> the anti-HIV-1 activities and cytotoxicities of compounds **1-4** were tested. The cytotoxicity assay against C8166 cells ( $\text{CC}_{50}$ ), and anti-HIV-1 activity were evaluated by the inhibition assay for the cytopathic effects of HIV-1 ( $\text{EC}_{50}$ ), using azidothymidine (AZT) as a positive control ( $\text{EC}_{50}$  = 0.034  $\mu\text{g mL}^{-1}$  and  $\text{CC}_{50}$  > 200  $\text{mg mL}^{-1}$ ).<sup>26</sup> The results are shown in Tables 3 and 4. The results revealed that compounds **1-4** showed moderate anti-HIV-1 activities with therapeutic index (TI) values above 61.7, 22.6, 57.7, and 27.9, respectively.

**Table 3.** Anti-HIV Activities of the compounds **1-4**

| Compounds | $\text{CC}_{50}$ / ( $\mu\text{g mL}^{-1}$ ) | $\text{EC}_{50}$ / ( $\mu\text{g mL}^{-1}$ ) | TI ( $\text{CC}_{50}/\text{EC}_{50}$ ) |
|-----------|--|--|--|
| <b>1</b>  | > 200  | 3.24   | > 61.7                                 |
| <b>2</b>  | 32.04  | 1.42   | 22.6                                   |
| <b>3</b>  | > 200  | 3.47   | > 57.7                                 |
| <b>4</b>  | > 200  | 7.15   | > 27.9                                 |

**Table 4.** Cytotoxicity data for the compounds **1-4** ( $\mu\text{g mL}^{-1}$ )

| Compounds | NB4  | A549 | SHSY5Y | PC3  | MCF7 |
|-----------|------|------|--------|------|------|
| <b>1</b>  | 7.9  | > 10 | 8.6    | > 10 | 8.7  |
| <b>2</b>  | 7.3  | > 10 | > 10   | 8.6  | > 10 |
| <b>3</b>  | > 10 | > 10 | > 10   | > 10 | > 10 |
| <b>4</b>  | 5.2  | 1.3  | 7.9    | > 10 | 5.6  |
| Taxol     | 0.03 | 0.02 | 0.2    | 0.2  | 0.1  |

The cytotoxicity tests for these compounds were performed using a previously reported procedure.<sup>27</sup> All of the experiments were performed in triplicate. In the MTT assay, the  $\text{IC}_{50}$  was defined as the concentration of the test compound resulting in a 50% reduction of absorbance compared with untreated cells. The cytotoxic activities against NB4, A549, SHSY5Y, PC3, and MCF7 tumor cell lines by MTT-assay (with taxol as the positive control) were tested. The results showed that compounds **1-4** have weak cytotoxic activities for some selected cell lines, with  $\text{IC}_{50}$  > 1.3  $\mu\text{g mL}^{-1}$ .

## Experimental Section

### General experimental procedures

Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. CD spectra were measured on a JASCO J-810 spectropolarimeter. A Tenor 27 spectrophotometer was used for scanning IR spectrometry. 1D and 2D NMR spectra were recorded on a DRX-500 NMR spectrometer with TMS as internal standard. Unless otherwise specified, chemical shifts ( $\delta$ ) are expressed in ppm with reference to the solvent signals. HRESIMS was performed on a VG Autospec-3000 spectrometer. Semi-preparative HPLC was performed on a Shimadzu LC-8A preparative liquid chromatograph with Zorbax PrepHT GF (21.2 mm  $\times$  25 cm) or Venusil MP  $\text{C}_{18}$  (20 mm  $\times$  25 cm) columns. Column chromatography was performed using silica gel (200-300 mesh, Qing-dao Marine Chemical, Inc., Qingdao, People's Republic of China), Lichroprep RP-18 gel (40-63  $\mu\text{m}$ , Merck, Darmstadt, Germany), and MCI gel (75-150  $\mu\text{m}$ , Mitsubishi Chemical Corporation, Tokyo, Japan). The fractions were monitored by TLC, and spots were visualized by heating silica gel plates sprayed with 5%  $\text{H}_2\text{SO}_4$  in EtOH.

### Plant material

The stems of *S. neglecta* were collected in Dali Prefecture, Yunnan Province, People's Republic of China, in July 2009. The identification of the plant material was done by Prof Xi-Wen Li of Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (KIB 09-9-36) has been deposited in 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 stems of *S. neglecta* (5.0 kg) were extracted four times with 70% acetone (4  $\times$  50 L) at room temperature and filtered, with the filtrate evaporated under reduced pressure and partitioned with EtOAc (3  $\times$  2 L). The EtOAc partition (385 g) was applied to silica gel (200-300 mesh) column chromatography, eluting with a  $\text{CHCl}_3$ -acetone gradient system (20:1, 9:1, 8:2, 7:3, 6:4, 5:5), to give five fractions A-E. The further separation of fraction B (32.6 g) by silica gel column chromatography, eluted with petroleum ether-acetone (20:1-1:2), yielded mixtures B1-B6. Fraction B2 (4.65 g) was subjected to silica gel column chromatography using petroleum ether-acetone and semi-

preparative HPLC (75% MeOH-H<sub>2</sub>O, flow rate 12 mL min<sup>-1</sup>) to give **6** (22.6 mg, 13 min), **10** (22.4 mg, 18 min), **11** (8.8 mg, 25 min), and **13** (86.2 mg, 28 min). Fraction B3 (2.8 g) was subjected to silica gel column chromatography using petroleum ether-acetone and semi-preparative HPLC (65% MeOH-H<sub>2</sub>O, flow rate 12 mL min<sup>-1</sup>) to give **4** (42.1 mg, 18 min), **5** (16.4 mg, 23 min), **7** (16.3 mg, 26 min), **8** (13.4 mg, 28 min), **9** (14.6 mg, 35 min), and **12** (43.5 mg, 30 min). Fraction B4 (2.7 g) was subjected to silica gel column chromatography using petroleum ether-acetone and semipreparative HPLC (60% MeOH-H<sub>2</sub>O, flow rate 12 mL min<sup>-1</sup>) to afford **1** (11.5 mg, 20 min), **2** (13.4 mg, 23 min) and **3** (15.4 mg, 28 min). Fraction C (60 g) was subjected to silica gel column chromatography using petroleum ether-acetone (20:1-6:4) for elution followed by a reversed-phase column (RP-18) eluting with MeOH-H<sub>2</sub>O (30%-90%) and then by Sephadex LH-20 using MeOH as eluant. Further purifications were performed by semipreparative HPLC and preparative HPLC separation (60% MeOH-H<sub>2</sub>O) to give compounds **14** (3.5 mg, 38 min), **15** (7.8 mg, 20 min), **16** (10.4 mg, 23 min), **17** (5 mg, 25 min), **18** (3.2 mg, 36 min), **19** (4 mg, 26 min), **20** (3 mg, 29 min), **21** (6 mg, 22 min), **22** (5 mg, 21 min), and **23** (7 mg, 41 min).

#### Anti-HIV-1 and cytotoxicity assays

The cytotoxicity assay against C8166 cells (CC<sub>50</sub>) was assessed using the MTT method, and anti-HIV-1 activity was evaluated by the inhibition assay for the cytopathic effects of HIV-1 (EC<sub>50</sub>).<sup>26</sup>

The cytotoxicity tests for these compounds were performed against NB4, A549, SHSY5Y, PC3, and MCF7 tumor cell lines by MTT-assay (with taxol as the positive control).<sup>27</sup>

#### Neglectaphenol A (**1**)

C<sub>23</sub>H<sub>28</sub>O<sub>7</sub>, obtained as yellow gum;  $[\alpha]_D^{24.5} = +38.2$  (*c* 0.25, MeOH); UV (MeOH)  $\lambda_{\max}/\text{nm}$  (log  $\epsilon$ ) 210 (4.18), 245 (3.26), 326 (1.22); CD (*c* 0.02, MeOH), nm ( $\Delta\epsilon$ ) 252 (-17.5), 225 (+7.83), 220 (+4.87); IR (KBr)  $\nu_{\max}/\text{cm}^{-1}$  3452, 2941, 2876, 2833, 1748, 1638, 1590, 1485, 1392, 1326, 1279, 1235, 1192, 1134, 1105, 1062, 1009, 876, 753; <sup>1</sup>H and <sup>13</sup>C NMR data (C<sub>5</sub>D<sub>5</sub>N, 500 and 125 MHz), Table 1; ESIMS (positive ion mode) *m/z* 439 [M+Na]<sup>+</sup>; HRESIMS (positive ion mode) *m/z* 439.1725 [M+Na]<sup>+</sup> (calcd. 439.1733 for C<sub>23</sub>H<sub>28</sub>NaO<sub>7</sub>).

#### Neglectaphenol B (**2**)

C<sub>23</sub>H<sub>28</sub>O<sub>7</sub>, obtained as yellow gum;  $[\alpha]_D^{24.8} = +32.1$  (*c* 0.25, MeOH); UV (MeOH)  $\lambda_{\max}/\text{nm}$  (log  $\epsilon$ ) 210 (4.22), 245 (3.18), 326 (0.97); IR (KBr)  $\nu_{\max}/\text{cm}^{-1}$  3458, 2962, 2851,

1746, 1635, 1576, 1483, 1462, 1385, 1334, 1276, 1233, 1194, 1076, 1016, 988, 884, 749; <sup>1</sup>H and <sup>13</sup>C NMR data (C<sub>5</sub>D<sub>5</sub>N, 500 and 125 MHz), Table 1; ESIMS (positive ion mode) *m/z* 439 [M+Na]<sup>+</sup>; HRESIMS (positive ion mode) *m/z* 439.1736 [M+Na]<sup>+</sup> (calcd. 439.1733 for C<sub>23</sub>H<sub>28</sub>NaO<sub>7</sub>).

#### Neglectaphenol C (**3**)

C<sub>24</sub>H<sub>32</sub>O<sub>7</sub>, obtained as yellow gum;  $[\alpha]_D^{24.9} = +28.6$  (*c* 0.20, MeOH); UV (MeOH)  $\lambda_{\max}/\text{nm}$  (log  $\epsilon$ ) 205 (4.24), 240 (3.81), 329 (0.86); CD (*c* 0.10, MeOH), nm ( $\Delta\epsilon$ ) 249 (-68.2), 240 (-39.5), 220 (+25.2), 210 (+6.3); IR (KBr)  $\nu_{\max}/\text{cm}^{-1}$  3438, 2942, 2925, 2876, 1624, 1579, 1495, 1453, 1408, 1328, 1276, 1097, 1069, 984, 862; <sup>1</sup>H NMR and <sup>13</sup>C NMR data (CDCl<sub>3</sub>, 500 and 125 MHz), Table 2; ESIMS (positive ion mode) *m/z* 455 [M+Na]<sup>+</sup>; HRESIMS (positive ion mode) *m/z* 455.2034 [M+Na]<sup>+</sup> (calcd. 455.2046 for C<sub>24</sub>H<sub>32</sub>NaO<sub>7</sub>).

#### Neglectaphenol D (**4**)

C<sub>22</sub>H<sub>26</sub>O<sub>7</sub>, obtained as yellow gum;  $[\alpha]_D^{25.0} = -45.2$  (*c* 0.25, MeOH); UV (MeOH)  $\lambda_{\max}/\text{nm}$  (log  $\epsilon$ ): 210 (4.15), 243 (3.32), 314 (0.59); CD (*c* 0.05, MeOH), nm ( $\Delta\epsilon$ ) 250 (-22.2), 240 (-16.5), 218 (+7.62), 210 (-2.24); IR (KBr)  $\nu_{\max}/\text{cm}^{-1}$  3441, 2925, 2847, 1612, 1586, 1465, 1372, 1323, 1083, 1046, 1028, 952, 860; <sup>1</sup>H NMR and <sup>13</sup>C NMR data (C<sub>5</sub>D<sub>5</sub>N, 500 and 125 MHz), Table 2; ESIMS (positive ion mode) *m/z* 425 [M+Na]<sup>+</sup>; HRESIMS (positive ion mode) *m/z* 425.1577 [M+Na]<sup>+</sup> (calcd. 425.1576 for C<sub>22</sub>H<sub>26</sub>NaO<sub>7</sub>).

### Supplementary Information

<sup>1</sup>H and <sup>13</sup>C NMR, HSQC, HMBC COSY, ROESY, HRESIMS, and CD spectra of **1**, <sup>1</sup>H and <sup>13</sup>C NMR, spectra of **2-4**, are available free of charge at <http://jbcbs.sbg.org.br> as PDF file.

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