Bioactive Protopanaxatriol Type Saponins Isolated from the Roots of *Panax Notoginseng* (Burk.) F. H. Chen

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**Abstract:** Seven new protopanaxatriol type saponins, 20*S*-sanchirhinosides A 1 (1), A 2 (2), A 3 (3), A 4 (4), A 5 (5), and A 6 (6), and sanchirhinoside B (7) were obtained as minor constituents from the root extract of *Panax notoginseng* (Burkill, F. H. Chen), which showed protection effects against antimycin A induced mitochondrial oxidative stress. Their structures were elucidated by chemical and spectroscopic methods (IR, HRESI-TOF-MS, 1D and 2D NMR). Among them, compounds 4, 6 and 7 showed significant protective effects against antimycin A-induced L6 cell injury.

**Keywords:** *Panax notoginseng*; root; protopanaxatriol type saponins; L6 cell; mitochondrial oxidative stress
1. Introduction

Reactive oxygen species (ROS) cause protein and DNA injuries and further induce pathological changes, such as heart failure [1], neuronal injury [2] and ischemia reperfusion [3]. A lot of natural products show potential ROS scavenging effects and are used as antioxidant agents. *Panax notoginseng* (Burkill, F. H. Chen), have been cultivated in China for more than 400 years. As a traditional Chinese medicine, whose root components have several medicinal properties and are used for stenching the blood, dispersion of gore and reduction of the pain caused by blood diseases, etc. The main components in this plant were identified to be saponins, flavonoids, dencichine and polysaccharides [4]. During the course of our characterization studies on the bioactive constituents from the roots of *P. notoginseng*, the 70% EtOH extract showed significant protective effects against antimycin A-induced L6 cell injuries. Seven new protopanaxatriol type saponins: 20S-sanchirhinosides A₁ (1), A₂ (2), A₃ (3), A₄ (4), A₅ (5), and A₆ (6) and sanchirhinoside B (7) were obtained as minor constituents from it. In this paper, we report the protect effects of *P. notoginseng* 70% EtOH extract and new compounds 1–7 against antimycin A-induced mitochondrial oxidative stress.

2. Results and Discussion

The dried roots of *P. notoginseng* were refluxed with 70% ethanol-water. Evaporation of the solvent under reduced pressure provided a 70% ethanol-water extract. The extract were subjected to column chromatography (CC) and finally HPLC to give seven new protopanaxatriol type saponins: 20S-sanchirhinosides A₁–A₆ (1–6), and sanchirhinoside B (7) (Figure 1).

![Figure 1. The structures of compounds 1–7.](image)

20(S)-Sanchirhinoside A₁ (1) was isolated as a white powder, \([\alpha]^{25}_{D} + 12.6^\circ (\text{MeOH})\). The IR spectrum showed absorption bands at 3,365, 1,717, and 1,654 cm⁻¹ ascribable to hydroxyl, \(\alpha,\beta\)-unsaturated ester, and olefin functions, respectively. The molecular formula, \(C_{40}H_{66}O_{10}\) of 1 was determined by
positive-ion HRESI-TOF-MS (m/z 729.4543 [M + Na]+, calcd. for C_{40}H_{66}O_{10}Na 729.4548). The
\(^1\)H-NMR spectrum of \(1\) (Table 1, in C\(_5\)D\(_5\)N) showed signals assignable to nine methyls
[\(\delta\) 0.84, 1.07, 1.26, 1.43, 1.56, 1.64, 1.68, 2.08 (3H each, all s, H\(_3\)-30, 19, 18, 21, 27, 26, 28), 1.77
(3H, br. d, ca. \(J = 7\) Hz, H\(_3\)-4\(''\))], three methines bearing oxygen functions [\(\delta\) 3.51 (1H, dd, \(J = 5.0, 12.0\)
Hz, H-3), 3.93 (1H, m, H-12), 4.40 (1H, ddd, \(J = 3.5, 10.5, 10.5\) Hz, H-6)], one trisubstituted olefin
[\(\delta\) 5.33 (1H, t, \(J = 7.0\) Hz, H-24)], one \(\alpha, \beta\)-unsaturated ester moiety [\(\delta\) 6.06 (1H, br. d, ca.
\(J = 16\) Hz, H-2\(''\)), 7.12 (1H, dq, \(J = 7.0, 15.5\) Hz, H-3\(''\))], together with an anomeric proton signal at \(\delta\) 5.06
(1H, d, \(J = 7.5\) Hz, H-1'). The \(^1\)C-NMR spectrum displayed 40 carbons, including 30 carbons for the aglycon, six
carbons for the sugar unit and four for a butenoyl group. Taken together the \(^1\)H- and \(^1\)C-NMR spectra
suggested that \(1\) was a dammarane-type triterpene saponin derivative. The chemical shift of \(\delta\) C 61.5
(C-5) indicated that \(1\) was a protopanaxatriol type saponin [\(\delta\) C\(\sim\) 56 and \(\sim\) 61 (C-5) for protopanaxadiol
and protopanaxatriol type saponins, respectively]. In conjunction with analysis of the HSQC spectrum,
the \(^1\)H- and \(^1\)C-NMR data for \(1\) were assigned as shown in Tables 1 (in C\(_5\)D\(_5\)N) and 2 (determined in
CD\(_3\)OD). The \(^1\)H \(^1\)H COSY experiment on \(1\) indicated the presence of the partial structure written in
bold lines. In HMBC experiment, long-range correlations were observed between the following protons
and carbons: H\(_3\)-18 and C-7\(−\)9, 14; H\(_3\)-19 and C-1, 5, 9, 10; H\(_3\)-21 and C-17, 20, 22; H\(_3\)-26 and C-24, 25,
27; H\(_3\)-27 and C-24\(−\)26; H\(_3\)-28 and C-3\(−\)5, 29; H\(_3\)-29 and C-3\(−\)5, 28; H\(_3\)-30 and C-8, 13\(−\)15; H-1' and
C-6; H-6' and C-1"; H-2", 3" and C-1"; H\(_3\)-4" and C-2", 3" (Figure 2). The stereochemistry of C-20 in \(1\) was
clarified by comparing the chemical shifts of 13-, 16-, 17-, and 21\(\sim\)24-carbons of it [\(\delta\) 23.1 (C-23),
27.0 (C-21), 27.1 (C-16), 35.9 (C-22), 43.1 (C-22), 48.7 (C-13), 50.5 (C-17), 125.9 (C-24)] [5], and 20(S)-
gensenoside Rh1 [\(\delta\) 23.0 (C-23), 26.9 (C-21), 27.1 (C-16), 35.9 (C-22), 48.3 (C-13), 54.8 (C-17), 126.4
(C-24)] [6], which was measured in the same solvent (C\(_5\)D\(_5\)N) as \(1\), the stereostructure of the 20-position in \(1\) was confirmed
to be S orientation.

| No. | \(\delta\) _H_(J in Hz) | No. | \(\delta\) _C_ | \(\delta\) _H_(J in Hz) |
|-----|------------------------|-----|-------------|------------------------|
| 1   | 39.5, 1.05 (m), 1.74 (m) | 22  | 35.9, 1.71 (m), 2.08 (m) |
| 2   | 27.9, 1.85 (m), 1.90 (m) | 23  | 23.1, 2.32 (m), 2.62 (m) |
| 3   | 78.7, 3.51 (dd, 5.0, 12.0) | 24  | 126.3, 5.33 (t, 7.0) |
| 4   | 40.3, — — | 25  | 130.8, — — |
| 5   | 61.5, 1.43 (d, 11.5) | 26  | 25.8, 1.68 (s) |
| 6   | 80.0, 4.40 (ddd, 3.5, 10.5, 10.5) | 27  | 17.7, 1.64 (s) |
| 7   | 45.7, 1.97 (dd, 10.5, 10.5) 2.35 (m) | 28  | 31.6, 2.08 (s) |
| 8   | 41.3, — — | 29  | 16.5, 1.56 (s) |
| 9   | 50.3, 1.59 (m) | 1', 106.2, 5.06 (d, 7.5) |
| 10  | 39.8, — — | 2', 75.4, 4.06 (dd, 7.5, 9.0) |
| 11  | 32.1, 1.59 (m), 2.15 (m) | 3', 79.2, 4.22 (dd, 9.0, 9.0) |
| 12  | 71.1, 3.93 (m) | 4', 71.6, 4.00 (dd, 9.0, 9.0) |
| 13  | 48.3, 2.10 (dd, 10.5, 10.5) | 5', 75.2, 4.07 (m) |
| 14  | 51.7, — — | 6', 65.2, 4.77 (dd, 6.5, 12.0) |
| 15  | 32.2, 1.59 (m), 2.15 (m) | 5.11 (br. d, ca. 12) |
Table 1. Cont.

| No. | $\delta_C$ | $\delta_H$ ($J$ in Hz) | No. | $\delta_C$ | $\delta_H$ ($J$ in Hz) |
|-----|------------|------------------------|-----|------------|------------------------|
| 16  | 27.1       | 1.43 (m), 1.87 (m)     | 17  | 54.8       | 2.35 (m)               |
| 17  |           |                        | 18  | 17.5       | 1.26 (s)               |
| 18  |           |                        | 19  | 17.7       | 1.07 (s)               |
| 19  |           |                        | 20  | 73.0       | —                      |
| 20  |           |                        | 21  | 27.0       | 1.43 (s)               |

Table 2. $^1$H- and $^{13}$C-NMR data for compound 1 in CD$_3$OD (500 MHz for $^1$H and 125 MHz for $^{13}$C).

| No. | $\delta_C$ | $\delta_H$ ($J$ in Hz) | No. | $\delta_C$ | $\delta_H$ ($J$ in Hz) |
|-----|------------|------------------------|-----|------------|------------------------|
| 1   | 40.2       | 1.06 (m), 1.75 (m)     | 22  | 36.3       | 1.37 (m), 1.54 (m)     |
| 2   | 27.6       | 1.57 (m), 1.63 (m)     | 23  | 23.3       | 1.98 (m), 2.15 (m)     |
| 3   | 79.9       | 3.10 (dd, 5.0, 10.5)   | 24  | 126.2      | 5.14 (t, 7.0)          |
| 4   | 40.4       | —                      | 25  | 132.1      | —                      |
| 5   | 61.9       | 1.11 (d, 10.5)         | 26  | 26.0       | 1.68 (s)               |
| 6   | 80.7       | 4.07 (dd, 3.0, 10.5)   | 27  | 17.84      | 1.62 (s)               |
| 7   | 45.9       | 1.59 (m), 2.00 (m)     | 28  | 31.3       | 1.34 (s)               |
| 8   | 42.0       | —                      | 29  | 16.3       | 0.97 (s)               |
| 9   | 50.9       | 1.45 (m)               | 30  | 17.1       | 0.91 (s)               |
| 10  | 40.5       | —                      | 1'  | 105.7      | 4.43 (d, 7.5)          |
| 11  | 32.0       | 1.20 (m), 1.85 (m)     | 2'  | 75.5       | 3.21 (dd, 7.5, 9.0)    |
| 12  | 72.0       | 3.53 (m)               | 3'  | 78.7       | 3.35 (dd, 9.0, 9.0)    |
| 13  | 48.5       | 1.72 (dd, 11.0, 11.0)  | 4'  | 71.8       | 3.23 (dd, 9.0, 9.0)    |
| 14  | 52.5       | —                      | 5'  | 75.3       | 3.52 (m)               |
| 15  | 32.2       | 1.02 (m), 1.49 (m)     | 6'  | 65.3       | 4.16 (dd, 6.0, 11.5)   |
| 16  | 27.4       | 1.28 (m), 1.86 (m)     | 1'  | 105.7      | 4.43 (d, 7.5)          |
| 17  | 55.1       | 2.03 (m)               | 1'' | 168.0      | —                      |
| 18  | 17.7       | 1.06 (s)               | 2'' | 123.5      | 5.88 (dd, 2.0, 15.0)   |
| 19  | 17.78      | 0.99 (s)               | 3'' | 146.5      | 7.00 (dq, 7.0, 15.0)   |
| 20  | 74.4       | —                      | 4'' | 18.3       | 1.88 (dd, 2.0, 7.0)    |
| 21  | 26.5       | 3.63 (1H, m, overlapped)|

Figure 2. The main $^1$H $^1$H COSY and HMBC correlations of 1 and 2.
Acid hydrolysis yielded D-glucose, which was identified by HPLC analysis by its retention time and optical rotation using chiral detection [7,8]. On the basis of above mentioned evidence, the structure of 1 was characterized to be 20(S)-sanchirhinoside A1.

20(S)-Sanchirhinoside A2 (2) was obtained as white powder with positive rotation ([α]D25 + 7.4°). The molecular formula, C43H72O14, of 2 was determined by positive-ion HRESI-TOF-MS (m/z 835.4832 [M + Na]+, calcd for C43H72O14Na 835.4814). Acid hydrolysis of 2 yielded D-glucose and D-xylose, which was identified by the same method as 1 [7,8]. The 1H and 13C (C5D5N, Table 3) and various 2D NMR experiments including 1H-1H COSY, HSQC, and HMBC spectra of 2 indicated the presence of a 20S-protopanaxatriol type aglycon [9] [δH 0.92, 1.00, 1.23, 1.40, 1.44, 1.64, 1.66, 2.06 (3H each, all s, H3-30, 19, 18, 29, 21, 27, 26, 28), 1.40 (1H, d, J = 11.0 Hz, H-5), 3.49 (1H, dd, J = 5.0, 11.5 Hz, H-3), 3.94 (1H, m, H-12), 4.34 (1H, m, H-6); δC 23.0 (C-23), 26.8 (C-16), 27.1 (C-21), 35.9 (C-22), 48.4 (C-13), 54.8 (C-17), 126.3 (C-24); a β-D-glucopyranosyl [δ 5.00 (1H, d, J = 7.5 Hz, H-1')]; a β-D-xylopyranosyl [δ 5.76 (1H, d, J = 7.0 Hz, H-1'')]; together with an acetyl group [δ 2.08 (3H, s, H3-2'')]; δC 21.0 (C-2''), 170.9 (C-1'')]. Furthermore, in the HMBC experiments, long-range correlations between the following protons and carbons were observed: H-1' and C-6; H-1'' and C-2'; H-6' and C-1'' (Figure 2). Consequently, the structure of 2 was determined and named as 20(S)-sanchirhinoside A2.

Table 3. 1H- and 13C-NMR data for compound 2 in C5D5N (500 MHz for 1H and 125 MHz for 13C).
(S)-Sanchirhinosides A₃ (3) and A₄ (4) were both obtained as white powders with positive rotation ([α]D⁺²⁵ + 19.7° for 3, and +23.2° for 4, respectively, both in MeOH). The same molecular formula, C₄₁H₇₀O₁₃, of 3 and 4 were determined by positive-ion HRESI-TOF-MS (m/z 793.4720 [M + Na]+ for 3, 793.4715 [M + Na]+ for 4, respectively, calcd for C₄₁H₇₀O₁₃Na 793.4709). With acid hydrolysis with 1 M HCl, both of them gave D-glucose and L-arabinose [7,8]. Compared with 20S-gensenoside Rh₁ [6] showed it to be similar except for the signals of an α-L-arabinopyranosyl moiety in the 1H and 13C (C₅D₅N, Table 4) data of 3 [δH 4.96 (1H, d, J = 8.0 Hz, H-1''); δC 66.9 (C-5''), 69.6 (C-4''), 72.6 (C-2''), 75.3 (C-3''), 98.7 (C-1'')]. On the other hand, the 13C-NMR chemical shift of the carbon in the 20-position was shifted from 73.0 [6] to 83.0, which indicated that C-20 was linked with a sugar. Furthermore, in the HMBC experiments, long-range correlations between H-1' and C-6, H-1'' and C-20 were observed (Figure 3). Meanwhile, the 1H- and 13C-NMR (C₅D₅N, Table 5) and various 2D NMR experiments including 1H 1H COSY, HSQC, and HMBC spectra of 4 showed the same fragments as 3, including a 20S-protopanaxatriol type aglycon [δH 0.94, 1.03, 1.16, 1.48, 1.60, 1.60, 1.62, 1.98 (3H each, all s, H₃-30, 19, 18, 29, 27, 26, 21, 28), 1.40 (1H, d, J = 10.5 Hz, H-5)], 3.48 (1H, dd, J = 10.5, 9.0 Hz, H-12), 4.18 (1H, m, H-12), 4.37 (1H, m, H-12), 5.28 (t, 7.0 Hz, H-1'')]. In the HMBC experiments, long-range correlations between H-1' and C-6, H-1'' and C-20 were observed (Figure 3). On the basis of above mentioned evidence, the structures of 3 and 4 were elucidated as 20(S)-sanchirhinosides A₃ and A₄, respectively, as shown in Figure 3.

Table 4. 1H- and 13C-NMR data for compound 3 in C₅D₅N (500 MHz for 1H and 125 MHz for 13C).

| No. | δC | δH (J in Hz) | No. | δC | δH (J in Hz) |
|-----|----|-------------|-----|----|-------------|
| 1   | 39.5 | 1.02 (m), 1.74 (m) | 23  | 23.2 | 2.22 (m), 2.50 (m) |
| 2   | 28.0 | 1.85 (m), 1.93 (m) | 24  | 125.9 | 5.28 (t, 7.0) |
| 3   | 78.7 | 3.50 (dd, 5.0, 11.5) | 25  | 131.1 | — |
| 4   | 40.4 | — | 26  | 25.8 | 1.62 (s) |
| 5   | 61.4 | 1.41 (d, 10.5) | 27  | 17.8 | 1.63 (s) |
| 6   | 80.2 | 4.42 (ddd, 3.0, 10.5, 10.5) | 28  | 31.8 | 2.08 (s) |
| 7   | 45.2 | 1.94 (m), 2.50 (m) | 29  | 16.4 | 1.61 (s) |
| 8   | 41.1 | — | 30  | 17.2 | 0.81 (s) |
| 9   | 50.0 | 1.51 (m) | 1'  | 106.0 | 5.02 (d, 8.0) |
| 10  | 39.7 | — | 2'  | 75.5 | 4.09 (dd, 8.0, 8.0) |
| 11  | 31.0 | 1.51 (m), 2.05 (m) | 3'  | 79.7 | 4.25 (m) |
| 12  | 70.1 | 4.11 (m) | 4'  | 71.9 | 4.21 (dd, 8.0, 9.0) |
| 13  | 49.2 | 1.98 (dd, 10.5, 10.5) | 5'  | 78.2 | 3.95 (m) |
| 14  | 51.3 | — | 6'  | 63.1 | 4.37 (dd, 5.0, 12.0) |
| 15  | 30.6 | 1.06 (m), 1.65 (m) | 5.02 (d, 8.0) |
| 16  | 26.6 | 1.30 (m), 1.75 (m) | 1'' | 98.7 | 4.96 (d, 8.0) |
| 17  | 51.5 | 2.48 (m) | 2'' | 72.6 | 4.38 (dd, 8.0, 8.5) |
| 18  | 17.60 | 1.17 (s) | 3'' | 75.3 | 4.15 (dd, 3.0, 8.5) |
| 19  | 17.55 | 1.03 (s) | 4'' | 69.6 | 4.27 (m) |
| 20  | 83.0 | — | 5'' | 66.9 | 3.75 (dd, 3.0, 11.0) |
| 21  | 22.2 | 1.56 (s) | 22  | 36.1 | 1.79 (m), 2.38 (m) |
| 23  | 3.63 (1H, m, overlapped) | 24  | 4.26 (m) |
Figure 3. The main $^1$H $^1$H COSY and HMBC correlations of 3 and 4.

Table 5. $^1$H- and $^{13}$C-NMR data for compound 4 in C$_5$D$_5$N (500 MHz for $^1$H and 125 MHz for $^{13}$C).

| No. | $\delta$C | $\delta$H ($J$ in Hz) | No. | $\delta$C | $\delta$H ($J$ in Hz) |
|-----|----------|---------------------|-----|----------|---------------------|
| 1   | 39.5     | 1.01 (m), 1.73 (m)  | 23  | 23.2     | 2.23 (m), 2.50 (m)  |
| 2   | 27.9     | 1.84 (m), 1.91 (m)  | 24  | 126.0    | 5.26 (t, 7.0)       |
| 3   | 78.6     | 3.48 (dd, 5.5, 10.5)| 25  | 131.0    | —                   |
| 4   | 40.2     | —                   | 26  | 25.8     | 1.60 (s)            |
| 5   | 61.4     | 1.40 (d, 10.5)      | 27  | 17.8     | 1.60 (s)            |
| 6   | 79.8     | 4.37 (m)            | 28  | 31.7     | 1.98 (s)            |
| 7   | 45.4     | 1.97 (m), 2.39 (m)  | 29  | 16.6     | 1.48 (s)            |
| 8   | 41.2     | —                   | 30  | 17.3     | 0.94 (s)            |
| 9   | 50.0     | 1.55 (m)            | 1'  | 106.4    | 4.98 (d, 8.0)       |
| 10  | 39.7     | —                   | 2'  | 72.6     | 4.51 (dd, 8.0, 8.5) |
| 11  | 31.0     | 1.55 (m), 2.09 (m)  | 3'  | 75.0     | 4.24 (dd, 3.0, 8.5) |
| 12  | 70.2     | 4.18 (m)            | 4'  | 69.1     | 4.39 (m)            |
| 13  | 49.3     | 2.00 (dd, 10.5, 10.5)| 5'  | 66.1     | 3.86 (dd, 3.0, 13.0)|
| 14  | 51.4     | —                   |     |          | 4.38 (m)            |
| 15  | 30.8     | 0.99 (m), 1.61 (m)  | 1'' | 98.3     | 5.20 (d, 7.5)       |
| 16  | 26.6     | 1.36 (m), 1.82 (m)  | 2'' | 75.2     | 4.01 (dd, 7.5, 8.5) |
| 17  | 51.6     | 2.55 (m)            | 3'' | 79.4     | 4.25 (dd, 8.5, 8.5) |
| 18  | 17.6     | 1.16 (s)            | 4'' | 71.7     | 4.18 (dd, 8.5, 9.0) |
| 19  | 17.5     | 1.03 (s)            | 5'' | 78.3     | 3.94 (m)            |
| 20  | 83.3     | —                   | 6'' | 62.9     | 4.34 (dd, 5.0, 11.5)|
| 21  | 22.4     | 1.62 (s)            |     |          | 4.50 (dd, 1.5, 11.5)|
| 22  | 36.2     | 1.84 (m), 2.41 (m)  |     |          |                    |

20(S)-Sanchirhinosides A$_5$ (5) and A$_6$ (6) were both isolated as white powders with positive optical rotations ($[\alpha]_D^{25} + 105.3^\circ$ for 5, and +3.1° for 6, respectively, both in MeOH). The molecular formula, C$_{47}$H$_{80}$O$_{18}$, of 5 was determined from positive-ion HRESI-TOF-MS (m/z 955.5248 [M + Na]$^+$, calcd. for C$_{47}$H$_{80}$O$_{18}$Na 955.5237). On the other hand, the molecular formula, C$_{53}$H$_{90}$O$_{23}$, of 6 (m/z 1117.5725 [M + Na]$^+$, calcd for C$_{53}$H$_{90}$O$_{23}$Na 1117.5765), was determined from HRESI-TOF-MS, too. Acid hydrolysis of 5 and 6 with 1 M HCl liberated D-glucose (from 5 and 6), D-xylose (from 6), and L-arabinose (from 5) [7,8]. Both the $^1$H- and $^{13}$C-NMR spectra of 5 and 6 (C$_5$D$_5$N, Table 6 for 5, and Table 7 for 6) indicated the presence of a 20S-protopanaxatriol type aglycon [9]. In conjunction with
analysis of HSQC and HSQC-TOCSY spectra, the $^1$H- and $^{13}$C-NMR data for 5 and 6 were assigned. Meanwhile, in the HMBC experiment for compound 5, the long-range correlations were observed between the following proton and carbon pairs: $\delta_H 5.10$ (1H, d, $J = 7.5$ Hz, H-1') and $\delta_C 78.8$ (C-6); $\delta_H 6.60$ (1H, d, $J = 2.5$ Hz, H-1'') and $\delta_C 79.2$ (C-2'); $\delta_H 5.16$ (1H, d, $J = 7.5$ Hz, H-1'''') and $\delta_C 83.3$ (C-20) (Figure 4). On the other hand, the correlations between $\delta_H 4.93$ (1H, d, $J = 7.5$ Hz, H-1') and $\delta_C 79.5$ (C-6); $\delta_H 5.76$ (1H, d, $J = 7.0$ Hz, H-1'') and $\delta_C 80.2$ (C-2'); $\delta_H 5.11$ (1H, d, $J = 7.0$ Hz, H-1''') and $\delta_C 83.5$ (C-20); $\delta_H 5.09$ (1H, d, $J = 7.5$ Hz, H-1''''') and $\delta_C 70.3$ (C-6''') were observed in HMBC experiment on compound 6. Consequently, compounds 5 and 6 were determined as 20($S$)-sanchirhinosides A$_5$ and A$_6$, respectively.

Table 6. $^1$H- and $^{13}$C-NMR data for compound 5 in C$_5$D$_5$N (500 MHz for $^1$H and 125 MHz for $^{13}$C).

| No. | $\delta_C$ | $\delta_H$ ($J$ in Hz) | No. | $\delta_C$ | $\delta_H$ ($J$ in Hz) |
|-----|-------------|------------------------|-----|-------------|------------------------|
| 1   | 39.5        | 0.96 (m), 1.69 (m)     | 26  | 25.8        | 1.61 (s)               |
| 2   | 27.8        | 1.75 (m), 1.85 (m)     | 27  | 17.8        | 1.61 (s)               |
| 3   | 78.7        | 3.48 (dd, 5.0, 11.5)   | 28  | 32.0        | 2.13 (s)               |
| 4   | 40.2        | —                      | 29  | 17.15       | 1.49 (s)               |
| 5   | 61.2        | 1.37 (d, 10.5)         | 30  | 17.21       | 0.83 (s)               |
| 6   | 78.8        | 4.41 (m)               | 1'  | 103.9       | 5.10 (d, 7.5)          |
| 7   | 45.5        | 1.92 (m), 2.41 (m)     | 2'  | 79.2        | 4.30 (dd, 7.5, 8.5)    |
| 8   | 41.2        | —                      | 3'  | 78.3        | 4.17 (m)               |
| 9   | 49.9        | 1.49 (m)               | 4'  | 72.0        | 4.17 (m)               |
| 10  | 39.7        | —                      | 5'  | 77.9        | 3.88 (m)               |
| 11  | 30.9        | 1.48 (m), 2.05 (m)     | 6'  | 62.8        | 4.32 (m)               |
| 12  | 70.3        | 4.10 (m)               |     |             | 4.48 (br. d, 11)       |
| 13  | 49.0        | 1.97 (dd, 10.5, 10.5)  | 1'' | 108.6       | 6.60 (d, 2.5)          |
| 14  | 51.4        | —                      | 2'' | 82.2        | 5.12 (br. s)           |
| 15  | 30.7        | 1.03 (m), 1.64 (m)     | 3'' | 77.6        | 4.93 (br. s)           |
| 16  | 26.6        | 1.28 (m), 1.75 (m)     | 4'' | 86.0        | 4.93 (br. s)           |
| 17  | 51.7        | 2.47 (m)               | 5'' | 62.4        | 4.18 (m)               |
| 18  | 17.4        | 1.17 (s)               |     |             | 4.30 (br. d, ca. 12)   |
| 19  | 17.5        | 0.96 (s)               | 1'''| 98.3        | 5.16 (d, 7.5)          |
| 20  | 83.3        | —                      | 2'''| 75.2        | 4.00 (dd, 7.5, 8.5)    |
| 21  | 22.4        | 1.60 (s)               | 3'''| 79.2        | 4.24 (dd, 8.5, 8.5)    |
| 22  | 36.0        | 1.81 (m), 2.39 (m)     | 4'''| 71.6        | 4.19 (dd, 8.5, 9.0)    |
| 23  | 23.3        | 2.25 (m), 2.50 (m)     | 5'''| 78.3        | 3.92 (m)               |
| 24  | 126.0       | 5.27 (t, 7.0)          | 6'''| 62.9        | 4.32 (m)               |
| 25  | 131.0       | —                      |     |             | 4.48 (br. d, ca. 11)   |
Figure 4. The main $^1$H $^1$H COSY and HMBC correlations of 5 and 6.

Table 7. $^1$H- and $^{13}$C-NMR data for compound 6 in C$_5$D$_5$N (500 MHz for $^1$H and 125 MHz for $^{13}$C).

| No. | $\delta$ C | $\delta$ H ($J$ in Hz) | No. | $\delta$ C | $\delta$ H ($J$ in Hz) |
|-----|------------|----------------|-----|------------|----------------|
| 1   | 39.5       | 0.94 (m), 1.71 (m) | 1'  | 103.6      | 4.93 (d, 7.5) |
| 2   | 27.8       | 1.81 (m)           | 2'  | 80.2       | 4.39 (dd, 7.5, 8.5) |
| 3   | 78.9       | 3.48 (dd, 5.0, 11.0) | 3'  | 79.9       | 4.35 (dd, 8.5, 8.5) |
| 4   | 40.2       | —                  | 4'  | 71.8       | 4.18 (m)     |
| 5   | 61.3       | 1.37 (d, 10.0)     | 5'  | 78.0       | 3.83 (m)     |
| 6   | 79.5       | 4.32 (m)           | 6'  | 62.9       | 4.31 (m)     |
| 7   | 45.0       | 1.93 (m), 2.35 (m) | 7'  | 4.57 (br. d, ca. 11) |
| 8   | 41.2       | —                  | 1'' | 104.9      | 5.76 (d, 7.0) |
| 9   | 50.0       | 1.48 (dd, 11.0, 11.0) | 2'' | 75.9       | 4.16 (dd, 7.0, 8.5) |
| 10  | 39.7       | —                  | 3'' | 78.8       | 4.25 (m)     |
| 11  | 30.9       | 1.50 (m), 2.04 (m) | 4'' | 71.3       | 4.25 (m)     |
| 12  | 70.2       | 4.16 (m)           | 5'' | 67.3       | 3.66 (dd, 10.5, 10.5) |
| 13  | 49.2       | 1.98 (dd, 10.5, 10.5) | 6'' | 70.3       | 4.31 (m)     |
| 14  | 51.4       | —                  | 7'' | 4.33 (m)   |
| 15  | 30.7       | 1.07 (m), 1.61 (m) | 5'' | 71.6       | 4.05 (m)     |
| 16  | 26.6       | 1.28 (m), 1.72 (m) | 4'' | 71.6       | 4.05 (m)     |
| 17  | 51.6       | 2.51 (m)           | 5'' | 77.1       | 4.06 (m)     |
| 18  | 17.59      | 1.15 (s)           | 6'' | 70.3       | 4.31 (m)     |
| 19  | 17.55      | 0.97 (s)           | 5''' | 4.72 (br. d, ca. 11) |
| 20  | 83.5       | —                  | 6''' | 4.33 (m)   |
| 21  | 22.3       | 1.63 (s)           | 1''' | 105.4      | 5.09 (d, 7.5) |
| 22  | 36.2       | 3.63 (1H, m, overlapped) | 2''' | 75.3       | 4.04 (m)     |
| 23  | 23.2       | 1.80 (m), 2.40 (m) | 3''' | 78.36      | 4.21 (m)     |
| 24  | 126.0      | 5.32 (t, 7.0)     | 4''' | 71.7       | 4.21 (m)     |
| 25  | 131.1      | —                  | 5''' | 78.41      | 3.92 (m)     |
| 26  | 25.8       | 1.61 (s)           | 6''' | 62.8       | 4.36 (m)     |
| 27  | 18.0       | 1.67 (s)           | 7''' | 4.51 (br. d, ca. 12) |
| 28  | 31.7       | 2.06 (s)           | 8''' | 4.51 (br. d, ca. 12) |
| 29  | 16.7       | 1.46 (s)           | 9''' | 4.51 (br. d, ca. 12) |
| 30  | 17.2       | 0.80 (s)           | 10''' | 4.51 (br. d, ca. 12) |
Sanchirhinoside B (7), \([\alpha]^{25}_D + 14.7^\circ\) (MeOH), was isolated as a white powder. The molecular formula, \(C_{42}H_{70}O_{13}\), of 7 was determined by positive-ion HRESI-TOF-MS \((m/z\ 805.4700 [M + Na]^+)\), calcd. for \(C_{42}H_{70}O_{13}Na\ 805.4709\). The \(^1\)H-, \(^{13}\)C-NMR (C\(_5\)D\(_5\)N, Table 8) and various 2D NMR experiments, including \(^1\)H \(^1\)H COSY, HSQC, and HMBC of 7 suggested the presence of eight methyls, two olefinic protons, three methines bearing oxygen functions, together with two anomic proton signals, which indicated that 7 was a dammarane-type triterpene saponin derivative with two double bonds. Comparison of the \(^1\)H- and \(^{13}\)C-NMR spectra of 7 with those of ginsenoside Rh4 [10] indicated that the two compounds had the same C-17 side chain. The stereochemistry of the double bond at C-20(22) was determined by a NOESY experiment. In the NOESY spectrum for 7, the correlation signal between \(\delta_H\) 1.77 (3H, s, H\(_3\)-21) and \(\delta_H\) 1.74, 2.81 (1H each, both m, H\(_2\)-23) was observed (Figure 5). Consequently, the configuration of double bond at C-20(22) was supposed to be \(E\). Furthermore, in HMBC experiment, long-range correlations were observed between \(\delta_H\) 5.01 (H-1') and \(\delta_C\) 80.0 (C-6); \(\delta_H\) 4.98 (H-1'') and \(\delta_C\) 77.1 (C-12). Finally, acid hydrolysis of 7 only liberated D-glucose [7,8]. Therefore, the structure of 7 was concluded to be sanchirhinoside B as shown in Figure 5.

Table 8. \(^1\)H- and \(^{13}\)C-NMR data for compound 7 in C\(_5\)D\(_5\)N (500 MHz for \(^1\)H and 125 MHz for \(^{13}\)C).

| No. | \(\delta_C\) | \(\delta_H\) \((J \text{ in Hz})\) | No. | \(\delta_C\) | \(\delta_H\) \((J \text{ in Hz})\) |
|-----|-------------|-------------------------------|-----|-------------|-------------------------------|
| 1   | 39.2        | 0.88 (m), 1.51 (m)            | 23  | 27.8        | 1.74 (m), 2.81 (m)            |
| 2   | 28.0        | 1.84 (m)                      | 24  | 124.8       | 5.41 (t, 7.0)                 |
| 3   | 78.6        | 3.52 (dd, 5.0, 11.0)          | 25  | 130.4       | —                             |
| 4   | 40.4        | —                             | 26  | 25.9        | 1.71 (s)                      |
| 5   | 61.5        | 1.37 (d, 10.5)                | 27  | 17.9        | 1.62 (s)                      |
| 6   | 80.0        | 4.38 (m)                      | 28  | 31.8        | 2.06 (s)                      |
| 7   | 45.1        | 1.88 (m), 2.47 (m)            | 29  | 16.3        | 1.59 (s)                      |
| 8   | 41.2        | —                             | 30  | 16.9        | 0.73 (s)                      |
| 9   | 50.4        | 1.43 (m)                      | 1'  | 105.9       | 5.01 (d, 7.5)                 |
| 10  | 39.8        | —                             | 2'  | 75.5        | 4.09 (dd, 7.5, 8.0)           |
| 11  | 28.1        | 1.22 (m), 2.15 (m)            | 3'  | 79.7        | 4.24 (dd, 8.0, 9.0)           |
| 12  | 77.1        | 4.12 (m)                      | 4'  | 72.5        | 4.11 (dd, 8.0, 9.0)           |
| 13  | 48.9        | 1.98 (dd, 10.5, 10.5)         | 5'  | 77.9        | 3.98 (m)                      |
| 14  | 51.1        | —                             | 6'  | 63.1        | 4.37 (dd, 5.0, 12.0)          |
| 15  | 32.7        | 1.10 (m), 1.66 (m)            | 1'' | 101.2       | 4.51 (dd, 2.0, 12.0)          |
| 16  | 29.4        | 1.44 (m), 1.78 (m)            | 1'' | 101.2       | 4.98 (d, 7.5)                 |
| 17  | 49.6        | 2.77 (m)                      | 2'' | 75.3        | 3.90 (dd, 7.5, 8.0)           |
| 18  | 17.2        | 1.09 (s)                      | 3'' | 78.6        | 4.27 (dd, 8.0, 9.0)           |
| 19  | 17.7        | 0.91 (s)                      | 4'' | 71.8        | 4.22 (dd, 9.0, 9.0)           |
| 20  | 138.4       | —                             | 5'' | 78.2        | 3.94 (m)                      |
| 21  | 13.6        | 1.77 (s)                      | 6'' | 63.7        | 4.36 (dd, 5.0, 12.0)          |
| 22  | 123.4       | 3.63 (1H, m, overlapped)      | 5.55 (t, 7.0) | 4.59 (dd, 2.0, 12.0)          |
Furthermore, the protective effects of *P. notoginseng* 70% EtOH extract and new compounds 1–7 against antimycin A-induced mitochondrial oxidative stress were determined. The 70% ethanolic extract and compounds 4, 6 and 7 showed significant protective effects against antimycin A-induced L6 cell injury (Table 9).

**Table 9.** Cell survival rate of *P. notoginseng* extract and compounds 1–7 on L6 cells treated with antimycin A.

| Sample          | Cell survival rate (%) |
|-----------------|------------------------|
| Normal          | 100.0 ± 0.0 **         |
| Control         | 45.9 ± 0.1             |
| Probucol        | 56.1 ± 1.1 **          |
| *P. notoginseng* ext. | 55.3 ± 1.2 *       |
| 1               | 50.8 ± 1.9             |
| 2               | 56.8 ± 2.5             |
| 3               | 54.2 ± 1.5             |
| 4               | 59.3 ± 2.1 *           |
| 5               | 57.2 ± 3.1             |
| 6               | 59.0 ± 2.1 *           |
| 7               | 57.4 ± 1.6 *           |

Values represent the mean ± SD of determinations (*n* = 8). * p < 0.05; ** p < 0.01 vs. control group. Administered concentration of probucol and 1–7 were 10 μmol/L, *P. notoginseng* ext. was 10 μg/mL. N = 8.

3. Experimental

3.1. General

Optical rotations were measured on a Rudolph Autopol® IV automatic polarimeter. IR spectra were recorded on a Varian 640-IR FT-IR spectrophotometer. UV spectra were obtained on a Varian Cary 50 UV-Vis spectrophotometer. NMR spectra were determined on a Bruker 500 MHz NMR spectrometer at 500 MHz for 1H- and 125 MHz for 13C-NMR, with TMS as an internal standard. Positive- and
Negative-ion HRESI-TOF-MS were recorded on an Agilent Technologies 6520 Accurate-Mass Q-Tof LC/MS spectrometer. Column chromatographies were performed on macroporous resin D101 (Haiguang Chemical Co., Ltd., Tianjin, China), silica gel (48–75 μm, Qingdao Haiyang Chemical Co., Ltd., Qingdao, China), Sephadex LH-20 (Ge Healthcare Bio-Sciences, Uppsala, Sweden), and ODS (40–63 μm, YMC Co., Ltd., Tokyo, Japan). A Cosmosil 5C18-MS-II (20 mm i.d. × 250 mm, Nakalai Tesque, Inc., Tokyo, Japan) preparative HPLC (PHPLC) column was used to purify the constituents. TLC plates pre-coated with silica gel GF254 (Tianjin Silida Technology Co., Ltd., Tianjin, China) were used to detect the purity of isolates by spraying with 10% aqueous H2SO4-EtOH, followed by heating.

3.2. Plant Material

The dried roots of *P. notoginseng* (Burkill, F. H. Chen) were collected from Wenshan, Guangxi province, China and identified by Dr. Li Tianxiang. The voucher specimen was deposited at the Academy of Traditional Chinese Medicine of Tianjin University of TCM (No. 20120505).

3.3. Extraction and Isolation

The dried roots of *P. notoginseng* (5.0 kg) were refluxed twice with 70% ethanol-water (volume) for 2 times. Evaporation of the solvent under reduced pressure provided a 70% ethanol-water extract (480.2 g). The residue was dissolved in H2O, then subjected to D101 CC [EtOH-H2O (0:100 → 50:50 → 100:0, v/v)] to afford three fractions (Fr. 1–3). Fraction 3 (120.0 g) was subjected to silica gel CC [CHCl3 → CHCl3-MeOH (100:3 → 100:7, v/v) → CHCl3-MeOH-H2O (10:3:1 → 7:3:1 → 6:4:1, v/v/v, lower layer)] to give 12 fractions (Fr. 1–12). Fraction 7 (8.0 g) was subjected to normal phase silica gel CC [CHCl3 → CHCl3-MeOH-H2O (40:3:1 → 30:3:1 → 20:3:1 → 10:3:1, v/v/v, lower layer) → MeOH] to yield fourteen fractions (Fr. 7-1-1–7-1-14). Fraction 7-6 (97.9 mg) was purified by prepared HPLC (PHPLC) [MeOH-H2O (70:30, v/v)], and sanchirhinoside A1 (1, 2.9 mg) was obtained. Fraction 8 (4.0 g) was isolated by ODS CC [MeOH-H2O (40:60 → 50:50 → 60:40 → 70:30 → 80:20 → 100:0, v/v)] to give 11 fractions (Fr. 8-1–8-11). Fractions 8-5 (46.6 mg), 8-6 (80.5 mg), and 8-8 (40.2 mg) were purified by PHPLC [MeOH-H2O (60:40, v/v)] to yield sanchirhinosides A4 (4, 1.6 mg), B (7, 3.3 mg), and A2 (2, 7.7 mg), respectively. Fraction 9 (16.0 g) was subjected to ODS CC [MeOH-H2O (30:70 → 40:60 → 50:50 → 60:40 → 70:30 → 100:0, v/v)] to afford nine fractions (Fr. 9-1–9-9). Fraction 9-7 (113.8 mg) was purified by PHPLC [MeOH-H2O (60:40, v/v)], and sanchirhinoside A3 (3, 7.6 mg) was obtained. Fraction 10 (3.6 g) was separated by ODS CC [MeOH-H2O (10:90 → 20:80 → 30:70 → 40:60 → 50:50 → 60:40 → 70:30 → 80:20 → 100:0, v/v)] to afford 15 fractions (Fr. 10-1–10-15). Fraction 10-7 (393.8 mg) was purified by PHPLC [MeOH-H2O (50:50, v/v)] to give sanchirhinoside A5 (5, 8.2 mg). Fraction 12 (10.0 g) was subjected to ODS CC [MeOH-H2O (50:50, v/v)] to give sanchirhinoside A5 (5, 8.2 mg). Fraction 12 (10.0 g) was subjected to ODS CC [MeOH-H2O (10:90 → 20:80 → 30:70 → 40:60 → 50:50 → 60:40 → 100:0, v/v)] to give 13 fractions (Fr. 12-1–12-13). Fraction 12-9 (107.8 mg) was further purified by silica gel CC [CHCl3-MeOH-H2O (7:3:1, v/v/v, lower layer) to yield sanchirhinoside A6 (6, 12.7 mg).

20S-Sanchirhinoside A1 (1): White powder. [α]D25 + 12.6° (c = 0.12, MeOH); IR νmax (KBr) cm⁻¹: 3,365, 2,928, 2,872, 1,717, 1,654, 1,457, 1,375, 1,316, 1,188, 1,085, 1,045. 1H-NMR (500 MHz, CD3OD) and 13C-NMR (125 MHz, CD3OD) spectroscopic data, see Table 1; 1H-NMR (500 MHz, CD3OD) and 13C-NMR (125 MHz, CD3OD) spectroscopic data, see Table 2. HRESI-TOF-MS: Positive-ion mode
m/z 729.4543 [M + Na]⁺ (calcd’ for C₄₀H₆₆O₁₀Na 729.4548); Negative-ion mode m/z 741.4364 [M + Cl]⁻ (calcd for C₄₀H₆₆O₁₀Cl 741.4350).

20S-Sanchirhinoside A₂ (2): White powder. [α]D²⁵ +7.4° (c = 0.33, MeOH); IR νmax (KBr) cm⁻¹: 3,367, 2,931, 2,876, 1,733, 1,642, 1,456, 1,373, 1,242, 1,160, 1,075, 1,043. ¹H NMR (500 MHz, C₅D₅N) and ¹³C-NMR (125 MHz, C₅D₅N) spectroscopic data, see Table 3. Positive-ion mode m/z 835.4832 [M + Na]⁺ (calcd. for C₄₃H₇₂O₁₄Na 835.4814); Negative-ion mode m/z 847.4568 [M + Cl]⁻ (calcd. for C₄₃H₇₂O₁₄Cl 847.4616).

20S-Sanchirhinoside A₃ (3): White powder. [α]D²⁵ + 19.7° (c = 0.36, MeOH); IR νmax (KBr) cm⁻¹: 3,367, 2,927, 2,875, 1,647, 1,457, 1,386, 1,253, 1,074, 1,027. ¹H NMR (500 MHz, C₅D₅N) and ¹³C-NMR (125 MHz, C₅D₅N) spectroscopic data, see Table 4. Positive-ion mode m/z 793.4720 [M + Na]⁺ (calcd. for C₄₁H₇₀O₁₃Na 793.4709); Negative-ion mode m/z 815.4779 [M + COOH]⁻ (calcd. for C₄₂H₇₁O₁₅ 815.4798).

20S-Sanchirhinoside A₄ (4): White powder. [α]D²⁵ + 23.2° (c = 0.08, MeOH); IR νmax (KBr) cm⁻¹: 3,366, 2,929, 2,872, 1,643, 1,457, 1,386, 1,255, 1,127, 1,073, 1,043. ¹H-NMR (500 MHz, C₅D₅N) and ¹³C-NMR (125 MHz, C₅D₅N) spectroscopic data, see Table 5. Positive-ion mode m/z 955.5248 [M + Na]⁺, calcd. for C₄₇H₈₀O₁₈Na 955.5237; Negative-ion mode m/z 967.4950 [M + COOH]⁻ (calcd. for C₄₇H₈₀O₁₈Cl 967.5039).

20S-Sanchirhinoside A₅ (5): White powder. [α]D²⁵ + 105.3° (c = 0.41, MeOH); IR νmax (KBr) cm⁻¹: 3,367, 2,930, 2,875, 1,647, 1,457, 1,386, 1,310, 1,073, 1,042. ¹H-NMR (500 MHz, C₅D₅N) and ¹³C-NMR (125 MHz, C₅D₅N) spectroscopic data, see Table 6. Positive-ion mode m/z 1117.5725 [M + Na]⁺, calcd. for C₅₃H₉₀O₂₃Na 1117.5765; Negative-ion mode m/z 1093.5731 [M – H]⁻ (calcd. for C₅₃H₈₉O₂₃ 1093.5800).

20S-Sanchirhinoside A₆ (6): White powder. [α]D²⁵ + 3.1° (c = 0.55, MeOH); IR νmax (KBr) cm⁻¹: 3,367, 2,929, 2,878, 1,645, 1,456, 1,386, 1,307, 1,074, 1,043. ¹H-NMR (500 MHz, C₅D₅N) and ¹³C-NMR (125 MHz, C₅D₅N) spectroscopic data, see Table 7. Positive-ion mode m/z 805.4700 [M + Na]⁺, calcd. for C₄₂H₇₀O₁₄Na 805.4709; Negative-ion mode m/z 817.4518 [M + Cl]⁻ (calcd. for C₄₂H₇₀O₁₅Cl 817.4510).

Sanchirhinoside B (7): White powder. [α]D²⁵ + 14.7° (c = 0.12, MeOH); IR νmax (KBr) cm⁻¹: 3,367, 2,927, 2,874, 1,653, 1,457, 1,395, 1,151, 1,072, 1,024. ¹H-NMR (500 MHz, C₅D₅N) and ¹³C-NMR (125 MHz, C₅D₅N) spectroscopic data, see Table 8. Positive-ion mode m/z 805.4700 [M + Na]⁺, calcd. for C₄₂H₇₀O₁₄Na 805.4709; Negative-ion mode m/z 817.4518 [M + Cl]⁻ (calcd. for C₄₂H₇₀O₁₅Cl 817.4510).

3.4. Acid Hydrolysis of 1–7

A solution of new compounds 1–7 (each 1.5 mg) in 1 M HCl (1 mL) was heated under reflux for 3 h, respectively. The reaction mixture was neutralized with Amberlite IRA-400 (OH⁻ form) and removed by filtration. The aqueous layer was subjected to the HPLC analysis under the following condition,
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respectively: HPLC column, Kaseisorb LC NH2-60-5, 4.6 mm i.d. × 250 mm (Tokyo Kasei Co. Ltd., Tokyo, Japan); detection, optical rotation [Chiralyser (IBZ Messtechnik GMBH, Hannover, Germany)]; mobile phase, CH3CN-H2O (75:25, v/v); flow rate 1.0 mL/min. As results, D-xylose (from 2, 6), L-arabinose (from 3–5), D-glucose (from 1–7) and were confirmed by comparison of the retention times with the authentic samples \[t_R: 8.8 \text{ min (D-xylose)}, 10.2 \text{ min (L-arabinose)}, \text{ and } 13.1 \text{ min (D-glucose), all of them showed positive optical rotations].

3.5. Mitochondrial Oxidative Stress Protect Effects Assay

Antimycin A was used to induce mitochondrial oxidative stress [11]. Briefly, L6 cells (Cell Resource Center, IBMS, CAMS/PUMC, Beijing, China) were plated at a density of 5 × 10^4 cells/well in Dulbecco’s modified Eagle’s medium (DMEM, Thermo Scientific, UT, USA) supplemented with 10% calf serum (Thermo Scientific) in a 96-well plate and were incubated at 37 °C for 24 h. Cells were treated with or without 10 µmol/L sample DMSO solution (final DMSO concentration was 0.5%). One hour later, medium was removed and 100 µg/mL antimycin A (Sigma Co. Ltd, MO, USA) in 200 µL DMEM was added to each well, The MTT assay was performed 24 h later to detect the cell survival rate. Probucol was used as positive control.

3.6. Statistical Analysis

Values are expressed as mean ± S.D. All the grouped data were statistically performed with SPSS 11.0. Significant differences between means were evaluated by one-way analysis of variance (ANOVA) and Tukey’s Studentized range test was used for post hoc evaluations. \(p < 0.05\) was considered to indicate statistical significance.

4. Conclusions

Antimycin A is known to cause the leakage of superoxide radicals from cell mitochondria by inhibiting mitochondrial electron transport [12]. Compared with normal group, 100 µg/mL antimycin A induced significant L6 cell injury, while 10 µM probucol showed increased cell survival rate effects compared with the antimycin treated group. From the bioactive 70% EtOH extract of \textit{P. notoginseng} roots, seven new protopanaxatriol type saponins, 20S-sanchirhinosides A1–A6 (1–6), and sanchirhinoside B (7) were obtained. Among the new compounds, 4, 6 and 7 showed significant protective effects against antimycin A-induced L6 cell injury. This research will benefit investigation of trace bioactive chemical constituents of \textit{P. notoginseng} root. On the basis of the activity screening results, further studies of the antioxidant mechanisms of compounds 1–7 are necessary.

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Conflicts of Interest

The authors declare no conflict of interest.

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Sample Availability: Samples of compounds 1–7 are available from the authors.

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