Supplementary Information

Rapid preparation of rare ginsenosides by acid transformation and their structure-activity relationships against cancer cells

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[1] These authors contributed equally to this work.

Structure identification of dehydroxylated products

1.1 Structure identification of compound 1.

Compound 1 was obtained as a white powder and can be soluble in methanol. The TOF-MS spectrum showed an $[M-H]^-$ ion at *m/z* 619.4227, and together with an $[M+HCOO]^-$ ion at *m/z* 665.4283, this indicated a molecular formula of $C_{36}H_{60}O_8$.

^{13}C-NMR (500 MHz, C_5D_5N) δ: 40.040 (C-1), 28.430 (C-2), 79.097 (C-3), 41.810 (C-4), 61.965 (C-5), 80.519 (C-6), 45.866 (C-7), 40.860 (C-8), 51.160 (C-9), 40.249 (C-10), 33.227 (C-11), 72.973 (C-12), 52.630 (C-13),51.652 (C-14), 33.011 (C-15), 31.202 (C-16), 48.754 (C-17), 17.863 (C-18), 18.227 (C-19), 155.981 (C-20), 108.655 (C-21), 34.273 (C-22), 27.574 (C-23), 125.837 (C-24), 131.691 (C-25), 26.214 (C-26), 17.863 (C-27), 32.200 (C-28), 16.828 (C-29), 17.274 (C-30), 106.500 (C-1'), 75.963 (C-2'), 80.119 (C-3'), 72.411 (C-4'), 78.582 (C-5'), 63.635 (C-6'). These results are in agreement with data of Rk3 from literature[1]. Compound 1 was identified as Rk3.

1.2 Structure identification of compound 2.

Compound 2 was a white powder and can be soluble in methanol. It had the molecular formula $C_{36}H_{60}O_8$ determined from the quasi-molecular peaks at *m/z* 619.4219 $[M-H]^-$ and *m/z* 665.4274 $[M+HCOO]^-$ in its TOF-MS spectrum.

^{13}C-NMR (500 MHz, C_5D_5N) δ: 39.320 (C-1), 27.717 (C-2), 78.378 (C-3), 40.146 (C-4), 61.246 (C-5), 79.812 (C-6), 45.154 (C-7), 41.162 (C-8), 50.371 (C-9), 39.559 (C-10), 32.054 (C-11), 72.373 (C-12), 50.512 (C-13),50.627 (C-14), 32.335

(C-15), 28.573 (C-16), 50.193 (C-17), 17.182 (C-18), 17.502 (C-19), 139.926 (C-20), 12.892 (C-21), 123.052 (C-22), 27.217 (C-23), 123.448 (C-24), 131.032 (C-25), 25.428 (C-26), 17.502 (C-27), 31.497 (C-28), 16.124 (C-29), 16.609 (C-30), 105.792 (C-1'), 75.250 (C-2'), 79.418 (C-3'), 71.698 (C-4'), 77.878 (C-5'), 62.934 (C-6'). These results are in agreement with data of Rh4 from literatures[1,2]. Compound 2 was identified as Rh4.

1.3 Structure identification of compound 3

Compound 3 was a white powder and can be soluble in methanol. It had the molecular formula $C_{42}H_{70}O_{12}$ deduced from an $[M-H]^-$ molecular peak at *m/z* 765.4782 and an $[M+HCOO]^-$ molecular peak at *m/z* 811.4829 in its negative TOF-MS.

^{13}C-NMR (500MHz, C_5D_5N) δ: 39.801 (C-1), 27.260 (C-2), 89.430 (C-3), 40.215 (C-4), 56.937 (C-5), 18.949 (C-6), 35.860 (C-7), 40.717 (C-8), 48.728 (C-9), 37.539 (C-10), 33.092 (C-11), 72.971 (C-12), 53.005 (C-13),51.707 (C-14), 33.174 (C-15), 31.254 (C-16), 51.359 (C-17), 17.078 (C-18), 17.078 (C-19), 156.074 (C-20), 108.638 (C-21), 34.395 (C-22), 27.589 (C-23), 125.823 (C-24), 131.693 (C-25), 26.209 (C-26), 18.216 (C-27), 28.614 (C-28), 17.477 (C-29), 17.477 (C-30), 105.584 (C-1'), 84.028 (C-2'), 78.670 (C-3'), 72.172 (C-4'), 78.471 (C-5'), 63.280 (C-6'), 106.560 (C-1''), 77.597 (C-2''), 78.854 (C-3''), 72.249 (C-4''), 78.555 (C-5''), 63.392 (C-6''). These results are in agreement with data of Rk1 from literature[1]. Compound 3 was identified as Rk1.

1.4 Structure identification of compound 4

Compound 4 was obtained as a white powder and can be soluble in methanol. The TOF-MS spectrum showed an $[M-H]^-$ ion at *m/z* 765.4806, and together with an $[M+HCOO]^-$ ion at *m/z* 811.4861, this indicated a molecular formula of $C_{42}H_{70}O_{12}$.

^{13}C-NMR (500MHz, C_5D_5N) δ: 39.319 (C-1), 26.778 (C-2), 88.952 (C-3), 39.735 (C-4), 56.450 (C-5), 18.465 (C-6), 35.381 (C-7), 40.294 (C-8), 50.798 (C-9), 37.064 (C-10), 32.225 (C-11), 72.627 (C-12), 50.459 (C-13), 51.070 (C-14), 32.225 (C-15), 28.839 (C-16), 50.922 (C-17), 16.609 (C-18), 16.609 (C-19), 140.213 (C-20), 13 (C-21), 123.305 (C-22), 27.461 (C-23), 123.701 (C-24), 131.280 (C-25), 25.673 (C-26), 17.717 (C-27), 28.137 (C-28), 17.046 (C-29), 17.046 (C-30), 105.114 (C-1'), 83.524 (C-2'), 78.208 (C-3'), 71.682 (C-4'), 77.981 (C-5'), 62.777 (C-6'), 105.114 (C-1''), 77.124 (C-2''), 78.366 (C-3''), 71.744 (C-4''), 78.088 (C-5''), 62.899 (C-6''). These results are in agreement with data of Rg5 from literature[1]. Compound 4 was identified as Rg5.

1.5 Structure identification of compound 5

Compound 5 was a white powder and can be soluble in methanol. It had the molecular formula $C_{36}H_{60}O_7$ determined from the quasi-molecular peaks at *m/z* 603.4283 $[M-H]^-$ and *m/z* 645.4348 $[M+HCOO]^-$ in its TOF-MS spectrum.

^{13}C-NMR (500MHz, C_5D_5N) δ: 39.812 (C-1), 27.254 (C-2), 89.295 (C-3), 40.206 (C-4), 56.949 (C-5), 18.988 (C-6), 35.897 (C-7), 40.829 (C-8), 51.378 (C-9),

37.627 (C-10), 33.095 (C-11), 72.965 (C-12), 53.017 (C-13),51.728 (C-14), 34.207 (C-15), 28.896 (C-16), 48.752 (C-17), 16.364 (C-18), 16.948 (C-19), 140.313 (C-20), 125.049 (C-21), 33.207 (C-22), 27.605 (C-23), 125.845 (C-24), 140.313 (C-25), 26.229 (C-26), 18.253 (C-27), 28.673 (C-28), 17.290 (C-29), 17.509 (C-30), 107.434 (C-1'), 76.313 (C-2'), 79.279 (C-3'), 72.453 (C-4'), 78.833 (C-5'), 63.637 (C-6'). These results are in agreement with data of Rk2 from literature[1]. Compound 5 was identified as Rk2.

1.6 Structure identification of compound 6

Compound 6 was a white powder and can be soluble in methanol. It had the molecular formula $C_{36}H_{60}O_7$ deduced from an $[M-H]^-$ molecular peak at *m/z* 603.4254 and an $[M+HCOO]^-$ molecular peak at *m/z* 645.4307 in its negative TOF-MS.

^{13}C-NMR (500MHz, C_5D_5N) δ: 39.802 (C-1), 28.657 (C-2), 89.282 (C-3), 40.796 (C-4), 56.937 (C-5), 16.936 (C-6), 35.859 (C-7), 40.190 (C-8), 51.288 (C-9), 37.599 (C-10), 32.712 (C-11), 73.087 (C-12), 50.945 (C-13), 51.564 (C-14), 33.135 (C-15), 27.244 (C-16), 51.400 (C-17), 18.694 (C-18), 17.271 (C-19), 140.693 (C-20), 13.654 (C-21), 124.299 (C-22), 27.947 (C-23), 123.534 (C-24), 131.767 (C-25), 26.149 (C-26), 18.196 (C-27), 29.323 (C-28), 16.340 (C-29), 17.547 (C-30), 107.435 (C-1'), 76.302 (C-2'), 79.266 (C-3'), 72.450 (C-4'), 78.832 (C-5'), 63.634 (C-6'). These results are in agreement with data of Rh3 from literature[1]. Compound 6 was identified as Rh3.

Reference

1 Park, I. H. *et al.* Three new dammarane glycosides from heat processed ginseng. *Arch. Pharm. Res.* **25**, 428-432 (2002).

2 Baek, N. I. *et al.* Ginsenoside Rh4, a genuine dammarane glycoside from Korean red ginseng. *Planta Med.* **62**, 86-87 (1996).

Figure Legends

Figure S1 ^{13}C-NMR spectra of compound 1.

Figure S2 ^{13}C-NMR spectra of compound 2.

Figure S3 ^{13}C-NMR spectra of compound 3.

Figure S4 ^{13}C-NMR spectra of compound 4.

Figure S5 ^{13}C-NMR spectra of compound 5.

Figure S6 ^{13}C-NMR spectra of compound 6.

Figure S7 Antiproliferation effect of 23 ginsenosides on six human cancer cells (HCT-116, HepG2, MCF-7, Hela, PANC-1, and A549). The antiproliferative effect was determined by the MTT assay and calculated by comparison with the control after exposure to 5, 10, 20, 40, and 80 μM concentration of ginsenosides for 24 h. The data are expressed as the mean ± SD.

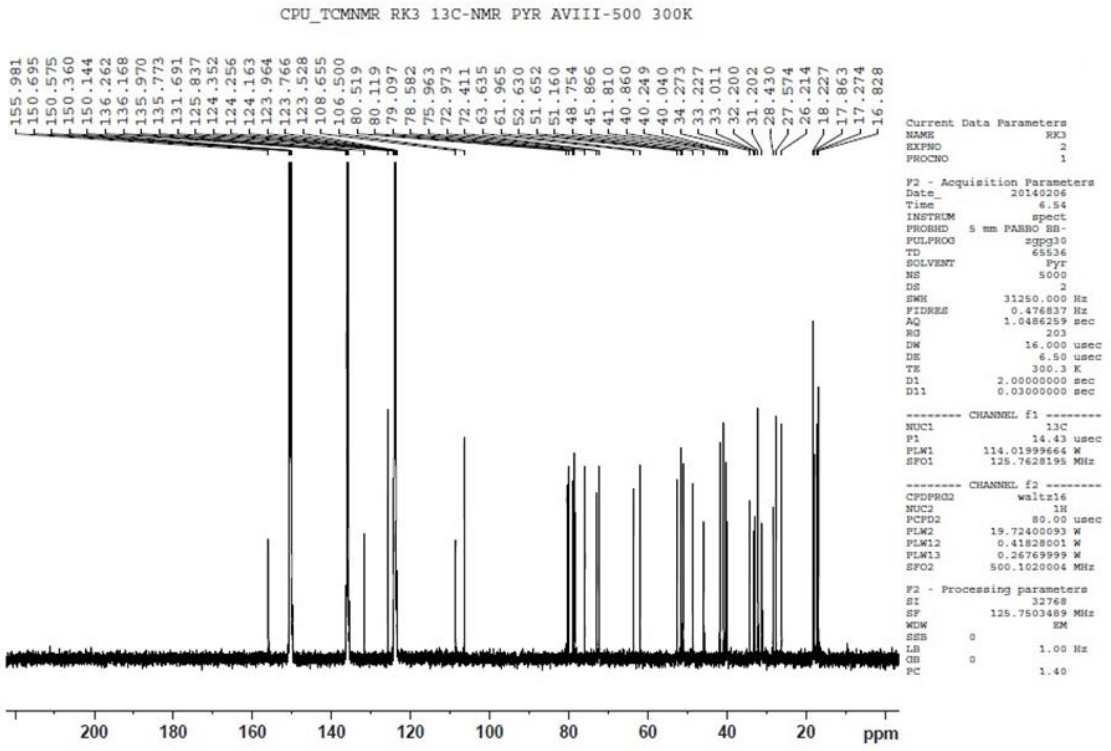
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Figure S1

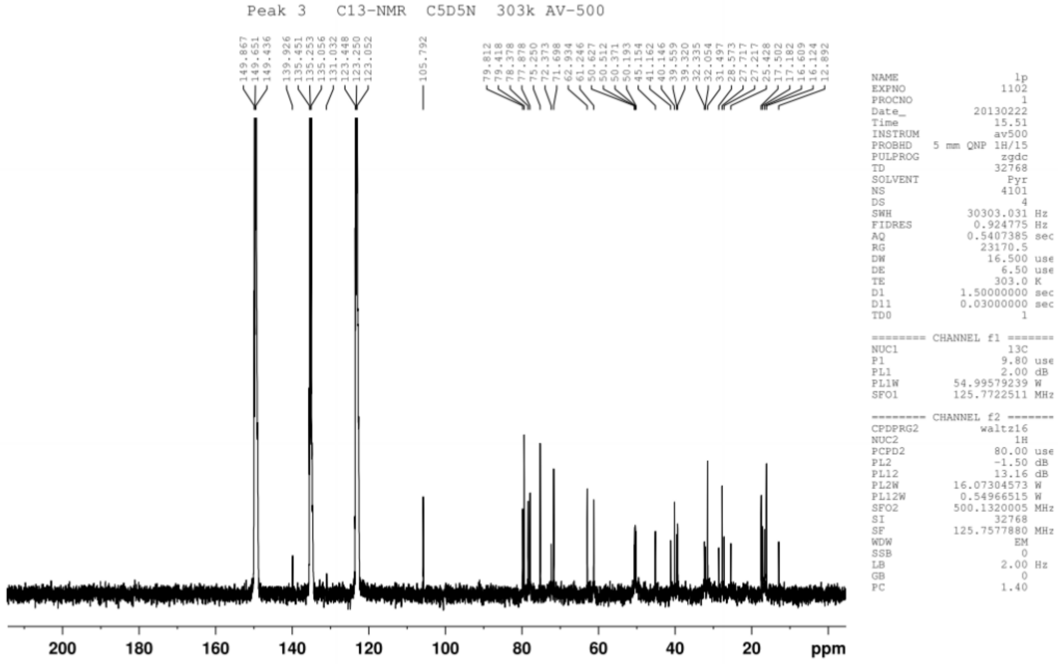
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Figure S2

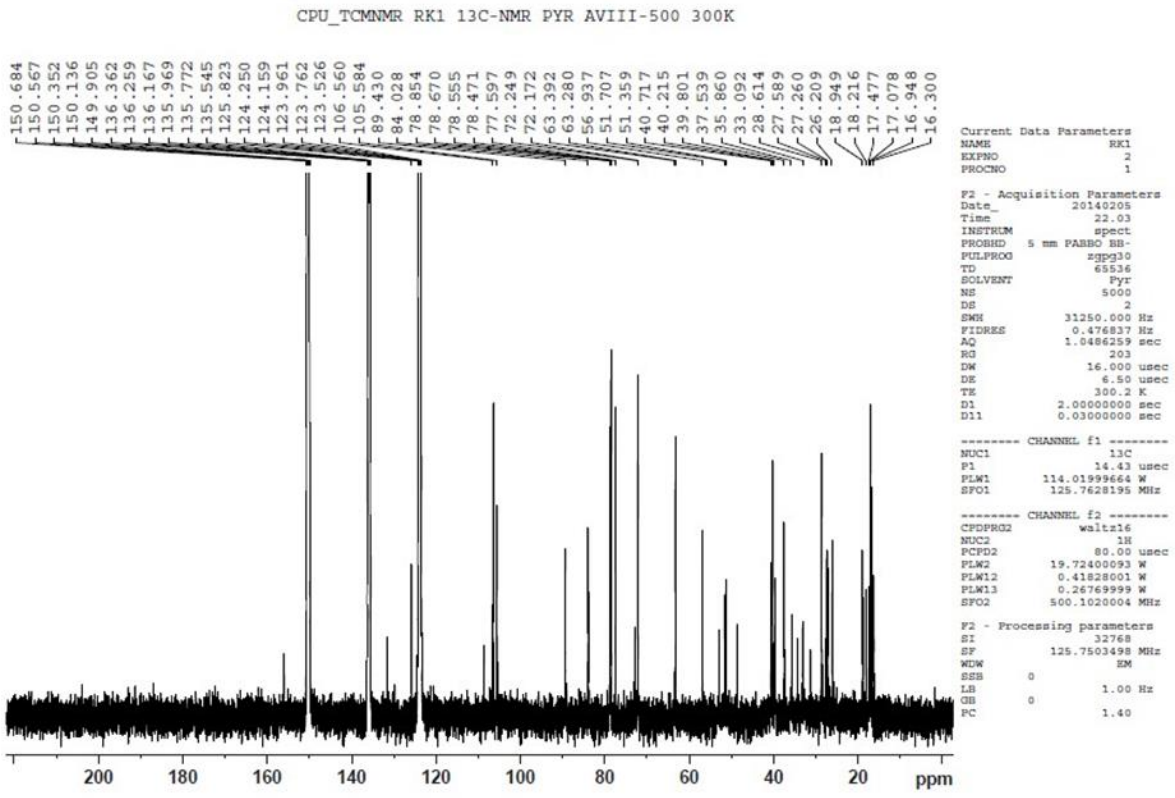
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Figure S3

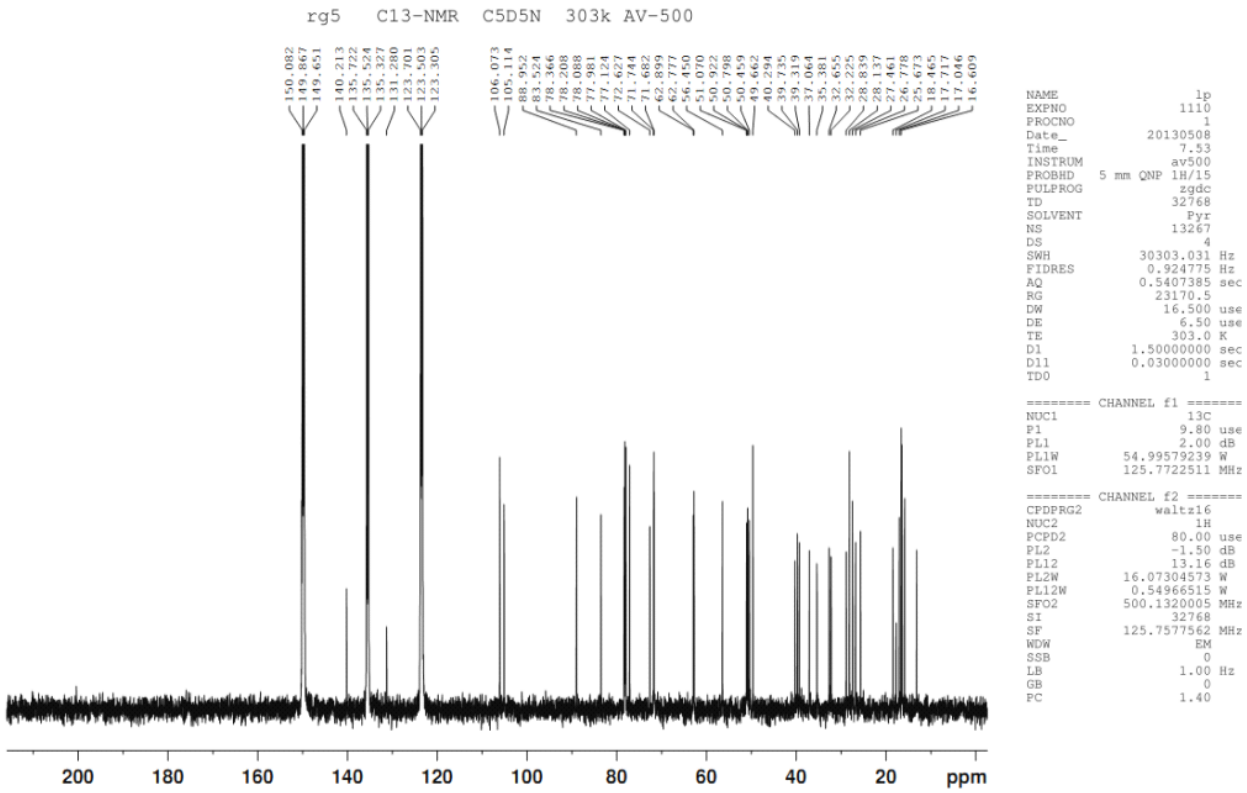
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Figure S4

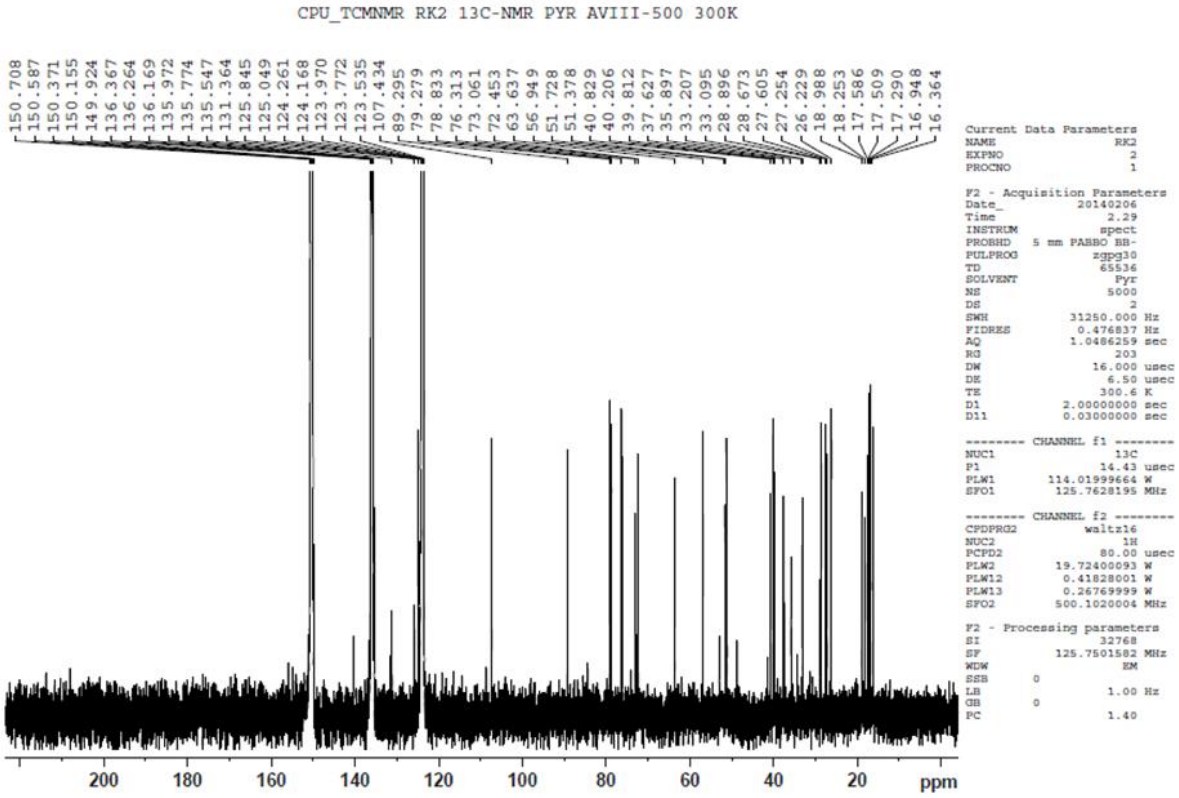
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Figure S5

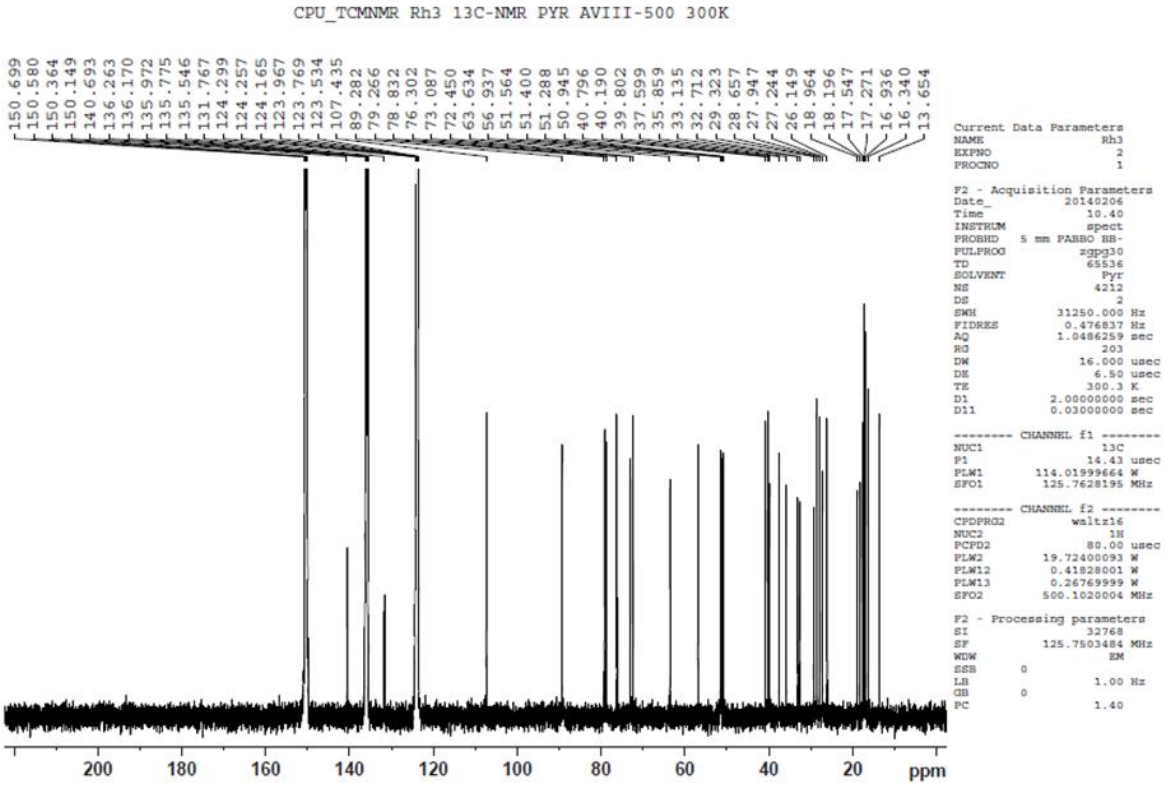
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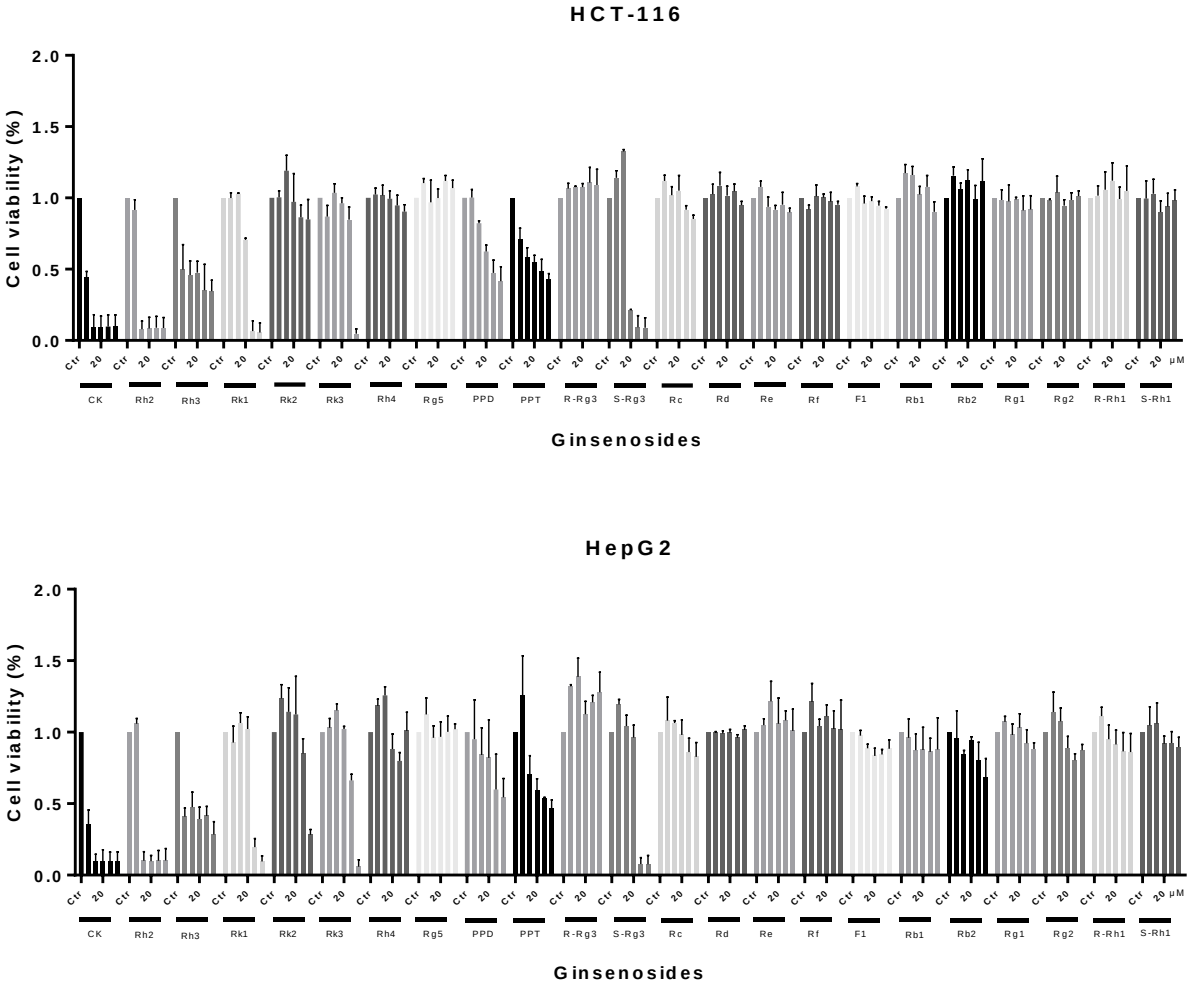
Figure S6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Rk3
Rh4
Rg5
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S-Rg3
Rc
Rd
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Rf
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Rb2
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Rg2
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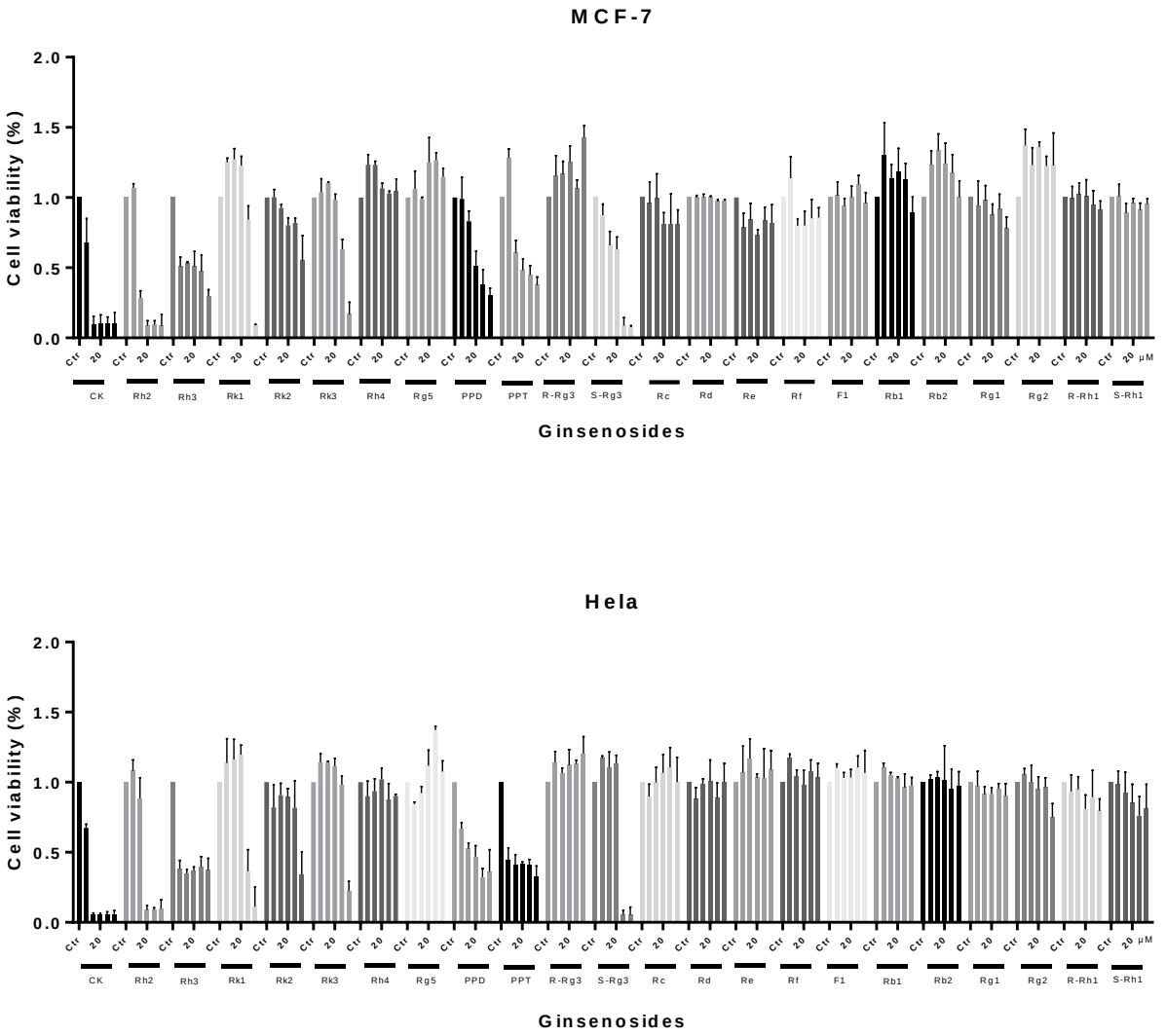
Ginsenosides
HepG2
Cell viability (%)
Ginsenosides


MCF-7
Cell viability (%)
2.0
1.5
1.0
0.5
0.0
Ctr
20
μM
CK
Rh2
Rh3
Rk1
Rk2
Rk3
Rh4
Rg5
PPD
PPT
R-Rg3
S-Rg3
Rc
Rd
Re
Rf
F1
Rb1
Rb2
Rg1
Rg2
R-Rh1
S-Rh1
Ginsenosides
Hela
Cell viability (%)
Ginsenosides

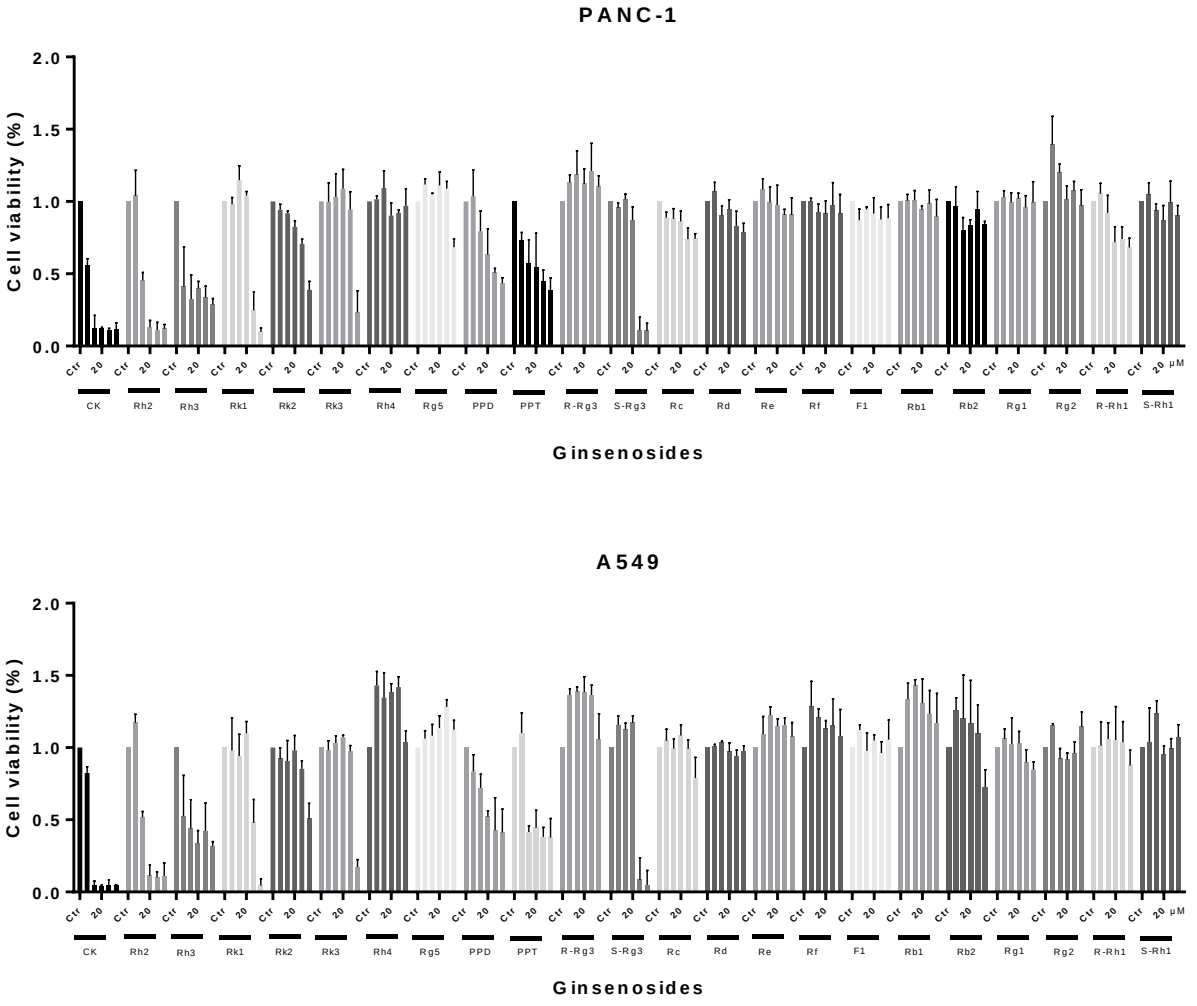

PANC-1
Cell viability (%)
2.0
1.5
1.0
0.5
0.0
Ctr
20
μM
CK
Rh2
Rh3
Rk1
Rk2
Rk3
Rh4
Rg5
PPD
PPT
R-Rg3
S-Rg3
Rc
Rd
Re
Rf
F1
Rb1
Rb2
Rg1
Rg2
R-Rh1
S-Rh1
Ginsenosides
A549
Cell viability (%)
Ginsenosides

Figure S7