Supporting Information

Schistosomicidal and Antioxidant Flavonoids from *Astragalus englerianus*

Bei Chao-Jiang Xiao, Yu Zhang, Lin Qiu, Xiang Dong Jiang

Affiliation

Institute of Materia Medica, College of Pharmacy and Chemistry, Dali University, Dali, P. R. China

Correspondence

*Prof. Dr. Bei Jiang*

Institute of Materia Medica
College of Pharmacy and Chemistry
Dali University
Xueren Road 2
Dali
China
Phone: +86 872 225 7316
Fax: +86 872 225 7401
dalinorthjiang@163.com
Experimental Section

Extraction and isolation

(3R)-sativan (4): Colorless cluster crystals (petroleum ether–ethyl acetate). \([\alpha]_D^{16.2}-11.36\) (c 0.96, MeOH); \(^1\)H NMR (400 MHz, CD\(_3\)COCD\(_3\)) \(\delta\): 8.33 (1H, brs, 7-OH), 7.07 (1H, d, \(J = 8.2\) Hz, H-6'), 6.89 (1H, d, \(J = 8.2\) Hz, H-5), 6.57 (1H, d, \(J = 2.3\) Hz, H-3'), 6.49 (1H, dd, \(J = 8.2, 2.3\) Hz, H-5'), 6.41 (1H, dd, \(J = 8.2, 2.4\) Hz, H-6), 6.34 (1H, d, \(J = 2.4\) Hz, H-8), 4.20 (1H, ddd, \(J = 10.4, 3.2, 1.9\) Hz, H-2a), 3.93 (1H, d, \(J = 10.4\) Hz, H-2b), 3.83 and 3.77 (each 3H, s, 2×-OCH\(_3\)), 3.47 (1H, m, H-3), 2.92 (1H, dd, \(J = 15.5, 10.8\) Hz, H-4a), 2.77 (1H, ddd, \(J = 15.5, 5.1, 1.9\) Hz, H-4b); \(^{13}\)C NMR (100 MHz, CD\(_3\)COCD\(_3\)) \(\delta\): 70.6 (t, C-2), 32.4 (d, C-3), 31.2 (t, C-4), 131.1 (d, C-5), 108.9 (d, C-6), 157.6 (s, C-7), 103.8 (d, C-8), 156.1 (s, C-9), 114.3 (s, C-10), 122.5 (s, C-1'), 159.2 (s, C-2'), 99.4 (d, C-3'), 160.8 (s, C-4'), 105.5 (d, C-5'), 128.4 (d, C-6'), 55.9 and 55.7 (q, 2×-OCH\(_3\)).

(6aR,11aR)-maackiain (5): Colorless cluster crystals (petroleum ether–ethyl acetate). \([\alpha]_D^{14.1}-239.46\) (c 0.27, acetone); ESI-MS \(m/z\): 307 [M + Na]\(^+\). \(^1\)H NMR (400 MHz, CD\(_3\)COCD\(_3\)) \(\delta\): 8.63 (1H, brs, 3-OH), 7.31 (1H, d, \(J = 8.4\) Hz, H-1), 6.90 (1H, s, H-7), 6.57 (1H, dd, \(J = 8.4, 2.4\) Hz, H-2), 6.41 (1H, s, H-10), 6.37 (1H, d, \(J = 2.4\) Hz, H-4), 5.94, 5.92 (each 1H, d, \(J = 0.8\) Hz, -OCH\(_2\)-), 5.50 (1H, d, \(J = 5.5\) Hz, H-11a), 4.28 (1H, dd, \(J = 10.4, 4.5\) Hz, H-6 a), 3.62 (1H, t, \(J = 10.4\) Hz, H-6 b), 3.57 (1H, m, H-6a); \(^{13}\)C NMR (100 MHz, CD\(_3\)COCD\(_3\)) \(\delta\): 133.1 (d, C-1), 110.5 (d, C-2), 159.7 (s, C-3), 103.9 (d, C-4), 157.7 (s, C-4a), 67.0 (t, C-6), 41.1 (d, C-6a), 119.5 (s, C-6b), 105.9 (d, C-7), 142.5 (s, C-8), 148.9 (s, C-9), 94.0 (d, C-10), 155.3 (s, C-10a), 79.4 (d, C-11a), 112.5 (s, C-11b), 102.1 (t, -OCH\(_2\)-).

Schistosomicidal assay
The schistosomicidal assay was performed according to a previous method. Briefly, *Schistosoma japonicum* worms harvested from rabbits were washed in RPMI 1640 medium. Worms were incubated in a 24-well culture plate containing 1.9 mL of the same medium with 10% fetal bovine serum at 37°C in a 5% CO₂ atmosphere, six worms per well. After 2 h, 0.1 mL of different concentration sample solutions were added to the culture. Samples were dissolved in RPMI 1640 containing 2.5% dimethyl sulfoxide (DMSO). The control worms were assayed in RPMI 1640 medium with 2.5% DMSO as a negative control group and in 0.12 mM praziquantel (PZQ) as a positive control group.

Worm mortality and vigor reduction rates were monitored per 12 h for two days using an inverted microscope and a stereomicroscope. The worm mortality rate (MR%) and vigor reduction rate (VR%) were according to following formulas:

\[
MR\% = \frac{\text{Number of dead worms}}{\text{Number of all worms}} \times 100
\]

\[
VR\% = \left(\frac{S_C - S_T}{S_C}\right) \times 100
\]

where \(S_C\) is the total score for the negative control group worms incubated at the 0 h, and \(S_T\) is the total score for the trial group worms incubated in a drug-containing medium at certain periods. The scoring criteria is as follows: the worm is very active, its body is natural, deft, and hyaline (4 points); the worm is active, its body is weakly inflexible and translucent (3 points); the worm is inactive, its body is inflexible and translucent (2 points); only the head and tail or sucker of the worm show some motion, the body is stiff and opaque with white color (1 point); the worm did not move within two minutes, its body is stiff and opaque with white color, the worm is dead (0 point).

This study was conducted according to protocols approved by the institutional ethical committee of Dali University (approval No.: 2012-016). All animals were handled in strict accordance with good animal practice as defined by the Animals Use Ethics Committee of the Dali University and the Institute of Materia Medica; the study was conducted adhering to the institution’s guidelines for animal husbandry.
**DPPH free radical scavenging capability assay**

Antioxidant activity was tested by the DPPH method, which has been previously described. In short, the reaction mixture containing 50 μL of sample solution [different concentrations in dimethyl sulfoxide (DMSO)] and 150 μL of DPPH (100 μM) in methanol was put in a 96-well plate and incubated at 37°C for 30 min. Then the absorbance (A) was measured at 517 nm using an automated microplate reader. The percent of radical scavenging activity was determined by comparison with a DMSO-containing control. The inhibition percentage (I%) was derived from the equation:

\[
I\% = \left[\frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}}\right] \times 100
\]

where \(A_{\text{blank}}\) and \(A_{\text{sample}}\) are the absorbencies of the control and test sample, respectively. \(\text{IC}_{50}\) values represent the concentration of compounds to scavenge 50% of DPPH radicals and are expressed as means ± SD of four separate experiments. Vitamin C (Vc) was used as a positive control.
Legends for Supporting Information Figures

Fig. 1S $^1$H NMR spectrum for compound 1 (400 MHz, pyridine-$d_5$).
Fig. 2S DEPT and $^{13}$C NMR spectra for compound 1 (100 MHz, pyridine-$d_5$).
Fig. 3S HSQC spectrum for compound 1 ($f_2$ 400 MHz, $f_1$ 100 MHz, pyridine-$d_5$).
Fig. 4S HMBC spectrum for compound 1 ($f_2$ 400 MHz, $f_1$ 100 MHz, pyridine-$d_5$).
Fig. 5S $^1$H-$^1$H COSY spectrum for compound 1 (400 MHz, pyridine-$d_5$).
Fig. 6S IR spectrum for compound 1 (KBr pellet).
Fig. 7S UV spectrum for compound 1 (MeOH).
Fig. 8S EI-MS spectrum for compound 1.
Fig. 9S HR-EI-MS spectrum for compound 1.
Fig. 10S $^1$H NMR spectrum for compound 2 (400 MHz, CD$_3$COCD$_3$).
Fig. 11S DEPT and $^{13}$C NMR spectra for compound 2 (100 MHz, CD$_3$COCD$_3$).
Fig. 12S HSQC spectrum for compound 2 ($f_2$ 400 MHz, $f_1$ 100 MHz, CD$_3$COCD$_3$).
Fig. 13S HMBC spectrum for compound 2 ($f_2$ 400 MHz, $f_1$ 100 MHz, CD$_3$COCD$_3$).
Fig. 14S $^1$H-$^1$H COSY spectrum for compound 2 (400 MHz, CD$_3$COCD$_3$).
Fig. 15S IR spectrum for compound 2 (KBr pellet).
Fig. 16S UV spectrum for compound 2 (MeOH).
Fig. 17S EI-MS spectrum for compound 2.
Fig. 18S HR-EI-MS spectrum for compound 2.
Fig. 19S $^1$H NMR spectrum for compound 3 (400 MHz, CD$_3$OD).
Fig. 20S DEPT and $^{13}$C NMR spectra for compound 3 (100 MHz, CD$_3$OD).
Fig. 21S HSQC spectrum for compound 3 ($f_2$ 400 MHz, $f_1$ 100 MHz, CD$_3$OD).
Fig. 22S HMBC spectrum for compound 3 ($f_2$ 400 MHz, $f_1$ 100 MHz, CD$_3$OD).
Fig. 23S $^1$H-$^1$H COSY spectrum for compound 3 (400 MHz, CD$_3$OD).
Fig. 24S IR spectrum for compound 3 (KBr pellet).
Fig. 25S UV spectrum for compound 3 (MeOH).
Fig. 26S EI-MS spectrum for compound 3.
Fig. 27S HR-EI-MS spectrum for compound 3.
Fig. 1S $^1$H NMR spectrum for compound 1 (400 MHz, pyridine-$d_5$).

Fig. 2S DEPT and $^{13}$C NMR spectra for compound 1 (100 MHz, pyridine-$d_5$).
Fig. 3S HSQC spectrum for compound 1 (f$_2$ 400 MHz, f$_1$ 100 MHz, pyridine-$d_5$).

Fig. 4S HMBC spectrum for compound 1 (f$_2$ 400 MHz, f$_1$ 100 MHz, pyridine-$d_5$).
Fig. 5S $^1$H-$^1$H COSY spectrum for compound 1 (400 MHz, pyridine-$d_5$).

Fig. 6S IR spectrum for compound 1 (KBr pellet).
Fig. 7S UV spectrum for compound 1 (MeOH).

Fig. 8S EI-MS spectrum for compound 1.
Fig. 9S HR-EI-MS spectrum for compound 1.

Fig. 10S $^1$H NMR spectrum for compound 2 (400 MHz, CD$_3$COCD$_3$).
Fig. 11S DEPT and $^{13}$C NMR spectra for compound 2 (100 MHz, CD$_3$COCD$_3$).

Fig. 12S HSQC spectrum for compound 2 ($f_2$ 400 MHz, $f_1$ 100 MHz, CD$_3$COCD$_3$).
Fig. 13S HMBC spectrum for compound 2 ($f_2$ 400 MHz, $f_1$ 100 MHz, CD$_3$COCD$_3$).

Fig. 14S $^1$H-$^1$H COSY spectrum for compound 2 (400 MHz, CD$_3$COCD$_3$).
Fig. 15S IR spectrum for compound 2 (KBr pellet).

Fig. 16S UV spectrum for compound 2 (MeOH).
Fig. 17S EI-MS spectrum for compound 2.

Fig. 18S HR-EI-MS spectrum for compound 2.
Fig. 19S $^1$H NMR spectrum for compound 3 (400 MHz, CD$_3$OD).

Fig. 20S DEPT and $^{13}$C NMR spectra for compound 3 (100 MHz, CD$_3$OD).
Fig. 21S HSQC spectrum for compound 3 ($f_2$ 400 MHz, $f_1$ 100 MHz, CD$_3$OD).

Fig. 22S HMBC spectrum for compound 3 ($f_2$ 400 MHz, $f_1$ 100 MHz, CD$_3$OD).
Fig. 23S $^1$H-$^1$H COSY spectrum for compound 3 (400 MHz, CD$_3$OD).

Fig. 24S IR spectrum for compound 3 (KBr pellet).
Fig. 25S UV spectrum for compound 3 (MeOH).

Fig. 26S EI-MS spectrum for compound 3.
Fig. 27S HR-EI-MS spectrum for compound 3.