Supplementary material

Ginsenosides from Korean red ginseng modulate T cell function via the regulation of NF-AT-mediated IL-2 production.

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CONTENTS

General experimental procedures

Optical rotations were determined on a JASCO P-2000 polarimeter. IR spectra were obtained on a Bruker TENSOR 37 FT-IR spectrometer (Bruker Optics, Ettlingen, Germany). The high-resolution electrospray ionization mass spectra (HR-ESI-MS) were gained using an AGILENT 6530 Accurate-Mass Q-TOF LC/MS system. The ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) spectra were recorded on a Bruker Avance 600 MHz spectrometer (Billerica, MA). TMS was used as an internal standard. Column chromatography (CC) was performed on silica gel (Kieselgel 60, 70–230 mesh and 230–400 mesh, Merck, Darmstadt, Germany) and YMC RP-18 resins (30 - 50 μm, Fuji Silysia Chemical Ltd., Kasugai, Aichi, Japan). Thin layer chromatography (TLC) was performed on silica gel 60 F_{254} (1.05554.0001, Merck, Darmstadt, Germany) and RP-18 F_{254S} plates (1.15685.0001, Merck, Darmstadt, Germany) and compounds were visualized by spraying with aqueous 10% H_2SO_4 solution, followed by heating.

Extraction and isolation

Korean red ginseng (2.4 kg) was extracted three times in refluxing (H_2O). The water extract (600 g) was separated through a Diaion (HP-20) CC eluting with a gradient solvent mixture of MeOH in H_2O (0 %, 50 %, and 100 %) to yield three fractions (W1 to W3), based on TLC analysis. The fraction W2 (250 g) was crudely separated with silica gel CC eluting with gradient solvent systems of CH_2Cl_2-MeOH (25:1-1:0) to yield seven fractions (W2A-W2G), respectively. The fraction W2C (10 g) was then isolated by MPLC RP-18 CC using acetone-water (0.5:1, v/v) to give six subfractions (W2C1-W2C6). Purification of the subfraction W2C3 (3 g) using adsorbents Sephadex LH-20 resin, silica gel and the eluent CH_2Cl_2-MeOH (10:1) gave compounds **1** (8.7 mg), **2** (7.8 mg), and **3** (12.5 mg). Repeating the same steps above, subfraction W2C4 (2 g) was purified on a Sephadex LH-20 and silica gel columns with the eluent CH_2Cl_2-MeOH (10:1) to give compounds **4** (27.8 mg), and **5** (16.4 mg).

Similarly, the fraction W2E (12 g) was separated via MPLC RP-18 CC with a gradient solvent mixture of MeOH-H_2O (1:1-1:0, v/v) and purified with Sephadex LH-20 column by mixture of MeOH- acetone -H_2O (7.5:1:1, v/v/v) to obtain compounds **6** (15.7 mg), **9** (30.6 mg), and **11** (10.2 mg). Next, the fraction W2G was purified by silica gel CC using solvent mixture of CH_2Cl_2- EtOAc-MeOH (7:1:1, v/v/v) to yield four subfractions (W2G1-W2G4). The subfraction W2G2 was isolated by Sephadex LH-20 column and further purified with RP-18 column using MeOH-H_2O (4:1) to afford compounds **7** (7.5 mg), **8** (20.3 mg), and **10** (9.7 mg).