Am J Cancer Res 2015;5(4):1308-1318
www.ajcr.us /ISSN:2156-6976/ajcr0006260

Original Article

Mogrol represents a novel leukemia therapeutic, via ERK and STAT3 inhibition

Can Liu[1,2,3*], Yan Zeng[2*], Long-Hai Dai[2*], Tian-Yu Cai[1], Yue-Ming Zhu[2], De-Quan Dou[1], Lan-Qing Ma[1,3], Yuan-Xia Sun[2]

[1]*Beijing University of Agriculture, Beijing 102206, China;* [2]*National Engineering Laboratory for Industrial Enzymes, Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, Tianjin 300308, China;* [3]*Key Laboratory of Urban Agriculture (North) of Ministry of Agriculture P. R. China, Beijing University of Agriculture, Beijing 102206, China.* [*]*Equal contributors.*

Received January 22, 2015; Accepted March 10, 2015; Epub March 15, 2015; Published April 1, 2015

Abstract: Unlike solid tumors, the primary strategy for leukemia treatment is chemotherapy. However, leukemia chemotherapy is associated with adverse drug effects and drug resistance. Therefore, it is imperative to identify novel agents that effectively treat leukemia while minimizing adverse effects. The Raf/MEK/extracellular regulated kinase (ERK) and signal transducer and activator of transcription 3 (STAT3) pathways have been implicated in leukemia carcinogenesis, and provide novel molecular targets for therapeutic intervention in cancer. Mogrol, a biometabolite of mogrosides found in *Siraitia grosvenorii*, has exhibited anti-cancer activities; however, the underlying mechanism of this effect remains unclear. To clarify its anti-cancer activity and mechanism of action, we treated K562 leukemia cells with mogrol. Mogrol suppressed leukemia cell growth via inhibition of the ERK1/2 and STAT3 pathways, in particular, through the suppression of p-ERK1/2 and p-STAT3. Inhibition of these pathways suppressed Bcl-2 expression, thereby inducing K562 cell apoptosis. Furthermore, mogrol enhanced p21 expression, resulting in G0/G1 cell cycle arrest. The findings provide new perspectives regarding the role of mogrol in leukemia treatment.

Keywords: Mogrosides, mogrol, apoptosis, ERK1/2, STAT3

Introduction

Leukemia is a malignant disease of blood cell-forming tissues. Unlike solid tumors, malignant hematological tumors cannot be cured by surgical treatment or radiation therapy. Therefore, chemotherapy is the main strategy for leukemia treatment [1]. However, over time, leukemia cells can show a reduced sensitivity to cytotoxic drugs, leading to multi drug resistance. Furthermore, currently available chemotherapeutics can induce adverse side effects [2]. Thus, it is imperative to identify novel chemotherapeutic agents that treat leukemia while minimizing adverse effects.

Increased knowledge of the molecular mechanisms underlying cancer may provide novel molecular targets for therapeutic intervention [3]. The ERK cascade is primarily activated by growth factors, and is critical for proliferation and survival [4]. Signal transducer and activator of transcription 3 (STAT3) is another well-known oncogene that is constitutively activated in many human cancers [5]. Emerging literature suggests that ERK and STAT3 signaling may play a critical role in cancer formation and progression [6, 7]. Furthermore, aberrant activation of the ERK pathway [8, 9] and constitutively active STAT3 is frequently found in leukemia [10, 11]. Thus, targeting STAT3 or ERK with novel, low-toxicity anticancer compounds could be promising for leukemia treatment.

Mogrosides (triterpene glycosides) are non-caloric sweeteners derived from *Siraitia grosvenorii*, a traditional Chinese fruit used in folk medicine. A previous study showed that oral administration of mogrosides had no significant adverse effects during clinical observations [12]. Furthermore, mogrosides can inhibit lipopolysaccharide-induced inflammation [13] and have an anti-oxidative effect on lipid peroxidation [14]. These data indicate that mogroside supplementation may prevent complications and attenuate pathological conditions in type 2 diabetes [15]. Additionally, mogrosides effectively inhibited tumor growth in a two-stage car-

cinogenesis test, using a mouse skin tumor induced by 7,12-dimethylbenz(a)anthracene (DMBA) [16].

Based on these data, mogrosides may represent a novel class of bioactive plant compounds with anticancer effects. However, its clinical applications are limited, because the mechanism of its anticancer effects remains unclear. It is worthwhile to note that mogrosides are digested to mogrol *in vivo*, and thus mogrol may induce the pharmacological effects of mogrosides. This paper reports the anti-leukemia effects of mogrol and its mechanism of anticancer action.

Materials and methods

Cell culture

The human leukemia cell line, K562, was obtained from Shanghai Cell Biology Institute (Shanghai, China). Cells were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum (Gibco; Grand Island, NY, USA), 100 U/mL penicillin, and 100 μg/mL streptomycin in a humidified 5% CO_2 atmosphere at 37°C.

Preparation of mogrol

S. grosvenorii was provided by White Pagoda Pharmacy (Beijing, China). Mogrol was prepared as follows. First, mogrosides were extracted from *S. grosvenorii* as previously described [17]. Dry *S. grosvenorii* fruit was pulverized to 60-80 mesh and extracted with ethanol. Mogrosides were then obtained by chromatographic separation with Amberlite XAD-16 resin (Rohm & Haas Co.; Philadelphia, PA, USA) and ion-exchange resin D213 (Dandong Chemical Factory; Liaoning, China). Mogrol was produced by hydrolysis of the obtained mogrosides.

Hydrolysis was performed using 0.5N HCl at 95-100°C for 10 h. Mogrosides were degraded to mogrol and glucose, and mogrol was purified using a preparative high performance liquid chromatography (HPLC) system. HPLC separation was conducted on an Agilent 1200 Series instrument (Agilent Technologies Co., California, USA) coupled to a reverse-phase Ultimate C18 column (Welch, Shanghai, China) at 30°C. The flow rates were 1 mL/min for analytic HPLC and 10 mL/min for semi-preparative HPLC. Detection was performed at a wavelength of 214 nm.

Structural analysis

The structure of prepared mogrol from *Siraitia grosvenorii* was determined by spectrum. Agilent 1260 HPLC system coupled to a Bruker-micrOTOF-II mass spectrometer with an electrospray ionization (ESI) probe was used for LC-ESI-MS analysis. The isolated product from *Siraitia grosvenorii* were subjected to a reverse-phase Ultimate C18 column (4.6×250 mm, 5 um particle, Welch, Shanghai, China) with the flow rate of 1 mL/min and UV wavelength of 214 nm. The chemical structure of mogrol was further confirmed by NMR analysis. 1H and 13C NMR spectra were obtained with a Bruker DMX-600 NMR spectrometer operating at 600 MHz. For structural analysis, mogrol was dissolved in pyridine-d5 with 1% tetramethylsilane (TMS) as an internal standard.

Cell viability assay

Cell viability was determined with a 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Leukemia cells were plated in triplicate into a 96-well plate. After overnight incubation, they were treated with various concentrations of mogrol (0, 0.1, 1, 10, 100, 200 and 250 μM) for 24 h and 48 h. The percentage of viable cells was calculated as the ratio (A490) of treated cells over control cells. Triplicate experiments were performed.

Morphology of apoptotic cells

Hoechst 33258 staining was used to observe morphological changes in K562 cells. After K562 cells were treated with mogrol, the cells were collected and fixed with 4% formaldehyde in PBS for 10 min. After being stained with Hoechst 33258 for 10 min in the dark at room temperature, the cells were washed with PBS twice and smeared onto cover slides. Nuclear morphologic changes were observed and imaged under a fluorescence microscope at a magnification of 400× (Olympus IX-70, Tokyo, Japan) [18].

Cell-cycle distribution

Cell-cycle distribution after treatment with mogrol was determined by flow cytometry. K562 cells were analyzed for alterations in cell cycle distribution after treatment with mogrol for 24 h. After the cells were fixed in 70% methanol at 4°C, the samples were treated with RNase A and stained with propidium iodide for analysis

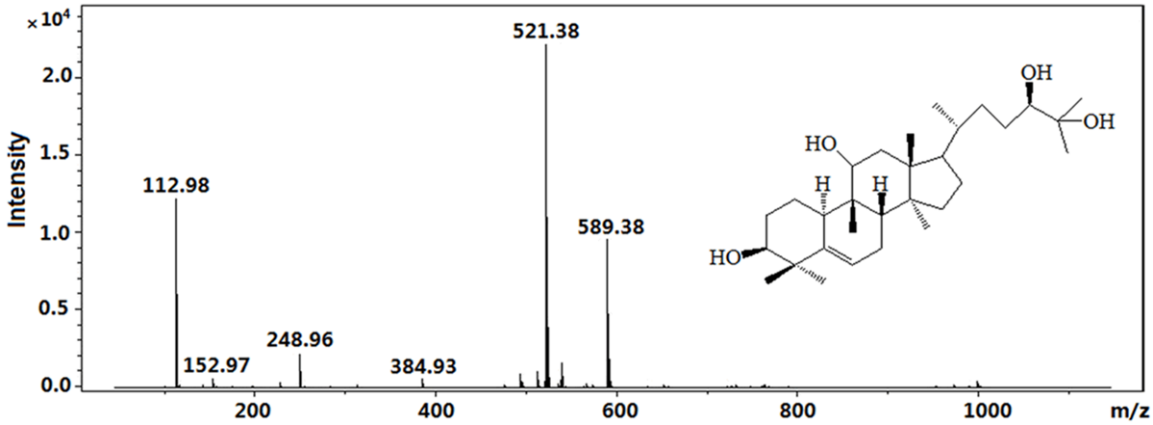

Figure 1. Mass spectrum of isolated mogrol from *S. grosvenorii*. Electrospray ionization (ESI) parameters were conducted as follows: the scan range was 100-1000 m/z in negative ion mode, spray voltage was 4500 V, capillary temperature was 400°C, dry gas was 6 mL/min, dry temperature was 180°C, nebulizer pressure was 1 bar.

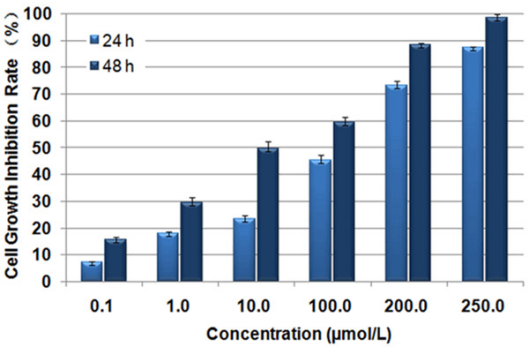

Figure 2. Inhibitory effect of mogrol on K562 cell proliferation after 24 or 48 h, as determined by MTT assay. Data from three independent experiments are presented as the mean ± SD.

by flow cytometry (FACS Calibur BD Flow Cytometer).

Analysis of apoptosis

Annexin V-FITC/propidium iodide (PI) assay was used for the measurement of cellular apoptosis. After K562 cells were treated with mogrol for 24 h, 1×10^6 cells were washed in 200 μL PBS, centrifuged, and resuspended in 100 μL binding buffer. Next, the cells were incubated for 10 min at 37°C with 2 μL of Annexin V-FITC and 5 μL of PI, and subsequently analyzed by flow cytometry. Results were evaluated with CellQuest software (BD Biosciences, San Jose, CA, USA).

Western blot analysis

The cells were harvested on ice and allowed to lyse for 25 min with vortexing every 5 min. About 15 μg of protein from the cell lysates was separated by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred onto a PVDF membrane. Antibodies against STAT3, phosphor (p)-STAT3 (Tyr705), ERK1/2, phosphor (p)- ERK1/2, P21, Bcl-2, and β-actin were used for the western blot analysis. The antibodies were purchased from Sigma-Aldrich Co. LLC [19].

Statistical analysis

All assays were performed in triplicate with 3 independent experiments and data were presented as mean ± standard deviation (SD). Statistical analysis was performed using SPSS for Windows, version 11.5 (IBM Inc., Armonk, NY, USA). Results were statistically analyzed with Student's *t*-test and $p < 0.05$ was considered significant.

Results

Spectroscopic identification of mogrol

Mogrol, a pure white compound, was obtained from *S. grosvenorii.* The mass spectrometric (MS) fragmentation pattern of the compound was identical to that of an authentic mogrol sample, with m/z ions at 521 $[M-H+HCOOH]^-$ and 589 $[M-H+TFA]^-$ (**Figure 1**). The structure of the pure compound was further determined by ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra (data not shown) (Figures S1, S2). The molecular formula of the isolated mogrol was determined to be $C_{30}H_{52}O_4$. This compound was used for the cell growth inhibition tests.

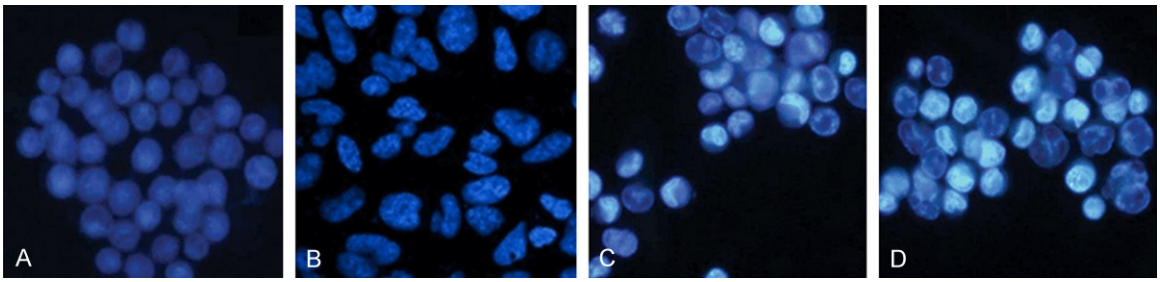

Figure 3. Fluorescence photomicrograph of K562 cells stained with Hoechst 33258. A. Control: untreated K562 cells show homogeneous nuclear staining. B-D. Mogrol-treated: cells were treated with, 10, 100, and 250 μmol/L mogrol for 24 h. Marked morphological changes including chromatin condensation and nuclear fragmentation (brightly stained regions) were observed.

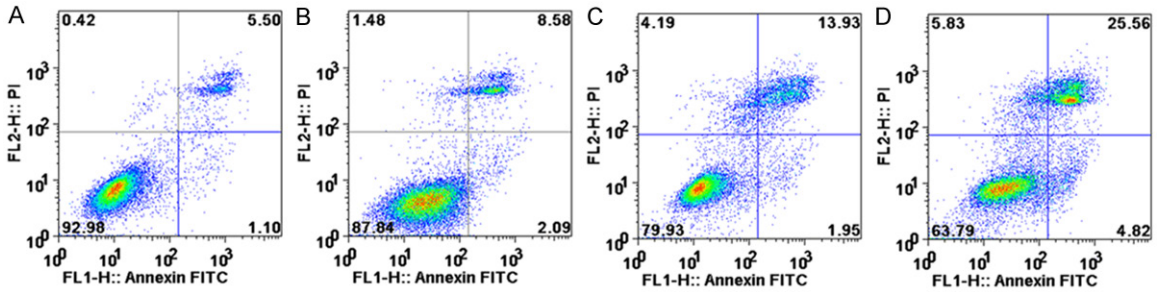

Figure 4. Apoptotic effects of mogrol on K562 cells as determined by flow cytometry. A-D. K562 cells were treated for 0, 10, 100, and 250 μmol/L of mogrol for 24 h.

Effect of mogrol on K562 cells proliferation

To evaluate the effects of mogrol oncell growth, K562 leukemia cells were exposed to 0-250 μmol/L mogrol for 24 or 48 h, and cell viability was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. As illustrated in **Figure 2**, mogrol significantly and dose- and time-dependently inhibited K562 cell growth. After 24 h treatment, the rate of inhibition was 7 to 88% at concentrations between 0.1 and 250 μmol/L. Furthermore, incubation with mogrol for 48 h induced dramatic cell mortality as compared to 24 h, decreasing the rate of inhibition from 50 to 22% in the presence of 10 μmol/L mogrol.

Morphology of apoptotic K562 cells

A Hoechst 33258 staining assay revealed morphological alterations in the nucleus of mogrol-treated K562 cells. In the untreated group, the nuclei maintained a regular shape, and were homogeneously stained. However, in the mogrol-treated group, condensed chromatin and irregular nuclei were observed by brilliant blue staining (**Figure 3**). Mogrol dose-dependently increased the number of apoptotic cells. These results provided evidence for mogrol-induced apoptosis in K562 cells.

Detection of apoptosis

To assess the mechanism responsible for the growth-inhibitory activity of mogrol, cell apoptosis detection was performed in K562 cells. The population of necrotic or post-apoptotic cells was significantly elevated (from 5.50% to 25.56%) in cells treated with mogrol (0, 10, 100, and 250 μmol/L) for 24 h (**Figure 4**). Mogrol induced apoptosis in K562 cells in a dose-dependent manner, relative to control cells.

Detection of cell-cycle distribution

Cell-cycle distribution of K562 cancer cells after treatment with mogrol was measured by flow cytometry. Mogrol treatment inhibited a cell cycle progression in K562 cells, which was reflected by a distinct increase of the percentage of cells in the G0/G1 phase and a significant reduction in the S and G2/M phase (**Figure 5**). The percentage of K562 cells in the G0/G1 phase increased from 36.48% to 77.41% with increasing mogrol concentrations. Mogrol cau-

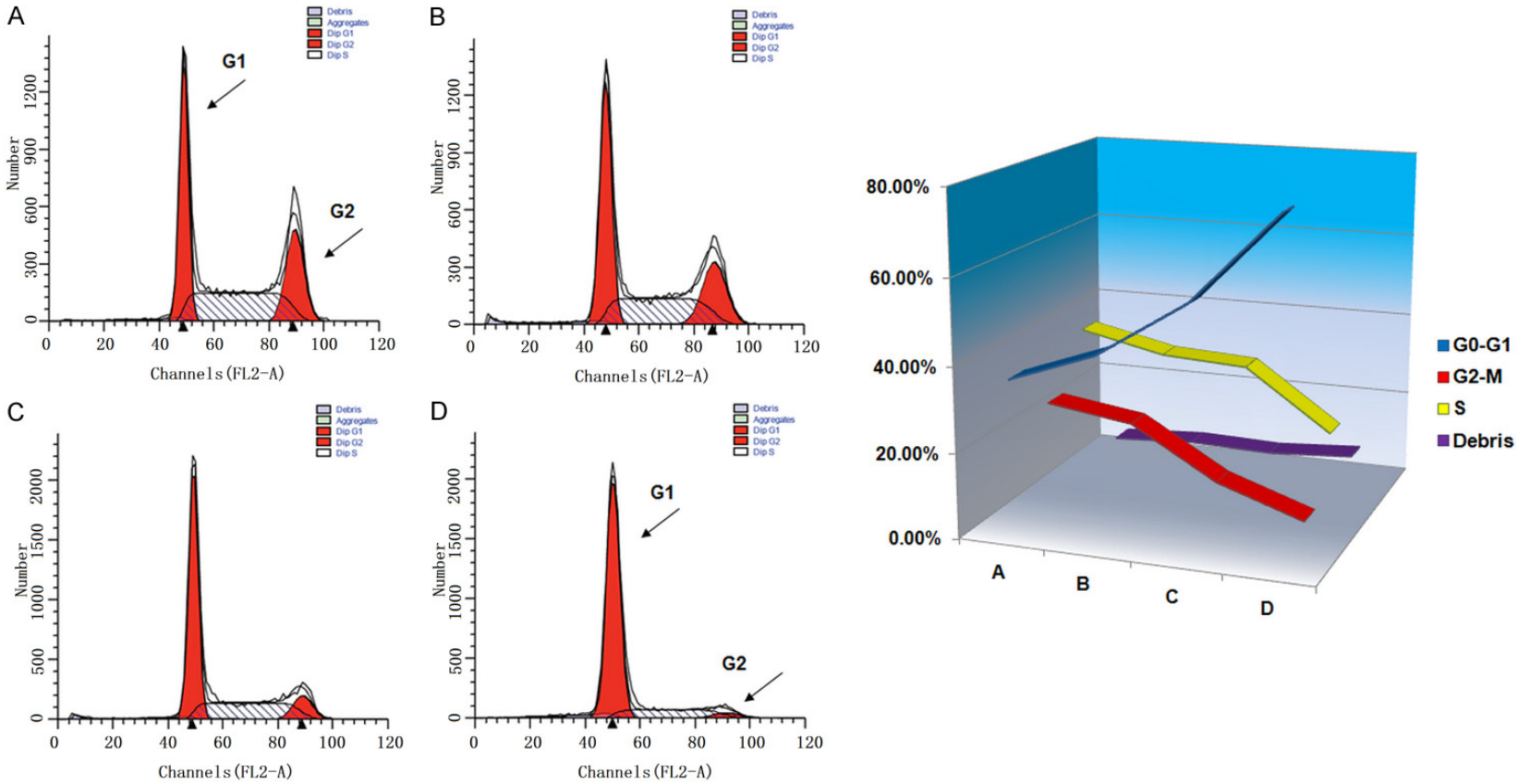

Figure 5. Mogrol induced G1 phase cell cycle arrest in K562 cells. Cells were treated with mogrol for 24 at indicated concentrations. Cells were stained with Annexin V-FITC and PI and analyzed by flow cytometry. A-D. K562 cells treated with 0, 10, 100, and 250 μmol/L of mogrol for 24 h.

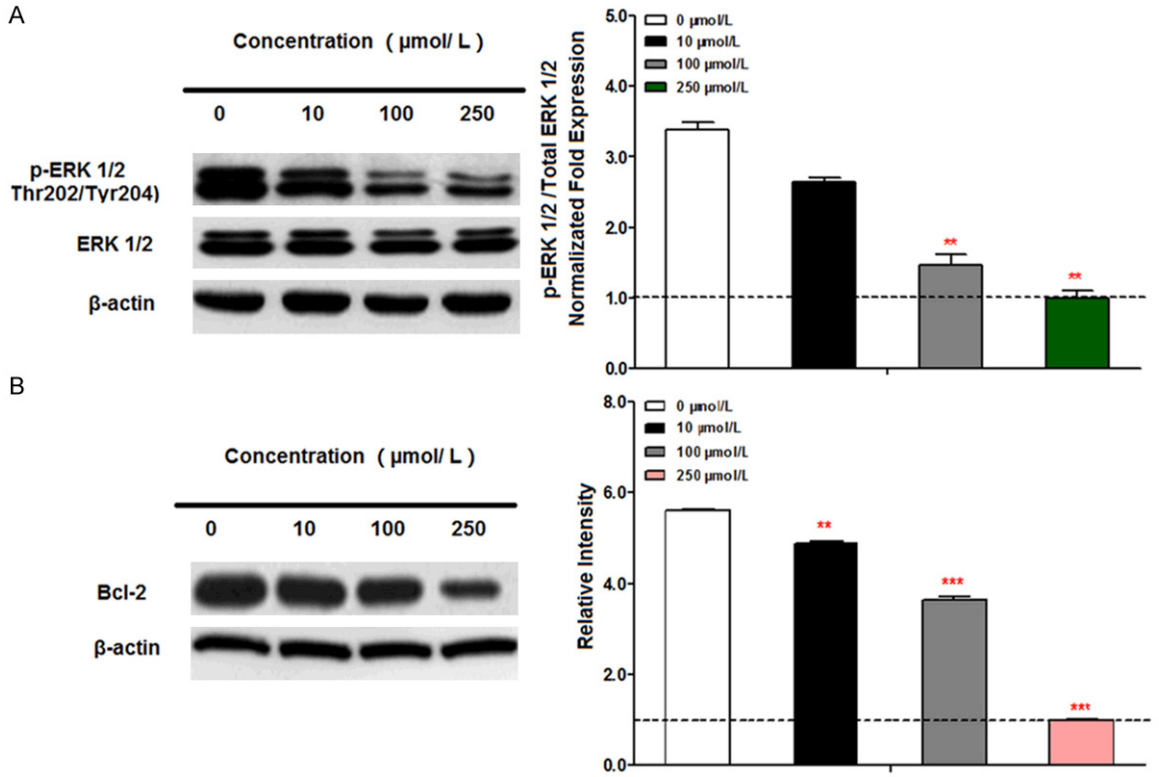

Figure 6. Effects of mogrol on p-ERK1/2 and Bcl-2 expression. K562 cells were treated with mogrol for 24 h. Cell lysates were then subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), followed by western blotting with anti-ERK1/2 (total and phosphorylated) and anti-Bcl-2 antibodies. Proteins were quantified by densitometric analysis of the western blotting results. The expression of β-actin served as a loading control. A. Effects of mogrol on p-ERK expression; B. Effects of mogrol on Bcl-2 expression. Data are presented as the mean ± S.D. Results were statistically analyzed by the Student's *t*-test (**P*<0.05, ***P*<0.01, and ****P*<0.001 were versus control values).

sed a dose-dependent growth arrest in the G0/G1 phase of the cell cycle.

Effects of mogrol on ERK phosphorylation and Bcl-2 expression

The ERK signaling pathway is activated in response to cell stress, and is implicated in cell death and survival signaling [20]. To analyze whether inhibition of ERK phosphorylation is related to mogrol-induced apoptosis, we measured total and phosphorylated levels of this protein. As shown in **Figure 6A**, treatment with mogrol significantly decreased ERK phosphorylation as compared to control cells, whereas total ERK protein was not affected. Both the upper and lower bands, corresponding to p-ERK1 and p-ERK2, were decreased.

ERK activity is associated with the regulation of the pro-apoptotic protein Bcl-2 [21]. Bcl-2 is a critical determinant of, and is essential for, the induction of apoptosis [22]. To determine whether mogrol-induced apoptosis in K562 cells was associated Bcl, we evaluated the effect of mogrol on Bcl-2 expression. As illustrated in **Figure 6B**, Bcl-2 levels dose-dependently decreased in mogrol-treated K562 cells. Combined, these results indicate mogrol may inhibit leukemia cell growth via the modulation of ERK1/2 and Bcl-2.

Effects of mogrol on p-STAT3 and p21 expression

STAT3 is constitutively activated in many human cancers via tyrosine phosphorylation [23]. To determine whether mogrol suppresses STAT3 phosphorylation, dose-escalation studies were conducted in K562 cells. As illustrated in **Figure 7A**, mogrol dose-dependently reduced p-STAT3 levels in K562 cells, with little or no change in total STAT3 levels. These data suggest that mogrol-mediated inhibition of K562 cell growth may involve STAT3 signaling suppression.

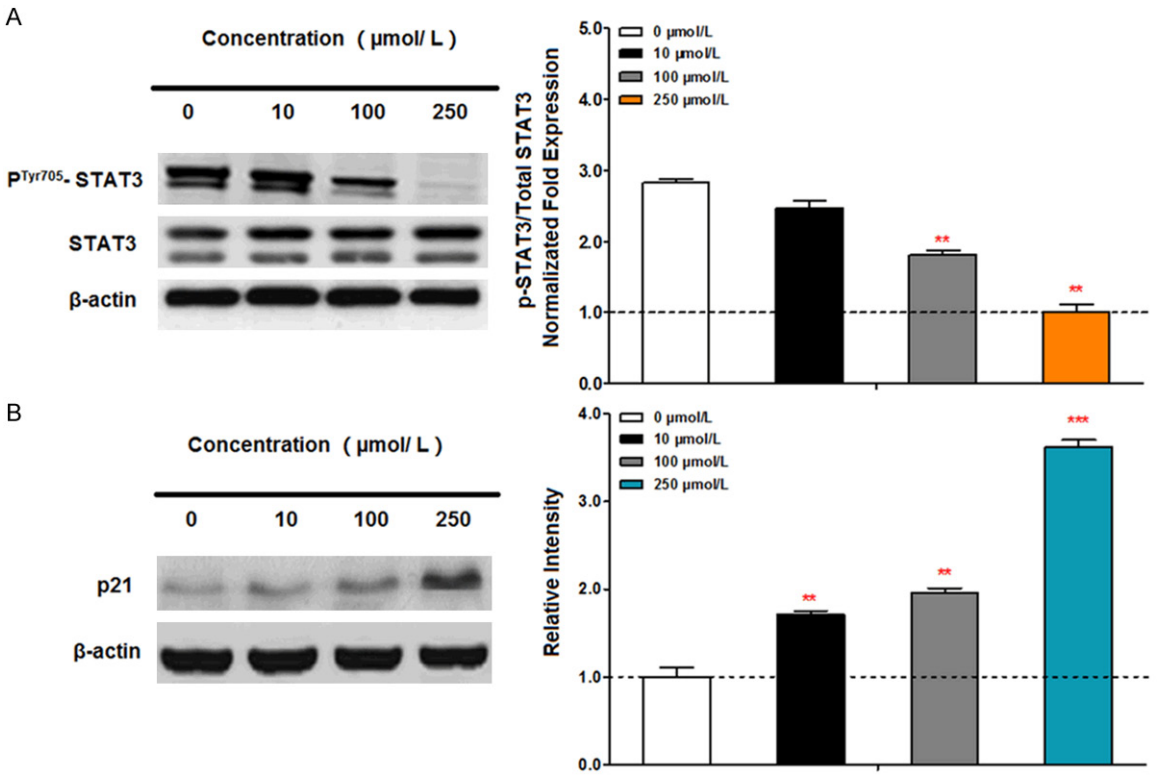

Figure 7. Effects of mogrol on p-STAT3 and p21 expression. K562 cells were treated with mogrol for 24 h. Cell lysates were then subjected to SDS-PAGE, followed by western blotting with anti-STAT3 (total and phosphorylated) or anti-p21 antibodies. Proteins were quantified by densitometry analysis. A. Effects of mogrol on pSTAT3 expression; B. Effects of mogrol on p21 expression. β-actin served as the loading control. Data are presented as the mean ± S.D. Results were statistically analyzed using the Student's *t*-test (**P*<0.05, ***P*<0.01, and ****P*< 0.001 versus control values).

Mogrol dose-dependently induced growth arrest in G0/G1 phase of the cell cycle (**Figure 5**), as determined by flow cytometry. p21 is a downstream gene of STAT3 that regulates cell cycle progression through G0/G1 phase [24]. To determine whether mogrol-induced cell cycle arrest was associated with p21 expression, we evaluated p21 protein expression by western blot.

As shown in **Figure 7B**, mogrol significantly and dose-dependently enhanced p21 protein expression in K562 cells. The maximum effect, a 3.5-fold increase versus control cells, was observed after 24 h exposure to 250 μmol/L mogrol. Combined, our western blot data suggests that mogrol regulates p-STAT3 and p21 expression in K562 cells.

Discussion

Mogrosides were approved by the Food and Drug Administration (FDA) as a food additive. *In vivo,* mogrosides are metabolized to mogrol (**Figure 8**). Previous work has shown that mogrosides can be digested without adverse effects, suggesting that mogrol may be safe for use [12]. Furthermore, mogrol may play a role in the pharmacological effects of mogrosides. However, the anticancer properties of this compound in leukemia cells remain unclear. In this study, we used K562 leukemia cells to evaluate the anticancer properties and molecular mechanisms of mogrol. We found that mogrol exerts a strong anticancer effect on K562 cells via the induction of apoptosis and cell cycle arrest.

Treatment with mogrol for 24 and 48 h markedly inhibited leukemia cell growth in a concentration- and time-dependent manner. Flow cytometry analysis revealed that mogrol induced apoptosis, which may mediate its growth inhibitory effects in K562 cells. However, the underlying molecular mechanism remained unclear.

Ras/Raf/ERK pathway activation is typically associated with cell proliferation [25-27], and

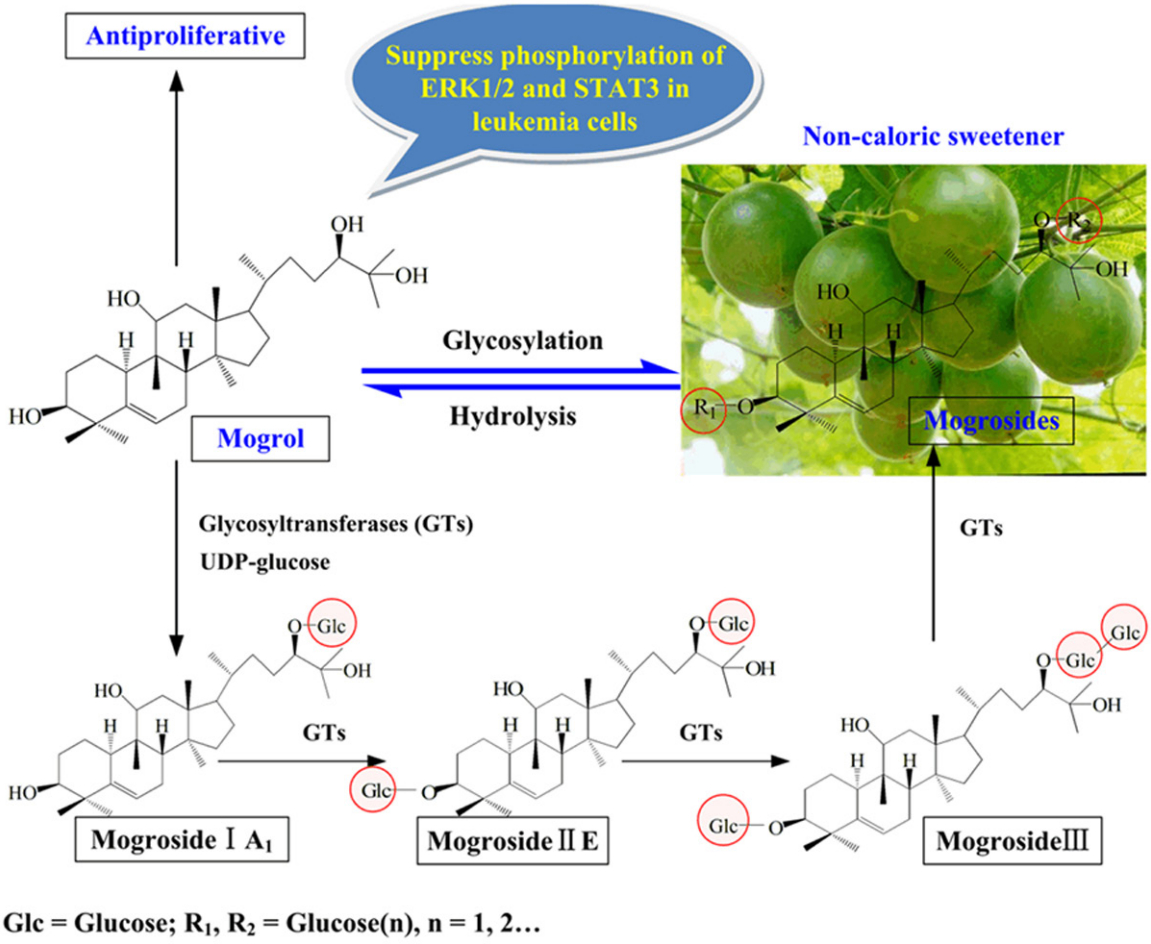

Figure 8. The interconversion of mogrol and mogrosides. Mogrol can be converted to mogrosides by glycosyltransferases in *Siraitia grosvenorii*, while mogrosides can be reconverted into mogrol by digestive enzymes in vivo.

ERK1/2 activation can provide tumor cells with a survival advantage [28]. Furthermore, aberrant activation of the ERK pathway is frequently observed in leukemia patients [8, 29]. Therefore, ERK inhibitors may be promising anticancer therapeutics [28]. Based on these data, we next evaluated whether mogrol inhibits cell growth via regulation of the ERK pathway in K562 cells. p-ERK levels were measured evaluated by western blot analysis, revealing that mogrol significantly suppressed ERK phosphorylation (**Figure 6**). As ERK plays a critical role in cellular proliferation [30], inactivation of the ERK pathway by mogrol may contribute to its anti-proliferative effects.

ERK activity is associated with the upregulation of pro-apoptotic proteins, such as Bcl-2 [31]. Previous work has suggested that reductions in intracellular Bcl-2 levels can induce apoptosis in several myeloid leukemias [32, 33]. Upon activation, ERK is translocated to the nucleus, where it mediates Bcl-2 transcription, thereby increasing anti-apoptotic protein levels [33]. Bcl-2 is considered a critical determinant of cell survival versus apoptosis. Our data indicated that Bcl-2 expression was dose-dependently decreased in mogrol-treated K562 cells. Since Bcl-2 is an oncogenic protein that inhibits programmed cell death [32, 33], decreased Bcl-2 expression may mediate mogrol-induced apoptosis. Our data suggests that mogrol inhibits p-ERK expression, thereby downregulating Bcl-2 expression to promote cancer cell apoptosis.

STAT3 plays a key role in cell-cycle transition [34, 35], and numerous studies have demonstrated constitutive STAT3 activation in human cancers [36, 37]. Furthermore, STAT3 is consti-

tutively active in K562 cells [38]. These studies suggest that STAT3 may represent a novel molecular target for cancer therapeutics [39]. Inhibition of Tyr705 phosphorylation on STAT3 could suppress aberrant STAT3 signaling [40-42]. Our western blot data revealed that mogrol dose-dependently reduced p-STAT3s level in K562 cells, suggesting that mogrol inhibits K562 growth via its anti-STAT3 activity.

The STAT3 pathway can also influence cycle progression by altering the expression of downstream cycle proteins, such as p21 [43]. Downregulation of p-STAT3 expression leads to p21 upregulation, which can thereby inhibit cell cycle progression through the G1/S phase [44]. Interestingly, p21 was upregulated in K562 cells when exposed to mogrol. Overexpression of p21 induces cell cycle arrest, consistent with our finding that the cell DNA was characteristic of G1 arrest following mogrol treatment (**Figure 5**).

Activated STAT specifically upregulates p21 messenger RNA [45]. After treatment with mogrol, a negative correlation was observed between p-STAT3 and p21 expression (**Figure 7**). Decreases in pSTAT3 were associated with increased p21 expression, consistent with a role for p-STAT3 in upregulation. These findings demonstrated mogrol-induced inhibition of STAT3 signaling in human leukemia cells. Further, mogrol-induced inhibition of STAT3 signally may induce G0/G1-phase arrest via p21 upregulation [46, 47].

Both ERK and STAT3 phosphorylation were reduced by mogrol in K562 cells. Our data suggest that the ERK and STAT3 pathway are coordinately involved in mogrol-mediated inhibition of K562 cells.

In conclusion, we showed that mogrol suppresses both the ERK and STAT3 signaling pathway in K562 cells. This is the first study to demonstrate a dual effect of mogrol on ERK and STAT3. Targeting both of these signaling pathways is an important strategy for anticancer therapeutic intervention. This work reveals the molecular mechanism of the anticancer activity of mogrol, and suggests that mogrol may be a promising anti-leukemia agent.

Acknowledgements

The authors acknowledge the support of the Tianjing Enterprise Postdoctoral Innovation Project special foundation, the foundation of "Da Bei Nong" (13Z K001). The Comprehensive Reforming Project to Promote Talents Training of BUA (BNRC&CC201406); "863" Program, (No: 2012AA021403).

Disclosure of conflict of interest

None.

Address correspondence to: Dequan Dou or Lanqing Ma, Beijing University of Agriculture, 7 Beinong Road, Changping, Beijing 102206, Peoples Republic of China. Tel: +86-10-80799163; Fax: +86-10-80799163; E-mail: doudequan@bac.edu.cn (DQD); Tel: +86-10-80797305; Fax: +86-10-80797305; E-mail: lqma@bac.edu.cn (LQM)

References

[1] Huang T, Lin J, Cao J, Zhang P, Bai Y, Chen G, Chen K. An exopolysaccharide from Trichoderma pseudokoningii and its apoptotic activity on human leukemia K562 cells. Carbohydr Polym 2012; 89: 701-708.

[2] Singh A, Dilnawaz F, Sahoo SK. Long circulating lectin conjugated paclitaxel loaded magnetic nanoparticles: a new theranostic avenue for leukemia therapy. PLoS One 2011; 6: e26803.

[3] Buettner R, Mora LB, Jove R. Activated STAT signaling in human tumors provides novel molecular targets for therapeutic intervention. Clin Cancer Res 2002; 8: 945-954.

[4] Shaul YD, Seger R. The MEK/ERK cascade: from signaling specificity to diverse functions. Bba-mol Cell Res 2007; 1773: 1213-1226.

[5] Mora LB, Buettner R, Seigne J, Diaz J, Ahmad N, Garcia R, Bowman T, Falcone R, Fairclough R, Cantor A. Constitutive activation of STAT3 in human prostate tumors and cell lines direct inhibition of STAT3 signaling induces apoptosis of prostate cancer cells. Cancer Res 2002; 62: 6659-6666.

[6] Monami G, Gonzalez EM, Hellman M, Gomella LG, Baffa R, Iozzo RV, Morrione A. Proepithelin promotes migration and invasion of 5637 bladder cancer cells through the activation of ERK1/2 and the formation of a paxillin/FAK/ERK complex. Cancer Res 2006; 66: 7103-7110.

[7] Chan KS, Sano S, Kiguchi K, Anders J, Komazawa N, Takeda J, DiGiovanni J. Disruption of Stat3 reveals a critical role in both the initiation and the promotion stages of epithelial carcinogenesis. J Clin Invest 2004; 114: 720-728.

[8] Kang CD, Yoo SD, Hwang BW, Kim KW, Kim DW, Kim CM, Kim SH, Chung BS. The inhibition

of ERK/MAPK not the activation of JNK/SAPK is primarily required to induce apoptosis in chronic myelogenous leukemic K562 cells. Leukemia Res 2000; 24: 527-534.
[9] Woessmann W, Mivechi NF. Role of ERK activation in growth and erythroid differentiation of K562 cells. Exp Cell Res 2001; 264: 193-200.
[10] Aoki Y, Feldman GM, Tosato G. Inhibition of STAT3 signaling induces apoptosis and decreases survivin expression in primary effusion lymphoma. Blood 2003; 101: 1535-1542.
[11] Zisen H, Jianwei W, Shudan S, Yaping W. Effects of angelica polysaccharide on expression and activation of JAK2 and STAT3 in K562 leukemia cells. Chinese Journal of Anatomy. 2009; 1: 008.
[12] Qin X, Xiaojian S, Ronggan L, Yuxian W, Zhunian T, Shouji G, Heimbach J. Subchronic 90-day oral (Gavage) toxicity study of a Luo Han Guo mogroside extract in dogs. Food Chem Toxicol 2006; 44: 2106-2109.
[13] Di R, Huang MT, Ho CT. Anti-inflammatory activities of mogrosides from Momordica grosvenori in murine macrophages and a murine ear edema model. J Agr Food Chem 2011; 59: 7474-7481.
[14] Suzuki YA, Tomoda M, Murata Y, Inui H, Sugiura M, Nakano Y. Antidiabetic effect of long-term supplementation with Siraitia grosvenori on the spontaneously diabetic Goto-Kakizaki rat. Brit J Nutr 2007; 97: 770-775.
[15] Xiangyang Q, Weijun C, Liegang L, Ping Y, Bijun X. Effect of a Siraitia grosvenori extract containing mogrosides on the cellular immune system of type 1 diabetes mellitus mice. Mol Nutr Food Res 2006; 50: 732-738.
[16] Takasaki M, Konoshima T, Murata Y, Sugiura M, Nishino H, Tokuda H, Matsumoto K, Kasai R, Yamasaki K. Anticarcinogenic activity of natural sweeteners, cucurbitane glycosides, from Momordica grosvenori. Cancer Lett 2003; 198: 37-42.
[17] Liu C, Liu J, Rong Y, Rong L. Preparation of productive and highly purified mogrosides from Siraitia grosvenorii. Afr J Biotechnol 2013; 10: 7021-7025.
[18] Chen T. In situ detection of mycoplasma contamination in cell cultures by fluorescent Hoechst 33258 stain. Exp Cell Res 1977; 104: 255-262.
[19] Shacter E, Williams JA, Lim M, Levine RL. Differential susceptibility of plasma proteins to oxidative modification: examination by western blot immunoassay. Free Radical Biol Med 1994; 17: 429-437.
[20] Wan PT, Garnett MJ, Roe SM, et al. Mechanism of activation of the RAF-ERK signaling pathway by oncogenic mutations of B-RAF. Cell 2004; 116: 855-867.
[21] Vaux D, Cory S, Adams J. Bcl-2 and cell survival. Nature 1988; 335: 440-442.
[22] Yang J, Liu X, Bhalla K, Kim CN, Ibrado AM, Cai J, Peng TI, Jones DP, Wang X. Prevention of apoptosis by Bcl-2: release of cytochrome c from mitochondria blocked. Science 1997; 275: 1129-1132.
[23] Garcia R, Bowman TL, Niu G, Yu H, Minton S, Muro-Cacho CA, Cox CE, Falcone R, Fairclough R, Parsons S. Constitutive activation of Stat3 by the Src and JAK tyrosine kinases participates in growth regulation of human breast carcinoma cells. Oncogene 2001; 20: 2499-2513.
[24] Coqueret O. New roles for p21 and p27 cell-cycle inhibitors: a function for each cell compartment? Trends Cell Biol 2003; 13: 65-70.
[25] Cagnol S, Chambard JC. ERK and cell death: Mechanisms of ERK-induced cell death–apoptosis, autophagy and senescence. FEBS J 2010; 277: 2-21.
[26] Johnstone RW, Ruefli AA, Lowe SW. Apoptosis: a link between cancer genetics and chemotherapy. Cell 2002; 108: 153-164.
[27] Sebolt-Leopold JS, Herrera R. Targeting the mitogen-activated protein kinase cascade to treat cancer. Nat Rev Cancer 2004; 4: 937-947.
[28] Cossa G, Lanzi C, Cassinelli G, Carenini N, Arrighetti N, Gatti L, Corna E, Zunino F, Zaffaroni N, Perego P. Differential outcome of MEK1/2 inhibitor-platinum combinations in platinum-sensitive and-resistant ovarian carcinoma cells. Cancer Lett 2014; 347: 212-224.
[29] Herrera R, Sebolt-Leopold JS. Unraveling the complexities of the Raf/MAP kinase pathway for pharmacological intervention. Trends Mol Med 2002; 8: S27-S31.
[30] Gerhardt D, Bertola G, Dietrich F, Figueiró F, Zanotto-Filho A, Moreira Fonseca JC, Morrone FB, Barrios CH, Battastini AMO, Salbego CG. Boldine induces cell cycle arrest and apoptosis in T24 human bladder cancer cell line via regulation of ERK, AKT, and GSK-3β. Urol Oncol-Semin Ori 2014: 36: e31-36, e39.
[31] Subramanian M, Shaha C. Up-regulation of Bcl-2 through ERK phosphorylation is associated with human macrophage survival in an estrogen microenvironment. J Immunol 2007; 179: 2330-2338.
[32] Perkins C, Kim CN, Fang G, Bhalla KN. Arsenic induces apoptosis of multidrug-resistant human myeloid leukemia cells that express Bcr-Abl or overexpress MDR, MRP, Bcl-2, or Bcl-xL. Blood 2000; 95: 1014-1022.
[33] Wang ZG, Rivi R, Delva L, König A, Scheinberg DA, Gambacorti-Passerini C, Gabrilove JL, Warrell RP, Pandolfi PP. Arsenic Trioxide and Melarsoprol Induce Programmed Cell Death in Myeloid Leukemia Cell Lines and Function in a

PML and PML-RAR Independent Manner. Blood 1998; 92: 1497-1504.
[34] Fukada T, Ohtani T, Yoshida Y, Shirogane T, Nishida K, Nakajima K, Hibi M, Hirano T. STAT3 orchestrates contradictory signals in cytokine induced G1 to S cell - cycle transition. EMBO J 1998; 17: 6670-6677.
[35] Nakatake M, Kakiuchi Y, Sasaki N, Murakami-Murofushi K, Yamada O. STAT3 and PKC differentially regulate telomerase activity during megakaryocytic differentiation of K562 cells. Cell Cycle 2007; 6: 1495-1500.
[36] Bowman T, Garcia R, Turkson J, Jove R. STATs in oncogenesis. Oncogene 2000; 19: 2474-2488.
[37] Blume-Jensen P, Hunter T. Oncogenic kinase signalling. Nature 2001; 411: 355-365.
[38] Mencalha AL, Binato R, Ferreira GM, Du Rocher B, Abdelhay E. Forkhead box M1 (FoxM1) gene is a new STAT3 transcriptional factor target and is essential for proliferation, survival and DNA repair of K562 cell line. PLoS One 2012; 7: e48160.
[39] Seidel HM, Lamb P, Rosen J. Pharmaceutical intervention in the JAK/STAT signaling pathway. Oncogene 2000; 19: 2645-2656.
[40] Darnell JE. STATs and gene regulation. Science 1997; 277: 1630-1635.
[41] Krasilnikov M, Ivanov VN, Dong J, Ronai ZE. ERK and PI3K negatively regulate STAT-transcriptional activities in human melanoma cells: implications towards sensitization to apoptosis. Oncogene 2003; 22: 4092-4101.
[42] Haspel R, Salditt-Georgieff M, Darnell J Jr. The rapid inactivation of nuclear tyrosine phosphorylated Stat1 depends upon a protein tyrosine phosphatase. EMBO J 1996; 15: 6262.
[43] Waldman T, Kinzler KW, Vogelstein B. p21 is necessary for the p53-mediated G1 arrest in human cancer cells. Cancer Res 1995; 55: 5187-5190.
[44] Harper JW, Elledge SJ, Keyomarsi K, Dynlacht B, Tsai LH, Zhang P, Dobrowolski S, Bai C, Connell-Crowley L, Swindell E. Inhibition of cyclin-dependent kinases by p21. Mol Biol Cell 1995; 6: 387-400.
[45] Chin YE, Kitagawa M, Su WC, You ZH, Iwamoto Y, Fu XY. Cell growth arrest and induction of cyclin-dependent kinase inhibitor p21WAF1/CIP1 mediated by STAT1. Science 1996; 272: 719-722.
[46] Ogryzko VV, Wong P, Howard BH. WAF1 retards S-phase progression primarily by inhibition of cyclin-dependent kinases. Mol Cell Biol 1997; 17: 4877-4882.
[47] Radhakrishnan SK, Feliciano CS, Najmabadi F, Haegebarth A, Kandel ES, Tyner AL, Gartel AL. Constitutive expression of E2F-1 leads to p21-dependent cell cycle arrest in S phase of the cell cycle. Oncogene 2004; 23: 4173-4176.

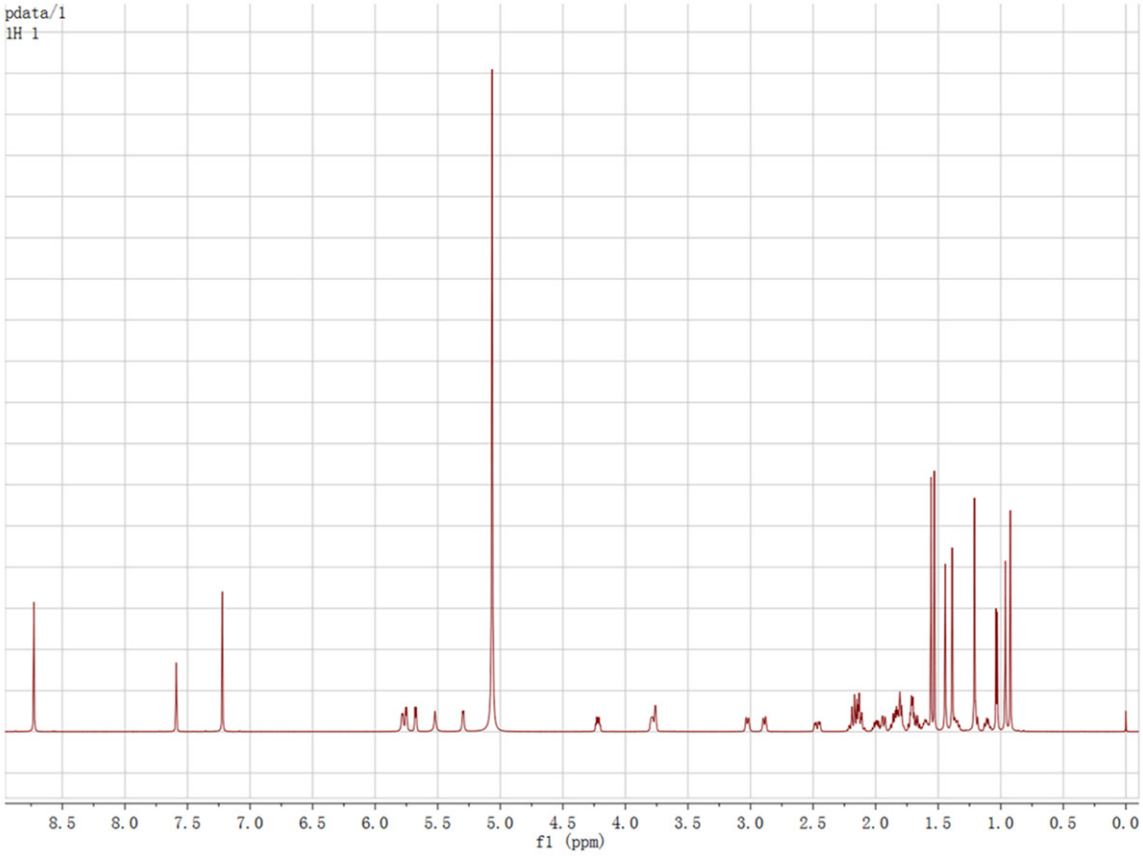

Figure S1. 1H NMR spctra of mogrol.

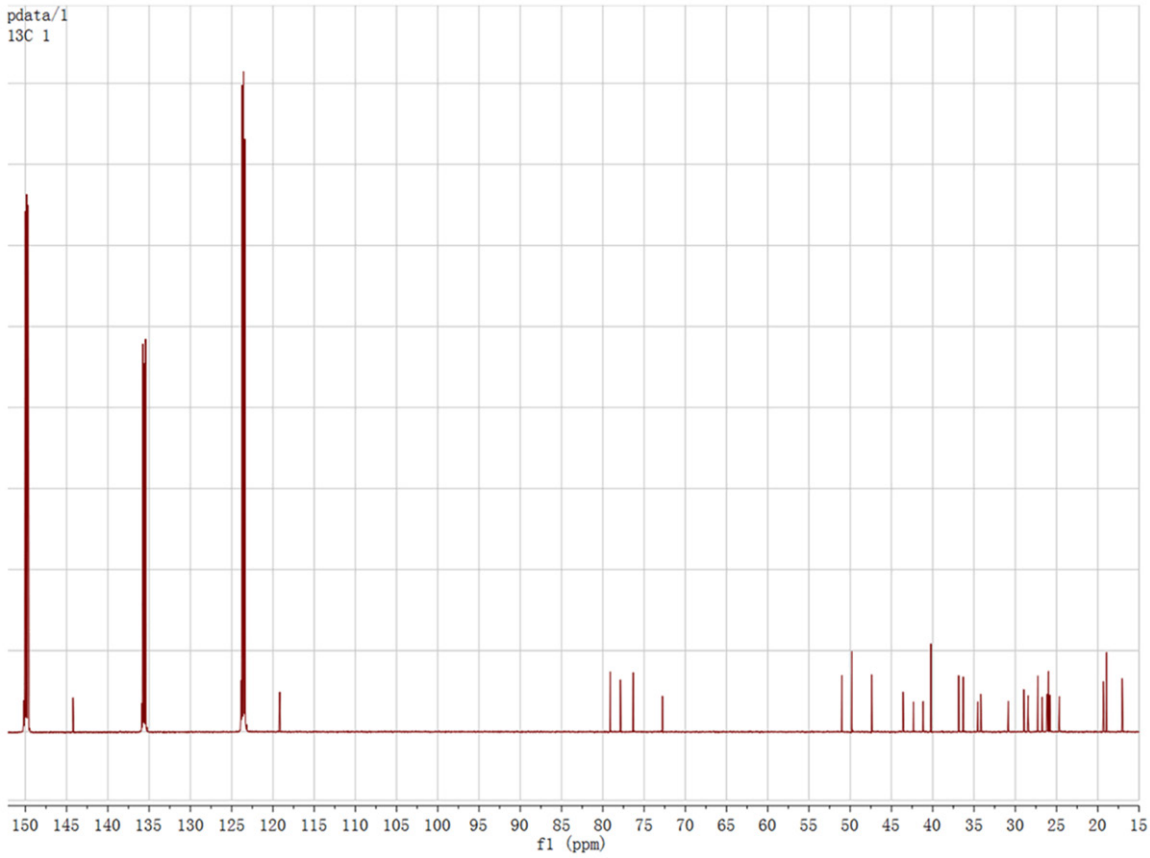

Figure S2. 13C NMR spctra of mogrol.