

Original Research

# A New Indole Derivative, LWX-473, Overcomes Glucocorticoid Resistance in Jurkat Cells by Activating Mediators of Apoptosis

Jingrui Song<sup>1,2,3,†</sup>, Kun Yang<sup>1,2,4,5,†</sup>, Babu Gajendran<sup>1,2,4</sup> , Krishnapriya M. Varier<sup>1,2,4</sup>,  
Wenxue Li<sup>1,2</sup>, Qin Liu<sup>1,2,4</sup>, Qing Rao<sup>1,2</sup>, Yubing Hang<sup>1,2</sup>, Xiangchun Shen<sup>1,2,4</sup>, Sheng Liu<sup>1,2</sup>,  
Lei Huang<sup>1,2,\*</sup>, Mei Xu<sup>1,2,\*</sup>, Yanmei Li<sup>1,2,\*</sup><sup>1</sup>State Key Laboratory for Functions and Applications of Medicinal Plants, Guizhou Medical University, 550014 Guiyang, Guizhou, China<sup>2</sup>Natural Products Research Center of Guizhou Province, 550014 Guiyang, Guizhou, China<sup>3</sup>School of Basic Medicine, Guizhou Medical University, 550025 Guiyang, Guizhou, China<sup>4</sup>School of Pharmaceutical Sciences, Guizhou Medical University, 550025 Guiyang, Guizhou, China<sup>5</sup>Department of Pharmacy, Guizhou Provincial People's Hospital, 550002 Guiyang, Guizhou, China\*Correspondence: [xinyanghuanglei@163.com](mailto:xinyanghuanglei@163.com) (Lei Huang); [774885024@qq.com](mailto:774885024@qq.com) (Mei Xu); [liyanmei518@hotmail.com](mailto:liyanmei518@hotmail.com) (Yanmei Li)

†These authors contributed equally.

Academic Editor: Taeg Kyu Kwon

Submitted: 21 August 2023 Revised: 28 November 2023 Accepted: 2 January 2024 Published: 23 April 2024

## Abstract

**Background:** Glucocorticoids (GCs) are commonly used as the primary chemotherapy for lymphoid malignancies, including acute lymphoblastic leukemia (ALL). However, the development of GC resistance limits their prolonged use. **Methods:** In this study, we investigated the potential of a newly synthesized indole derivative called LWX-473, in combination with the classic GC Dexamethasone (DEX), to enhance the responsiveness of Jurkat cells to GC treatment. **Results:** Our findings demonstrate that LWX-473 alone or in combination with DEX significantly improves GC-induced cell apoptosis and arrests the cell cycle in the G1 phase. Notably, the combination of LWX-473 and DEX exhibits superior efficacy in killing Jurkat cells compared to LWX-473 alone. Importantly, this compound demonstrates reduced toxicity towards normal cells. **Conclusions:** Our study reveals that LWX-473 has the ability to restore the sensitivity of Jurkat cells to DEX by modulating the mitochondrial membrane potential, activating the expression of DEX-liganded glucocorticoid receptor (GR), and inhibiting key molecules in the JAK/STAT signaling pathway. These findings suggest that LWX-473 could be a potential therapeutic agent for overcoming GC resistance in lymphoid malignancies.

**Keywords:** leukemia; glucocorticoid resistance; indole derivative; Jurkat cells; apoptosis

## 1. Introduction

Glucocorticoids (GCs) play a pivotal role in the metabolism of cancer cells by binding to the glucocorticoid receptor (GR) in the cytoplasm. GCs can homodimerize, translocate into the nucleus, interact with the glucocorticoid response element (GRE) to induce gene transcription, change the expression of various oncogenes, and induce cell cycle arrest and apoptosis [1,2]. The primary response of cancer patients to GCs corresponds to the overall clinical outcomes of therapy, but the development of initial or acquired drug resistance renders treatment ineffective or leads to chemoresistance [3].

Resistance to glucocorticoids is a complex process in many cancers, such as leukemia, lymphoma, and liver cancer [4]. It has been found that the level of the glucocorticoid receptor and its ligand determines the sensitivity of glucocorticoids in inducing leukemic cell apoptosis [5], thus affecting the effectiveness of GCs [6]. Researchers have also discovered that inhibiting the JAK/STAT pathway can overcome IL-7-induced resistance to glucocorticoids [7]. Additionally, the regulation of glucocorticoid resistance in pediatric T-cell acute lymphoblastic leukemia by increasing

BIM expression with PI3K/mTOR inhibitors [8] can assist in tumor therapy. Despite continuous discoveries regarding the mechanism of glucocorticoid resistance, finding new treatments or drugs to overcome this resistance remains a scientific challenge.

Indole compounds play an important role in the development of anticancer drugs. For example, some natural or synthetic indole compounds, such as vincristine, vinorelbine, semaxanib, and sunitinib, have been widely used in various clinical anti-tumor treatments [9,10]. Additionally, indole compounds have promising potential in addressing drug resistance. The indole alkaloid reserpine, which is used as an antihypertensive, increases tumor cell susceptibility to doxorubicin by inhibiting the efflux function of the P glycoprotein [11]. Newly synthesized indole compounds identified as double Aurora B/FLT3 inhibitors have nanomolar anti-leukemia activity and show good effects on sunitinib-resistant FLT3-ITD AML cells [12]. Therefore, the development of a new indole derivative to overcome glucocorticoid resistance in the treatment of leukemia holds great promise. In this study, a newly synthesized indole compound (LWX-473) was combined with



GCs to evaluate its efficiency in overpowering chemoresistance in Jurkat cells. This was achieved by inhibiting the PI3K/AKT/JAK2/Stat3 pathways and inducing apoptosis of Jurkat cells through activation of the GR receptor and the mitochondrial pathway, as well as arresting the cell cycle at their G1 phase.

## 2. Materials and Methods

### 2.1 Cell Culture, Reagents and Antibodies

The human cell lines Jurkat (acute T cell leukemia; STCC10904G) and CEM/C1 (T lymphoblast; STCC10910G) were purchased from Wuhan Servicebio Technology Co., Ltd, Wuhan, China. CEM-C7H2 (acute T lymphoblastic leukemia; GD-C60404492) and HL-7702 (normal liver; GD-C60306615) were purchased from Shanghai Guandao Bioengineering Co., Ltd, Shanghai, China. All cell lines were validated by STR profiling and tested negative for mycoplasma. All cells were cultured in RPMI 1640 medium (Gibco, Carlsbad, CA, USA) supplemented with 5% FBS (Biological Industries, Beijing, China) in a 37 °C humidified incubator with 5% CO<sub>2</sub>. The following antibodies were used in this study:  $\beta$ -actin (af7018, Affinity, Changzhou, China), Caspase-3 (9662S, Cell Signaling Technology (CST), Boston, MA, USA), Cleaved-Caspase-9 (7237S, CST), PARP (9542S, CST), Bcl-2 (4223S, CST), Bim (2933S, CST), GR (12041S, CST), P-GR (4161S, CST), NOTCH1 (4147, CST), CDK2 (ab32147, Abcam, Boston, MA, USA), Cyclin E1 (ab33911, Abcam), C-Myc (ab32072, Abcam), JAK2 (ab108596, Abcam), P-JAK2 (381556, ZEN-BIO), STAT3 (ab68153, Abcam), P-STAT3 (ab76315, Abcam), PI3K (4249S, CST), P-PI3K (4228S, CST), AKT (4685S, CST), and P-AKT (11054, SAB, College Park, MD, USA).

### 2.2 Synthesis of Methyl 3-((2-(1H-indol-3-yl) ethyl) (benzyl) amino)-5-chloro-2-oxoindoline-3-Carboxylate (LWX-473)

A solution of Methyl 3,5-dichloro-2-oxoindoline-3-carboxylate (1.0 g, 3.85 mmol) and N-benzyl-2-(1H-indol-3-yl) ethan-1-amine (0.96 g, 3.85 mmol) in 10 mL of MeCN was prepared. To this solution, DIPEA (2.01 mL, 11.54 mmol) was slowly added at 0 °C. The resulting mixture was then stirred at room temperature for 2 hours. Finally, the mixture was concentrated under reduced pressure. The crude product was purified by flash column chromatography (DCM/MeOH = 300:1–150:1) to obtain the product (1.15 g, yield 63%). Methyl 3-((2-(1H-indol-3-yl) ethyl) (benzyl) amino)-5-chloro-2-oxoindoline-3-carboxylate: white solid, m. p: 153.2–154.5 °C <sup>1</sup>H nuclear magnetic resonance (NMR, 600 MHz, CDCl<sub>3</sub>)  $\delta$  8.56 (br. s., 1 H), 7.85 (br. s., 1 H), 7.57 (d, J = 7.31 Hz, 2 H), 7.55 (d, J = 2.07 Hz, 1 H), 7.37 (t, J = 7.55 Hz, 2 H), 7.21–7.29 (m, 3 H), 7.11 (td, J = 7.35, 1.51 Hz, 1 H), 6.94–7.00 (m, 2 H), 6.80 (d, J = 8.26 Hz, 1 H), 6.67 (s, 1 H), 4.13 (s, 2 H), 3.75 (s, 3 H), 2.96–3.00 (m, 1 H), 2.86–2.87 (m, 1

H), 2.58 (td, J = 9.70, 5.72 Hz, 2 H); <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>)  $\delta$  176.9, 169.3, 140.2, 140.1, 136.1, 130.3, 128.7, 128.4, 128.3, 128.2, 127.3, 127.2, 126.0, 121.8, 121.7, 119.0, 118.5, 113.5, 111.8, 111.1, 76.6, 55.7, 53.4, 52.5, 25.3. High-resolution mass spectrometry (HRMS) (ESI) m/z calcd. for C<sub>27</sub>H<sub>24</sub>O<sub>3</sub>N<sub>3</sub>Cl [M + Na]<sup>+</sup>: 496.13984, found: 496.13843. Purity (>98%).

### 2.3 Cell Proliferation Assay and Cell Morphology Analysis

Cells (1.5 × 10<sup>4</sup> cells/well) were treated with different concentrations of both LWX-473 (2, 4, 8, 12, 16  $\mu$ M), with or without Dexamethasone (DEX) (100  $\mu$ M), in 96-well plates for 24, 48, and 72 h. After incubation, 10  $\mu$ L of MTT (5 mg/mL) was added to each well and incubated for 4 h. Later, 100  $\mu$ L of DMSO was added to dissolve the formazan crystals and quantified at 570 nm using a Synergy2 modular Multi-Mode Reader (BioTek, Winooski, VT, USA). The cell viability rates were evaluated using the following equation: survival rate (%) = (OD of treatment – OD of control)/OD of control × 100. Before the addition of the MTT reagent, the cellular morphology of the control or treated cells was recorded using a Nikon inverted microscope [13].

### 2.4 Flowcytometric Detection of Apoptosis

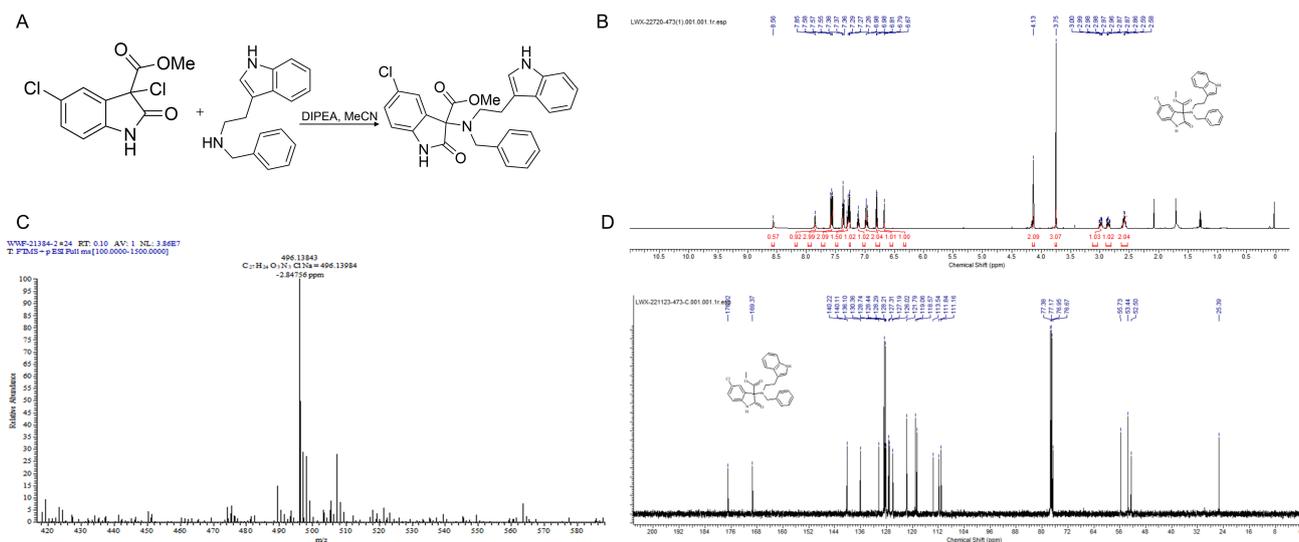
For apoptotic detection, cells (20 × 10<sup>4</sup> cells/well) were inoculated into a six-well plate and treated with different concentrations of LWX-473 (4, 8, 12  $\mu$ M) and/or DEX (100  $\mu$ M) for 48 h. Afterward, the cells were collected, washed in PBS, and stained using the Annexin V FITC/PI apoptosis detection Kit (BD Biosciences, Franklin Lakes, NJ, USA) according to the manufacturer's instructions. The stained cells were then analyzed using a Flow cytometer (ACEA NovoCyte, Miami, FL, USA) [14].

### 2.5 Cell Cycle Analysis

For the cell cycle analysis, Cells (20 × 10<sup>4</sup> cells/well) were treated with different concentrations of LWX-473 (4, 8, 12  $\mu$ M) and/or DEX (100  $\mu$ M) for 48 h. The cells were then collected and fixed with 70% ethanol overnight at –20 °C. Afterward, the cells were washed twice with PBS and incubated in the dark with Triton X-100, RNase A, and PI for 30 min at 37 °C. Finally, the cells were analyzed using a flow cytometer (ACEA NovoCyte) [15].

### 2.6 Hoechst 33258 Staining

The cells (2 × 10<sup>4</sup> cells/well) were seeded into a 6-well plate and treated with DMSO, DEX (100  $\mu$ M), LWX-473 (8  $\mu$ M), and DEX + LWX-473 (100 + 8  $\mu$ M) for 48 h. Afterward, the cells were collected and washed twice with precooled PBS. They were then stained with the Hoechst 33258 Staining Kit (Beyotime, China). Finally, a suitable volume of PBS was used to resuspend the cells, which were then dropped and mounted on a slide for observation of apoptotic bodies using a Nikon fluorescence microscope [14].



**Fig. 1. Synthesis and characterization of LWX-473.** (A) The synthesis of compound LWX-473. (B) The  $^1\text{H}$  NMR of compound LWX-473. (C) The HRMS of compound LWX-473. (D) The  $^{13}\text{C}$  NMR of compound LWX-473. LWX-473, methyl 3-((2-(1H-indol-3-yl)ethyl)(benzyl)amino)-5-chloro-2-oxoindoline-3-carboxylate; NMR, nuclear magnetic resonance; HRMS, high-resolution mass spectrometry.

### 2.7 The Mitochondrial Membrane Potential Assay

The mitochondrial membrane potential (MMP) variations were detected using the MMP assay kit with JC-1 (Beyotime, China) following the manufacturer's protocol. Briefly, the collected cells were incubated with JC-1 dye solution for 20 min. Afterward, the cells were collected and washed (600 g, 4 °C) in JC-1 dye buffer (1×) for 3–4 min. The cells were then resuspended in an appropriate amount of JC-1 dye buffer (1×) and observed under a Nikon fluorescence microscope [16].

### 2.8 Western Blot Analysis

Cells ( $5 \times 10^5$ /dish) were treated with DMSO, LWX-473 (8  $\mu\text{M}$ ), DEX (100  $\mu\text{M}$ ), and LWX-473 + DEX (8 + 100  $\mu\text{M}$ ) for 48 h. After incubation, the cells were collected and washed. An appropriate amount of cell lysate (RIPA:PMSF = 100:1) was added, and the mixture was incubated at 4 °C for 30 min. Cell debris was removed by centrifugation at 12,000 rpm for 15 min at 4 °C. Protein quantification was performed using the BCA assay (Solarbio, Beijing, China). The protein samples were then mixed with 5× loading buffer and boiled at 100 °C for 3–5 min. Equal amounts of protein from each sample were separated by sodium dodecyl sulfate -polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene fluoride (PVDF) membranes. The membranes were blocked with 5% bovine serum albumin (BSA) for 2 h and then incubated overnight with primary antibodies at 4 °C. After incubation with the secondary antibodies (H + L; DyLight™ 800 4X PEG Conjugate; CST, 5151, Boston, MA, USA), the membranes were scanned using an Odyssey scanner (Li-Cor Biosciences, Lincoln, NE, USA). Densitometry analy-

ses of the western blot bands were conducted using Image Studio software (Version 3.1, Texas Instruments, Dallas, TX, USA) [16,17].

### 2.9 Statistical Analysis

All statistical analyses were performed using the ANOVA test, followed by post-hoc analysis using GraphPad Prism9 Software (GraphPad Software, Inc., San Diego, CA, USA). All experiments were conducted in triplicates with three independent experiments. The data were expressed as mean  $\pm$  SD, and a *p*-value ( $*p < 0.05$ ,  $**p < 0.01$ ,  $***p < 0.001$ ,  $****p < 0.0001$ ) was considered statistically significant.

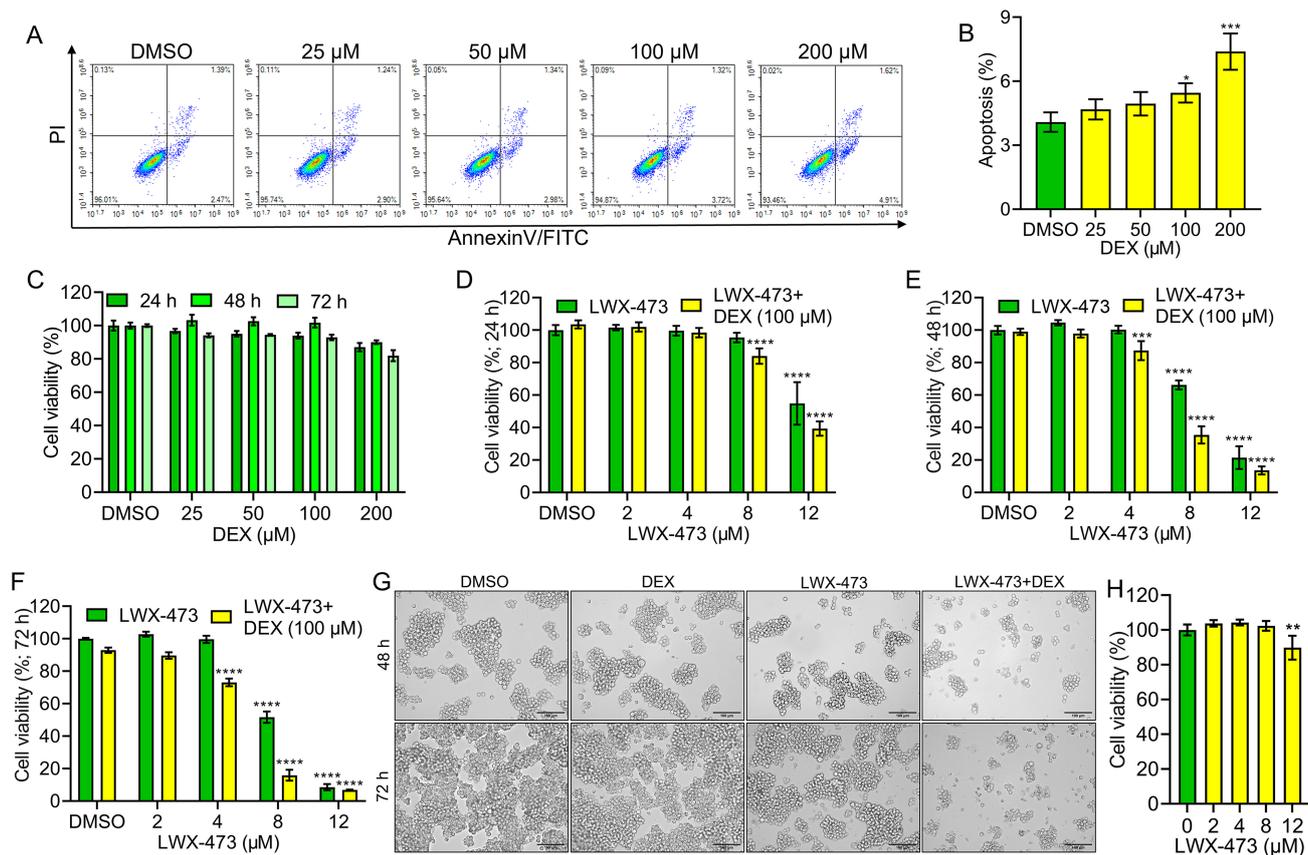
## 3. Results

### 3.1 Synthesis and Characterization of LWX-473

Natural indole compounds have shown promise in addressing drug resistance [18,19]. However, these compounds are naturally produced in small amounts. To overcome this limitation, a new indole derivative, methyl 3-((2-(1H-indol-3-yl)ethyl)(benzyl)amino)-5-chloro-2-oxoindoline-3-carboxylate (LWX-473), was designed and synthesized using a previously reported method. The synthesis involved the reaction of methyl 3,5-dichloro-2-oxoindoline-3-carboxylate with N-benzyl-2-(1H-indol-3-yl)ethan-1-amine, as depicted in Fig. 1A–D.

### 3.2 LWX-473 and DEX Inhibit the Growth of Jurkat Cells Synergistically

The T-cell leukemic Jurkat cells have been reported to exhibit resistance to glucocorticoids (GCs) due to qualitative (GR mutations) or quantitative defects in GR expres-



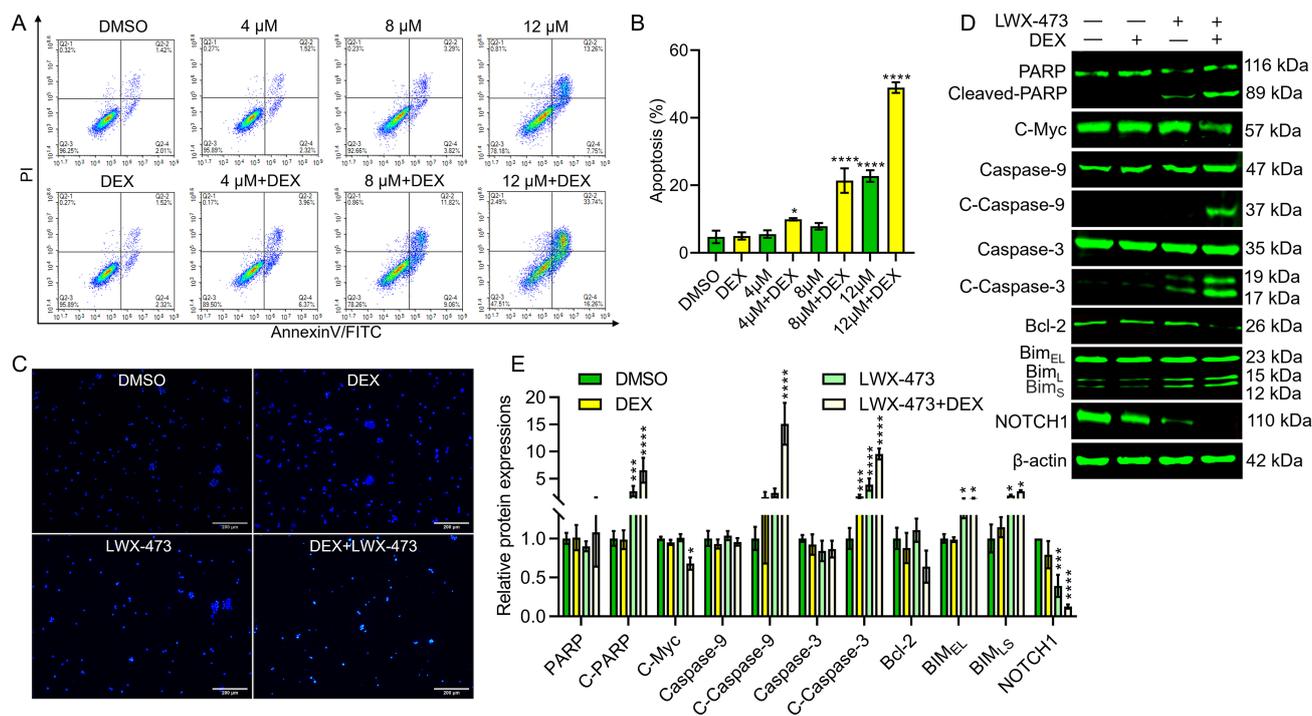
**Fig. 2. Effect of LWX-473 combined with Dexamethasone on the proliferation of Jurkat cells.** (A) Flow cytometric analysis of indicated Dexamethasone (DEX) concentrations on the proliferation of Jurkat cells. (B) Densitometry analysis of apoptotic effects of different DEX concentrations on Jurkat cells at 48 h. (C) Cell viability effect of different concentrations of DEX on Jurkat cells at indicated times. (D–F) The effect of various concentrations of LWX-473 on the proliferation of Jurkat cells in the presence of DEX (100 μM) for 24 (D), 48 (E), and 72 h (F) of incubation. (G) Microscopic images (20×) showing the changes in cell density and morphology of Jurkat cells treated with DEX or/and LWX-473 at the indicated time points. scale bar = 100 μm. (H) Proliferation of human non-tumorous liver cells, HL7702 with different concentrations of LWX-473 compound. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , \*\*\*\*  $p < 0.0001$  vs. control group. DMSO, dimethylsulfoxide.

sion [20]. To investigate the resistance of Jurkat cells to DEX-induced cell death, we treated the cells with different doses of DEX for varying durations and observed their response. The results showed that even at a high dose of 100 μM, DEX was unable to induce cell death in Jurkat cells (Fig. 2A–C). To assess the sensitivity of the novel indole derivative LWX-473 to DEX, we treated Jurkat cells with LWX-473 alone, DEX alone, or a combination of LWX-473 and DEX for different durations (24, 48, and 72 h). The results demonstrated that LWX-473 inhibited the growth of Jurkat cells in a time- and dose-dependent manner. Notably, the co-treatment of LWX-473 with DEX exhibited a more pronounced growth inhibition compared to LWX-473 alone at 48 and 72 h. These findings suggested a synergistic growth inhibition effect of LWX-473 and DEX on Jurkat, CEM/C1, and CEM-C7H2 cells (Fig. 2D–F; **Supplementary Fig. 1**). Morphological changes such as cellular loss, membrane bubbling, and cell death, leading to a loss of cell viability, further contributed to the synergis-

tic effect of LWX-473 and DEX in Jurkat cells (Fig. 2G). Importantly, LWX-473 displayed minimal toxicity on non-tumorous human normal liver cells HL7702, indicating its potential selectivity towards cancer cells (Fig. 2H).

### 3.3 LWX-473 Combined with DEX Induces Apoptosis of Jurkat Cells

Glucocorticoid resistance in leukemia cells allows them to evade normal apoptosis processes and survive. Therefore, we investigated whether LWX-473 could induce apoptosis in Jurkat cells. We observed that LWX-473 could induce apoptosis in a dose-dependent manner. Moreover, when combined with DEX, LWX-473 exhibited a more pronounced induction of apoptosis, overcoming the drug resistance effect of DEX (Fig. 3A,B). Hoechst staining further confirmed these findings, as treatment with DEX or LWX-473 alone did not significantly induce apoptosis, whereas the combination of DEX and LWX-473 resulted in significant apoptosis and cellular fragmentation, characterized by



**Fig. 3. LWX-473 combined with DEX induces apoptosis of Jurkat cells.** (A) Flow cytometric analysis to detect the effect of LWX-473 with or without DEX on apoptosis of Jurkat cells. (B) Apoptotic effects of different combinational concentrations on Jurkat cells at 48 h of incubation. (C) Jurkat cells treated with various indicated combinations were stained with Hoechst (20 $\times$ ) and analyzed for apoptotic bodies. scale bar = 200  $\mu$ m. (D) Western blot images showing variations in indicated protein levels after the intervention of different indicated drug combinations,  $\beta$ -actin was used as loading control. (E) Graph showing variations in protein expression levels of incited proteins after the indicated cotreatments. Data were represented as mean  $\pm$  standard deviation (SD), \*  $p < 0.05$ , \*\*\*  $p < 0.001$ , \*\*\*\*  $p < 0.0001$  vs. the control group.

the presence of apoptotic bodies (Fig. 3C). Furthermore, we examined the levels of various apoptotic markers and found that the combination of DEX and LWX-473 significantly increased the levels of cleaved PARP, cleaved caspase3, cleaved caspase9, BIMEL, and BIMSL. Additionally, it significantly reduced the levels of NOTCH1, c-Myc, and Bcl-2, promoting the apoptotic effects, compared to the control (Fig. 3D,E). These results suggest that the combination of DEX and LWX-473 enhances apoptosis in Jurkat cells by modulating key apoptotic proteins.

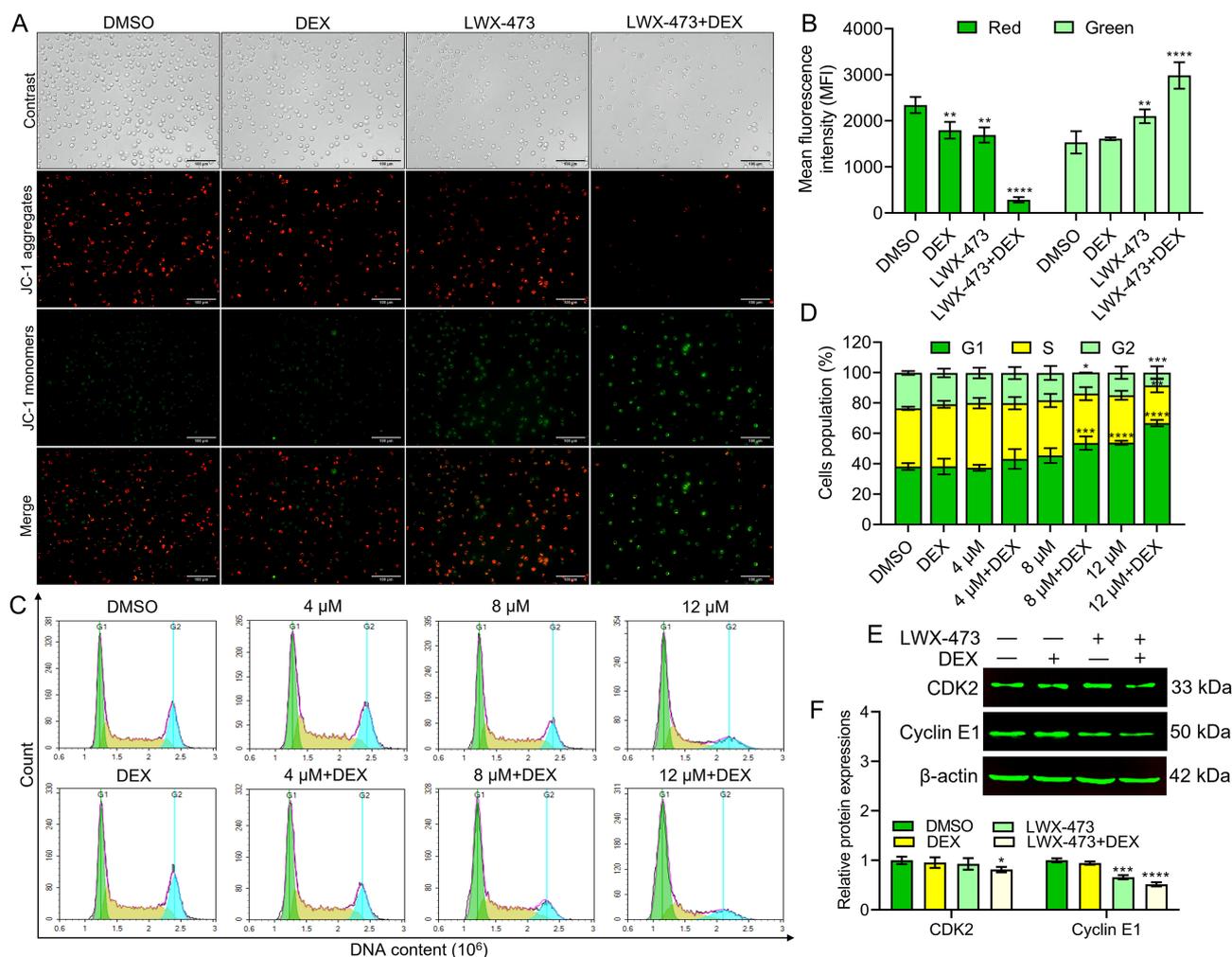
### 3.4 Co-Treatment Induced G1 Phase Cell Cycle Arrest and MMP Changes in Jurkat Cells

As an early event in apoptosis, there is a decrease in mitochondrial membrane potential (MMP) [21]. To assess the effect of the combination of LWX-473 and DEX on MMP, we used JC-1, a fluorescent probe to detect MMP. In Fig. 4A, compared to the other groups, the combination group exhibited stronger green fluorescence (JC-1 monomers) and weaker red fluorescence (JC-1 aggregates), indicating a significant decrease in MMP (Fig. 4B). Furthermore, it is known that DEX can induce G1 phase cell cycle arrest in cancer cells [22]. To investigate whether the co-administration of LWX-473 and DEX affects the cell cy-

cle of Jurkat cells, we treated the cells with different concentrations of LWX-473 and DEX individually. The results demonstrated that LWX-473 could dose-dependently induce G1 phase cell cycle arrest in Jurkat cells (Fig. 4C,D). Additionally, we examined G1 phase-related proteins using western blot analysis. When LWX-473 was combined with DEX, the levels of CDK2 and cyclin E1 proteins were significantly downregulated (Fig. 4E,F) in Jurkat cells, indicating the blockade of the cell cycle in the G1 phase.

### 3.5 LWX-473 Enhances Glucocorticoid Sensitivity by Inhibiting JAK2/STAT3/PI3K/AKT Signaling

Inhibition of the JAK/STAT pathway has been shown to enhance the sensitivity of DEX-induced apoptosis. To investigate the effect of LWX-473 and DEX on this pathway, we analyzed the protein expression of phosphorylated JAK2, STAT3, PI3K, and AKT. We observed that the combination of LWX-473 and DEX significantly reduced the phosphorylation of JAK2. Neither DEX nor LWX-473 alone had an impact on the expression of phosphorylated JAK2 protein compared to the control (Fig. 5A). Furthermore, we found that co-treatment with LWX-473 and DEX significantly reduced the phosphorylation of PI3K and AKT proteins, indicating their involvement in the regulation of



**Fig. 4. LWX-473 combined with DEX induced mitochondrial membrane potential (MMP) changes and G1 phase cell cycle arrest of Jurkat cells.** (A) Cells treated with indicated combinations for 48 h were stained with JC-1 and analyzed by fluorescent microscope (40×) to differentiate the JC-1 monomers and polymers. scale bar = 100 μm. (B) Cells treated at indicated concentrations were analyzed for green and red fluorescence stating mitochondrial damage. (C) Flow cytometric analysis to detect the effect of LWX-473 (4, 8, 12 μM) with or without DEX (100 μM) on the cell cycle of Jurkat cells. (D) Densitometry of the cells undergoing arrest at various stages of the cell cycle 48 h of incubation. (E) Western blot images showing variations in indicated protein levels after the intervention of different indicated drug combinations, β-actin was used as the loading control. (F) Graph showing variations in protein expression levels of incited proteins after the indicated cotreatments. Data were represented as mean ± SD, \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , \*\*\*\*  $p < 0.0001$  vs. the control group.

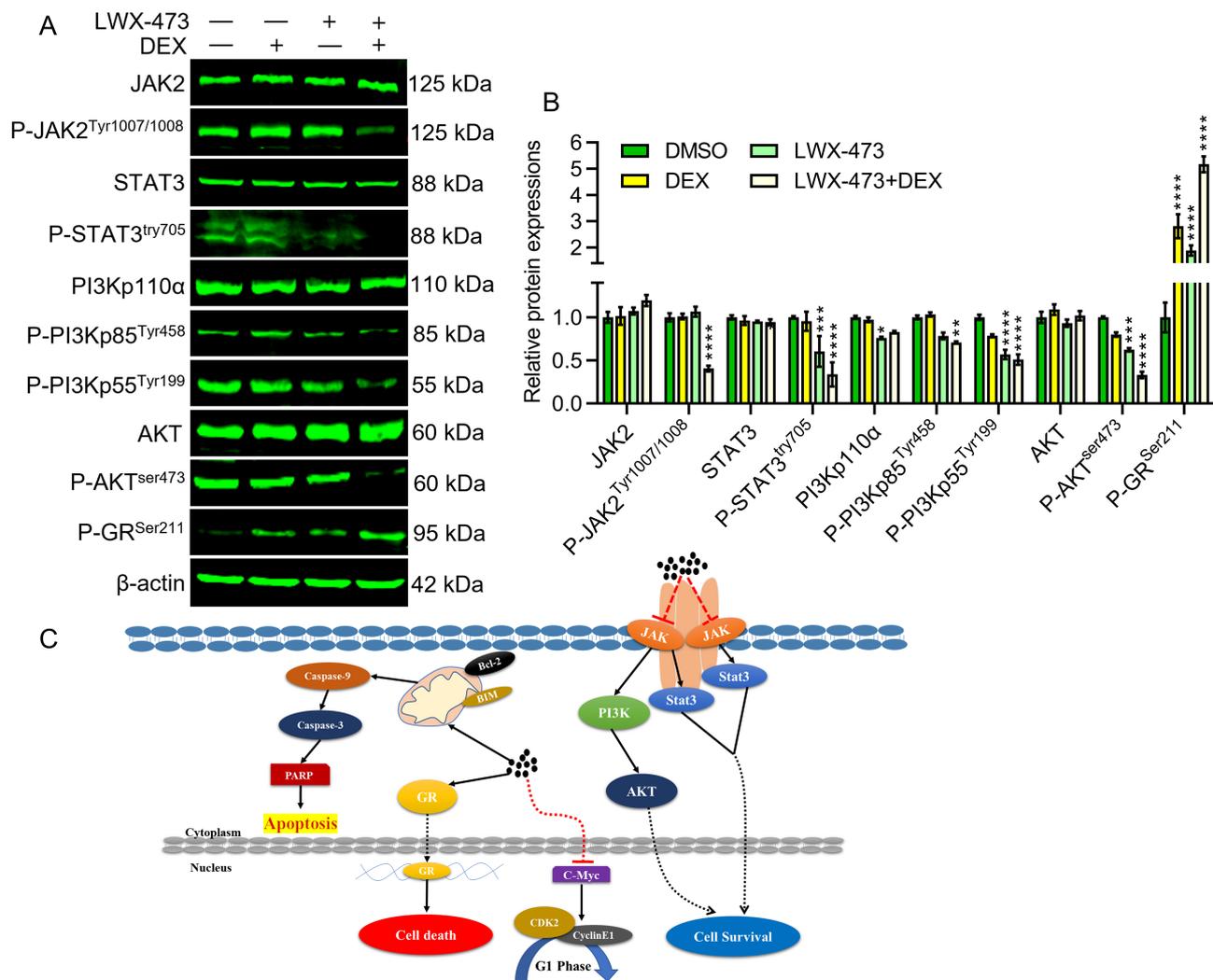
this pathway (Fig. 5B). These findings suggest that the combinational treatment of LWX-473 and DEX can overcome glucocorticoid resistance in Jurkat cells through the JAK2/STAT3/PI3K/AKT signaling pathway. Additionally, during co-treatment, there was an increased expression of phosphorylated GR (Ser211), confirming the escape of cells from the chemoresistance of glucocorticoids in Jurkat cells.

#### 4. Discussion

GCs regulate cell proliferation, inflammatory response, and metabolism by binding to the glucocorticoid receptor (GR) [23]. Moreover, resistance to glucocorticoids forms a complex process in leukemia. Thus, therapeutic

agents to overcome the resistance by enhancing apoptosis in leukemic cells are needed for anti-cancer therapy. This study investigated a novel indole compound (LWX-473), which was studied to enhance the apoptotic effect in the presence of a well-known GC, DEX.

The apoptosis, or programmed cell death, by employing cell cycle arrest contributes to the first line of chemotherapy. It can even lead to mitochondrial damage by increasing the level of cleavage of caspases and PARPs. In addition, the Bcl-2 family is related to glucocorticoid resistance. It has been reported that the increase in BIM could effectively overcome glucocorticoid resistance [24,25]. Similarly, the combination of drugs, LWX-473



**Fig. 5. LWX-473 enhances glucocorticoid DEX sensitivity of Jurkat cells by inhibiting JAK2/STAT3/PI3K/AKT pathway.** (A) Western blot images showing variations in indicated protein levels after the intervention of different indicated drug combinations,  $\beta$ -actin was used as the loading control. (B) Graph showing variations in protein expression levels of incited proteins after the indicated cotreatments. (C) Signal pathway diagram of LWX-473 overcoming glucocorticoid resistance in Jurkat cells. Data were represented as mean  $\pm$  SD, \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , \*\*\*\*  $p < 0.0001$  vs. the control group.

and DEX, could downregulate the anti-apoptotic Bcl-2 and upregulate the apoptosis-promoting BIM isomers. Similar effects were seen in the apoptosis and cell cycle analysis. In co-administration, we can decipher enhanced apoptosis by accelerating G1 cell cycle arrest. The level of BIM is closely related to the increase of glucocorticoid resistance, while the decrease of Bcl-2 can accelerate mitochondrial membrane potential damage and apoptosis in Jurkat cells.

The *c-Myc* gene is one of the essential members of the *Myc* gene family, and the activation of the *c-Myc* gene is closely related to the occurrence and development of many cancers. Targeted inhibition of the *c-Myc* pathway reduces tumor growth and restores antitumor immunity [24,25]. In addition, many studies have shown that *c-Myc* can affect the process of the cell cycle and interact with cyclin to arrest the tumor cell cycle [26,27]. The *c-Myc* gene is abnormally

activated in a variety of cancers and can regulate the cell cycle of tumor cells to make them divide rapidly [28]. However, in our study, LWX-473 combined with DEX significantly reduced the *c-Myc* protein and arrested Jurkat cell proliferation at its G1 phase, causing a significant reduction in NOTCH1, CDK2, and cyclin E1. This suggests that LWX-473's mechanism of action is potentially NOTCH1-dependent.

The abnormal activation of the Janus kinase/signal transducer and activator of the transcription (JAK/STAT) signaling pathway can cause many diseases. Mutations or overexpression of the JAK2/STAT3 pathway can be observed in children and adults with acute lymphoblastic leukemia [29,30]. Moreover, it can also restore the sensitivity of leukemia cells to glucocorticoids in leukemia treatment [31]. Additionally, studies have shown that the PI3K

pathway is involved in generating drug resistance, and the FDA has approved many PI3K inhibitors. Furthermore, studies have shown that the abnormal activation of JAK kinase can activate the PI3K pathway [32]. PI3K (phosphatidylinositol 3-kinase) participates in cell growth, survival, and metabolism. Increased activity of PI3K kinase is usually associated with various cancers [33]. Some studies have shown that the inhibition of PI3K and glucocorticoids cooperate in treating T-lymphoid leukemia [34,35]. Moreover, glucocorticoids can activate GR receptors to regulate target genes, which is a classic way for glucocorticoids to induce leukemia cells and an effective way to overcome glucocorticoid resistance [36]. This study found that the phosphorylation of JAK2 and STAT3, as well as PI3K/AKT pathway proteins, were significantly reduced in the presence of both DEX and LWX-473. However, the individual presence of either DEX or LWX-473 did not offer significant inhibition.

In the absence of glucocorticoids, the glucocorticoid receptor (GR) is located in the cytoplasm in an inactive form, bound to regulatory chaperones such as FKBP52, HSP90, and HSP70. Upon binding to glucocorticoids, GR is released from the chaperone complex and translocated to the nucleus as a dimer to bind specific DNA sequences called glucocorticoid response elements (GRE), thereby enhancing or inhibiting the transcription of specific target genes [37]. It has been proven that GR-mediated transcriptional activation is regulated by phosphorylation, and phosphorylation of Ser211 is a biomarker for GR activation *in vivo* [38,39]. Our experimental results show that LWX-473 enhances the sensitivity of Jurkat cells to DEX and improves apoptotic machinery in leukemia cells through the mitochondrial pathways and phosphorylation of GR (Ser221), inhibiting cellular proliferation.

## 5. Conclusions

When the synthesized indole compound LWX-473 was administered along with the glucocorticoid DEX, there was a significant enhancement in the apoptotic machinery of the chemotherapeutic drug. In other words, this co-administration was advantageous due to its efficiency in overcoming DEX resistance. It can enhance apoptosis by promoting cell cycle arrest and activating apoptotic molecules through the inhibition of anti-apoptotic mediators. Furthermore, the combinational therapy could enhance the inhibition of JAK2/STAT3/PI3K/AKT pathways by activating the phosphorylation of GR, contributing to the suppression of chemoresistance during anti-cancer LWX-473 treatments (Fig. 5C). These findings offer a novel arena of drug development with potential enrichment strategies to bypass the chemoresistance of glucocorticoids.

## Availability of Data and Materials

All data generated or analyzed during this study are included in this published article.

## Author Contributions

JS and KY designed the research study, performed the experiments, and wrote the original draft. KMV, WL, QL, QR, YH, XS, and SL performed formal analysis. XS, SL, LH, BG, MX, XH, and YL provided writing revisions. LH, BG, MX, XH, and YL conception of the work, supervised and contributed to writing, interpretation of data, funding acquisition, review, and editing. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

## Ethics Approval and Consent to Participate

Not applicable.

## Acknowledgment

The authors thank to Yaacov Ben David, State Key Laboratory for Functions and Applications of Medicinal Plants, Guizhou Medical University who provide a platform to finish some experiments.

## Funding

This research was funded by the grants from the National Natural Science Foundation of China (82360035, 81960546, 81872772, 82160808 & 82260040), The Science and Technology Department of Guizhou Province (QKHPTRC [2020]5008, QKHZC[2022]YB192).

## Conflict of Interest

The authors declare no conflict of interest.

## Supplementary Material

Supplementary material associated with this article can be found, in the online version, at <https://doi.org/10.31083/j.fbl2904163>.

## References

- [1] Vandewalle J, Luypaert A, De Bosscher K, Libert C. Therapeutic Mechanisms of Glucocorticoids. *Trends in Endocrinology and Metabolism: TEM*. 2018; 29: 42–54.
- [2] Schlossmacher G, Stevens A, White A. Glucocorticoid receptor-mediated apoptosis: mechanisms of resistance in cancer cells. *The Journal of Endocrinology*. 2011; 211: 17–25.
- [3] Mörnicke A, Reiter A, Zimmermann M, Gardner H, Stanulla M, Dördelmann M, *et al*. Risk-adjusted therapy of acute lymphoblastic leukemia can decrease treatment burden and improve survival: treatment results of 2169 unselected pediatric and adolescent patients enrolled in the trial ALL-BFM 95. *Blood*. 2008; 111: 4477–4489.
- [4] Inaba H, Pui CH. Glucocorticoid use in acute lymphoblastic leukaemia. *The Lancet. Oncology*. 2010; 11: 1096–1106.
- [5] Gruber G, Carlet M, Türtscher E, Meister B, Irving JAE, Ploner C, *et al*. Levels of glucocorticoid receptor and its ligand determine sensitivity and kinetics of glucocorticoid-induced leukemia apoptosis. *Leukemia*. 2009; 23: 820–823.

- [6] Liu H, Li Z, Qiu F, Li C, Lin X, He Y, *et al.* Association Between *NR3C1* Mutations and Glucocorticoid Resistance in Children With Acute Lymphoblastic Leukemia. *Frontiers in Pharmacology*. 2021; 12: 634956.
- [7] Delgado-Martin C, Meyer LK, Huang BJ, Shimano KA, Zinter MS, Nguyen JV, *et al.* JAK/STAT pathway inhibition overcomes IL7-induced glucocorticoid resistance in a subset of human T-cell acute lymphoblastic leukemias. *Leukemia*. 2017; 31: 2568–2576.
- [8] Hall CP, Reynolds CP, Kang MH. Modulation of Glucocorticoid Resistance in Pediatric T-cell Acute Lymphoblastic Leukemia by Increasing BIM Expression with the PI3K/mTOR Inhibitor BEZ235. *Clinical Cancer Research: an Official Journal of the American Association for Cancer Research*. 2016; 22: 621–632.
- [9] Dhyani P, Quispe C, Sharma E, Bahukhandi A, Sati P, Attri DC, *et al.* Anticancer potential of alkaloids: a key emphasis to colchicine, vinblastine, vincristine, vindesine, vinorelbine and vincamine. *Cancer Cell International*. 2022; 22: 206.
- [10] Chow LQM, Eckhardt SG. Sunitinib: from rational design to clinical efficacy. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology*. 2007; 25: 884–896.
- [11] Zeino M, Paulsen MS, Zehl M, Urban E, Kopp B, Efferth T. Identification of new P-glycoprotein inhibitors derived from cardiotonic steroids. *Biochemical Pharmacology*. 2015; 93: 11–24.
- [12] Jagtap AD, Chang PT, Liu JR, Wang HC, Kondekar NB, Shen LJ, *et al.* Novel acylureidoindolin-2-one derivatives as dual Aurora B/FLT3 inhibitors for the treatment of acute myeloid leukemia. *European Journal of Medicinal Chemistry*. 2014; 85: 268–288.
- [13] Gajendran B, Durai P, Madhu Varier K, Chinnasamy A. A novel phytosterol isolated from *Datura innoxia*, RinoxiaB is a potential cure colon cancer agent by targeting BAX/Bcl2 pathway. *Bioorganic & Medicinal Chemistry*. 2020; 28: 115242.
- [14] Long Q, Xiao X, Yi P, Liu Y, Varier KM, Rao Q, *et al.* L20, a Calothrixin B analog, induces intrinsic apoptosis on HEL cells through ROS/ $\gamma$ -H2AX/p38 MAPK pathway. *Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie*. 2021; 137: 111336.
- [15] Yang J, Chen L, Yan Y, Qiu J, Chen J, Song J, *et al.* BW18, a C-21 steroidal glycoside, exerts an excellent anti-leukemia activity through inducing S phase cell cycle arrest and apoptosis via MAPK pathway in K562 cells. *Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie*. 2019; 112: 108603.
- [16] Varier KM, Dan G, Liu W, Wu G, Xiao C, Lei H, *et al.* Stilbene B10 induces apoptosis and tumor suppression in lymphoid Raji cells by BTK-mediated regulation of the KRAS/HDAC1/EP300/PEBP1 axis. *Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie*. 2022; 156: 113887.
- [17] Yu F, Gajendran B, Wang N, Sample KM, Liu W, Wang C, *et al.* ERK activation via A1542/3 limonoids attenuates erythro leukemia through transcriptional stimulation of cholesterol biosynthesis genes. *BMC Cancer*. 2021; 21: 680.
- [18] Liu Y, Cui Y, Lu L, Gong Y, Han W, Piao G. Natural indole-containing alkaloids and their antibacterial activities. *Archiv Der Pharmazie*. 2020; 353: e2000120.
- [19] Xu M, Peng R, Min Q, Hui S, Chen X, Yang G, *et al.* Bisindole natural products: A vital source for the development of new anticancer drugs. *European Journal of Medicinal Chemistry*. 2022; 243: 114748.
- [20] Riml S, Schmidt S, Ausserlechner MJ, Geley S, Kofler R. Glucocorticoid receptor heterozygosity combined with lack of receptor auto-induction causes glucocorticoid resistance in Jurkat acute lymphoblastic leukemia cells. *Cell Death and Differentiation*. 2004; 11: S65–S72.
- [21] Bock FJ, Tait SWG. Mitochondria as multifaceted regulators of cell death. *Nature Reviews. Molecular Cell Biology*. 2020; 21: 85–100.
- [22] Funakoshi Y, Shiono H, Inoue M, Kadota Y, Ohta M, Matsuda H, *et al.* Glucocorticoids induce G1 cell cycle arrest in human neoplastic thymic epithelial cells. *Journal of Cancer Research and Clinical Oncology*. 2005; 131: 314–322.
- [23] Yamamoto KR. Steroid receptor regulated transcription of specific genes and gene networks. *Annual Review of Genetics*. 1985; 19: 209–252.
- [24] Toscan CE, Jing D, Mayoh C, Lock RB. Reversal of glucocorticoid resistance in paediatric acute lymphoblastic leukaemia is dependent on restoring BIM expression. *British Journal of Cancer*. 2020; 122: 1769–1781.
- [25] Malyukova A, Brown S, Papa R, O'Brien R, Giles J, Trahair TN, *et al.* FBXW7 regulates glucocorticoid response in T-cell acute lymphoblastic leukaemia by targeting the glucocorticoid receptor for degradation. *Leukemia*. 2013; 27: 1053–1062.
- [26] Li Y, Wang Y, Li J, Ling Z, Chen W, Zhang L, *et al.* Tacrolimus inhibits oral carcinogenesis through cell cycle control. *Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie*. 2021; 139: 111545.
- [27] Seth A, Gupta S, Davis RJ. Cell cycle regulation of the c-Myc transcriptional activation domain. *Molecular and Cellular Biology*. 1993; 13: 4125–4136.
- [28] Gao Y, Miles SL, Dasgupta P, Rankin GO, Cutler S, Chen YC. Trichodermin Induces G0/G1 Cell Cycle Arrest by Inhibiting c-Myc in Ovarian Cancer Cells and Tumor Xenograft-Bearing Mice. *International Journal of Molecular Sciences*. 2021; 22: 5022.
- [29] Hu X, Li J, Fu M, Zhao X, Wang W. The JAK/STAT signaling pathway: from bench to clinic. *Signal Transduction and Targeted Therapy*. 2021; 6: 402.
- [30] Vainchenker W, Constantinescu SN. JAK/STAT signaling in hematological malignancies. *Oncogene*. 2013; 32: 2601–2613.
- [31] Fang D, Kong LY, Cai J, Li S, Liu XD, Han JS, *et al.* Interleukin-6-mediated functional upregulation of TRPV1 receptors in dorsal root ganglion neurons through the activation of JAK/PI3K signaling pathway: roles in the development of bone cancer pain in a rat model. *Pain*. 2015; 156: 1124–1144.
- [32] Jampasri S, Reabroi S, Tungmunthum D, Parichatikanond W, Pinthong D. Plumbagin Suppresses Breast Cancer Progression by Downregulating HIF-1 $\alpha$  Expression via a PI3K/Akt/mTOR Independent Pathway under Hypoxic Condition. *Molecules (Basel, Switzerland)*. 2022; 27: 5716.
- [33] Goncalves MD, Hopkins BD, Cantley LC. Phosphatidylinositol 3-Kinase, Growth Disorders, and Cancer. *The New England Journal of Medicine*. 2018; 379: 2052–2062.
- [34] Silveira AB, Laranjeira ABA, Rodrigues GOL, Leal PC, Cardoso BA, Barata JT, *et al.* PI3K inhibition synergizes with glucocorticoids but antagonizes with methotrexate in T-cell acute lymphoblastic leukemia. *Oncotarget*. 2015; 6: 13105–13118.
- [35] Evangelisti C, Cappellini A, Oliveira M, Fragoso R, Barata JT, Bertaina A, *et al.* Phosphatidylinositol 3-kinase inhibition potentiates glucocorticoid response in B-cell acute lymphoblastic leukemia. *Journal of Cellular Physiology*. 2018; 233: 1796–1811.
- [36] Shi Y, Wang G, Muhowski EM, McCaw L, Wang C, Bjarnason G, *et al.* Ibrutinib reprograms the glucocorticoid receptor in chronic lymphocytic leukemia cells. *Leukemia*. 2019; 33: 1650–1662.
- [37] Necela BM, Cidlowski JA. Crystallization of the human glucocorticoid receptor ligand binding domain: a step towards selective glucocorticoids. *Trends in Pharmacological Sciences*. 2003; 24: 58–61.
- [38] Wang Z, Frederick J, Garabedian MJ. Deciphering the phosphorylation “code” of the glucocorticoid receptor in vivo. *The Journal of Biological Chemistry*. 2002; 277: 26573–26580.
- [39] Ismaili N, Garabedian MJ. Modulation of glucocorticoid receptor function via phosphorylation. *Annals of the New York Academy of Sciences*. 2004; 1024: 86–101.