ACCSCIENCE PUBLISHING

Eurasian Journal of Medicine and Oncology

ORIGINAL RESEARCH ARTICLE

Novel compound NHWD-870 suppresses melanoma proliferation by inducing cell cycle arrest and apoptosis

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Citation: Liu Y, Sun H, Yin M, Xue Y, Hu R. Novel compound NHWD-870 suppresses melanoma proliferation by inducing cell cycle arrest and apoptosis. *Eurasian J Med Oncol.* 2026;10(5):025010554. doi: 10.36922/EJMO025010554

Received: December 30, 2025

Revised: February 1, 2026

Accepted: February 4, 2026

Published online: March 31, 2026

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Abstract

Introduction: Malignant melanoma is the most aggressive form of skin cancer worldwide. Although various novel therapies, including targeted therapies, immunotherapies, and epigenetic therapies, have been developed in recent decades to provide new treatment options, most patients with melanoma either respond poorly or develop resistance quickly. Thus, new therapeutic options are urgently needed. Bromodomain and extra-terminal domain (BET) inhibition has emerged as a potential therapeutic strategy in cancer treatment.

Objectives: We aimed to study the effects of NHWD-870, a novel BET inhibitor analogue of BMS-986158, on melanoma proliferation.

Methods: We examined the effects of BMS-986158 and NHWD-870 on tumor proliferation *in vitro* and *in vivo* using the Cell Counting Kit-8 assay, Ki67-positive cell analysis, colony formation assay, and a xenograft mouse model. Flow cytometry and Western blotting were performed to investigate the effects of NHWD-870 on melanoma cell cycle and apoptosis.

Results: We found that NHWD-870 exhibited improved ability to suppress melanoma cell proliferation both *in vitro* and *in vivo* compared to BMS-986158. Mechanistically, NHWD-870 treatment induced G2/M cell cycle arrest and apoptosis, accompanied by decreased cyclin B1 levels.

Conclusion: Our results suggest that NHWD-870 exhibits effective antineoplastic potency. It effectively inhibits melanoma progression, demonstrating greater efficacy than BMS-986158. These findings support further development of NHWD-870 as a potential new therapeutic candidate for melanoma treatment.

Keywords: Bromodomain and extra-terminal domain inhibitor; Cell proliferation; Melanoma; Cell cycle; Apoptosis

1. Introduction

Malignant melanoma is the most aggressive skin cancer and is associated with increased morbidity and mortality rates worldwide.[1,2] The incidence of cutaneous melanoma is rising.[3] It is the fifth most common type of cancer in men and the sixth most common in women. Until the approval of targeted therapies and immunotherapies over the last decade, such as B-Raf proto-oncogene, serine/threonine kinase (BRAF) inhibitors, mitogen-activated protein kinase kinase inhibitors, and immune checkpoint inhibitors, surgical removal and chemotherapy were the only available treatments for melanoma. Surgery remains the primary approach, where wide excision is performed for primary tumors with the margin determined by tumor thickness; however, the appropriate margin for high-risk tumors is still being explored.[4] Sentinel lymph node biopsy is important for detecting regional metastasis, but the value of completion lymph node dissection has been questioned.[5] Advanced treatments have dramatically improved the survival of patients with melanoma. However, resistance to targeted agents develops quickly,[6] while most melanoma patients do not respond well to immunotherapies.[7] Anti-PD-1 and BRAF-targeted therapies are commonly used but face issues such as a lack of overall survival benefit and drug resistance.[8] In advanced melanoma, targeted therapies and immunotherapies have limitations, including resistance and biomarker deficiency.[9] Thus, despite substantial improvements in melanoma treatment over recent years, new therapeutic options are still urgently needed.

Bromodomains (BRDs) are a group of evolutionarily conserved domains that recognize acetylated lysine residues in histones. There are 42 known BRD-containing proteins in the human genome. These proteins regulate gene expression by recognizing acetylated histones, binding gene loci, recruiting other factors, and altering local epigenetic features.[10] Bromodomain and extra-terminal (BET) proteins, including BRD2, BRD3, BRD4, and BRDT, are a subfamily of BRD proteins that share a common domain architecture, comprising two N-terminal bromodomains (BD1 and BD2) and an extra-terminal domain. Current studies indicate that BET family proteins are widely expressed in human tissues and exert regulatory functions in gene transcription under physiological conditions, modulating normal cellular processes such as cell growth. Under pathological conditions, BET proteins are implicated in the pathogenesis and progression of various diseases, including inflammation, epilepsy, chronic obstructive pulmonary disease, pulmonary fibrosis, and tumors.[11-13] BRD4 is the best-characterized BET protein and is reported to be aberrantly expressed in cancer, influencing tumor initiation and progression. Blocking the protein–protein interaction between BRD4 and acetyl-lysine in histones with small-molecule inhibitors can effectively prevent tumor cell proliferation.[14,15]

Highly selective BRD4 inhibitors act by specifically recognizing and binding to the hydrophobic pocket of BET proteins, thereby completely or partially blocking the formation of the corresponding protein complexes. This mechanism downregulates oncogene transcription, including those involving MYC and BCL2-family genes, thereby affecting tumor initiation, invasion, and metastasis.[16] To date, a series of small-molecule BRD4 inhibitors has been developed and shown to suppress the growth of multiple tumor types *in vivo*. Several of these agents have entered clinical trials for hematologic malignancies and solid tumors, including acute myeloid leukemia, multiple myeloma, lung adenocarcinoma, and melanoma.[14,17,18] Promising results have been observed in human clinical studies for some of these inhibitors,[19,20] underscoring BRD4 as a promising therapeutic target for cancer treatment.

The discovery and optimization of BET bromodomain inhibitors represent a significant trajectory in oncology drug development. Early contributions include methyltriazolodiazepine derivatives disclosed by Mitsubishi Tanabe Pharma in 2009, which showed modest activity against BRD4 and potential in refractory cancers.[21] A major breakthrough followed in 2010 with JQ1, a selective thienotriazolodiazepine inhibitor that competitively occupies the acetyl-lysine-binding sites on BET proteins.[22] JQ1 demonstrated robust antitumor efficacy in preclinical models of NMC, establishing a valuable chemical scaffold for subsequent drug design.[23,24] Inspired by JQ1, first-generation clinical candidates such as CPI-0610, OTX-015, and I-BET762 were developed. However, their therapeutic impact in solid tumors was limited due to suboptimal clinical responses and dose-limiting toxicities. These challenges are likely linked to the ubiquitous expression of BRD4 protein across critical normal tissues, including hematopoietic, immune, and central nervous systems,[25] highlighting the risk of on-target adverse effects.

To overcome these limitations, next-generation inhibitors with distinct chemotypes have been pursued. BMS-986158, a structurally divergent compound developed through structure-based design, exhibits a promising preclinical profile and is currently under evaluation in Phase I/II trials for solid tumors.[22,23,26] This evolution underscores a central goal in the field: to develop agents with greater potency and a wider therapeutic window. Consequently, ongoing research is intensely focused on discovering novel, high-efficiency, and low-toxicity BET inhibitors to improve outcomes for patients with advanced malignancies. We thus designed a new series of compounds based on BMS-986158 and identified a new BET inhibitor, NHWD-870. In our recent study, NHWD-870 directly inhibited tumor cell proliferation via the noncanonical NF-κB/SPP1 pathway and blocked the proliferation of tumor-associated macrophages (TAMs).[27,28] However, its effect on the cell cycle and apoptosis remains unclear. Here, we show that NHWD-870 demonstrates greater ability—compared to BMS-986158—to suppress melanoma cell proliferation *in vitro* and *in vivo* by inducing cell cycle arrest at the G2/M stage and apoptosis. Overall, our study suggests that NHWD-870 exhibits effective antineoplastic potency and is a promising novel candidate drug for melanoma treatment.

2. Materials and methods

2.1. Cell culture

The melanoma cell line A375 was purchased from ATCC (United States of America [USA]) and was cultured in high-glucose Dulbecco's modified Eagle medium (DMEM) (06-1055-57-1ACS, BI, Israel) supplemented with 10% fetal bovine serum (04-001-1ACS, BI, Israel) and antibiotics. All cells were incubated at 37 °C in a humidified atmosphere containing 5% CO_2 (Thermo Scientific, USA).

2.2. NHWD-870

We thank Ningbo Wenda Pharma (China) for generously providing NHWD-870.

2.3. Colony formation assays

A375 cells were plated at 800 cells/well in 6-well plates, 24 h before treatment with NHWD-870. The cell culture medium was replaced with fresh medium containing NHWD-870 every 3 days. Two weeks after treatment, the cells were fixed with methanol (Cat# G1101, Servicebio, China) for 20 min and then stained with 0.25% crystal violet (Cat# 0121, Beyotime Biotechnology, China) in 20% methanol for 20 min. Plates were rinsed with water until background staining disappeared.

2.4. Cell Counting Kit-8 cell proliferation assay

A375 cells were plated at 800 cells/well in 96-well plates, 24 h before treatment with NHWD-870. At indicated time points, the medium was replaced with medium containing 10% Cell Counting Kit-8 reagent (Cat# B34302, Bimake, USA). Absorbance at 450 nm was measured (SpectraMax i3x, Molecular Devices, China) after 2.5 h of incubation.

2.5. Ki67-positive cell analysis

A375 cells were plated at a density of 5×10^5 cells/well in 6-well plates. After 12 h, the cells were treated with 2 nM or 4 nM NHWD-870. Medium without NHWD-870 was added to the blank and control cells. After 3 days, A375 cells were trypsinized (TBD Science, China) to prepare single-cell suspensions, centrifuged at 1000 rpm (L530R, Cence, China) for 5 min, and washed with phosphate-buffered solution. Cell fixation and permeabilization were performed using the Foxp3/Transcription Factor Staining Buffer set (#00-5523-00, Thermo Fisher Scientific, USA). Cells were then stained with Fixable Viability Dye from the Zombie Aqua Fixable Viability Kit (#423102, Biolegend, USA) and anti-Ki67–fluorescein isothiocyanate (FITC) monoclonal antibody (#11-5698-80, Thermo Fisher Scientific, USA). Ki67-positive cells were detected by flow cytometry and analyzed using FlowJo software (FlowJo Software version 7.6.1, Tree Star, Inc., USA).

2.6. Cell cycle analysis

A375 cells were plated at a density of 5×10^5 cells/well in 6-well plates. After 12 h, the cells were treated with 0.5, 2, or 8 nM NHWD-870. Medium without NHWD-870 was added to the blank and control cells. After 3 days of treatment, A375 cells were trypsinized to prepare single-cell suspensions. A375 cells were centrifuged at 1000 rpm for 5 min and washed with phosphate-buffered solution. The cells were then fixed in 70% ethanol on ice for 3 h and stained with propidium iodide (PI) solution (Becton, Dickinson and Company, USA) according to the manufacturer's instructions. Cell cycle analysis was performed using flow cytometry, and the data were assessed using ModFit software (ModFit LT for Windows Version 3.2, Verity Software House, USA).

2.7. Apoptosis assay

A375 cells were plated at a density of 2×10^5 cells/well in 6-well plates. After 12 h, cells were treated with 8 nM of NHWD-870. Medium without NHWD-870 was added to the blank and control cells. After 3 days of treatment, the medium was removed, and the A375 cells were trypsinized without ethylenediaminetetraacetic acid to prepare

single-cell suspensions. Each suspension was collected and centrifuged at 1000 rpm for 5 min. The collected cells were incubated with annexin V/PI staining solution (Becton, Dickinson and Company, USA) according to the manufacturer's instructions. Melanoma cell apoptosis was assessed using flow cytometry and analyzed using FlowJo software (FlowJo Software version 7.6.1, Tree Star, Inc., USA).

2.8. Western blot analysis

Cells were lysed in radioimmunoprecipitation assay lysis buffer supplemented with 1% protease inhibitor cocktail (B14002, Bimake, USA). After centrifugation, protein concentration of the lysates was quantified using the BCA Protein Assay Kit (Pierce, USA). Equal quantities of protein samples were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membranes, which were blocked with 5% nonfat milk (Solarbio, China). Membranes were probed overnight at 4 °C with primary antibodies: cyclin B1 (1:1000; #sc-245, Santa Cruz Biotechnology, USA); CDC2 (1:1000; #28439, CST, USA); GAPDH (1:3000; #6004-1-Ig, ProteinTech, China), and then incubated for 2 h at room temperature with the respective secondary antibodies (horseradish peroxidase [HRP]-conjugated goat anti-mouse IgG (heavy and light chains [H+L]) (1:5000; #AS003, Abclonal, China); HRP-conjugated goat anti-rabbit IgG (H+L) (1:5000; #AS014, Abclonal, China). Protein expression was visualized using ECL Select Western blot detection reagent (GE Healthcare, United Kingdom).

2.9. Animal study

A total of 15 female BALB/c nude mice were purchased from Shanghai Sippe-Bk Lab Animal Co., Ltd. (China). All mice were bred and housed in a temperature- and humidity-controlled room with a 12-h light–dark cycle. The animals were provided with autoclaved tap water and autoclaved standard laboratory chow *ad libitum*. During the study, the health and behavior of each mouse were closely monitored and evaluated at least once per day by trained and experienced animal research personnel at the Department of Laboratory Animals, Chongqing University Three Gorges Hospital. Mice were euthanized at the end point or if moribund with rapid weight loss, hunched posture, and labored breathing. For tumor studies, 5×10^6 A375 cells in 100 µl DMEM were injected subcutaneously into the right flank of each female BALB/c nude mouse. After 10 days, when tumors reached approximately 100 mm^3, BMS-986158 (2 mg/kg; $n = 5$), NHWD-870 (2 mg/kg; $n = 5$), or corn oil (C; $n = 5$) was administered orally once daily for five consecutive days, followed by a two-day recovery. Tumor size and body weight were recorded every three days. Tumor size was calculated as ([length × width^2]/2) using Vernier caliper measurements. The fifteen nude mice were euthanized at the end of the experiment (day 18). All mice were sacrificed by cervical dislocation after intraperitoneal injection with 0.1% pentobarbital sodium (30 mg/kg) (Sigma-Aldrich, China). Efforts were made to minimize animal suffering and to reduce the number of animals used. All animal experimental procedures were approved by the Ethics Committee of Chongqing University Three Gorges Hospital (Chongqing, China) and complied with the Animal Research: Reporting of *In Vivo* Experiments guidelines and the European Union Directive 2010/63/EU for animal experiments.

2.10. Statistical analysis

Data are expressed as the mean ± SEM. Statistical analysis was performed using an unpaired Student's *t*-test or one-way analysis of variance followed by Tukey's multiple comparison using SPSS software (version 19.0, IBM SPSS, USA). Statistical significance was set at $p < 0.05$.

3. Results

3.1. NHWD-870 is an analogue of the bromodomain and extra-terminal inhibitor BMS-986158

BMS-986158 is a bromodomain and BET family protein inhibitor that has demonstrated potent antitumor activity in preclinical models, prompting its evaluation in Phase I/II clinical trials across various malignancies. To enhance its therapeutic potential, particularly in terms of efficacy, a medicinal chemistry campaign was undertaken to optimize the structure of BMS-986158. From this effort, NHWD-870 was identified as a superior analog exhibiting the most potent biological activity in preliminary screenings (Figure 1A). To quantitatively compare the antiproliferative effects of the parent compound and its optimized derivative, we conducted *in vitro* assays using the human melanoma A375 cell line. Cell viability was measured via the CCK-8 assay following treatment with either BMS-986158 or NHWD-870. Both compounds significantly suppressed the proliferation of A375 cells compared to the vehicle control. Notably, NHWD-870 exhibited substantially enhanced potency, inhibiting cell growth at approximately one-third of the concentration required for BMS-986158 to achieve a comparable effect, indicating an approximate three-fold increase in activity (Figure 1B). This marked improvement underscores the success of the structure-optimization strategy and warrants further investigation into the pharmacological properties and mechanism of action of NHWD-870.

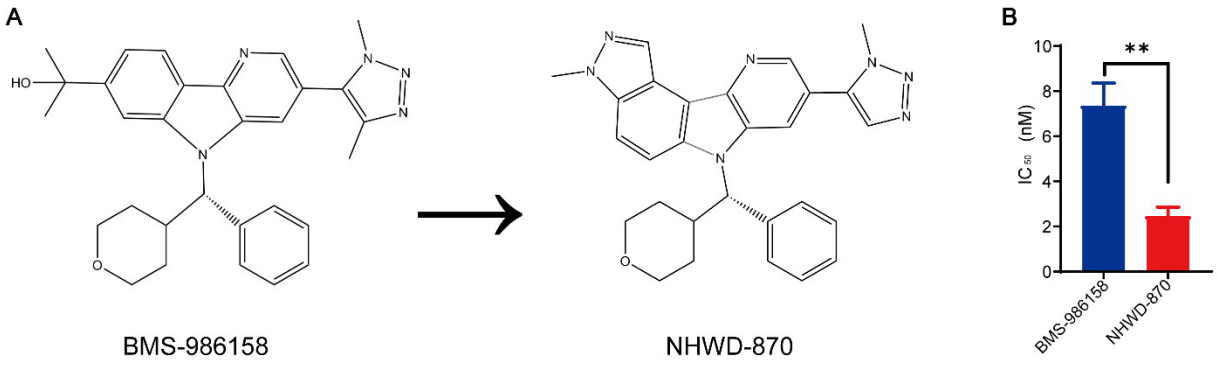


Figure 1. BET inhibitor BMS-986158 and its analogue NHWD-870. (A) Chemical structures of the BET inhibitor BMS-986158 and its analogue NHWD-870. (B) IC_{50} values of BMS-986158 and NHWD-870 in A375 cells, determined by the CCK-8 assay. All quantification graphs represent the mean ± SEM. $^{**}p < 0.01$; IC_{50} denotes half maximal inhibitory concentration.
Abbreviations: BET: Bromodomain and extra-terminal; CCK-8: Cell Counting Kit-8.

3.2. NHWD-870 inhibits tumor cell proliferation in melanoma cells

To further investigate the antiproliferative effects of NHWD-870, we performed a colony formation assay, which assesses the long-term clonogenic survival capacity of tumor cells. As shown in Figure 2A, treatment with NHWD-870 led to a pronounced and significant reduction in both the number and size of colonies formed by A375 melanoma cells compared to the vehicle-treated control group. This result demonstrates the compound's ability to sustainably inhibit the proliferative potential of these cells over an extended period. To gain deeper insight into the mechanism underlying this growth suppression, we analyzed Ki67 expression, a well-established cellular marker associated with active phases of the cell cycle (G1, S, G2, and mitosis), using flow cytometry. Following 3 days of treatment with NHWD-870, a dramatic decrease in the proportion of Ki67-positive A375 cells was observed. Quantitative analysis revealed that while 63.7% of cells in the control group were actively proliferating (Ki67-positive), this fraction dropped to 21.5% in the NHWD-870-treated population (Figure 2B and 2C). Collectively, the concordant data from the colony formation assay and the Ki67 proliferation marker analysis provide robust and complementary evidence that NHWD-870 potently impairs the proliferative capacity of A375 melanoma cells, not only by reducing their long-term clonogenic survival but also by forcing a larger proportion of the cell population into a non-proliferative state.

3.3. NHWD-870 induces cell cycle arrest at the G2/M stage and apoptosis in melanoma cells

Since cell cycle arrest is a primary mechanism underlying the suppression of cellular proliferation, we systematically investigated the ability of NHWD-870 to perturb the cell cycle in A375 melanoma cells. Flow cytometric analysis of DNA content was employed to determine the distribution of cells across different cell cycle phases following treatment. This analysis revealed a significant accumulation of cells in the G2/M phase upon exposure to NHWD-870. Specifically, the proportion of A375 cells in G2/M increased from 6.2% in the vehicle-treated control group to 13.6% in the NHWD-870-treated group, indicating a potent arrest at this critical checkpoint (Figure 3A and 3B). Furthermore, this G2/M phase arrest was demonstrated to be dose-dependent, with higher concentrations of NHWD-870 leading to a more pronounced accumulation of cells in this phase, thereby confirming a specific, concentration-responsive disruption of cell cycle progression.

To elucidate the molecular mechanism driving this arrest, we examined the expression of key regulatory proteins governing the G2/M transition. Western blot analysis was performed to assess the levels of cyclin B1 and its catalytic partner, CDC2, which together form the cyclin-dependent kinase complex essential for mitotic entry. Our results showed that treatment with NHWD-870 led to a significant, dose-dependent downregulation of cyclin B1 protein expression (Figure 3C). In contrast, the expression level of CDC2 remained largely unchanged under the same treatment conditions. This selective suppression of cyclin B1 strongly suggests that the observed G2/M phase arrest is mechanistically linked to the disruption of the active cyclin B1/CDC2 complex. The reduced availability of cyclin B1 likely impedes CDC2 activation, thereby preventing the cell from progressing from the G2 phase into mitosis. Collectively, these data indicate that the antiproliferative effect of NHWD-870 in A375 cells may be mediated by G2/M cell cycle arrest, specifically through

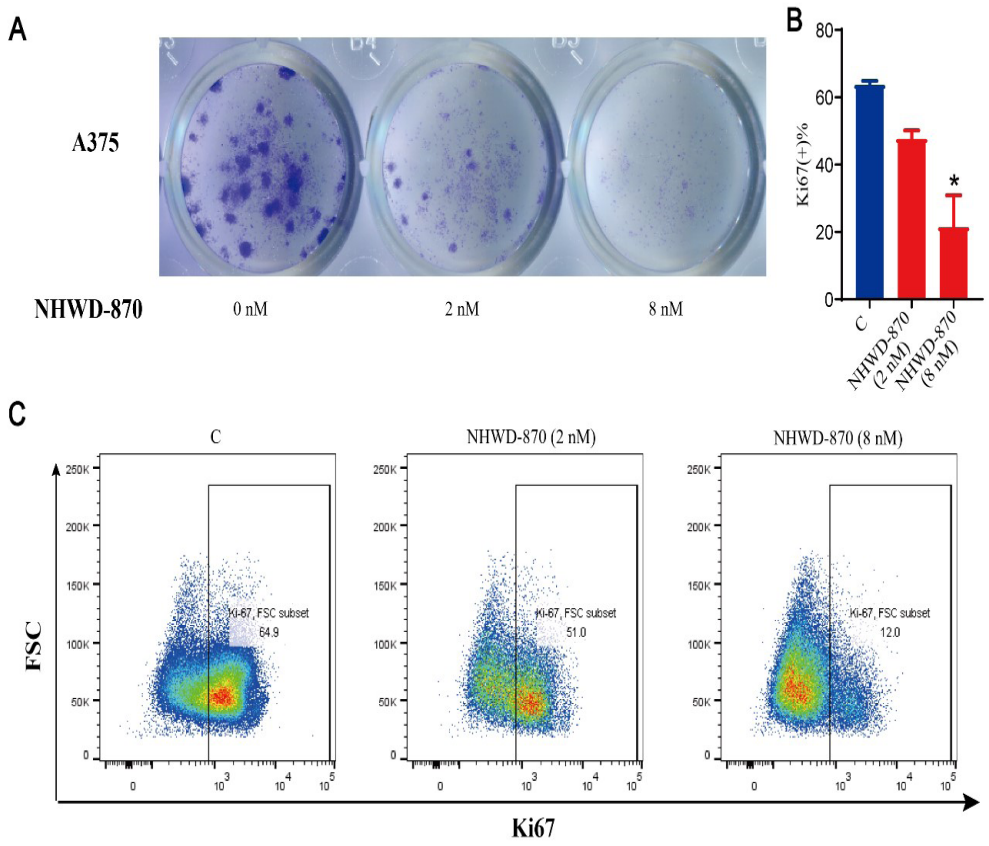


Figure 2. NHWD-870 inhibits melanoma tumorigenesis and proliferation. (A) Colony formation of A375 cells treated with different concentrations of NHWD-870. (B–C) Ki67 expression in A375 cells treated with NHWD-870 was determined by flow cytometry. The bar graphs show the percentage of Ki67 (+) cells. All quantification graphs show the mean ± SEM. *$p < 0.05$.

the downregulation of cyclin B1.

To further delineate the mechanism by which NHWD-870 inhibits melanoma cell growth, we investigated its potential to induce programmed cell death, or apoptosis. Apoptosis is a critical process for eliminating malignant cells, and its induction is a key attribute of many effective anticancer agents. To this end, we employed a well-established flow cytometry assay using dual staining with FITC-conjugated annexin V and PI. This method allows discrimination of viable cells (annexin V−/PI−), early apoptotic cells (annexin V+/PI−), which have externalized phosphatidylserine but maintain membrane integrity, and late apoptotic or necrotic cells (annexin V+/PI+). After treatment with NHWD-870, no significant difference in the proportion of late apoptotic cells was observed between treated and control cells; however, the percentages of total apoptotic cells and early apoptotic cells increased from 3.6% to 17.6% and from 0.8% to 13.6%, respectively (Figure 3D). These results indicate that NHWD-870 increases apoptosis in A375 melanoma cells.

3.4. NHWD-870 reduces melanoma tumor growth *in vivo*

To evaluate the *in vivo* antitumor efficacy of NHWD-870, we established a xenograft mouse model by inoculating nude mice with A375 human melanoma cells. Once the tumors reached a palpable size, the mice were randomly assigned to three treatment groups (n = 5 per group) to ensure an unbiased assessment of therapeutic response. The treatment regimen consisted of daily oral administration for five consecutive days, followed by a two-day recovery period, with this cycle repeated as outlined in Figure 4A. The experimental groups received either 2 mg/kg of BMS-986158, 2 mg/kg of NHWD-870, or an equivalent volume of corn oil as the vehicle control.

Throughout the study, animal body weight was meticulously monitored every three days as a primary indicator of systemic tolerability and treatment-related toxicity. Encouragingly, no significant body weight loss or other overt signs of distress were observed in any group, suggesting that both compounds were well-tolerated at the

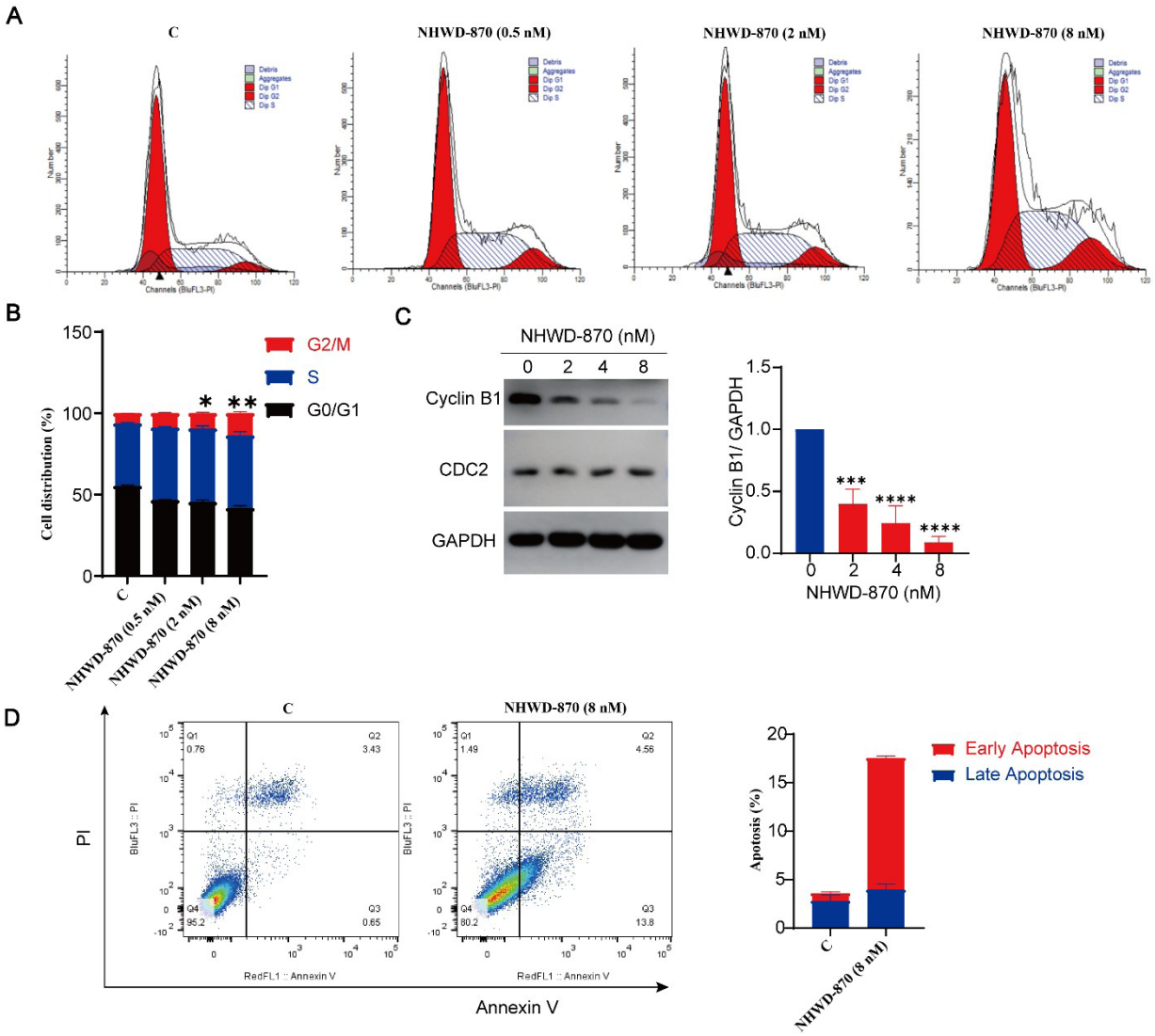


Figure 3. NHWD-870 induces G2/M cell cycle arrest and apoptosis. (A) Cell cycle distribution in A375 cells determined by flow cytometry. (B) Histograms showing the changes in the G2/M phase of A375 cells after treatment with NHWD-870. (C) Cells were treated with NHWD-870 for 3 days, and protein expression levels were determined by western blot analysis. (D) The percentage of apoptotic cells in A375 cells was analyzed by flow cytometry. The bar graphs show the percentage of apoptotic cells. All quantification graphs represent the mean ± SEM. * denotes $p < 0.05$; ** denotes $p < 0.01$.
Abbreviation: C: Control; PI: Propidium iodide.

administered dose. The antitumor effect was quantitatively assessed by measurements of tumor volume. Statistical analysis of the tumor growth curves revealed significant suppression of tumor progression in both the BMS-986158 and NHWD-870 treatment groups compared with the control cohort ($p < 0.05$), confirming the *in vivo* activity of the parent BET inhibitor. Strikingly, direct comparison between the two active treatment arms demonstrated that NHWD-870 induced a markedly greater reduction in tumor size and growth rate than BMS-986158 at the same dosage (Figure 4B–E). The final tumor sizes harvested at the endpoint of the experiment corroborated these volumetric measurements, further solidifying the quantitative findings.

In conclusion, this *in vivo* study demonstrates that NHWD-870 possesses remarkable efficacy in inhibiting melanoma growth. Its activity significantly surpasses that of its predecessor, BMS-986158, within the same experimental framework. These compelling results, showing potent efficacy coupled with an acceptable tolerability profile in a preclinical model, strongly support

the further development of NHWD-870 as a promising next-generation therapeutic candidate for melanoma and potentially other malignancies.

4. Discussion

Although classical chemotherapeutics have shown progress in the treatment of melanoma, they offer limited overall survival benefits, low response rates, and are associated with extensive side effects.[29] The approval of targeted therapies and immunotherapies has improved patient survival; however, resistance develops rapidly. A high percentage of melanoma patients still have no targeted therapeutic options, highlighting the urgent need for highly efficacious treatments.[20]

Bromodomain and extra-terminal domain proteins are epigenome readers that play key roles in connecting chromatin remodeling and transcriptional regulation. BRD2 and BRD4 are overexpressed in melanoma, whereas

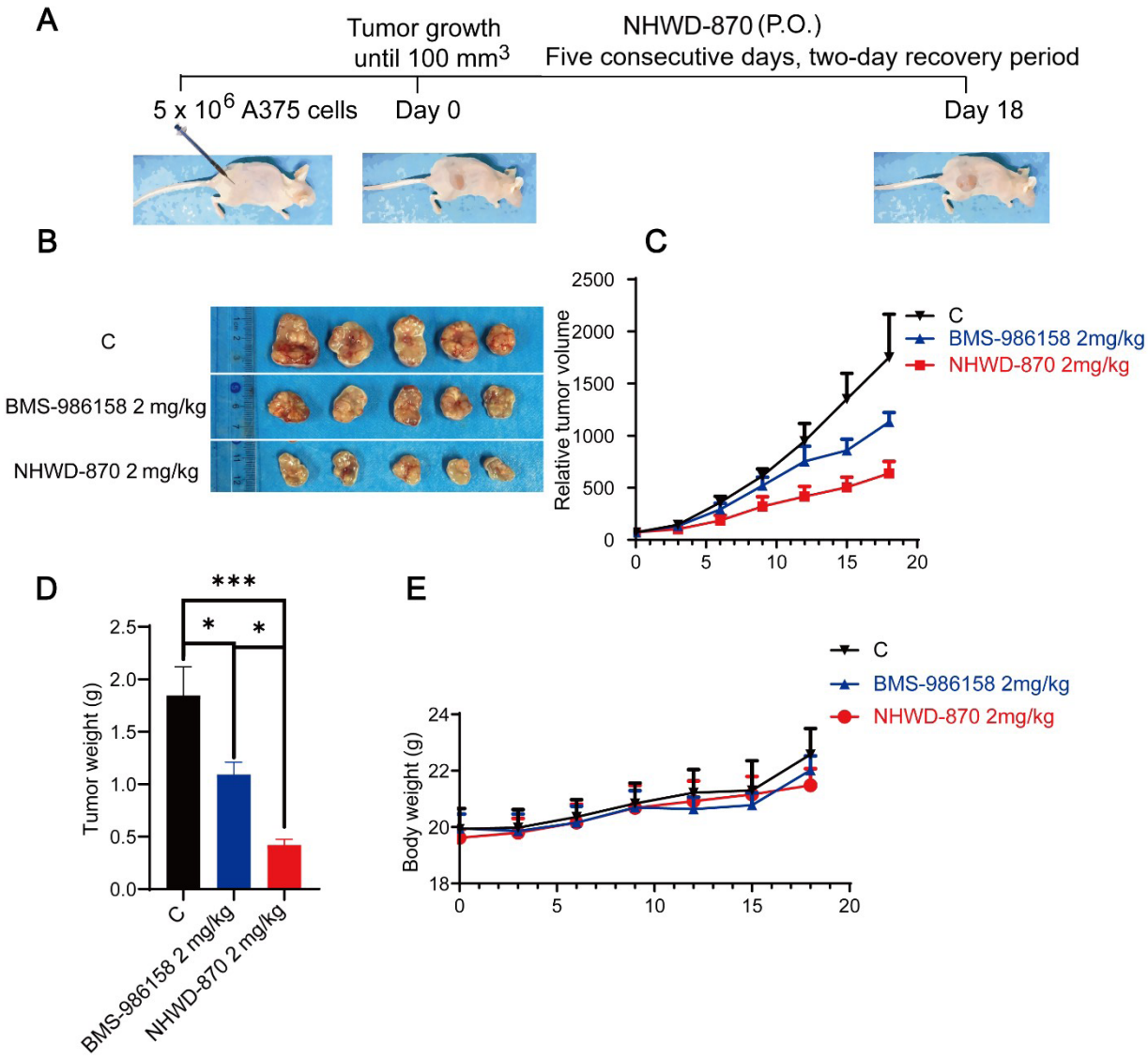


Figure 4. NHWD-870 reduces melanoma growth *in vivo*. (A) Schematic of the animal experimental protocol. (B) Representative images of resected subcutaneous xenografted tumors. (C) Tumor growth curves of A375 tumor-bearing nude mice treated with the corn oil (control), 2 mg/kg BMS-986158, or 2 mg/kg NHWD-870 for 18 days post-subcutaneous implantation. BMS-986158 and NHWD-870 were administered orally daily for 5 consecutive days, followed by a two-day recovery period. Tumor size (D) and body weight (E) were measured every 3 days. All tumors were removed and weighed on day 18. All quantification graphs represent the mean ± SEM. *denotes $p < 0.05$, *** denotes $p < 0.001$.
Abbreviation: C: Control; P.O.: Oral (by mouth).

silencing BRD2 or BRD4 induces melanoma growth arrest.[14,30] BET inhibitors have demonstrated antitumor effects and are thus considered promising drug candidates for melanoma treatment. Most BET inhibitors in the clinic, including OTX-015, GSK525762, CPI-0610, and MT1, share structural similarity with JQ-1. These compounds exhibit relatively low potency and are associated with toxicities such as diarrhea, fatigue, nausea, and skin changes, which can hinder patient compliance.[31] In contrast, BMS-986158 has a distinct core structure and may have different pharmacological effects. Based on BMS-986158, NHWD-870 was designed as a new BET inhibitor. Compared with classical chemotherapeutics, NHWD-870 showed higher efficacy in BRD-overexpressing melanoma. As an oral medication, NHWD-870 provides a safe and convenient therapeutic approach. NHWD-870 has also been recently reported to be more efficacious than JQ1 in pancreatic ductal adenocarcinoma.[32] Our recent study showed that it directly inhibits tumor cell proliferation and blocks TAM proliferation.[27,28] We further found that NHWD-870 inhibited melanoma cell proliferation more strongly than BMS-986158. Colony formation assays also showed that NHWD-870 inhibited the colony-forming ability of A375 melanoma cells in a dose-dependent manner.

To investigate the antiproliferative effects of NHWD-870, we analyzed cell cycle progression by flow cytometry. The G2/M checkpoint is a critical regulatory node that allows DNA-damaged cells to repair their DNA; failure to repair it triggers cell death.[33,34] We found that NHWD-870 induced G2/M phase cell cycle arrest in a dose-dependent manner in A375 melanoma cells. Cyclin B1 plays a key role in G2/M cell cycle progression,[35] and targeting cyclin B1 has been shown to affect several cancer types, including melanoma.[36-38] In this study, NHWD-870 treatment decreased cyclin B1 protein levels in melanoma cells in a dose-dependent manner, suggesting that the observed G2/M phase cell cycle arrest induced by NHWD-870 likely results from reduced cyclin B1 expression. This mechanism may be related to BET inhibitor-mediated suppression of the transcription of some critical cell cycle genes, including *MYC*, *ERK1*, *SKP2*, and *CDK6*.[39] In addition to cell cycle arrest, apoptosis is closely associated with tumor development and treatment response.[35] Our results showed that NHWD-870 increased apoptosis, especially early apoptosis, in melanoma cells. This finding is consistent with previous studies showing that BET inhibition can modulate *MYC*- and *BCL2*/*BCL6*-related transcriptional programs.[40,41] Collectively, we demonstrate that NHWD-870 is a promising therapeutic option for melanoma.

5. Conclusion

In conclusion, our study reveals the antitumor effects of NHWD-870 in melanoma, both *in vivo* and *in vitro*. We found that NHWD-870 significantly induces G2/M cell cycle arrest and apoptosis. However, additional investigations are required to further elucidate the underlying molecular mechanisms. Overall, NHWD-870 exhibits effective antineoplastic potency and may represent a more promising therapeutic candidate than BMS-986158 for melanoma treatment.

Acknowledgments

We thank Ningbo Wenda Pharma (Ninghai, Zhejiang, China) for generously providing NHWD-870.

Funding

This research was funded by Natural Science Foundation of the New Era Longjiang Outstanding Master's and Doctoral Dissertation Funding Program (grant no: LJYXL2022-074), Basic Scientific Research Funds for Central Universities (grant no: 2024IAIS-ZD001), Science and Technology Innovation Key R&D Program of Chongqing (grant no: CSTB2023TIAD-STX0011), The National Natural Science Foundation of China (grant no: 82404107), and Natural Science Foundation of Chongqing, China (grant no: CSTB2024NSCQ-MSX0171).

Conflict of interest

Mingzhu Yin is the Editor in Chief of this journal, but was not in any way involved in the editorial and peer-review process conducted for this paper, directly or indirectly. Separately, other authors declared that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Author contributions

Conceptualization: Mingzhu Yin, Yadong Xue
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Formal analysis: Yingying Liu
Investigation: Hongyin Sun
Methodology: Yingying Liu, Hongyin Sun, Rui Hu
Software: Yingying Liu
Validation: Yingying Liu, Hongyin Sun, Rui Hu
Writing–original draft: Hongyin Sun, Rui Hu
Writing–review & editing: Mingzhu Yin, Yadong Xue

Ethics approval and consent to participate

The animal study protocol was approved by the Ethics

Committee of Chongqing University Three Gorges Hospital (protocol code SXYYDW2023-001, approved on July 21, 2023).

Consent for publication

Not applicable.

Availability of data

The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding authors.

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Appendix

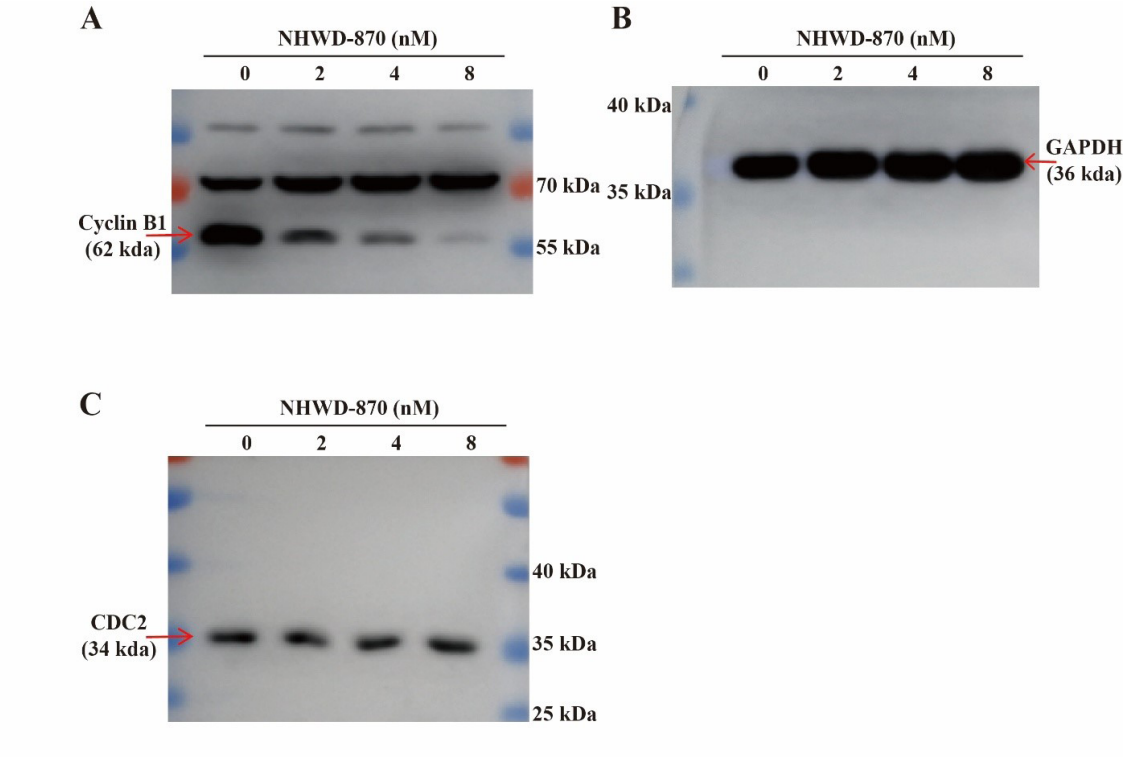


Figure A1. Original Western blot images for Cyclin B1, GAPDH, and CDC2 shown in Figure 3C. (A) Western blot for Cyclin B1 (62 kDa). (B) Western blot for GAPDH (36 kDa). (C) Western blot for CDC2 (34 kDa).