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RESEARCH ARTICLE

Compound C620-0696, a new potent inhibitor targeting BPTF, the chromatin-remodeling factor in non-small-cell lung cancer

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Abstract **Bromodomain PHD-finger transcription factor (BPTF) is the largest subunit of the nucleosome remodeling factor and plays an important role in chromatin remodeling for gene activation through its association with histone acetylation or methylation. BPTF is also involved in oncogene transcription in diverse progressions of cancers. Despite clinical trials for inhibitors of bromodomain and extra-terminal family proteins in human cancers, no potent and selective inhibitor targeting the BPTF bromodomain has been discovered. In this study, we identified a potential inhibitor, namely, C620-0696, by computational docking modeling to target bromodomain. Results of biolayer interferometry revealed that compound C620-0696 exhibited high binding affinity to the BPTF bromodomain. Moreover, C620-0696 was cytotoxic in BPTF with a high expression of non-small-cell lung cancer (NSCLC) cells. It suppressed the expression of the BPTF target gene c-MYC, which is known as an oncogenic transcriptional regulator in various cancers. C620-0696 also partially inhibited the migration and colony formation of NSCLC cells owing to apoptosis induction and cell cycle blockage. Thus, our study presents an effective strategy to target a bromodomain factor-mediated tumorigenesis in cancers with small molecules, supporting further exploration of the use of these inhibitors in oncology.**

Keywords BPTF; small molecule; epigenetics; non-small-cell lung cancer

Introduction

Lung cancer is the most common cause of death all over the world, with non-small-cell lung cancer (NSCLC) accounting for 78% of all related deaths [1]. Genetic changes of driver genes have been identified in NSCLC, including KRAS, EGFR, ALK, ROS1, BRAF, and HER2 [2]. Other than genomic changes, epigenetics is a current challenge and a new therapeutic option; it has heritable changes without DNA sequence alteration and appears to be crucial in various diseases, including cancer, inflammation, and metabolic disease [3]. Epigenetic alterations, such as changes in DNA methylation, histone modifications, and chromatin organization (e.g., nucleosome remodeling), affect gene expression and cellular gene function as well as play an important role in the onset and progression of cancers [4–8]. Targeting epigenetic regulators, such as DNA methylation and histone deacetylase inhibitor, to treat human cancers has proven to be successful [9–13]. Bromodomains are readers that can recognize acetyl-lysine marks in histone tails, which serve a key role in transcriptional activation by binding to specific histone modification sites and recruiting different transcription machineries [14–17]. In addition, evaluations into many small molecule inhibitors of bromodomain and extra-terminal family proteins (i.e., BRD2, 3, 4) are underway in clinical therapeutics [18], exhibiting potent anti-tumor effects in cancers, such as hematologic malignancies, lung cancer, breast cancer, and colon cancer [19–22]. Thus,

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epigenetic therapy has gradually become a potential treatment solution.

Bromodomain PHD-finger transcription factor (BPTF) mainly regulates gene transcription and mediates histone modification essential for the development of key tissues in chromatin remodeling [23–25]. A high BPTF expression is significantly associated with tumor progression in colorectal cancer [26] and promotes tumor cell proliferation and metastasis in melanoma [27,28]. BPTF can also repress T-cell-mediated anticancer immunity by regulating the expression of the major histocompatibility locus genes in two tumor models [29]. c-MYC is an oncogene that plays a key role in cancer pathogenesis by regulating the transcriptional program influencing cell proliferation [30–32]. c-MYC transcription is associated with histone lysine acetylation. Therefore, inhibiting the acetyl-lysine recognition domains has become a potential therapeutic strategy [33–36]. The bromodomain of BPTF is known to specifically recognize histone H4 acetylated lysine 16 (H4K16ac) by binding to acetylated lysine residues, which perform the essential regulation of the chromatin structure in development [37–39]. Knockdown BPTF reduces c-MYC-driven cell proliferation in mouse embryonic fibroblasts [40]. The inhibition of bromodomain transcription factors is already considered a potential therapeutic approach in clinical trials [41]. Recently, high BPTF expression was also verified to be closely associated with poor prognoses in lung adenocarcinomas, displaying an essential role in tumor cell growth and survival [42]. However, to date, no selective inhibitor targeting BPTF bromodomain in human cancers, especially in NSCLC, has been identified, motivating us to discover potential and effective molecules to interrupt the BPTF function in tumorigenesis.

In this study, we investigated a potential and efficacious inhibitor by targeting the bromodomain for suppressing tumor growth mediated by high BPTF expression in NSCLC cells. We performed a molecular docking-based virtual screening from a small molecule database to screen BPTF inhibitors. The biolayer interferometry (BLI) assay found that a potential inhibitor compound, C620-0696, binds directly to BPTF and exhibits effective cytotoxicity to BPTF overexpression in NSCLC cell lines.

Materials and methods

Cell culture

A549 and H358 cell lines were obtained from ATCC. A549 and H358 cells were cultured by RPMI 1640 medium, which was supplemented with 10% fetal bovine serum and 100 units/mL penicillin and 100 μg/mL streptomycin. The cells were grown through incubation at 37 °C with 5% CO_2.

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

All compounds (TopScience, Shanghai, China) were dissolved in dimethyl sulfoxide (DMSO). A549 and H358 cells were plated in a 96-well plate, with 3000 cells plated per well. The cells were treated with different concentrations. After 72 h, 10 μL of MTT solution were added to each well and incubated at 37 °C for 4 h. Then, 100 μL of the SDS solution (10% SDS and 0.1 mmol/L HCL) was added to each well and incubated at 37 °C for another 4 h. Finally, the absorbance of the plate was measured by an absorbance reader (Tecan, Morrisville, NC, USA).

Colony formation assay

Cell survival was assessed by colony formation as previously described. A549 and H358 cells were seeded onto a 6-well plate, with 500–1000 cells plated per well. The cells were then exposed to various doses of C620-0696. After seven days, colonies were fixed with 4% paraformaldehyde for 15 min and stained with crystal violet for 10–15 min. Finally, the staining solution was slowly washed off with water and the cells were air-dried.

Wound healing assay

A549 and H358 cells were seeded onto 6-well plates. When the cell confluence reached $> 90\%$, scratch wounds were made by using 200 μL tips to scrape the cell layer across each plate. Wounded cultures were incubated in the medium for 48 h and then visualized through an Olympus inverted microscope to assess the cell migration ability.

Western blot analysis

Total cell proteins were extracted by a RIPA lysis buffer containing protease (Roche) and phosphatase (Roche) inhibitors. The primary antibodies of PARP-1, c-MYC, and cyclin D1 were from Cell Signaling Technology (Danvers, MA, USA). The primary antibody of GAPDH was from Santa Cruz (Dallas, TX, USA). The primary antibody of BPTF was from EMD Millipore Corporation (Billerica, MA, USA). The secondary antibodies were from Odyssey (Belfast, ME, USA). LI-COR Odyssey scanner (Belfast, ME, USA) was used to detect the Western blot result.

Kinetic binding analysis by BLI

The Octet Red96 system (Forte Bio, Pall) was used to determine the binding between C620-0696 and BPTF. BPTF protein was loaded to saturation onto anti-His capture sensors (ForteBio) and then placed for 2 min in

wells containing C620-0696 (concentrations: 3.125, 6.25, 12.5, 25, 50, 100, and 200 μmol/L). The baseline and dissociation steps were carried out in the kinetic buffer as per the instrument manufacturer's recommendations.

Molecular docking-based virtual screening

The free BPTF structure was derived from Protein Data Bank (PDB ID: 2f6n) and prepared with the Protein Preparation Wizard in Maestro (Schrodinger, NY, USA; Schrodinger 2015). Given that the structures of BPTF and BRD4 were highly similar and the structure of the BPTF-inhibitor complex was unavailable, we first aligned the BPTF (PDB ID: 2f6n) and BRD4-benzoisoxazoloazepine 3 complex (PDB ID: 5hm0). Next, the grid box in the BPTF structure was generated on the size and center of benzoisoxazoloazepine 3. For the ligands, a total of 1 668 608 compounds from ChemDiv and Specs databases were prepared with a ligand preparation module. Three-level (HTVS, SP, and XP) molecular docking-based virtual screening was successively performed using a Glide module, similar to our previous work [43]. The top 10% compounds ranked by gscore were clustered into 200 groups. Through visual inspection of the binding poses of the BPTF inhibitor, 150 compounds were selected for the experimental validation. All the compounds were purchased from TopScience Company (Shanghai, China).

Statistical analysis

Statistical analysis was conducted using Graph Prism 5.0. Significant differences between data sets were assessed via one-way analysis of variance. The descriptive analytical data were presented as mean ± SD.

Results

Compound C620-0696 was identified through its binding affinity with BPTF.

The BPTF bromodomain can recognize histone H4K16ac and further repress the c-MYC transcriptional activation. A report states that BRD4 and BPTF have similar acetylated-lysine binding pockets that have found an inhibitor of BPTF bromodomain [44]. In the present study, 150 compounds were obtained through molecular docking-based virtual screening. To discover the potential inhibitors that may be able to interact directly with BPTF, we first conducted BLI assays [44] to assess the binding affinity between these 150 compounds and BPTF.

The bromodomain was immobilized on the streptavidin biosensors and then mixed with a different concentration of each compound at 3.125, 6.25, 12.5, 25, 50, 100 or 200 μmol/L. The results of steady-state analysis showed that only C620-0696 exhibited a binding affinity for BPTF, with a K_D value of 35.5 μmol/L (Fig. 1B).

Molecular docking was then performed to identify the binding mode between C620-0696 and BPTF. The obtained docking score was −9.18 kcal/mol. As shown in Fig. 1C, the aromatic plane of C620-0696 was inserted into a hydrophobic pocket composed of residues Pro92, Asp101, Tyr105, Asn148, and Phe154. When interacting with the bromodomain of BPTF, C620-0696 formed five hydrogen bonds with residues Pro92, Asp101, Tyr105, and Asn148 (Fig. 1C). In addition, C620-0696 formed pai–pai stacking interactions with Phe154, which further strengthened the binding interactions between BPTF and C620-0696.

C620-0696 is cytotoxic in NSCLC cells with high BPTF expression

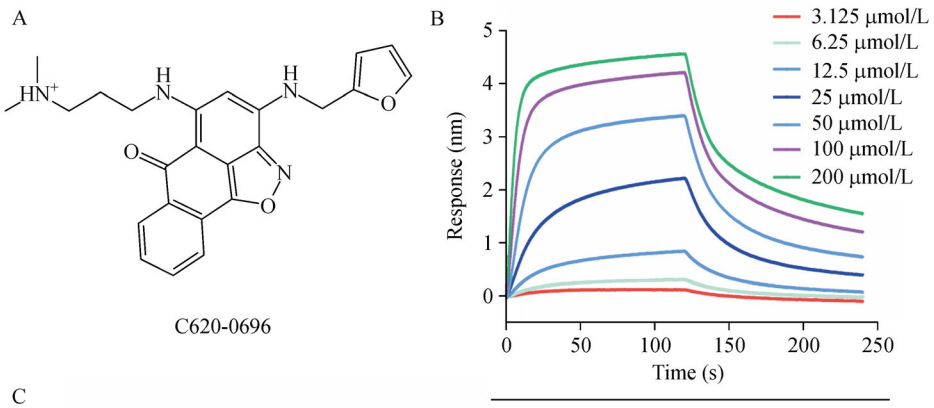
With C620-0696 bound to the BPTF bromodomain, we further studied its cytotoxic potency in NSCLC cells with BPTF overexpression. Previous studies have implicated BPTF overexpression in NSCLC cell lines. Therefore, to investigate whether C620-0696 could inhibit BPTF function *in vitro*, we first selected BPTF highly expressing in NSCLC cell lines (A549 and H358), compared to the human bronchial epithelial cell (BEAS-2B), as the cell models for this study (Fig. 2A). C620-0696 was found to inhibit cell viability with an IC_{50} of 11.2 and 6.72 μmol/L at 72 h of treatment (Fig. 2B – 2D). These results indicated that C620-0696 exhibits a significantly cytotoxic effect on NSCLC with high BPTF expression.

C620-0696 inhibits the migratory capacity of NSCLC cells

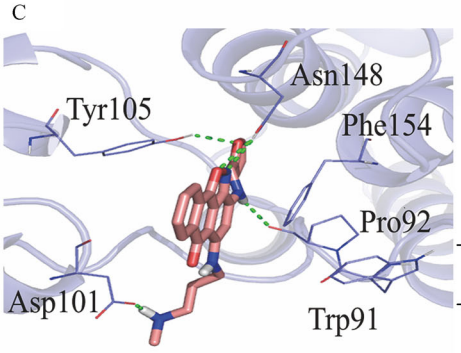
Compound C620-0696 showed cytotoxicity in A549 and H358 cells. Thus, we wanted to evaluate the impact of C620-0696 on cell migration and cellular processes in NSCLC. We also employed the wound healing assay to assess the directional cell motility. The results clearly indicated that over time A549 and H358 cells migrated slower under C620-0696 treatment compared with the control cells (Fig. 3), supporting the finding that the inhibition of BPTF challenges the migratory capacity of NSCLC cells.

C620-0696 inhibits the colony formation of NSCLC cells

Colony formation assay was conducted to test if C620-0696 could affect the growth behavior of A549 and H358 cells. A significant loss in colony formation was observed in A549 and H358 cells treated with C620-0696 (Fig. 4). These results suggested that C620-0696 blocks cell growth and proliferation.

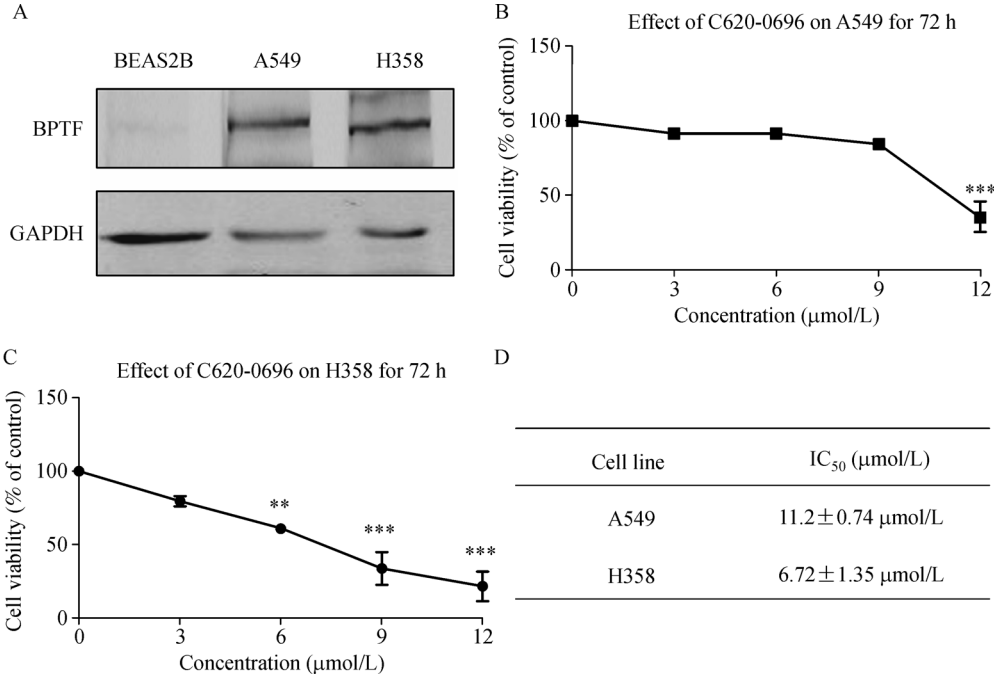


K_D (μmol/L)	K_{on} (L·mol^{-1}·s^{-1})	K_{off} (s^{-1})
35.5	4.78E+02	1.70E−02



System	Docking score (kcal/mol)
BPTF	−9.187

Fig. 1 (A) Chemical structure of C620-0696. (B) Determination of the binding affinity of the BPTF bromodomain with C620-0696 by BLI assay. The binding affinity (K_D) of the bromodomain for C620-0696 was determined by the rate constants of $K_{on} = 4.78 \times 10^2$ L·mol^{-1}·s^{-1} and $K_{off} = 1.70 \times 10^{-2}$ s^{-1}. (C) The detailed binding mode between C620-0696 and the BPTF bromodomain. Green dashed lines represent hydrogen bonds.



Cell line	IC_{50} (μmol/L)
A549	11.2±0.74 μmol/L
H358	6.72±1.35 μmol/L

Fig. 2 (A) BPTF expression was analyzed by Western blot in BEAS-2B, A549, and H358 cell lines. (B, C) Cell viability was measured in A549 and H358 cells treated with C620-0696 at 3, 6, 9, 12 μmol/L for 72 h. (D) The IC_{50} value was calculated in A549 and H358 cells treated with C620-0696. All data were presented as mean ± SD. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$.

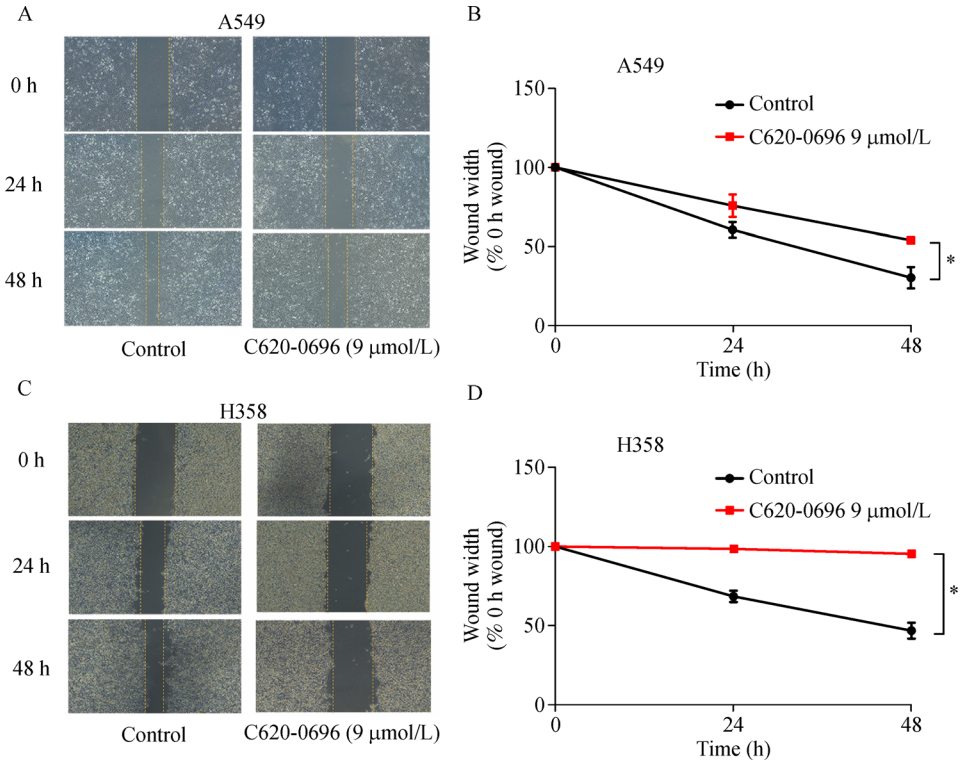


Fig. 3 (A, C) Motility of C620-0696-treated cells or control cells captured after 24 and 48 h post injury, respectively. Dashed yellow lines show the images of a representative wound (10×). (B, D) Rate of wound healing of A549 and H358 cells. All data were presented as mean ± SD. *$P < 0.05$.

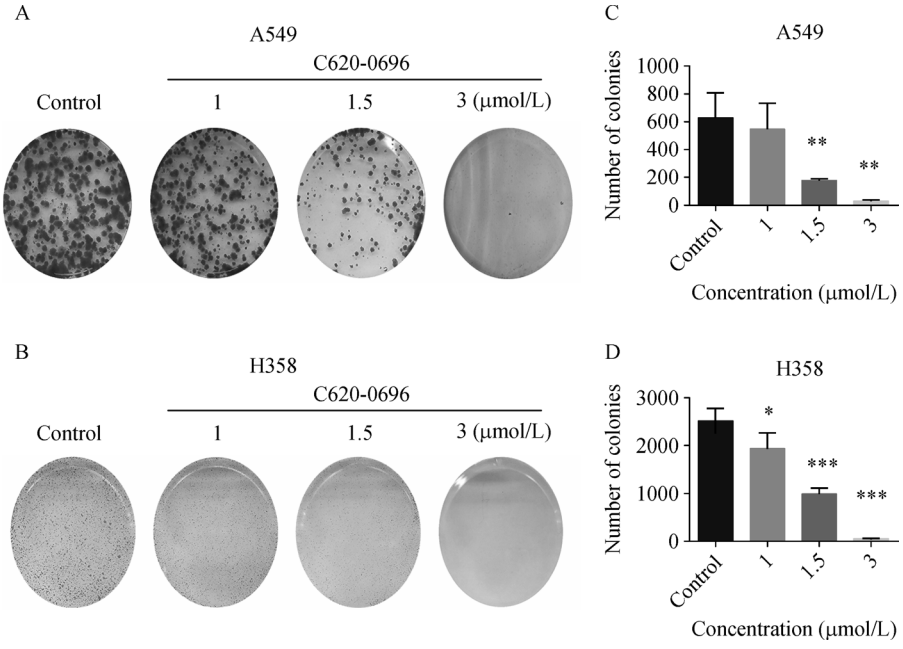


Fig. 4 (A, B) A549 and H358 cells of colony formation assay data after treatment with C620-0696 (Control, 1, 1.5, and 3 μmol/L). (C, D) Statistical analysis of colony formation assay. All data were presented as mean ± SD. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$.

C620-0696 induces apoptosis and suppresses cell cycle in NSCLC cells

BPTF knockdown was previously reported as capable of restricting cell proliferation and inducing apoptosis in lung cancer [42]. To ascertain whether the induction of apoptosis also contributed to C620-0696-mediated growth inhibition in NSCLC cells, we detected cleaved Poly (ADP-ribose) polymerase 1 (PARP-1) in C620-0696-treated H358 cells. C620-0696 up to 9 μmol/L significantly increased the levels of cleaved PARP-1 (Fig. 5). It also reduced the expression of cyclin D1 related to cell cycle in H358 cells and suppressed the expression of an oncogenic transcriptional regulator c-MYC (Fig. 5), which is a BPTF target gene in tumorigenesis. These data suggested that C620-0696 may inhibit BPTF transcriptional regulation of its target oncogenic genes, thereby leading to cell apoptosis induction and cell cycle suppression in NSCLC.

Discussion

Targeting epigenetic regulators such as DNA methylation and histone deacetylase inhibitor to treat human cancers has been proven to be successful [9,10]. Researchers reported that a combined treatment with DNA methyltransferase and histone deacetylase inhibitors could prolong the survival of patients with NSCLC [45]. Meanwhile, bromodomain as a reader can recognize acetyl-lysine sites and transcription activities [18]. In the past few years, researchers have found some small molecule bromodomain inhibitors, such as JQ1, RVX-208, and OTX015, some of which are under phase I/II clinical trials [14,15,18,22]. Therefore, identifying an effective inhibitor that targets the bromodomain is of value. As a transcription factor, BPTF plays a key role in transcriptional modification and has high expression in many human cancers, especially lung adenocarcinomas [23,46]. Compared with normal cells, BPTF is highly expressed in NSCLC cell lines and tumor tissues [42]. BPTF is a new lung adenocarcinoma susceptibility locus and is associated with lung premalignance [47,48]. However, effective BPTF inhibitors have not been discovered fully yet. Recently, researchers reported uncovering the first BPTF small molecule inhibitor AU1 through protein-observed fluorine NMR [44]. The report provides suitable ligands for developing BPTF inhibitors.

In this work, we identified C620-0696 as a BPTF bromodomain inhibitor through virtual screening. C620-0696 exhibited a high binding affinity to the bromodomain of BPTF by using BLI, which has a K_D value of 35.5 μmol/L. These results suggested that C620-0696 is a potent inhibitor targeting the bromodomain of chromatin-remodeling factor BPTF. In addition, cell cytotoxicity in A549 and H358 cells was found to be induced after treatment with C620-0696, but the AU1 did not show the cytotoxic effect in A549 and H358 cells [44]. These results showed that C620-0696 has good cytotoxic effect. Many studies have found that BPTF overexpression is associated with cancer cell proliferation and apoptosis suppression in various tumors [28,30,42]. In our study, we found that C620-0696 induces cell death by increasing the level of PARP-1 and the expression of cyclin D1, resulting in cell cycle arrest. It likewise inhibits migratory capacity and colony formation in NSCLC cells by inhibiting the BPTF bromodomain.

C620-0696 could also inhibit the binding between the BPTF bromodomain and H4K16ac, which could repress c-MYC transcription activation. The inhibition of the interaction between bromodomain and acetylated lysine reduces the c-MYC target gene level and transcription inhibition of the c-MYC gene itself [33,40]. Decreasing the c-MYC expression can lead to the resistance of cancer cell apoptosis and cell cycle progress [31,49]. In sum, our result demonstrates that C620-0696 is a potent inhibitor of BPTF bromodomain.

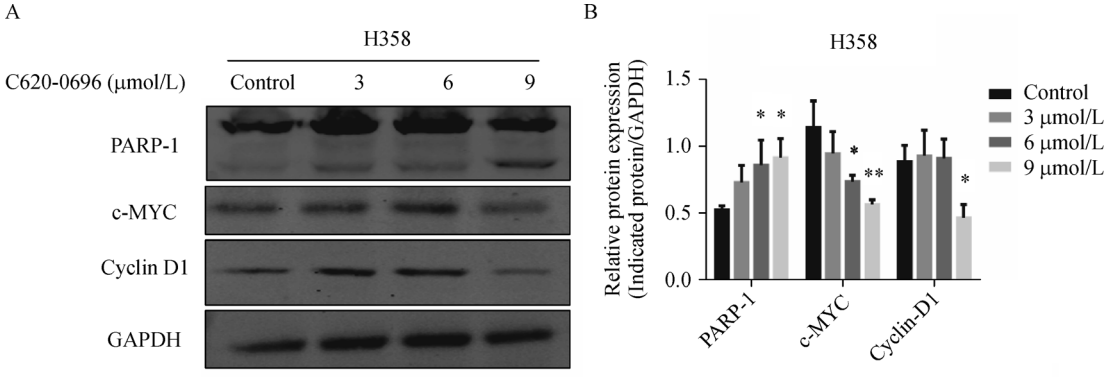


Fig. 5 (A) H358 cells were treated with C620-0696 at Control, 3, 6, and 9 μmol/L for 24 h. Western blot was used to detect the expression levels of PARP-1, c-MYC, and cyclin D1, while GAPDH was used as the loading control. (B) Statistical analysis of colony formation assay. All data were presented as mean ± SD. *$P < 0.05$, **$P < 0.01$.

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Compliance with ethics guidelines

Jiahui Xu, Qianqian Wang, Elaine Lai Han Leung, Ying Li, Xingxing Fan, Qibiao Wu, Xiaojun Yao, and Liang Liu declare that they have no conflicts of interest. This article does not contain any studies with human or animal subjects.

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