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*Original Article*

# Superior activity of a modified histone deacetylase inhibitor in inhibiting growth of human gastric cells by promoting p21$^{WAF1}$ expression

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**Abstract:** Objective: To investigate the application of a modified histone deacetylase inhibitor named D3 for inhibiting cancer cell proliferation and inducing apoptosis. Material and methods: We designed to investigate its exact mechanism of D3 anti-cancer function. Activity of D3 against human gastric cancer cells (BGC-823, SGC-7901) and normal gastric epithelial cell line (GES-1) were investigated in vitro by using proliferation assays, cell cycle assays, apoptosis assays, RNA interference, promoter acetylation assays, as well as analysis of related molecules. Result: D3 strongly inhibited the proliferation of gastric cancer cells compared with SAHA. The 50% growth-inhibitory concentrations of D3 for GES-1, BGC-823, SGC-7901 treated for 48 h were 3.2±0.09 μM, 3.77±0.1 μM, 4.97±0.2 μM while that of SAHA were 12.9±0.26 μM, 28.93±0.4 μM, 27.1±0.4 μM respectively. We found that D3 caused more significant G1 cell cycle arrest and apoptosis than SAHA in a density-dependent manner. In addition, levels of Bcl-xl and Bcl-2 mRNA and protein in BGC823 cells decreased after treatment with either SAHA or D3; moreover, D3 had a higher inhibition activity. D3 induced hyperacetylation of histone H3 and H4 around the promoter region of *p21$^{WAF1}$*. Conclusion: D3 could represent a novel pharmacological agent with potential benefit for patients with gastric cancer.

**Keywords:** Histone deacetylase inhibitor, gastric cancer, *p21$^{WAF1}$*, anticancer

## Introduction

Traditionally, cancer has been regarded as cell proliferation infinitely caused by gene mutation, gain of hyperactivation of oncogenes or loss of tumor suppressor gene [1, 2]. However, there is growing evidence that the remodelling of nucleosome is a key epigenetic mechanism for modulating genome expression which is also crucial to the onset and progression of cancer [3]. Several reversible posttranslational changes of histone proteins have been described such as lysine ubiquitination serine phosphorylation, proline isomerization, and so on [4, 5]. There is antiproliferative and anticancer effects of HDAC inhibitors (HDACIs), which have been demonstrated both in cellular and animal models and in clinical trials. In 2006, the FDA approved suberoylanilidehydroxamic acid (SAHA; vorinostat) which became the first HDACi for clinically use in the treatment of cutaneous T-cell lymphoma [6, 7].

The balance between two groups of enzymes, histone acetyltransferases and histone deacetylases (HDACs), determine the pattern of histone acetylation. Disruption of the balance is possibly associated with development of cancer [8]. HDACIs promote acetylation of histone proteins, allowing the transcriptional machinery to access DNA and modulating/enhancing gene transcription, and also of nonhistone protein substrates, such as transcription factors, DNA-binding proteins, DNA-repair proteins, signal-transduction factors, and chaperone proteins [9]. SAHA is a synthetic hydroxamic acid, which have a high affinity for biometals. SAHA been used to investigate the mechanism of HDACis in the control of cancer on account of it is the first approved by FDA to use clinically. HDACis inhibit cancer cells proliferation by inducing upregulation of *p21$^{WAF1}$* who is an inhibitor of cyclin-dependent kinases (CDKs) and inhibits cell cycle progression in the G1 phase of the cell cycle and can be induced within 2

**Table 1.** Primers and their sequence used in this study

| Transcripts | Sequence (5'-3') | Product size (bp) |
|---|---|---|
| β-actin | F: ATCATGTTTGAGACCTTCAACA | 300 |
| | R: CATCTCTTGCTCGAAGTCCA | |
| BCL-2 | F: CGACTTCGCCGAGATGTCCAGCCAG | 364 |
| | R: ACTTGTGGCCCAGATAGGCACCCAG | |
| BCL-xl | F: GGAACAATGCAGCAGCCGAG | 141 |
| | R: GTAGAGTGGATGGTCAGTGT | |
| *p21$^{WAF1}$* | F: TGTCCGTCAGAACCCATG | 219 |
| | R: TGGGAAGGTAGAGCTTGG | |

h of culture of transformed cells with HDACis [10-12]. Another aspect of tumor cell growth inhibition of HDACi is downregulation of anti-apoptotic members of the B-cell lymphoma 2 protein (bcl-2) family who has been implicated in the lethality of these agents toward diverse transformed cell types [13, 14]. Bcl-2 overexpression seem to be a molecular hallmark related with apoptosis resistance of gastric carcinomas associated with EBV [15, 16]. Bcl-2 expression may be important for prognostic outcome or a useful target for therapeutic intervention [17].

In our previous study, we designed and synthesized a series of novel aromatic group-modificatiryhydroxamic acid derivatives as HDACis. Through several rounds of structural improvement, D3 emerged excellent inhibition of HDACs and potent growth inhibition in multiple tumor cell lines. Our present study investigated the anticancer activity and molecular mechanisms of action of D3. Compared with SAHA, D3 showed significantly increased HDACi activity. Importantly, D3-induced cell cycle arrest in human gastric cancer cells by upregulating *p21$^{WAF1}$* and downregulating bcl-xl and bcl-2 more significantly than did SAHA. Our results suggested that D3 is a promising novel HDACi with high efficiency and low toxicity for the treatment of gastric malignancies.

## Materials and methods

### *Cell culture and reagents*

Human gastric cancer cell line BGC823 and SGC7961 were purchased from Cell Bank of the Committee on Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China) A previously reported human gastric epithelial cell line GES-1 cell line was also included in here as control [18]. All the cell lines were cultured in RPMI 1640 medium (Invitrogen, Carsbad, CA, USA) supplemented with 10% fetal bovine serum and incubated at 5% $CO_2$, 37°C and 95% humidity. D3, D18 and SAHA were stored at -80°C dissolved as 5 mmol/L stock in dimethyl sulfoxide (DMSO) and diluted with PBS or culture medium as needed. For all cell-based assays, control cells were incubated in medium with equivalent amounts of DMSO, but lacking HDACis.

### *In-vitro cytotoxicity assays*

A WST-1 assay was performed according to the manufacturer's instructions. In brief, cells were administered with different concentrations of SAHA or D3, D18 and incubated for 48 h; 20 ml of WST-1 (Roche, Mannheim, Germany) was added and cultured for another 4 h. The absorbance at 450 nm was determined using a microplate reader (Synergy 2; BioTek, Winooski, Vermont, USA) and the inhibition rate was calculated as (1-$OD_{\text{treated cells}}$/$OD_{\text{control cells}}$) ×100%. Finally, dose-effect histograms were constructed and $IC_{50}$ values were calculated.

### *RNA extraction and semi-quantitative RT-PCR*

RNA was extracted using the TRIzol RNA isolation kit (Invitrogen; Carlsbad, California, USA) ollowing manufacturer's instruction. RNA isolation and semiquantitative reverse transcription PCR were performed as described previously [19, 20]. The concentrations were quantified by NanoDrop 2000 (Nanodrop, Wilmington, DE, USA). The sequences of the PCR primers are listed in **Table 1**.

### *Western blot analyses*

BGC823 cells were treated with SAHA or D3 (2 mmol/l) for the indicated times. Western blotting was carried out as described previously [20-22]. The following antibodies were used: anti-p21$^{WAF1}$ mAb, anti-p27$^{Kip1}$ mAb (Epitomics, Burlingame, California, USA), anti-bcl-2 mAb (Santa Cruz Biotechnology, Santa Cruz, CA, USA), antiacetyl histone H3 (AcH3) mAb, anti-acetyl histone H4 (AcH4) mAb (Millipore, Temecula, California, USA), anti-bcr-ablmAb, and anti-p53 mAb (BBI; Sangon Biotech, Shanghai, China).

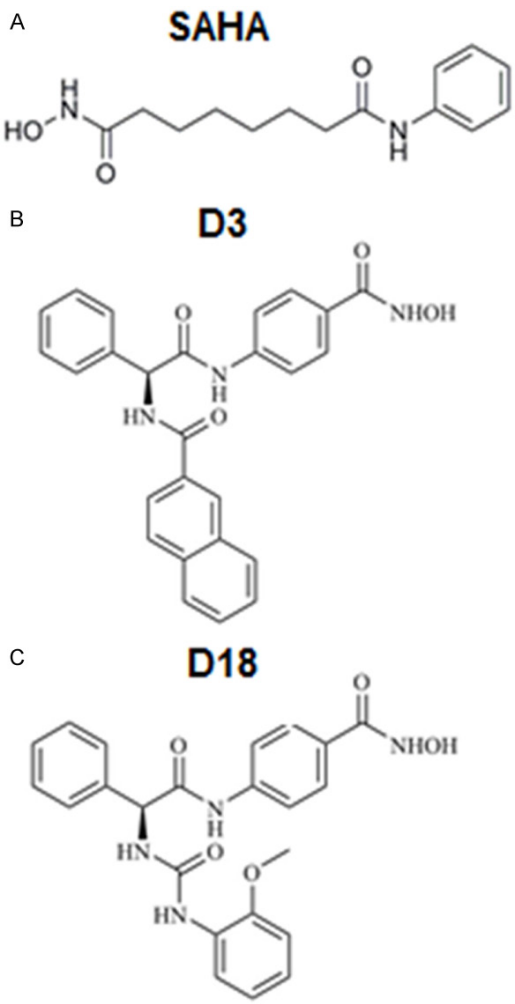


**Figure 1.** Schematic illustration of molecular structure of SAHA, D3 and D18. A. Molecular structure of SAHA. B. Molecular structure of D3. C. Molecular structure of D18.

*Apoptosis assay*

Apoptotic cells were quantified with fluorescein isothiocyanate (FITC) annexin V Apoptosis Detection Kit I (BD Biosciences, Franklin Lakes, New Jersey, USA) according to the manufacturer's instructions. Briefly, after 48 h of culture, cells were washed and labeled with annexin V-FITC and propidium iodide (PI) [23, 24]. Stained cells were analysis by flow cytometry (FACS Calibur; BD Biosciences).

*Cell cycle analysis*

BGC823 cells were cultured in the presence of 2 mmol/l SAHA, D3 or vehicle control for 24 h. Cells were harvested and fixed in 75% ethanol at 4°C overnight. After washing with PBS, the cells were resuspended in PBS containing 250 mg/ml RNase A (Sigma, St. Louis, Missouri, USA) for 30 min at 37°C and stained with 50 mg/ml PI (Sigma) for 10 min at 4°C in the dark [21]. The DNA content of cells was measured using the Cell Quest Program (BD Biosciences, San Jose, California, USA) on a flow cytometer. Proportions of cells in G1, S, and G2/M phases were analyzed using Mod Fit Software (Verity Software House, Topsham, Maine, USA).

*Transwell cell invasion assay*

To evaluated the the capability of D3 on inhibiting cell invasion, the Transwell cell invasion assay had been conducted as previously described with modifications [14]. Briefly, the D3 was used to treating the cells with different dose (2.5 μM and 5 μM). After 24 hours treatment, cells was trypsinized and stained with trypan blue for cell counting. Then totally 100 uL cell suspension medium had been added into Transwell chamber and cultured for another 48 h. Then those chambers were stained with hematoxylin. Six microscopic fields were randomly selected from each chamber and captured for quantification of cell numbers.

*Clonogenic cell survival assay*

Clonogenic cell survival assay was conducted as previously described with modifications as well [15]. Briefly, miR-152 mimic or scramble control transfected cells was trypsinized and counted for viable cells. Totally $1\times10^4$ cells were seeded into 100 mm cell culture dishes and maintained for one week. Then the cell colonies were stained with gentian violet for pictures.

*Statistical analysis*

Experimental results were plotted and analyzed for statistical significance with Prism5 software (GraphPad Software Inc., CA). Significant differences were determined using Student's *t*-test and one way analysis of variance. A *P* value of 0.05 was considered as statistically significant.

## Results

*D3 has more potent anti-proliferative activity than SAHA in vitro*

In previous studies, we had designed a series of small molecules HDACi that had anti-tumor activity. We expected to get more molecules

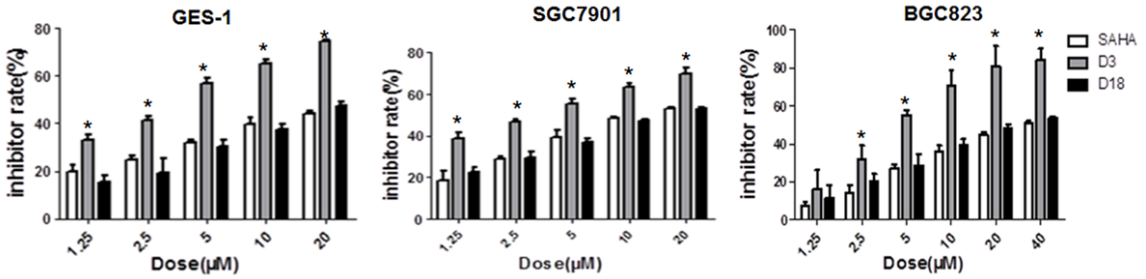


**Figure 2.** Effects of SAHA, D3 and D18 on cell proliferation of GES-1, SGC7901 and BGC823 cells. GES-1, SGC7901 and BGC823 cells was treated with indicated concentration of SAHA, D3 and D18, cell proliferation for each cell lines were determined by the WST-1 assay. The data represent the mean ± SD of three independent experiments. *P<0.05, versus vehicle control group.

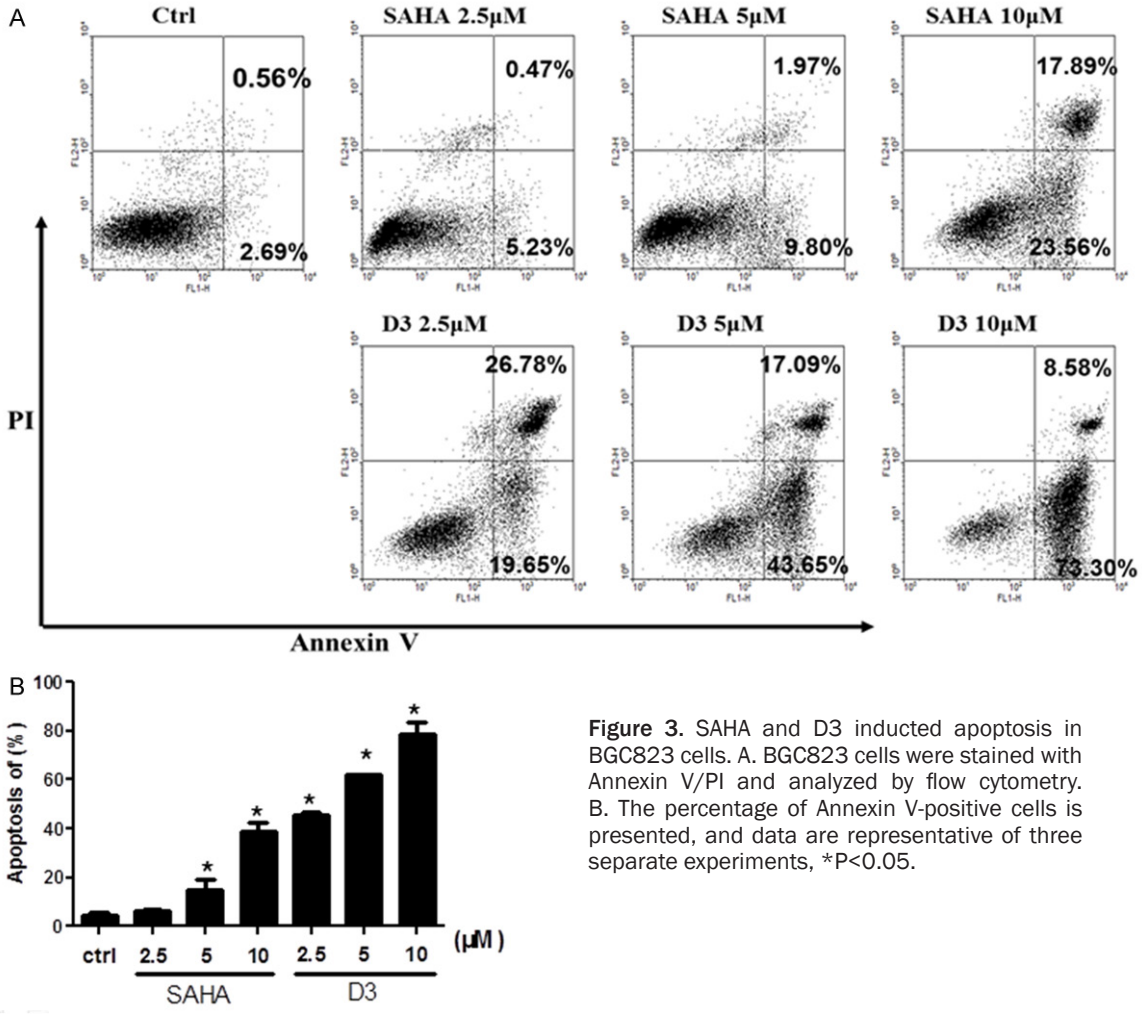


**Figure 3.** SAHA and D3 inducted apoptosis in BGC823 cells. A. BGC823 cells were stained with Annexin V/PI and analyzed by flow cytometry. B. The percentage of Annexin V-positive cells is presented, and data are representative of three separate experiments, *P<0.05.

that had better tumor cell growth inhibition by introduction of aromatic group which was helpful to improve the activity of compounds. Derivatives D3 and D18 were obtained by modifying structure in the end (**Figure 1A-C**).

Our previous studies indicated that D3 and D18 showed superior antitumor potency in both breast cancer and hematological tumors compared with SAHA (Data not show). To determine the molecular mechanisms and to further eval-

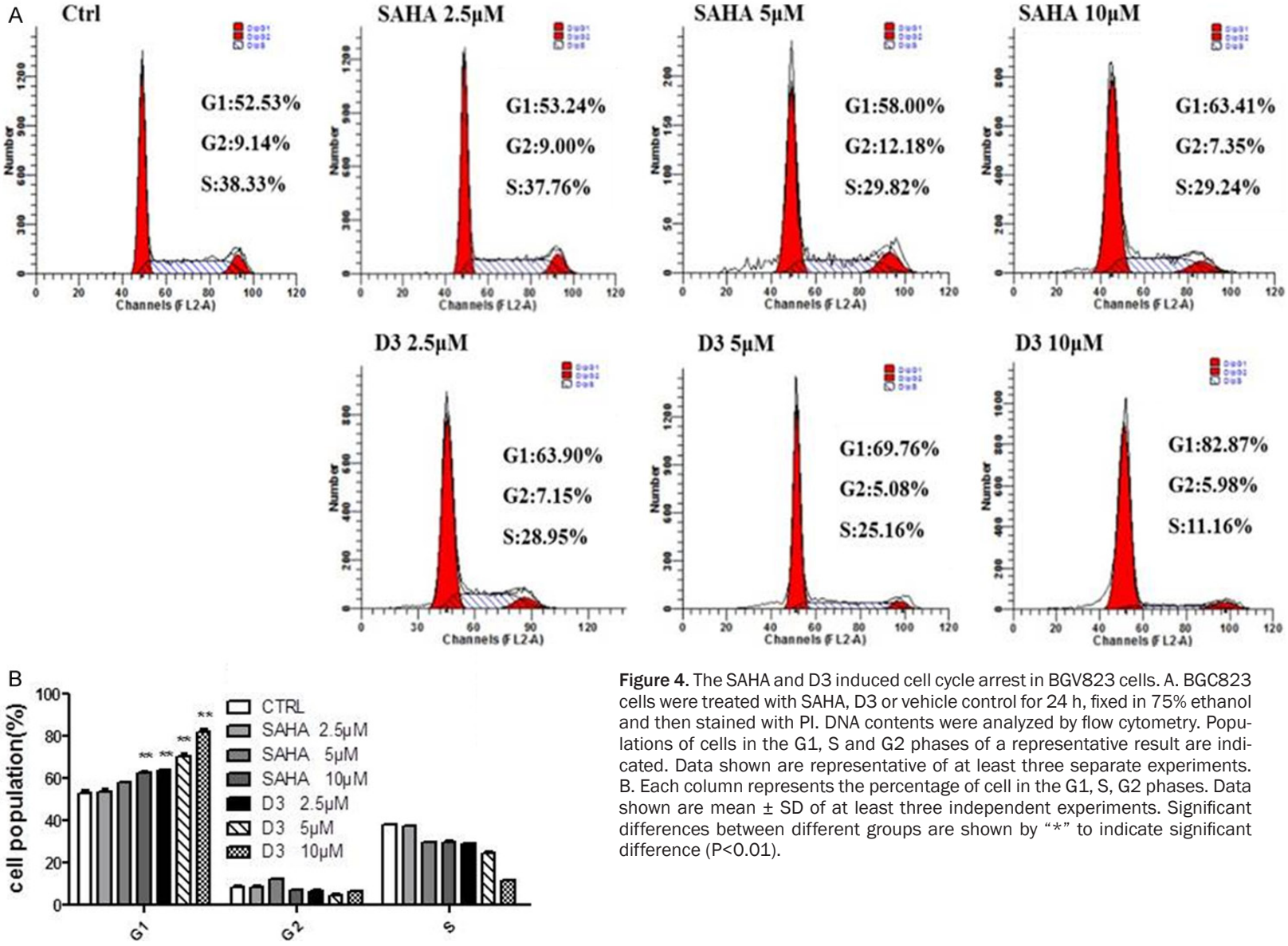


**Figure 4.** The SAHA and D3 induced cell cycle arrest in BGV823 cells. A. BGC823 cells were treated with SAHA, D3 or vehicle control for 24 h, fixed in 75% ethanol and then stained with PI. DNA contents were analyzed by flow cytometry. Populations of cells in the G1, S and G2 phases of a representative result are indicated. Data shown are representative of at least three separate experiments. B. Each column represents the percentage of cell in the G1, S, G2 phases. Data shown are mean ± SD of at least three independent experiments. Significant differences between different groups are shown by "*" to indicate significant difference ($P<0.01$).

uate whether D3 or D18 have antiproliferative activity against gastric cancer, GES-1, BGC-823 and SGC-7901 cells were treated with D3, D18 or SAHA at different concentrations (0-20 μM) for 48 h. The rate of inhibition of cell growth was determined using the WST-1 assay. The results indicated that, compared with SAHA and D18, D3 significantly inhibited the proliferation of cells after treatment for 48 h mostly (**Figure 2**). Further tests found that D3 yielded an $IC_{50}$ of GES-1, BGC-823 and |SGC-7901 cells were 3.2±0.09 μM, 3.77±0.1 μM and 4.97±0.2 μM respectively, and that of D18 were 14.4±0.62 μM, 22.7±0.2 μM and 23.5±0.35 μM, while that of SAHA were 12.9±0.26 μM, 28.93±0.4 μM and 27.1±0.4 μM. Results suggested that D3 exerts a most potent inhibitory effect on gastric cancer than SAHA and D18.

*D3 cause more significant cell apoptosis than SAHA*

To determine whether the superior growth inhibition by D3 is caused by cell cycle arrest and/or apoptosis, BGC823 cells which were most sensitive to HDACi were treated with SAHA or D3 for 48 h. As shown in **Figure 3**, the number of BGC823 cells treated with SAHA stained for annexin V increased from 5.18±1.87 to 6.39±1.09% (2.5 μM), 18.52±3.86% (5 μM) and 42.05±2.73% (10 μM) in a concentration-dependent manner compared with the vehicle control. For BGC823 cells treated with D3, the rate of annexin V-stained apoptotic cells increased from 6.23±1.87 to 46.18±1.24% (2.5 μM), 63.17±0.53% (5 μM) and 79.05±4.72% (10 μM). There were significant differences in induced rates of apoptosis between SAHA and D3 treatment. These results suggested that at a moderate dose range (2.5 μM), SAHA orD3 cause considerable apoptosis. When the HDACi concentration increased to 5 μM, BGC823 cells undergo severe apoptosis. Therefore, in subsequent experiments, we chose 2.5 μM and 5 μM as the experimental concentration for observing the mRNA and protein expression of some apoptosis-regulating genes after treated with both HDACis.

*D3 induces G1-phase arrest more significantly than SAHA*

In order to explore the mechanism on the stronger growth inhibition of gastric cancer cells mediated by D3, we studied the effect of SAHA and D3 on the cell cycle in BGC823 cells. As the incubation density with D3 at 24 h increased from 2.5 μM to 10 μM, the percent of BGC823 cells in the G1 phase increased from 59.68±6.72 to 75.43±8.83% in a density-dependent manner, with a concomitant decrease in S-phase cells (**Figure 4**). While the corresponding number of BGC823 cells treated with SAHA increased from 43.78±7.04 to 54.36±5.28%. Compared with SAHA, D3 showed superior G1-phase arrest activity. These results showed that the mechanism of superior cell growth inhibition of D3 observed in the WST-1 cell-proliferation assay was mediated not only by apoptosis but also cell cycle arrest.

*D3 treatment in BGC823 cell inhibiting cell invasion and proliferation*

As our data have demonstrated that D3 treatment could promote cell apoptosis and cell cycle arrest, we further examine if D3 treatment affect the cell invasion ability as well its capability of clone formation since invasiveness of transformed cells is a necessary step for tumor progression [25]. In Clonogenic Cell Survival Assay which is alternative way to evaluate the cell proliferation capability, we observed a very strong increasing of colonies formed by BGC823 cell with D3 treatment (**Figure 5**). Moreover, In the cell invasion assay, cell staining results demonstrated a stronger invasion inhibition by D3 treatment in BGC823 cell as well (**Figure 6A**). Statistical analysis demonstrated the cell counts was increased from 147 per field to more than 200 (**Figure 6B**).

*D3 induces the expression of apoptosis-regulating genes more significantly than SAHA*

Increases in cellular levels of p21$^{WAF1}$ have been shown to correlate with increased inactivation of cyclin-dependent kinases, thus leading to G1-phase arrest [26-28]. Increases in cellular p27$^{Kip1}$ [29, 30] and p57$^{Kip2}$ [31-33] have also been correlated with inhibition of cyclin-dependent kinase activity. The Bcl-2 gene families is the cell apoptosis related gene gained intensive study whose expression and regulation is one of the key factors that affect the cell apoptosis and play an important role in signal transduction pathways. Antiapoptotic genes of Bcl-2 mainly include the Bcl-2, the Bcl-xl, the Bcl-w, Mcl-1, and promoting apoptosis

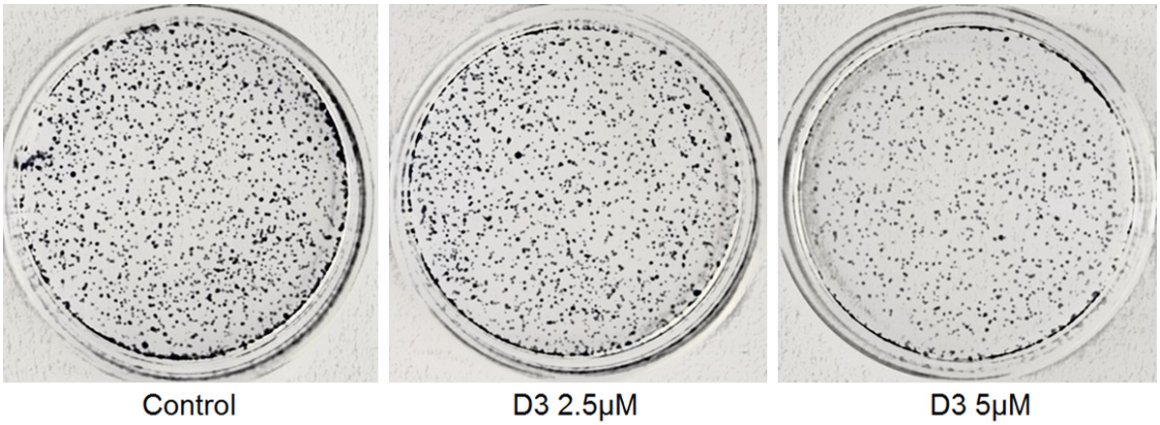


**Figure 5.** D3 inhibit cell proliferation in Clonogenic Cell Survival Assay. BGC823 cells were treated with D3 (2.5 μM and 5 μM) or left untreated for 24 h, then the cells was trypsinized seeded into 100 mm cell culture dishes and maintained for one week. Then the cell colonies were stained with gentian violet.

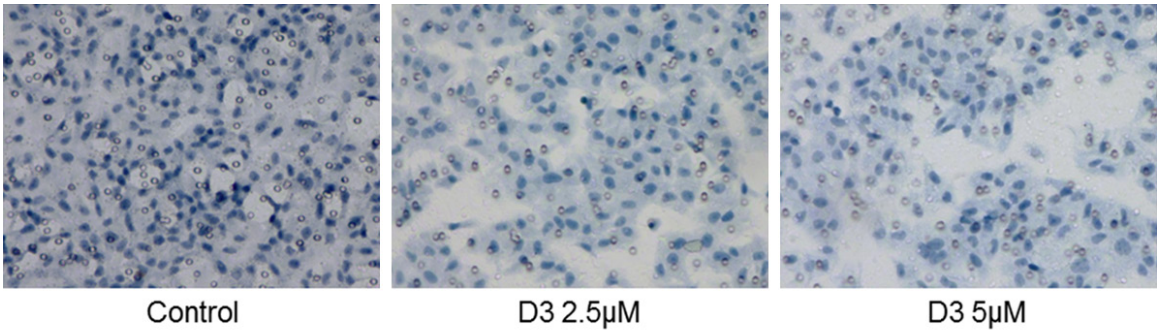


**Figure 6.** D3 inhibits cell invasion of BGC823 cell. A. The typical microscopic fields picture of BGC823 cells treated with D3. The BGC823 cells were treated with D3 (2.5 μM and 5 μM) or left untreated for 24 h, then the cells was trypsinized and seeded into Transwell chamber and cultured for another 48 h. Then those chambers were stained with hematoxylin. B. The quatification analysis of cell count from 3 randomly selected picture of each chamber. Data shown are mean ± SD. Significant differences between different groups are shown by "*" to indicate significant difference ($P<0.01$).

genes including Bax, Bak, Bad, Bid and Bim [34]. To understand the mechanism of D3-induced cell cycle arrest and apoptosis, we detected the effect of SAHA and D3 on the expression of various cell cycle and apoptosis regulators in BGC823 cells. As shown in **Figure 7**, D3 enhanced the mRNA expression of p21$^{WAF1}$ and downregulated the expression of Bcl-xl and Bcl-2 more significantly, and also had a certain concentration dependence. However, no changes were observed in the mRNA expression of p27$^{Kip1}$ and p57$^{Kip2}$ gene, other Kip family CDK inhibitor gene (**Figure 7**). SAHA also had a same function to the mRNA expression of p21$^{WAF1}$, Bcl-xl and Bcl-2, but its effect was weaker compared with D3. In addition, upon exposure to SAHA or D3, a same trend of the protein expression change of p21$^{WAF1}$, Bcl-xl and Bcl-2 was observed (**Figure 8**). This data showed that the mechanism of superior cell cycle arrest and apoptosis of D3 was mediated by a greater ability to upregulate p21$^{WAF1}$ and downregulate Bcl-xl and Bcl-2 compared with SAHA.

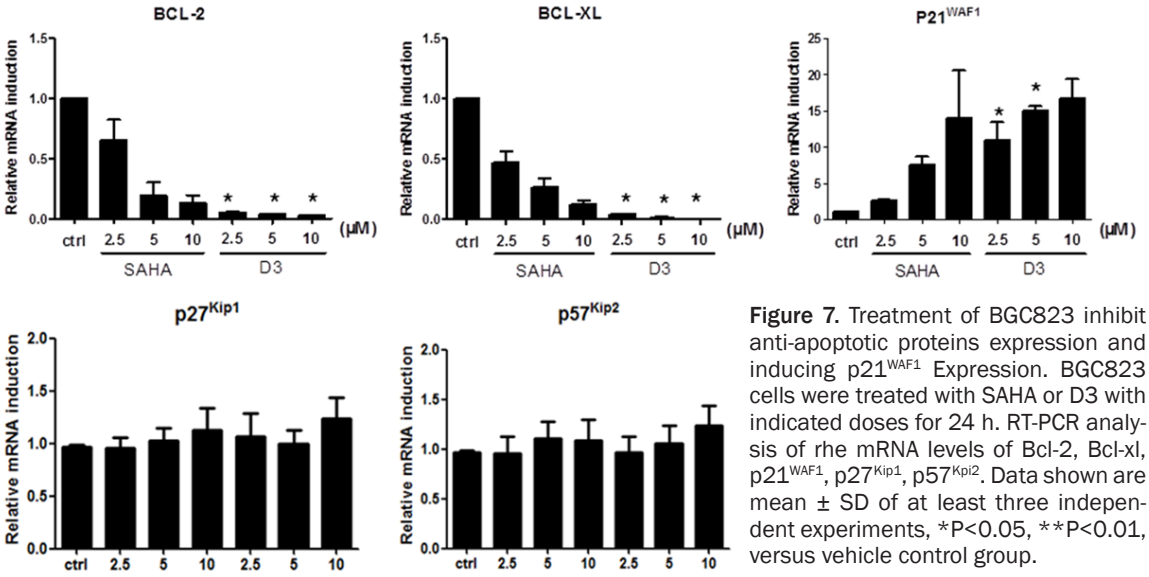


**Figure 7.** Treatment of BGC823 inhibit anti-apoptotic proteins expression and inducing p21$^{WAF1}$ Expression. BGC823 cells were treated with SAHA or D3 with indicated doses for 24 h. RT-PCR analysis of rhe mRNA levels of Bcl-2, Bcl-xl, p21$^{WAF1}$, p27$^{Kip1}$, p57$^{Kpi2}$. Data shown are mean ± SD of at least three independent experiments, *P<0.05, **P<0.01, versus vehicle control group.

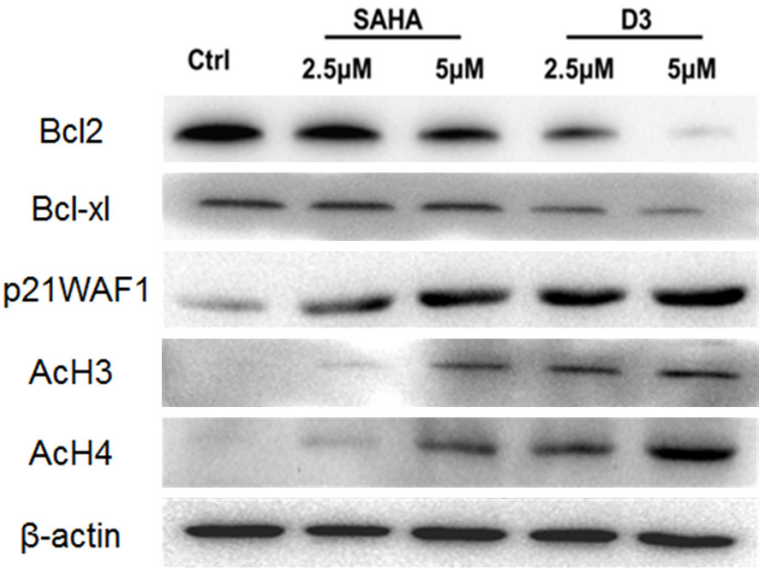


**Figure 8.** SAHA and D3 treatment inhibit anti-apoptotic protein but increases acetylated histone and p21$^{WAF1}$. After BGC823 cells were treated with SAHA or D3 with indicated doses for 48 h. Western blot analysis revealed a increase in acetylated histone 3 (AcH3), acetylated histone 4 (AcH4), p21$^{WAF1}$ protein and a decrease in bcl-2, bcl-xl.

*D3 induces accumulation of acetylated histones in chromatin more significantly than SAHA*

In order to confirm the inhibition of drug to histone acetylation enzyme, we further determined the levels of histone acetylation in BGC823 cells after SAHA or D3 treatment. As shown in **Figure 8**, no AcH3 or AcH4 was detectable in BGC823 cells treated with vehicle control (DMSO), indicating excessive removal of acetyl groups from the histone proteins by overactive HDACs. After cells were treated with HDACis, protein expression of *p21$^{WAF1}$* upregulation was mainly enhanced as the levels of AcH3 and AcH4 protein increased. But after two kinds of histone acetylation enzyme inhibitors treatment, the expression of total AcH3 and AcH4 quantity increase obviously, and also had the concentration dependence. Compared with the SAHA, D3 has more effective induction of histone acetylation.

## Discussion

Recently, histone deacetylase inhibitors (HDACi) has been regard as a potential strategy to cancer by induction of apoptosis and/or inhibition of cell proliferation which have been proposed as the major antitumor responses of them [35]. Very recently, hundreds of clinical trials are investigating the clinical use of BuA, PBuA, SAHA, apicidin, MS-275, and other HDAC inhibitors for the treatment of various human malignancies, both as single agents and in combination chemotherapeutic protocols [36-38]. However,

long-term or high doses use of SAHA led to several clinical adverse events, such as diarrhea, fatigue, nausea, and anorexia [39]. Besides, not all tumor cells are sensitive to SAHA. SAHA received approval to treat cutaneous T-cell lymphoma, which is a rare cancer. Therefore, identify and develop novel HDACis has been a direction of our efforts.

Despite each of HDACis with its unique structure their, most of them share a common pharmacophore which mainly contains three parts: a zinc ion-binding group and a surface recognition domain, joined by a linker domain of appropriate length [21]. In our previous study, we designed and synthesized a novel series of novel aromatic group-modificatiryhydroxamic acid derivatives as HDACis. Through several rounds of structural optimization, D3 showed excellent HDAC inhibition and potent growth inhibition in multiple tumor cell lines. In this study, we further found that this drug showed an enhanced growth-inhibitory effect against human gastric cancer cell lines by cell cycle arrest and apoptosis.

Compared with SAHA, D3 does induce severe apoptosis and cell cycle arrest in a density-dependent manner, suggesting that the antiproliferative effect of D3 is not only blocking the proliferation in G1-phase, but also directly related to cell death. Treating gastric carcinoma by forcing malignant cells to undergo terminal differentiation and promote cell apoptosis is a promising alternative therapeutic strategy for gastric carcinoma. Cell cycle arrest in cell proliferation inhibition is an essential early event in cell differentiation. The upregulation of *p21$^{WAF1}$* and downregulation of bcl-2 and bcl-xl is likely to be a contributing factor to the apoptosisi promotion and G1-phase arrest accompanied by a decrease in their S-phase content. Moreover, there are two sides of *p21$^{Cip1/WAF1}$* in cell cycle regulation: negative regulator of G1 phase cell cycle progression and regulation of the progression to terminal differentiation [40, 41]. The bcl-2 oncogene family has been implicated as the cause of several malignancy and become a critical molecular target. Our results showed that D3 markedly decreased the expression of bcl-2 and bcl-xl mRNA and protein in BGC823 cells. Moreover, D3 had a superior ability to deplete bcl-2 and bcl-xl and inhibit their kinase activity in BGC823 cells compared with SAHA. A number of genes were found to be related to treatment response of patient-derived cells cultures to combination treatments. Others showed that the gene FBXW7 was related to SAHA-induced cell death and Bcl-2 inhibition [42]. This points to a biological rationale in bcl-2/bcl-xl functioning to be predictive of treatment response to HDACi. The use of these genes as potential markers for patient to HDACi treatments should be explored in future studies. In mantle cell lymphoma, the efficacy of SAHA was enhanced by the BH-3 mimetic ABT-263 [43]. In contrast, another study did find that HDACi efficacy to be not related to Bcl-2 family proteins [44, 45]. Thus, these relationships between Bcl-2 family proteins and HDACi responses are determined by either tumor type or HDACi characteristic.

The expression of acetylated histone H3 and H4 was markedly induced by D3, even at low concentrations. Hyperacetylation of histone H3 and H4 which around the promoter region of *p21$^{WAF1}$* may enhance *p21$^{WAF1}$* mRNA level and protein expression [21]. *P21$^{WAF1}$* was one of the first genes found to be induced by HDACis [46], an event associated with marked acetylation of the *p21$^{WAF1}$* promoter. However, others the HDACivorinostator SAHA was relatively ineffective in inducing *p21$^{WAF1}$* expression, despite causing increased acetylation of histone H3 [47, 48]. In our study, both D3 and SAHA induced a G1 cell cycle arrest of BGC823 cells in a concentration-dependent manner. Therefore, we examined the concentration-dependent histone acetylation. The results showed that the protein expression levels of H3/H4 decreased at both 2.5 μM and 5 μM. Similar to the H3/H4 protein expression level, D3-induced upregulation of bcl-2 and bcl-xl in BGC-823 cells more significantly at the 5 μM point than at 2.5 μM. This result strongly suggested that *p21$^{WAF1}$* upregulation is mainly the result of enhanced acetylation of histones H3 and H4 around the promoter region of *p21$^{WAF1}$*.

In summary, this study reports the identification and characterization of a new aromatic group-modificatiryhydroxamic acid derivatives as HDACi, D3, which is more competent than SAHA as an inducer of acetylation of histones H3 and H4 promoting *p21$^{WAF1}$* expression which lead to cell cycle arrest and apoptosis. This feature of D3 predicts a broader effectiveness for apoptosis induction and even for potential clini-

cal effectiveness. Studies to test the anticancer efficiency of D3 in vivo are in progress. The results suggested that D3 is a potential therapeutic strategy for the treatment of human gastric cancer.

## Disclosure of conflict of interest

None.

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