Anti-Proliferative Activity of Benzamide Analogs in A549 Tumor Cells in Vitro

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Abstract. Objective: To determine the antitumor activity of five benzamide derivatives with similar structure in A549 tumor cells. Methods: Cytotoxicity of the compounds in A549 tumor cells were monitored through an xCELLigence Real-Time Cell Analysis System. Results: Four benzamide analogs inhibited the growth of A549 tumor cells in a dose- and time-dependent manner. Conclusion: Four benzamide derivatives exhibited antiproliferative activity against A549 tumor cells in vitro and could be further developed in preclinical studies as therapeutic agents for non small cell lung cancer.

Introduction

Lung cancer is one of the most serious threats to human health [1]. Recent reports from many countries revealed that the incidence and mortality rates of lung cancer account for the first and second of all malignant tumors in male and female, respectively [2-4]. Non-small cell lung cancer (NSCLC) is the most common lung cancer and accounts for approximately 80% of the total lung cancer. NSCLC is less sensitive to conventional chemotherapy than other types, and its 5-year survival rate is as low as only 15% [5, 6]. Hence, it is urgent to develop novel therapeutics for NSCLC treatment.

Histone deacetylases (HDACs) are a class of proteases which play an important role in the structural modification of chromosome and the regulation of gene expression. Histone acetylation facilitates the dissociation of DNA and histone octamer and relaxes the nucleosome structure, which allows transcription factors and co-transcription factors to bind specific sites in DNA, resulting in the promotion of genes transcription. In tumor cells, over expression of HDACs would lead to enhanced deacetylation and increase the affinity between DNA and histones, which makes slack nucleosomes become very tight and down-regulates the expression of particular genes, especially some tumor suppressor genes. The close relation between deregulated HDACs expression and tumor highlight them as attractive targets for tumor therapies [7].

Histone deacetylase inhibitors (HDACi) increase histone acetylation, thereby regulate the expression of related genes and induce apoptosis and differentiation in

tumor cells. Since the advantages of relatively high selectivity and low toxicity, HDACi have attracted much attention from Drug R & D personnel as a class of potential anticancer drugs [8-10]. Benzamide compounds represent a novel class of HDACi which show inhibitory activity through binding to a hydrophobic pocket of the center region of the HDAC catalytic site [11, 12]. To date, two benzamide analogs MS-275 and MGCD0103 have entered clinical trials and shown promising results [13, 14]. Based on these results, we examined five benzamide analogs for antiproliferative activity in A549 tumor cells to search for valuable lead compounds for NSCLC treatment.

Materials and methods

Materials

Benzamide analogs (2A, 2B, 2C, 2D, and 2E) were obtained from College of Pharmacy, Zhejiang University of Technology, China. The compounds were provided in a powdery form and dissolved in DMSO, which was further diluted with RPMI1640 medium. For the analysis of tumor growth, Real-Time Cell Analyzer (RTCA) System (Roche) was utilized. RPMI1640 medium, fetal bovine serum (FBS), and penicillin/streptomycin were purchased from Life Technology; A549 tumor cell line (non small cell lung cancer) from American Type Culture Collection (ATCC).

Methods

Cytotoxicity assay using an RTCA system

Cytotoxicity assay was performed using a modified method as described in our previous report [15]. To obtain background levels, 95 μL of RPMI1640 medium were added to each well of 96-Plate first, followed by 100 μL of cell suspensions containing about 8,000 A549 tumor cells. Approximately 24 h after incubation, when the cells reached exponential growth phase, 5 μL of benzamide analogs (2A, 2B, 2C, 2D, and 2E) were added to each well at final concentrations of 10, 30, 60, 90, and 120 μM, respectively. Solvent control (1% DMSO) and blank control were also included. To monitor the viability of the cells in each well, impedance was automatically recorded every 15 min by the Real-Time Cell Analyzer System. All operation processes were performed in a humidified atmosphere at 37 ℃ with 5% CO_2.

Statistics.

All experiments were performed in triplicate. Data were presented as mean $\pm$ SD and percentage. Data were evaluated by t-test and one-way Analysis of Variance and $p < 0.05$ were considered significant.

Results and Discussion

RTCA measurement showed that cell survival rates were significantly decreased when A549 tumor cells were exposed to various concentrations of benzamide analogs for 24 h, compared to controls. Compounds 2A ($p = 0.0067$), 2C ($p < 0.0001$), 2D ($p = 0.0002$) and 2E ($p = 0.0416$) markedly inhibited the proliferation of A549 cells in a time- and dose-dependent manner (Figure1 and Table1). However, compound 2B did not inhibit the proliferation of the tumor cells effectively even at the highest concentration, 120 μM, used in this study, the p value is 0.4091. The results revealed that structurally distinct benzamide analogs (2A, 2C, 2D, and 2E) suppressed the proliferation of A549 tumor cells to different degrees.

Table 1. Cytotoxicity elicited by various concentrations of compounds 2A-2E

	AUC-24				
Compound(μM)	2A	2B	2C	2D	2E
Control	63.0	62.8	65.3	81.5	62.5
DMSO	62.6	63.0	60.2	77.9	56.3
10	54.0	52.0	52.0	71.5	46.8
30	43.1	46.9	48.7	62.0	40.2
60	36.4	46.0	47.6	54.1	39.6
90	35.0	42.3	36.0	32.7	33.0
120	30.8	41.5	27.7	26.4	32.2

AUC-24: Area under concentration-time curve 24 h after the addition of the compound

The IC50 values of the compound 2A-2E for A549 cells were obtained by using an xCELLigence System software (Figure 2). The five benzamide analogs inhibited the growth of A549 tumor cells to different degrees in the following order; compound 2D > compound 2C > compound 2A > compound 2E > compound 2B. Compounds 2C and 2D showed superior cytotoxicity to A549 cells with the IC50 of 11.5 and 8.4 μM, respectively, whereas compound 2B displayed a weak killing effect against A549 tumor cells. It is evident that there was a dichotomy in IC50 for A549 cells between compounds 2B and 2D, suggesting that subtle changes in structure of the benzamide analogs might cause significant difference in the inhibitory activity for A549 tumor cells.

Figure. 1. Effect of benzamide analogs 2A-2E on the proliferation of A549 tumor cells when

A549-2A   A549-2B   A549-2C

A549-2D   A549-2E
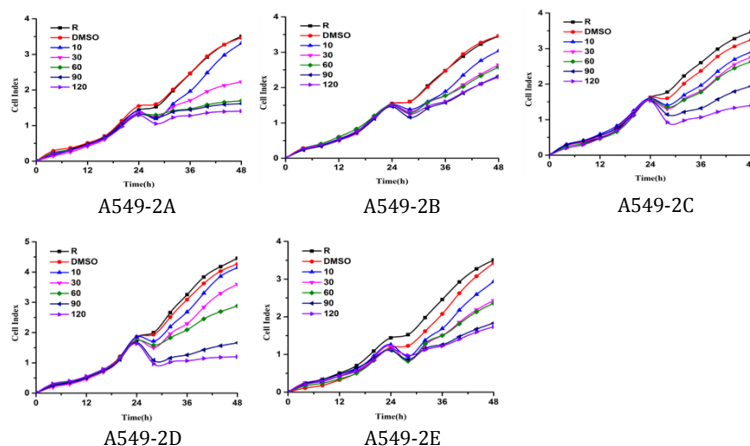
determined through an RTCA system. Cell Index: Relative cell number; R: neither compounds or DMSO is added; DMSO: 1% DMSO is added to cell; 10: 10 μM compound is added to cell; 30: 30 μM compound is added to cell; 60: 60 μM compound is added to cell; 90: 90 μM compound was added to cell; 120: 120 μM compound is added to cell
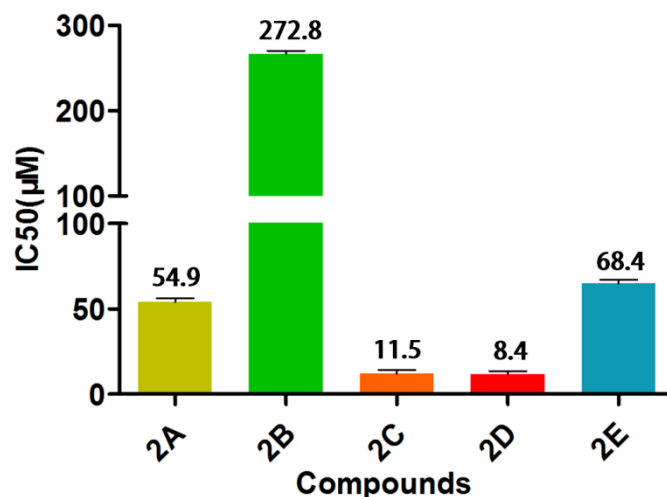
Figure.2. The IC50 of the compounds 2A-2E for A549 cells. IC50: half maximal inhibitory concentration; IC50(Compound 2A): 54.9 μM; IC50(Compound 2B): 272.8 μM; IC50(Compound 2C): 11.5 μM; IC50(Compound 2D): 8.4 μM; IC50(Compound 2E): 68.4 μM

Conclusion

Benzamide derivatives have recently received considerable attention because of their HDACi properties. All the five benzamide analogs except 2B examined in this study showed a potent inhibitory activity against A549 cells in a concentration- and time-dependent manner. Of the five analogs, compound 2D is the most effective against A549 cells. Our findings demonstrated that these benzamide compounds were potent HDACs inhibitors and could be promising candidates for further development of chemotherapeutic drugs for lung cancer. The antitumor activity elicited by these compounds may be attributed to their inhibitory activity against HDACs. In order to develop medicines for lung cancer, it is cardinal to further extend the investigation of drugs for lung cancer and elucidate the mechanism by which the benzamide derivatives efficiently inhibited the proliferation of lung cancer.

Acknowledgments

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