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2-(1-Hydroxethyl)-4,8-dihydrobenzo[1,2-b:5,4-b′]dithiophene-4,8-dione (BTP-11) enhances the ATRA-induced differentiation in human leukemia HL-60 cells

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ABSTRACT

2-(1-Hydroxethyl)-4,8-dihydrobenzo[1,2-b:5,4-b′]dithiophene-4,8-dione (**BTP-11**) is a potent enhancer for all-*trans* retinoic acid (ATRA)-induced differentiation in HL-60 cells. Combination of **BTP-11** and ATRA cut down the concentration of ATRA significantly, and that **BTP-11** promoted the progression of ATRA-induced into the terminal granulocytic differentiation. Further, Western blot analysis revealed that combination of **BTP-11** and ATRA decreased cyclin D/CDK4 and increased C/EBPε protein expression to arrest the cells into G0/G1 phase leading to granulocytic maturation. These results confirmed that **BTP-11** is a potent enhancer for ATRA-induced differentiation of HL-60 cells, and the great developmental potential of **BTP-11** will be expected.

1. Introduction

Differentiation of leukemia cells has promoted increasing research interest as an alternative or support to conventional cytotoxic chemotherapy [1]. In acute promyelocytic leukemia (APL), all-*trans* retinoic acid (ATRA) is a potent inducer of APL cell differentiation, and its use in anti-APL therapy markedly improved survival and prognosis of patients with this disease [2]. However, the therapeutic use of this compound is limited by a number of problems, which include serious systemic toxicity [3] and induced ATRA resistance [4]. To improve the APL treatment, several chemotherapeutic agents, such as arsenic trioxide (As_2O_3) [5] and cytarabine (Ara-C) [6], were also used in combination with ATRA, although As_2O_3 and Ara-C are highly toxic in nature. Recent studies had also indicated that combinatorial use of retinoid and non-retinoid compounds, such as histone deacetylase (HDAC) inhibitors and protein kinase A agonists, has higher therapeutic value in APL [7]. However, preclinical studies predicted that high concentration for currently used HDAC inhibitors, such as phenylbutyrate or valproic acid, will be required to achieve therapeutic effect [8,9]. Thus, the search of novel and potent enhancers for ATRA-induced differentiation in leukemia cells might be considered as a desirable therapeutic goal.

In prior work, we synthesized a series of benzodithiophenedione derivatives and found that many of these chemicals exhibited potent cytotoxicity and differentiation activity toward acute myeloid leukemia HL-60 cells [10–12]. In order to exposure the differentiating mechanisms of these series derivatives, 2-(1-hydroxethyl)-4,8-dihydrobenzo[1,2-b:5,4-b′]dithiophene-4,8-dione (**BTP-11**) was selected for further investigation. In this study, we found that there is a synergetic effect between **BTP-11** and ATRA on the cell differentiating activity of HL-60 cells. Further, the action mechanisms were described here. Our primary goal is to provide guidelines for designing animal and clinical test models of the combination used with **BTP-11** and ATRA in the future.

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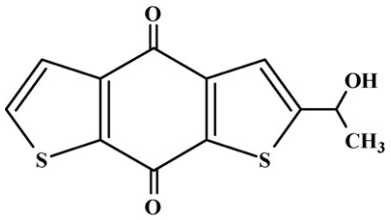

Fig. 1. Chemical structure of **BTP-11.**

2. Materials and methods

2.1. Chemicals and reagents

BTP-11 had been prepared in our laboratory (structure shown in Fig. 1) [12]. **BTP-11** was initially dissolved in DMSO and diluted to appropriate concentrations before each experiment. The final concentration of DMSO in the culture medium was kept below 0.1%. All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA) unless otherwise specified.

2.2. Cell culture

The human promyelocytic leukemia cells (HL-60, ATCC) were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum (GIBCO/BRL), penicillin (100 U/ml)/streptomycin (100 μg/ml)(GIBCO/BRL) and 1% *L*-glutamine (GIBCO/BRL) at 37 °C in a humidified atmosphere containing 5% CO_2. Logarithmically growing HL-60 cells were used for all experiments.

2.3. Cellular proliferation, apoptosis and differentiation

The total number of cells were assessed by the standard procedure of leukocyte counting using a hemocytometer, and cell viability was checked by the ability of viable cells to exclude trypan blue (Sigma). For the morphological observation of apoptosis, cells were stained with 4′-6-diamidine-2-phenylindole (DAPI), as described. For the cell differentiation, nitrobluetetrazolium (NBT)-reduction assay was performed on extracts of PMA-simulated cells as detailed in a previous study [13].

2.4. Cell cycle distribution analysis

Cell cycle analysis by flow cytometry was performed as described in the previous paper [14]. After treatment, the cells were collected, washed with cold PBS, and fixed with 70% ice-cold ethanol at −20 °C overnight. Then the cells were centrifuged and suspended in a staining solution containing 1% Triton-X 100 (Sigma), 0.1 mg/ml RNase (Sigma) and 4 μg/ml phopidium iodide (Sigma) for 30 min at 37 °C in the dark and analyzed on a fluorescence-activated cell sorter flow cytometry (FACS-caliber, Becton Dickinson, San Jose, CA, USA) and all histograms were analyzed by ModFit software.

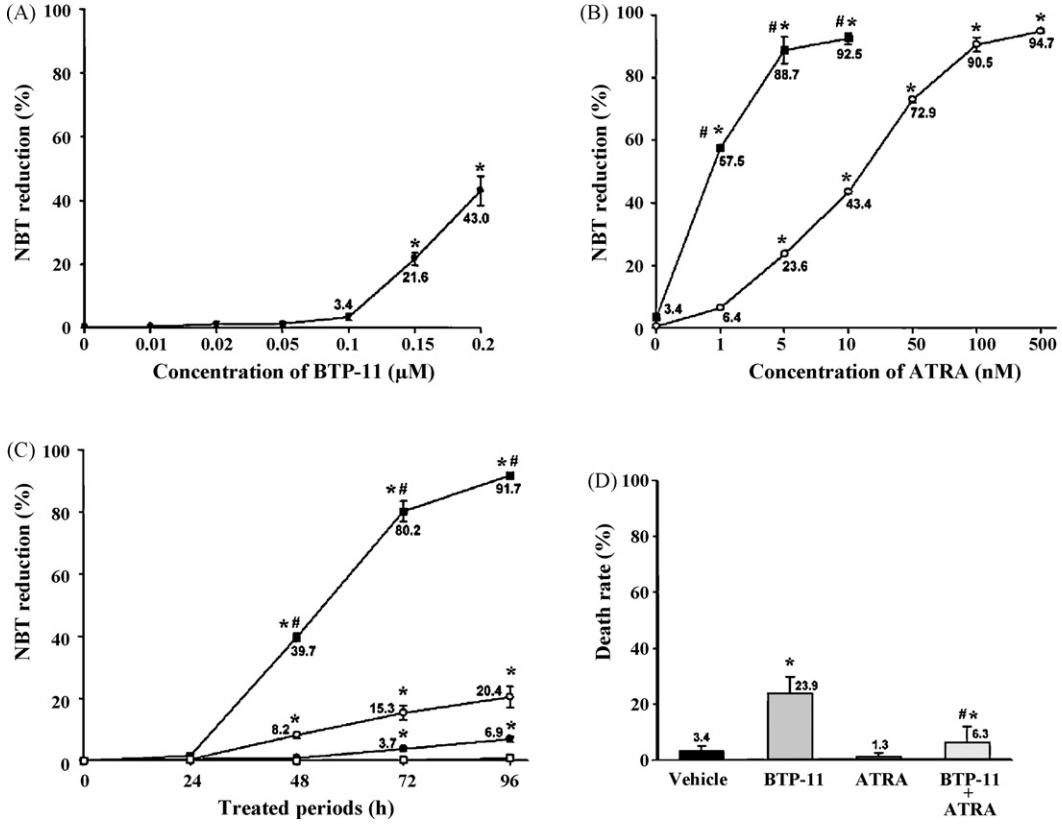

Fig. 2. Differentiation effect of BTP-11 in HL-60 cells. Cells (2×10^4) were treated with vehicle (□), BTP-11 (●), ATRA (○), or BTP-11 + ATRA (■) for the indicated periods. (A) Cells were treated with BTP-11 (0.01–0.2 μM) alone for 96 h. (B) Cells were treated with various concentrations of ATRA in the absence or presence of BTP-11 (0.1 μM) for 96 h. (C) Cells were treated with vehicle, BTP-11 (0.1 μM), ATRA (5 nM) or BTP-11 (0.1 μM) + ATRA (5 nM) for 24–96 h. Then the differentiation effect was determined by NBT-reduction assay. Each value represents as mean ± S.D. of four independent experiments, and the mean values were marked. (D) Cells were treated with vehicle, BTP-11 (0.1 μM), ATRA (5 nM) or BTP-11 (0.1 μM) + ATRA (5 nM) for 96 h. Then the death rate was determined by trypan blue exclusion. Each value represents as mean ± S.D. of four independent experiments, and the mean values were marked. *Significantly higher than the vehicle control; #significantly higher than exposure to BTP-11 or ATRA used alone. $P < 0.001$ according to the Student's *t* test.