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Design and antiproliferative activity of 2-(3,5-dihydroxyphenyl)-6-hydroxybenzothiazole (DHB) on PC-3 prostate cancer cell line

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Abstract: 2-(3,5-Dihydroxyphenyl)-6-hydroxybenzothiazole (**DHB**) has been synthesized and characterized. The antiproliferative activity of **DHB** against cancer cells lines had been previously reported. Herein, we show that **DHB** inhibits the growth of human prostate cancer PC-3 with an IC_{50} value of 45 μM in a dose/time dependent manner by using Sulforhodamine B (SRB) assay. The present analysis of PC-3 revealed suppression of clonogenic activity after exposure to **DHB** at a concentration of 40 μM for PC-3.

Keywords: Benzothiazole**,** Sulforhodamine B (SRB), PC-3 Prostate Cancer, Clonogenic activity.

Introduction

The benzothiazole nucleus is of particular interest especially within the realm of medicinal chemistry. Many useful therapeutic agents contain the benzothiazole moiety and have useful physiological activities such as neuroprotective [1,2], Antifungal [3], antibacterial [4], antiallergic [5] and antitumor activities [6-7]. Compound **DHB** is a "drug-like" analogue of resveratrol which is well-known dietary polyphenol displaying remarkable cancer chemopreventive properties [8]. Our previous studies proves that 2-(3,5-Dihydroxyphenyl)-6-hydroxybenzothiazole (**DHB,** Scheme **1**) is most active antiproliferative agent among its other derivatives against MDA-MB-231 breast cancer cells, displaying an IC_{50} value of 19.4 μM [9] and also showed the highest levels of vasorelaxing potency and efficacy in rat aortic rings pre-contracted with KCl 60 mM since it is widely known that high levels of membrane depolarization due to high extracellular concentrations of potassium ions can dramatically and non-specifically reduce the vasorelaxing effects of vasorelaxing agents acting through the activation of every type of membrane potassium channels [10]. The above controversial observations on the cytotoxicty activity and vasorelaxing potency of **DHB** led us to synthesis its following the method described by Bertini *et al* (Scheme **1**) [9]. Therefore, we decided to further investigate this compound and study in details the dose&time-dependent antiproliferation and morphological study and clonogenic activity of **DHB** on PC-3 prostate cancer cell lines which were originally derived from advanced androgen independent bone metastasized prostate cancer.

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Results and discussion

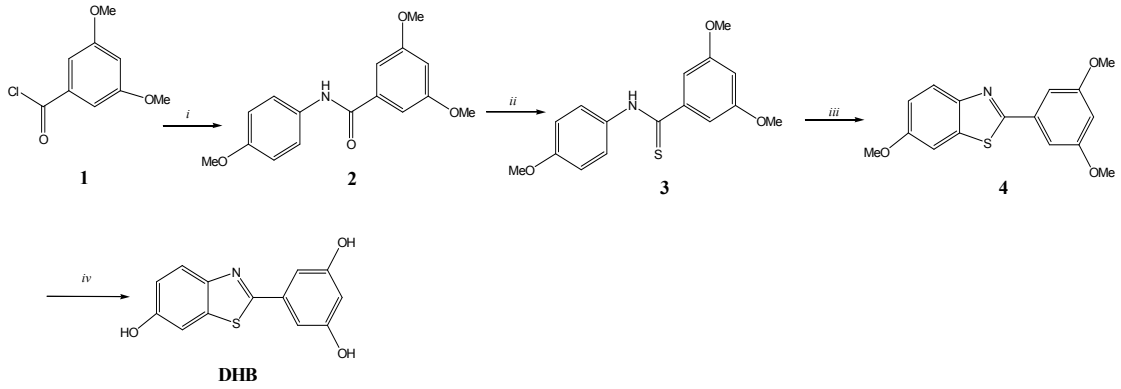
Chemical synthesis of 2-(3,5-Dihydroxyphenyl)-6-hydroxybenzothiazole (DHB)

DHB was prepared following the method described by Bertini *et al* [9]. Briefly, the synthesis of benzothiazole **DHB**, started with a condensation of 3,5-dimethoxybenzoyl chloride with *p*-anisidine, to form amide. Treatment of this amide with the Lawesson's reagent produced thioamide, which was then submitted to an oxidative cyclization promoted by potassium ferricyanide. The resulting trimethoxy-substituted benzothiazole was treated with BBr_3 to yield final product **DHB** with 49% yield (Scheme **1**).

Cytotoxic evaluation and cell morphology

Cells were treated with **DHB** at different concentrations, ranging from 1 μM to 200 μM for 3 days by the using SRB assay**.** The percentage growth inhibition was calculated by comparison of the absorbance of treated versus control cells. IC50 calculated for PC-3 was 45 μM **(**Figure**. 1A, B)** after 72 h treatment. Control group showed regular polygonal

shape and cell antennas were short. The cell morphology of treated cells was affected by **DHB** treatment and loss of adhesion, rounding, cell shrinkage, and detachment from the substratum were additionally observed **(**Figure**. 1C)**.



Scheme 1. Reagents and conditions: (*i*) *p*-anisidine, CH_2Cl_2, DMAP, PS-DIEA, rt, overnight [61%]; (*ii*) Lawesson's reagent, 130°C, 3 h [67%]; (*iii*) $K_3Fe(CN)_6$, aq NaOH 30%, EtOH, 85°C, 30 min, then rt overnight [74%]; (*iv*) BBr_3, CH_2Cl_2, -78°C to rt, overnight [49%].

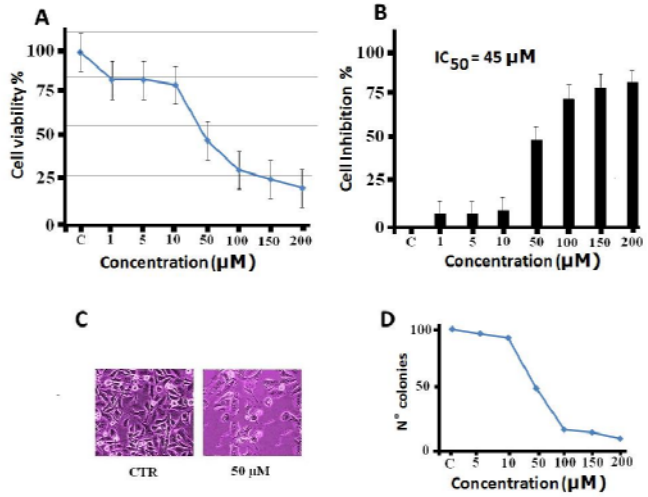


Figure 1. (**A**) Effects of **DHB** on the proliferation of PC-3; (**B**) Inhibition growth of DHB on PC-3; (**C**) Morphological analysis for the effects of **DHB** on PC-3 after 72 h incubation; (**D**) Dose-response curve of **DHB**-mediated inhibition of PC-3 cell colony formation.

Clonogenic assays

Analysis of clonogenic activity in PC-3 cells treated with **DHB** at concentarion of 0, 5, 10, 50, 100, 150 and 200 µM of **DHB** for 14 days revealed a complete inhibition of colony formation at 40 µM for PC-3 cells, whereas concentrations lower than 10 µM were almost ineffective **(Figure. 1D)**.

Experimental

Chemicals and materials

Trypsin, Trypan Blue, antibiotic and antimycotic agent, fetal bovine serum (FBS), sulforhodamine B (SRB) and dimethyl sulfoxide (DMSO), were purchased from Sigma Chemical Co. (St. Louis, MO).

Cell culture

Human prostate cancer PC-3 cell lines were supplied from ATCC and were maintained in the standard medium and grown as a monolayer in DMEM containing 10% FBS, 2 mM glutamine, 100 units/ml penicillin, and 100 µg/ml streptomycin. Cultures were

maintained at 37 °C with 5% CO_2 in a humidified atmosphere.

In vitro evaluation of cytotoxic activity

Growth activity of **DHB** *in vitro* was evaluated by the SRB assay [11]. **DHB** stock solution (10 mM in DMSO) was stored at 4°C and diluted with DMEM up to 0.1–1 mM range at room temperature before treatment. The final percentage of DMSO in the reaction mixture was less than 1% (v/v). Cancer cells (2.5 x 10^3 cells/ well) were plated in 96-wells plates and incubated in medium for 24 hours. Serial dilutions of individual compounds were added. The plates were incubated at 37 °C, for 72 hours prior to addition of **DHB**. The assay was terminated by the addition of 50 μL of ice-cold trichloroacetic acid (final concentration, 10% TCA) and incubated for 60 min at 4°C. The plates were washed five times with distilled water and air-dried. SRB solution (50 μL) at 0.4% (w/v) in 1% acetic acid was added to each of the wells, and plates were incubated for 30 min at room temperature. The residual dye was removed by washing five times with 1% acetic acid. The plates were air-dried or under hood. Bound stain was subsequently eluted with 10 mM Trizma base, and the absorbance was read on an ELISA plate reader at a wavelength of 540 nm and used as a relative measure of viable cell number. The percentage of growth inhibition was calculated by using the equation: percentage growth inhibition (1-A_t/A_c) x 100, where At and Ac represent the absorbance in treated and control cultures, respectively. IC_{50} was determined by interpolation from dose–response curves.

Evaluation of Cell morphology

PC-3 cells plated at about 20,000 cells/well on chamber-slides (8 wells) were treated with 0, 5, 10 and 50 μM of **DHB** for 72 hr. After rinsing in phosphate-buffered saline, cells were either fixed in methanol 100% and stained with 10% Giemsa and photographed using a Nikon camera attached to the microscope.

Clonogenic assay

PC-3 cells were plated at a density of 800 cells/well in 12-well plates and treated at concentarion of 0, 5, 10, 50, 100, 150 and 200 μM of **DHB** for 14 days. Plates were rinsed in phosphate-buffered saline and colonies were methanol-fixed and stained with 10% Giemsa and Clones were counted under a light microscope.

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