

Cytotoxic Activity of BornUSU I towards T47D Breast Cancer Cells

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Abstract

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AIM: The aim of this study was to determine cytotoxic activity of BornUSU I or Boronhafagama I (1,5-bis(4-hydroxyphenyl)-3-oxa-1,5-diaza-2,4-diboropentane-2,4-diol) as a boron derivate compounds which are boron neutron captured therapy (BNCT) candidates.

METHODS: The T47D cells were treated by BornUSU I, and Tamoxifen as a positive control. The in vitro study was using MTT method with the incubation period for 24h and 48h. All data were determined using viability of cells equation for showing each IC₅₀ value.

RESULTS: The IC₅₀ value of BornUSU I and Tamoxifen were 72.61 ± 0.82 µM and 10.62 ± 0.06 µM for 24 h incubation period, and for the 48 h incubation period were 44.63 ± 0.23 µM and 7.79 ± 0.05 µM. The 48 h incubation period results showed the lowest IC₅₀ value.

CONCLUSION: The results reveal that BornUSU I provide effective as anticancer, especially for breast cancer treatment.

Introduction

Breast cancer is the most incidence cancer and the second leading cause of cancer death among females [1]. Moreover, breast cancer ranks as the fifth cause of death from cancer overall (522,000 deaths), is the most frequent cause of cancer death in women in less developed countries (324,000 deaths, 14.3% of total), and the second cause of cancer death in developed countries (198,000 deaths, 15.4%) after lung cancer. A recent study published which breast cancer is leading in the estimated new cancer cases, and the second most common death cause among women suffering from cancer in the USA [2].

Boron Neutron Capture Therapy (BNCT) is an advanced form of radiotherapy technique which is potentially superior to all conventional techniques for cancer treatment, as it is targeted at killing individual cancerous cells with minimal damage to surrounding healthy cells [3]. Boronic compounds has been previously used in imaging and medicinal chemistry offering unique advantages associated with its low toxicity and stability [4].

BornUSU I (1,5-bis(4-hydroxyphenyl)-3-oxa-1,5-diaza-2,4-diboropentane-2,4-diol) or Boronhafagama I. The chemical structures of BornUSU I and tamoxifen are showed in Figure 1.

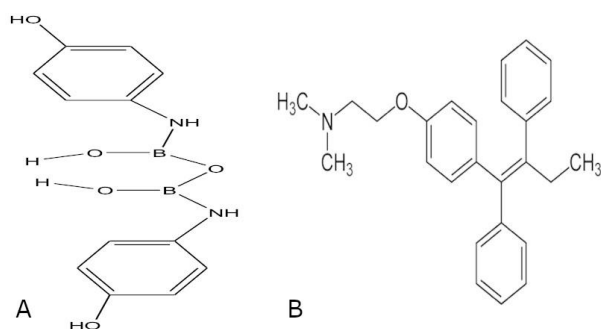


Figure 1: Structure of A) BornUSU I and B) Tamoxifen

Material and Methods

Chemicals Material

BornUSU I was obtained from Dr. Hari Purnomo, M.S., Apt. Chemicals used were distilled water from Water One-OneMed (Indonesia), DMSO and [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) from Sigma - Aldrich (USA).

Cytotoxicity Assay

The cells were treated by BornUSU I and tamoxifen. In this test, T47D cell line was grown in RPMI-1640 medium, medium containing 10% Fetal Bovine Serum (Gibco), 1% penicillin-streptomycin (Gibco), and fungizone 0.5% (Gibco) in a petridish in a humidified atmosphere (5% CO₂) at 37°C. The inoculums seeded at 1 x 10⁴ cells/mL at an optimal volume of 0.1 mL per well. After 24 h incubation, the medium was discharged and treated by BornUSU I and tamoxifen. After incubation 24 h and 48 h, the cells were incubated with 0.5 mg/mL MTT for 4 h in 37°C. Viable cells reacted with MTT to produce purple formazan crystals. After 4 h, SDS 10% as stopper (Sigma) in 0.01N HCl (Merck) was added to dissolve the formazan crystals. The cells were incubated for 24 h in room temperature and protected from light. After incubation, absorbance was measured using ELISA reader at λ 595 nm. The data which were absorbed from each well were converted to percentage of viable cells [5], [6], [7], [8], [9]. The equation to determine viability of cells:

$$\text{Viability} = \frac{\text{Abs of treatment} - \text{Abs of medium}}{\text{Abs of control cells} - \text{Abs of medium}} \times 100\%$$

Statistic Analysis

All cytotoxic data were analyzed using SPSS 21 software by probit analysis.

Results

Inhibitory Concentration 50% (IC₅₀)

MTT method was used to determine cell viability after incubation for 24h and 48h. The two different period of incubation was used to determine which one can give the lowest IC₅₀ value. Cytotoxic activity of BornUSU I and tamoxifen were showed in Table 1.

Table 1: IC₅₀ value of BornUSU I and tamoxifen towards T47D breast cancer cells

Treatment	IC ₅₀ (μM)	
	24h	48h
BornUSU I	72.61 ± 0.82	44.63 ± 0.23
Tamoxifen	10.62 ± 0.06	7.79 ± 0.05

Based on the results, it can be seen that T47D cell showed the inhibition of cell growth due to the influenced of BornUSU I and tamoxifen. The IC₅₀ values with BornUSU I treatment for each 24 h and 48 h incubation were obtained 72.61 ± 0.82 μM and 44.63 ± 0.23 μM, also the IC₅₀ values with tamoxifen treatment for each 24 h and 48 h incubation were obtained 10.62 ± 0.06 μM and 7.79 ± 0.05 μM. The results showed that IC₅₀ values for 48h incubation had smaller values than 24 h incubation.

Discussion

The principle of the MTT method is cellular reduction reaction based on the breakdown of yellow MTT tetrazolium salts into purplish blue formazan crystals. This color changing is an indicator of cell proliferation. The mitochondria of proliferating cells will absorb MTT so these cells will turn purple due to the formation of tetrazolium crystals (formazan) [10].

The results showed that IC₅₀ values for 48h incubation had smaller values than 24 h incubation. More decreasing of IC₅₀ value means the cytotoxic activity is higher [11]. Barry et al., in 2014, reported the need for an efficient delivery of boron agents to overcome the challenge of clinical development [12]. In this study, T47D cell line was used to determine BornUSU I had the cytotoxicity activity as the clinical developing of anticancer agent. T47D breast cancer cells have positive molecular characteristics of Estrogen Receptor alpha (ER-α) overexpression [13]. Tamoxifen has become a first-line therapy for patients with breast cancer risk with ER-α for both pre and postmenopausal patients, as well as for advanced breast cancer patients [14].

In conclusion, BornUSU I is boron derivate compounds. BornUSU I has the cytotoxic activity and it is potential to develop as more effective anticancer agent.

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