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Cell Cytotoxicity of Benzothiazole Derivatives Aagainst the Human Carcinoma Cell Line LungA549

Research Article

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Abstract : Three different benzothiazole derivatives have been synthesized and then were subjected to biological evaluation for the cytotoxicity study against human carcinoma cell line LungA549 by using *in vitro* 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) cytotoxicity assay. These compounds included *N*-(6-nitrobenzo[d]thiazol-2-yl)acetamide (**A**), *N*-(benzo[d]thiazol-2-yl)acetamide (**B**) and 6-nitrobenzo[d]thiazol-2-ol (**C**). In this study, compound **A** and compound **C** showed mild but interesting cytotoxic property against the carcinoma cell lines especially in 40 µg/mL dose with the IC_{50} values of 68 µg/mL and 121 µg/mL, respectively.

Keywords: *LungA549 cell line ● Benzothiazole ● Cytotoxicity ● Synthesis*

1. Introduction

Cancer is a major killer worldwide and now-a-days is a strong challenge to the medicinal chemists. More than 19 million cancer patients have been diagnosed worldwide in 2020 and almost 10 million cancer deaths have been reported by this time. In these malignant diseases, the dividing cells grow uncontrollably thereby leading to increased consumption of energy and defected cell functions through inadequate differentiation (Foye *et al*., 2008; Hyuna *et al*., 2021 and Zhu *et al*. 2017). These malignancies are most prevalent in lungs, breasts, prostrates and colon. While considering the cancer associated deaths, the first one is breast cancer (11.7%) followed by lung cancer (11.4%). The other major deaths are from colorectal cancer (10.0%), prostate cancer (7.3%) and stomach cancers (5.6%).

Diversified anticancer drugs are available for treating the various types of cancer patients. These drugs act either by killing the cancer cells or by affecting their metabolic functions and are often associated with cytotoxicity as well as genotoxicity to the normal cells leading to a lot of adverse effects to human body. Additionally, there are reports that the anticancer drugs become resistant with time (Aydemir *et al*., 2003 and Pranika *et al*., 2016). Although numerous researchers have put their efforts to predict the cancer pathophysiology and early detection of cancer, they could not reduce the overall mortality rates (Mallath *et al*., 2014). Obviously, because of the complexity of the robust biological networks, cancer cells proceed with metastasis and irreversible resistance to apoptotic signals (Rashid *et al*., 2012 and Nagula *et al*. 2019). As a result, there are continuous searches for discovering safer anti-cancer agents.

Heterocyclic compounds are of particular interest because of their capacity of forming the polar interactions in the binding site of the receptors. Accordingly, benzothiazole derivatives have been attracted by a lot of medicinal chemists for their drug discovery projects (Chatrabhuji *et*

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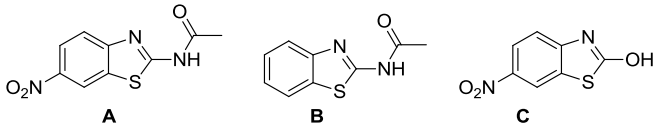
al., 2010 and Londhe *et al*., 2010). These properties have been correlated to diversified biological activities as reported in various journals (Evindar *et al*., 2006; Tang *et al*., 2003; Malik *et al*., 2010; Priyanka *et al*., 2010; Muttu *et al*., 2010 and Prabodh *et al*., 2013). Considering these encouraging facts, benzothiazole derivatives have been considered for this study.



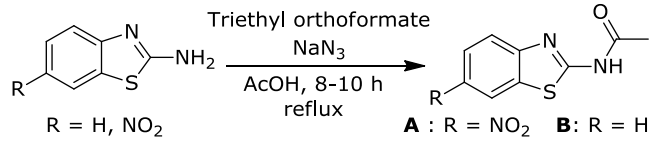
However, though diversified activities of benzothiazole derivatives have been reported in published journals, the cell cytotoxicity of benzothiazole derivatives (**A**, **B** and **C**) against the human carcinoma cell line LungA549 has not been reported so far. So, as per the initial focus point, these compounds have been considered for the cytotoxicity study by using *in vitro* 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) cytotoxicity assay and the observations have been reported here.

2. Materials and Methods

2.1. Test compound preparation

2.1.1. Synthesis of the test compounds

The targeted compounds, as shown in Scheme 1, were synthesized from respective 2-aminobenzothiazoles in research laboratory (Bepary *et al*., 2021) of the Department of Pharmacy, Jagannath University. After synthesis, the compounds were characterized before taking for the biological evaluation.



Scheme 1: Synthesis of *N*-(benzo[d]thiazol-2-yl) acetamides

2.1.2 Characterization of the synthesized compounds

Compounds were characterized by using the ^{1}H NMR by using Bruker 400 MHz NMR spectroscopy available in the Bangladesh Council of Scientific and Industrial Research (BCSIR). HRMS data was taken from Japan by using ion mode FAB+ and acetone as solvent.

2.2. *In vitro* study on cell line

Evaluation of cytotoxicity of pure compounds

Cytotoxic activity was studied against Human Lung Cancer cell line, LungA549, UK, using slight modification of the Trypan Blue Exclusion Method (Strober *et al*., 2001; Wilson *et al*., 2015; Khan *et al*., 2018; and Md *et al*., 2020). Cells were cultivated in 75 cm^2 flasks in 5% (v/v) CO_2 at 37°C with media described at Khan *et al*, 2018. According to the study design, cells were grouped into four with three replica for each concentration level. Treatment groups were evaluated with vehicle group against Human Lung Cancer cells split the day before experiments. The freshly prepared doses (5, 10, 20 and 40 µg/mL) were administered into 1 day before cultured T-flasks with approximately 2.5×10^6 cells. Negative control corresponds to the cells cultured in medium having 0.6% DMSO. After 24 h of incubation period, the cells were harvested using 0.5% trypsin. The number of dead cells was calculated by automated cell counter (LUNA-II™, South Korea, Berry *et al*., 1991) using trypan blue (0.4% w/v). Percentage of dead cells was calculated following the mathematical formula:

Percentage of dead cells =

(Number of dead cells)/ (Total number of cells)×100

2.3. Statistical Analysis

For antiproliferative assay, inhibition percentage at each concentration was compared with negative control and their statistical significance was tested with the one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test using GraphPad Prism version 9.00 for Windows, GraphPad Software. P values of less than 0.05 were considered statistically significant.

3. Results and Discussion

3.1. Characterization data of synthesized compounds

*N-(6-nitrobenzo[d]thiazol-2-yl) acetamide (**A**)*

^{1}H NMR: (400 MHz, DMSO-*d6*) δ 12.75 (s, 1H), 9.03 (s, 1H), 8.26 (d, *J* = 8.8 Hz, 1H), 7.87 (d, *J* = 8.8 Hz, 1H), 2.25 (s, 3H).

HRMS: (ESI) Observed m/z 237.9 $[M+H]^+$, (Calculated for $C_9H_7N_3O_3SH$+ 238.0286).

*N-(benzo[d]thiazol-2-yl)acetamide (**B**):*

^{1}H NMR: (400 MHz, DMSO-*d6*) δ 12.32 (s, 1H), 7.95 (d, *J* = 8.0 Hz, 1H), 7.72 (d, *J* = 8.0 Hz, 1H), 7.42 (t, *J* = 7.60 Hz, 1H), 7.29 (t, *J* = 7.60 Hz, 1H), 2.19 (s, 3H).

HRMS: (ESI) Observed m/z 193.0 [M+H]+ (Calculated for $C_9H_8N_2OSH$+ 193.0436).

*6-nitrobenzo[d]thiazol-2-ol (**C**):*

^{1}H NMR: (400 MHz, DMSO-*d6*) δ 12.56 (s, 1H), 8.62 (m, 1H), 8.16 (m, 1H), 7.26 (m, 1H).

HRMS: (ESI) Observed m/z 197.0 $[M+H]^+$ (Calculated for $C_7H_4N_2O_3SH$+ 195.9943).

3.2. Cell Viability Using MTT Assay

For observation of the cell viability from **A** in the MTT Assay method, four different doses (5 µg/mL, 10 µg/mL, 20 µg/mL and 40 µg/mL) were applied. Thus the applied

concentrations were 0.02-0.20 µM/mL for the test compounds. All the comparisons were made with the blank where only the vehicle was applied. From each dose group the average values were taken for subsequent analysis. For making the inference from the experiments, numbers of viable cells were counted and then the cell viability was calculated. As shown in Figure 1, when applied compound **A**, cell viability was very high in lower doses (5, 10 and 20 µg/mL), but there was a clear reduction in cell viability (79%) when applied 40 µg/mL doses (Graph 1&2). But after application of compound **B**, no visible reduction in cell viability was observed even from the highest dose (Graph 1).

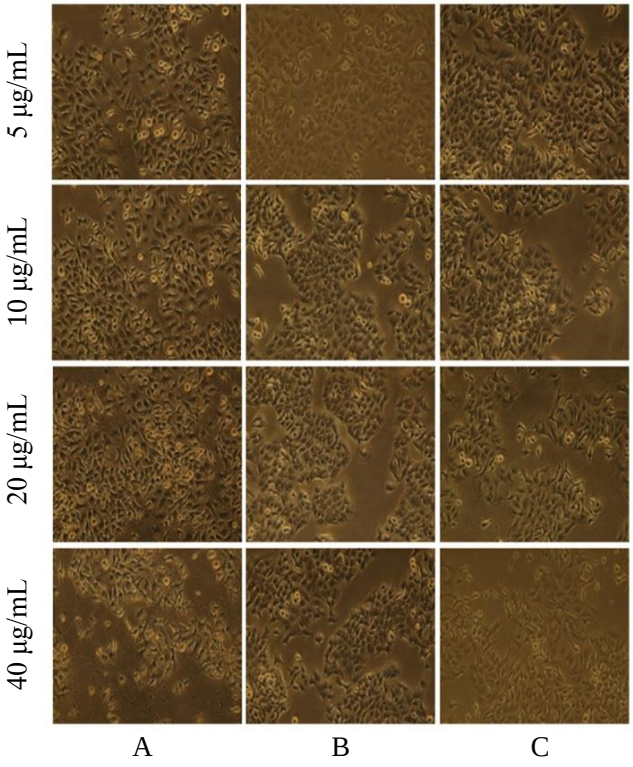
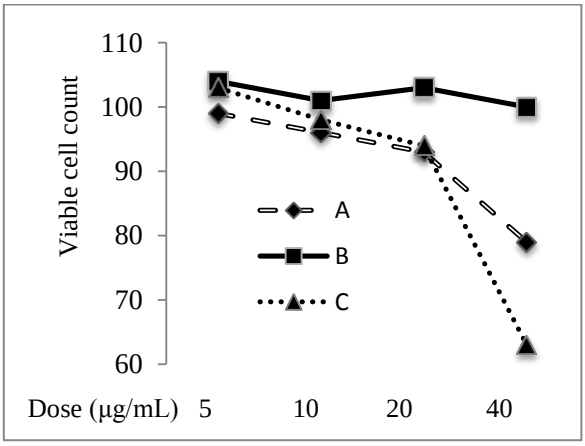
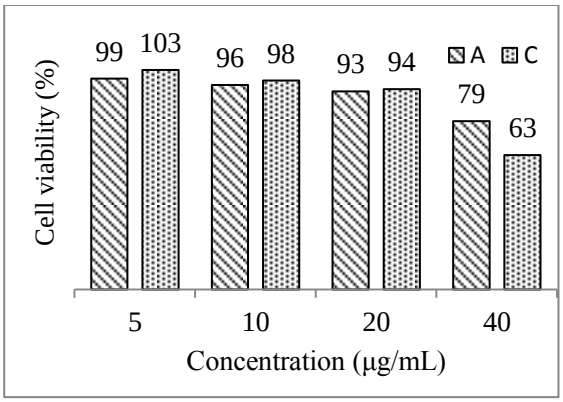


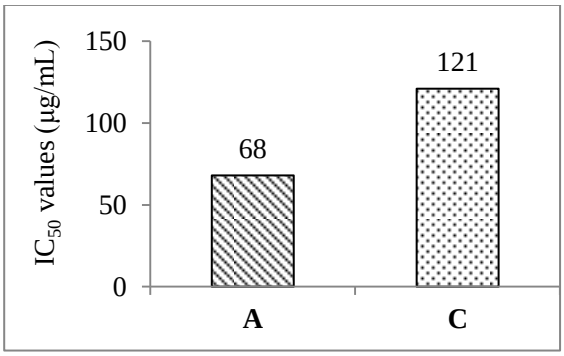
Figure 1: Cell cytotoxicity observed from various doses of **A**, **B** and **C**



Graph 1: Dose-dependent inhibition of Lung A549 Cell



Graph 2: Cell viability after application of **A**



Graph 3: IC_{50} values calculated for **A** and **C**

But the results were much better in case of compound **C**, whereas, the cell viability was reduced to 63% after application of 40 µg/mL dose (Graph 1&2). Thus there was a clear dose response relationship achieved after application of compound **A** and compound **C** as shown in Graphs 1&2 in Figure 1.

Finally the viability data obtained from compounds **A** and **C** were utilized for calculation of IC_{50} values. For this purpose, trend lines were generated first and then were extrapolated to get the values necessary for 50% inhibition (Graph 3).

5. Conclusions

Among the three benzothiazoles studied in this study, compound **A** and compound **C** offered mild but interesting dose-dependent reduction of the lung cancer cell line (Lung A549). Between these two derivatives, the 2-hydroxybenzothiazole was found to be more potent especially in higher dose. Thus it appears that the scaffold bears a potential as a pharmacophore for inhibiting the cancer cells. These compounds should be further derivatized for further exploration of the lead compound in this series. Even these could better be tested for any other cell line available for these types of assay to make further comprehensive inferences.

6. Acknowledgements

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Conflict of interest

Authors have no conflict of interest.

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