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Screening of Cytotoxicity Potential of 6-Haloaryl Benzimidazoquinazolines in Hela Cells

KEYWORDS: 6-haloaryl benzimidazo[1,2-c]quinazoline, anticancer screening, cytotoxicity, MTT assay.

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ABSTRACT *Cervical cancer is the second most common cancer in women worldwide, and vaccines are currently available. The vaccines can be prophylactic, but not therapeutic. So, in this study attempts was taken to screen synthesized benzimidazo [1,2-c] quinazoline derivatives antitumor potential towards the cervical cancer cells. In vitro anticancer screening was done by MTT assay. Out of all the compounds synthesized 6-(2,6-dichlorophenyl)-benzimidazo[1,2-c] quinazoline revealed 98.2 % cellular inhibition and IC50 value of about 6.4 µM. The percentage of cell inhibition demonstrated by the compound was more, whereas the IC50 values were less when compared with that of the standard drug etoposide.*

INTRODUCTION

Cancer is a disease which is associated with increase in cell number, alterations in mechanisms regulating new cell birth or cell proliferation. Certain types of cancer are associated with decreased rates of cell death, or apoptosis(1). Causes of cancer can be environmental exposures, lifestyle practices, medical interventions, genetic traits, viruses, familial susceptibility and aging(2). Cervical cancer is the fifth most common cancer in humans, the second most common cancer in women worldwide and the most common cancer cause of death in the developing countries. The bivalent and quadrivalent vaccines available are prophylactic, not therapeutic.

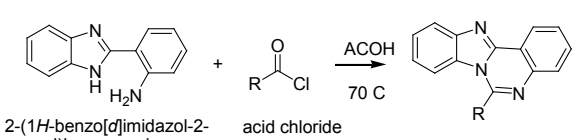
The studies already reported suggest that derivatives of 6-aryl benzimidazo quinazolines act as TNF-alpha production inhibitors(3), antifungal agents(4,5), potent antitumor agent (6).

Hence in this study we focussed on in vitro antitumor screening of the synthesized benzimidazoquinazolines in Hela cell lines.

MATERIALS AND METHODS

To 2-(2-aminophenyl) benzimidazole was added equal quantity of substituted acid chloride and 2 ml of glacial acetic acid. The mixture was stirred and heated for 16 hrs at 70°C(7). After reaction the crude compound was eluted by column chromatography using 40-50% of hexane in ethyl acetate, in gradient manner.

The reaction opted for synthesis is mentioned below.



2-(1*H*-benzo[*d*]imidazol-2-yl)benzenamine + acid chloride

CYTOTOXICITY ASSAY

The toxicity of the synthesized compounds towards cervical cancer cell lines was assessed by MTT assay method(8,9). MTT (3-(4,5-dimethylthiazol-2,5-diphenyltetrazolium bromide) is a yellow water soluble tetrazolium salt. Succinate-dehydrogenase is a mitochondrial enzyme present in living cells. This enzyme cleaves tetrazolium ring present in MTT converting it into purple colored formazan crystals which has absorption maxima at 570 nm. Amount of formazan produced will be directly proportional to the number of viable cells present. Etoposide was used as a positive standard for the assay.

A single layer of cells were removed from maintenance culture to prepare cell suspension. The suspension was diluted using eagles minimum essential medium with 5% fetal bovine serum to obtain a final concentration of $1x10^5$ cells/ml. To obtain the plating density of 10,000 cells/well in 96-well plate, 100 µl of the diluted suspension was added into each well. The plates were incubated at 37°C temperature, 5% Carbon dioxide and 95% air with 100% relative humidity to permit cell attachment to the well. After 24 hours incubation, 100 µl of diluted test samples were added. The plates were allowed for 48 hours of incubation for effective action. About 15 µl of 5 mg/ml MTT solution was added to each well and again incubated for 4 hours at 37°C. Later 100 µl of DMSO was added into each plate to solublize the crystals and the absorbance was measured at 570 nm using microplate reader. The cell inhibition percentage was determined as follows.

$$\% \text{ Cell Inhibition} = \frac{100 - \text{Ab (sample)}}{\text{Ab (control)}} \times 100$$

RESULTS AND DISCUSSION

The synthetic compounds were obtained by the reaction already reported at a yield in the range of 60.9 to 64.9 %. Characteristic IR peaks was observed around 3050 cm^{-1}, 1450 cm^{-1}, 1050 cm^{-1}, 1500 cm^{-1} and 760 cm^{-1}. In proton NMR spectra multiplets were developed in the range of δ 7.28-8.89 ppm. A single peak was obtained in the mass spectra at m/z ratio of 362.44(ES-), 364.10(ES+) and 362.39(ES-) for 6-(2,4-dichlorophenyl) - benzimidazo [1,2-c] quinazoline, 6-(3,5-dichlorophenyl) - benzimidazo [1,2-c] quinazoline, 6-(2,6-dichlorophenyl) - benzimidazo [1,2-c] quinazoline respectively.

For in vitro toxicity study five different concentrations of samples and standard were analysed against the HeLa cell lines. Each concentration of sample and standard were studied in triplicates and the average absorbance was

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considered. The percentage inhibition of the tumour cells was calculated (Table 1) and the percentage inhibition was plotted against concentration of the samples.

6-(2,4-dichlorophenyl) benzimidazo [1,2-c]quinazoline showed a moderate antitumor activity with the percentage of inhibition of about 69.5% at 100 µM. The compound 6 - (3,5-dichlorophenyl) - benzimidazo [1,2-c] quinazoline showed a very poor activity of about 19.4 % cell inhibition at the level of 100 µM. A compound in the same series 6-(2,6-dichlorophenyl) - benzimidazo [1,2-c] quinazoline exhibited a potent cellular inhibition of about 98.3% at the concentration of 100 µM, and this was more than the percentage of inhibition created by the standard etoposide at the same dose level.

IC50 values were determined for all the compounds. 6-(2,6-dichlorophenyl)- benzimidazo[1,2-c] quinazoline had IC50 value of about 6.4 µM comparable with the IC50 value of the standard etoposide which is about 1.3 µM.

Compound	Concentration (µM)	% cell inhibition	**IC50 (µM)**
Etoposide	0.05	3.951562	1.3
	0.5	31.48502	
	5	78.20268	
	50	88.91013	
	100	91.45953	
6-(2,4-dichlorophenyl)-benzimidazo[1,2-c] quinazoline	0.25	1.912046	77.74
	2.5	2.421925	
	25	4.907584	
	50	18.73805	
	100	69.471	
6-(3,5-dichlorophenyl)-benzimidazo[1,2-c] quinazoline	0.25	0.191205	> 100
	2.5	3.250478	
	25	4.525175	
	50	7.584449	
	100	19.3754	
6-(2,6-dichlorophenyl)-benzimidazo[1,2-c] quinazoline	0.25	5.226259	6.4
	2.5	32.56851	
	25	72.53027	
	50	82.79159	
	100	98.27916	

Table 1: Table showing the percentage of tumour cell inhibition at different doses.

CONCLUSION

The compound 6-(2,6-dichlorophenyl)-benzimidazo[1,2-c] quinazoline exhibited cellular inhibition of about 98.2%, at 100 µM dose level for cancer cells, which was found to be more than the cellular inhibition of the standard etoposide at the same dose level. The IC50 value for the compound was also comparable with that of the etoposide standard. Hence it can act as a good anticancer agent and also can serve as a new lead molecule for developing novel anticancer agents.

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