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Cytotoxicity and enzymes estimation of some newer benzimidazoles

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ABSTRACT

*The development of in-vitro cytotoxicity assays has been driven by the need to rapidly evaluate the potential toxicity of large numbers of compounds. In the present study the anticancer study has been performed to the synthesized compounds (**1-10**) by determination of total cell protein content through sulphoradamine B (SRB) assay on HEp-2 (Human larynx cancer cell line). Compounds **1, 2, 9** and **10** showed minimum cytotoxic concentration (CTC_{50}) at which 50 percent of cancer cell populations were inhibited and are comparable to the standard drug 5-Fluorouracil. Potent compounds of the series were selected for enzyme estimation to show any hepatic toxicity. All enzymes estimated were compared to the control. SGPT, SGOT values of compound **9** and alkaline phosphatase of compounds **9** and **10** were found to be moderately significant ($p < 0.01$).*

Key words: Cytotoxicity, hepatic toxicity, enzyme estimation, sulphoradamine B.

INTRODUCTION

Cytotoxicity is a subject of pharmaceutical study, particularly in the area of cancer research. Low cytotoxicity to healthy cells and high cytotoxicity to cancerous cells is the ultimate goal of many chemotherapy drugs. *In-vitro* cell based cytotoxicity assay is an easy and cost effective tool for hit ranking and lead optimization at the early stage of drug discovery.

The predictive value of *in-vitro* cytotoxicity tests is based on the idea of 'basal' cytotoxicity that toxic chemicals affect basic functions of cells which are common to all cells and that the toxicity can be measured by assessing cellular damage.

Several 2-substituted benzimidazoles have been reported as potential anticancer agents [1-4] and well-known for its diverse biological actions for example, anti-inflammatory [5], diuretic [6], antimicrobial [7], antiviral [8], antitumor [9], antiulcer [10], antioxidants [11], antiasthmatic [12] analgesic [13]. Some new benzimidazole derivatives at 2-position evaluated for cytotoxicity activity showed excellent results [9,14] .The most pronounced antiproliferative activity was shown with heterocyclic benzimidazole derivatives bearing amidino substituent at C-5 of benzimidazole ring [15]. These findings have inspired us to widen the list of 2-substituted benzimidazole derivatives with anticancer activity. In view of above compounds possessing anticancer activity, these structural contemplation gave impetus to prepare a series of 2-[(substituted)-phenylethylidine) hydrazine]-*N*-phenyl-1*H* benzo [*d*] imidazole-1-carbothioamide **(1-10)** by reaction of 2-mercaptobenzimidazole with hydrazine hydrate giving 2-hydrazinobenzimidazole which on reaction with substituted acetophenone derivatives gave benzimidazole hydrazones . Reaction of corresponding hydrazones with phenylisothiocynate in the presence of triethylamine and ethanol afforded the title compounds [16].

MATERIALS AND METHODS

Confluent monolayer cell cultures, TPVG solution (trypsin, versene and glucose in Phosphate Buffered Saline), Minimum Essential Medium (MEM), New born calf serum, inactivated, Microtitre plate (96 well), Drug dilutions, SRB dye (0.4% prepared in 1% acetic acid) (sigma chemical), 10mM tris base, 50% trichloro acetic acid , Micro plate reader (ELISA reader, Bio-rad). Cell cultures were obtained from National centre for cell sciences, Pune. HEp-2 cells were grown in Earl's Minimal essential medium supplemented with L-glutamine, 10% Fetal Bovine Serum, Penicillin, Streptomycin and Amphoterecin B and the cells were maintained at 37˚C in a humidified atmosphere with 5% CO_2 and subculture twice a week [17, 18].

Ethical clearance

The institutional animal ethical committee registration no. (882-ac/05/CPCSEA) approved experimental design performed in this study for the use of albino rats of either sex (150-200 g) as an animal model for biochemical studies (IAEC No. 014/10). Animals were obtained from animal house facility, Rajiv Academy for Pharmacy, Mathura.

Anticancer activity

Determination of total cell protein content by sulphoradamine B (SRB) assay

The monolayer cell culture was trypsinized and the cell count adjusted to 1.0 x 10^5 cell/ml using medium containing 10 % new born calf serum. To each well of the 96 well microtitre plate, 0.1 ml of diluted cell suspension (approximately 10,000 cells) was added. After 24 hours, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once and 100 µl of medium and different drug concentration added to the culture in microtitre plates. The plates were then incubated at 37°C for 3 days in 5% CO_2 atmosphere, microscopic examination was carried out and observations recorded every 24 hours. After 72 hours, 25 µl of 50% trichloro acetic acid was added to the wells gently such that it forms a thin layer over the drug dilution to form a overall concentration of 10%. The plates were incubated at 4°C for one hour. The culture plates were flicked and washed five times with tap water to remove traces of medium, drug and serum, and were then air-dried. The air-dried plates were stained with SRB for 30 minutes. The

unbound dye was then removed by rapidly washing four times with 1% acetic acid. The plates were then air-dried.

100 µl of 10 mM tris base was then added to the wells to solubilize the dye. The plates were shaken vigorously for 5 minutes. The absorbance was measured using micro plate reader at a wavelength of 540 nm.

Enzyme Estimation

Glutamate Pyruvate Transaminase (GPT) and Glutamate Oxalacetate Transaminase (GOT) are the amino transferase enzymes which catalyze the reversible reaction of amino acids and alpha-ketoglutaric acid by the transfer of the amino group. GPT is found in hepatocytes in highest concentration and it is considered to be more liver specific but GOT is present in large amounts in heart, liver muscle and kidney tissue. Consequently, the determination of serum GPT could serve as a valuable aid in different diagnosis [19]. Furthermore estimation of alkaline phosphate (ALP) involves hydrolysis of *p*-nitrophenyl phosphate by alkaline phosphatase to give *p*-nitrophenol, which gives yellow color in alkaline solution. The increase in absorbance due to its formation is directly proportional to alkaline phosphate (ALP) activity [20, 21].

Statistical Analysis

Results were expressed as mean ± (S.E.M). Statistical significance determined by one-way analysis of variance (ANOVA) by Graph Pad Prism version 3.0 software.

RESULTS AND DISCUSSION

All the newly synthesized compounds **(1-10)** were evaluated for anticancer activity. The anticancer activity was carried out by sulforadamine B (SRB) assay in which cell count adjusted to 1.0 x 10^5 cells/ml using medium containing 10% new born calf serum. Determination of cell viability was performed by sulforadamine B which is a bright pink aminoxanthene dye with two sulfonic groups. Under mild acidic conditions, SRB binds to protein basic amino acid residues in trichloro acetic acid (TCA) fixed cells to provide a sensitive index of cellular protein content that is linear over a cell density range of at least two orders of magnitude. Colour development in SRB assay is rapid, stable and visible. The developed colour was measured over a broad range of visible wavelength in either a spectrophotometer or a 96 well plate reader.

Our results revealed that, most of the synthesized compounds showed minimum cytotoxic concentration of HEp-2 cell line by SRB assay. Among the compounds tested, the cytotoxic concentrations (CTC_{50}) was found in order 25 > 27 > 45 > 49 > 56 > 64 > 65 > 93 > 35 > 39 µg/ml as shown in Table-1. Compounds **1, 2, 9** and **10** showed minimum cytotoxic concentrations (CTC_{50}) at which 50 percent of cancer cell populations were inhibited are shown in Fig 1. The significant inhibition concentration by compounds may be due to the presence of strong electronegative groups bromine at para position in compound **1** and fluorine at para position in compound **2**. In compound **8** replacement of fluorine by electron donating amine increases the inhibitory concentration. The presence of benzimidazole nucleus may be responsible for the moderate significant inhibition shown by compounds **3, 4** and **6.**

Enzyme estimation was performed for the most active compounds **1, 2, 9** and **10** and the data are presented in Table 2. All enzymes estimated were compared to the control. SGPT, SGOT values of compound **9** and alkaline phosphatase of compounds **9** and **10** were found to be moderately significant ($p < 0.01$).

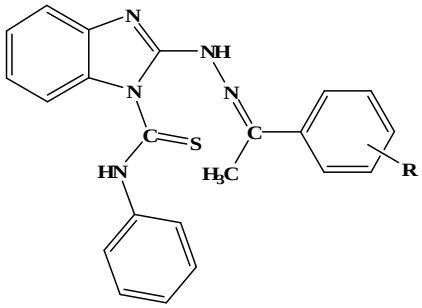

Table-1 : CTC_{50} value by SRB assay of compounds (1-10)

Compound No.	R	CTC_{50} (µg/ml)
1	*p*-Br	39
2	*p*-F	25
3	*o*-Cl	49
4	*p*-NO_2	56
5	*m*-Br	64
6	*p*-Cl	45
7	*m*-NO_2	93
8	*m*-NH_2	65
9	*p*-OCH_3	27
10	2,5-Dihydroxy	35
5-FU	37	

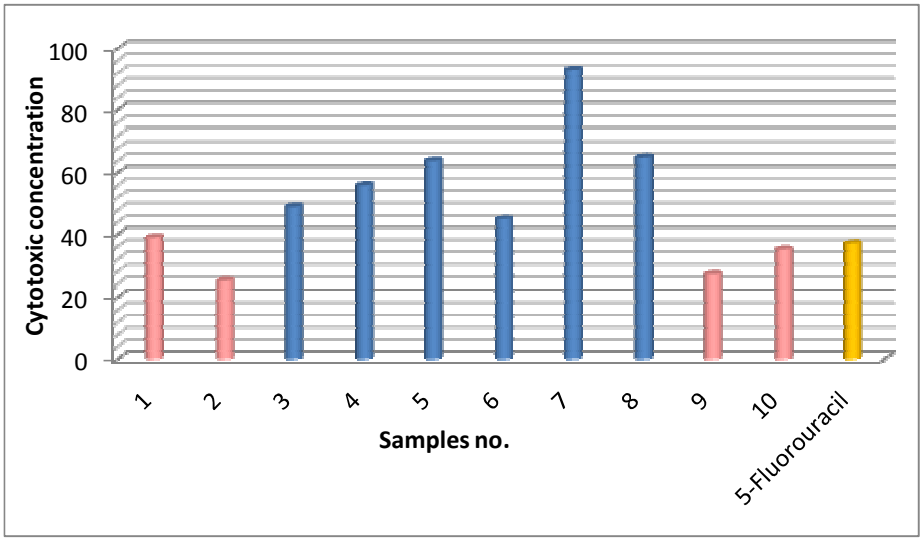

Fig. 1 Comparative CTC_{50} (µg/ml) of the compound with 5-Fluorourcil at which 50 percent of cancer cell populations were inhibited

Table-2 : Biochemical estimation (SGPT, SGOT and Alkaline Phosphatase) of potent compounds

Compound No.	Serum glutamate pyruvate transaminase (SGPT)	Serum glutamate oxaloacetate transaminase (SGOT)	Alkaline Phosphatase (ALP)
Control	47.14 ± 0.41	40.12 ± 0.34	14.47 ± 0.10
1	49.34 ± 0.32*	40.15 ± 0.41	15.26 ± 0.14
2	42.32 ± 0.48*	41.27 ± 0.39	19.41 ± 0.25*
9	53.56 ± 0.49**	56.28 ± 0.28**	28.38 ± 0.23**
10	48.54 ± 0.37	47.38 ± 0.37*	35.47 ± 0.28**

*Mean ±SEM values (n = 6). Significantly different from control: *p < 0.05, **p < 0.01.*

CONCLUSION

Compounds **1** and **2** showed minimum cytotoxic concentration (CTC_{50}) at which 50 percent of cancer cell populations were inhibited and were found to be comparable to the standard drug 5-Fluorouracil. It can be concluded that the presence of electron withdrawing groups (F and Br) and electron donating group ($-OCH_3$) may be responsible for the inhibition. But exact interaction of electron withdrawing and donating groups with human larynx cancer cells is not known.

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REFERENCES

[1] M. Prudhomme, Recent Pat. Anti-Canc. *Drug Discovery*, **2006**, 1, 55-68.
[2] D. Kumar, M. R. Jacob, M. B. Reynolds, S. M. Kervin, *Bioorg. Med. Chem.*, **2002**, 10, 3997-4004.
[3] M. Hranjec, M. Kralj, I. Piantanida, M. Sedic, L. Suman, K. Pavelic, G. Karminskizamola, *J. Med. Chem.*, **2007**, 50, 5696-5711.
[4] A. K. Piskin, Z. Ates-Alagoz, F. B. Atac, Y. Musdal, E. Buyukbingol, *Turk. J. Biochem.*, **2009**, 34, 39-43.
[5] J.T. Leonard, L. Jeyaseeli, M. Kumar, R Sivakumar, *Asian J. Chem.*, **2006**, 18, 1104.
[6] N. Srinivasan, A. Balaji, G. Nagrajan, R. Suthakaran, Y. Kumar, D. Jagdesh, *Asian J. Chem.*, **2008**, 20, 4934.
[7] M.B. Deshmukh, A.W. Suryavanshi, S.A. Deshmukh, S.S. Jagtap, *Ind. J. Chem.*, **2009**, 86B, 302.
[8] R.V. Devivar, E. kawashima, *J. Med. Chem.*, **1994**, 37, 2942.
[9] A. Gellis, H. Kovacic, N. Boufatah, P. Vanelle, *Eur. J. Med. Chem.*, **2008**, 43, 1858.
[10] J.B. Bariwal, A.K. Shah, M.K. Kathiravan, R.S. Somani, J.R. Jagtap, K.S. Jain, *Ind. J. Pharm. Ed. Res.*, **2008**, 42, 225.
[11] Z.A. Alagoz, C. Kus, T. Coban, *J. Enz. Inhib. Med. Chem.*, **2004**, 20, 325.
[12] B.B. Kumar, P.V. Rao, *Asian J. Chem.*, **2006**, 18, 3060.
[13] S.M. Sondhi, S. Rajvanshi, M. Johar, N. Bharti, A. Azam, A.K. Singh, *Eur. J. Med. Chem.*, **2002**, 37, 835.

[14] H.M. Refaat, *Eur. J. Med. Chem.*, **2010**, 45, 2949-2956.
[15] K. Starcevic, M. Kralj, K. Ester, I. Sabol, M. Grce, K. Pavelic, *Bioorg. Med. Chem.*, **2007**, 15, 4419-4426.
[16] B.P. Bhrigu, M.Pharm thesis, Uttar Pradesh Technical University (Lucknow, India, **2010**)
[17] R. Sivakumar, C. Alagesaboopathi, *Afri. J. Biotech.*, **2008**, 7, 22, 3984-3988.
[18] R.M. Patel, N.J. Patel, *Intern. J. Pharm. Res.*, **2010**, 2, 1, 1429-1433.
[19] S. Reitman, S. A. Frankel, *Am. J. Clin. Pathol.*, **1957**, 28, 56-63.
[20] E. J. King, A. R. Armstrong, *Can. Med. Assoc. J.*, **1934**, 31, 376-381.
[21] K. K. Pillai, J. S. Qadry, Biochemistry and Clinical Pathology, 1st Ed., (CBS Publishers and distributors, New Delhi, **1994)**, 143-153.