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# SYNTHESIS AND ANTICANCER ACTIVITY OF SOME NOVEL 2-SUBSTITUTED BENZOTHIAZOLE

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## ABSTRACT

The series of seven substituted 2-phenyl-benzothiazole and substituted 1, 3-benzothiazole-2 -yl-4-carbothiaote derivatives were synthesized. Substituted 2 -phenyl-benzothiazole were synthesised by condensing substituted benzoic acid with 2-amino thiophenol in the presence of phosphoric acid and 3-benzothiazole-2-yl-4-substituted carbothiaote derivatives were prepared by condensing 2-mercaptobenzo-thiazole with substituted acid chloride. Structures of all the compounds were characterized by spectral and elemental analysis. All the synthesised novel compounds were screened for anticancer activity. It was also found that compounds **1**, **2**, **6** and **7** showed very good anticancer activity whereas all the other compounds showed mild to moderate anticancer activity as compared to standard drug.

**Key words**: Synthesis, Anticancer activity, 2-Phenyl benzothiazole, 2-Mercaptobenzothiazole.

## INTRODUCTION

Cancer is currently second leading cause of death after cardiovascular disease. Consequently, there is great unmet medical need for new anticancer small molecule therapeutics. A tumour is an abnormal mass of tissue, the growth of which exceeds and is uncoordinated with that of normal tissue and continues in the same manner after cessation of the stimuli, which has initiated it. The past two decades have witnessed a remarkable revolution in the field of tumour chemotherapy[1, 2]. Wealth of basic knowledge with regard to molecular and cellular biology, better understanding of mechanism of cellular division, tumour immunology and detailed information of fundamental factors involved in both viral and chemical carcinogenesis and the improved investigative techniques have ultimately led to the introduction of a substantial number of newer antineoplastic agents. On the basis of exhaustive literature review, it has been found that 2-substituted benzothiazole have good

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potential to exhibit anticancer activity[3-10]. So, it was decided to synthesise some novel substituted benzothiazole derivatives to evaluate their anticancer activity.

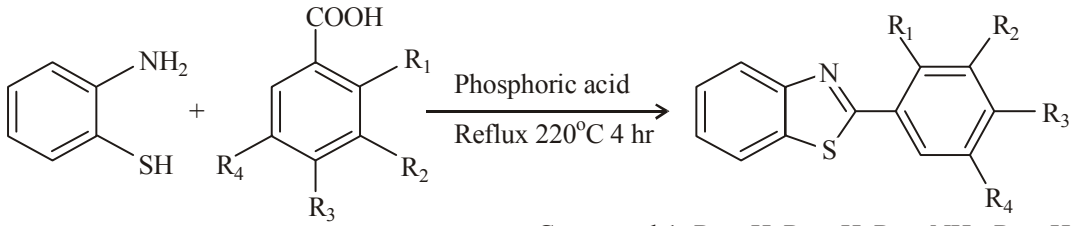
## EXPERIMENTAL

The melting range of the synthesized compounds was determined by LAB INDIA visual melting range apparatus. The UV-visible studies were performed by instrument Elico SL164 double beam spectrophotometer. The KBr pellets of the synthesized compounds were prepared by pressed-pellet technique. IR spectra were recorded in KBr disc on a FTIR 8300, (Shimadzu). Mass studies of the synthesized compounds were performed by using the instrument Shimadzu QP 500. The $^1$H NMR spectral study was performed by instrument R32 Perkin-Elmer. The solvent system used for the study was DMSO-$d_6$. Reaction progress was checked by TLC in solvent vapour saturated chamber on glass plates coated with silica gel G 254 (Merck) and followed by visualization under UV light. The solvent system used for thin layer chromatography was acetonitrile : methanol : buffer* (40 : 40 : 20).

### General method of synthesis of 2-substituted phenyl benzothiazole (Comp. - 1, 2, 4) (Scheme-1)

Equimolar quantities of o-aminothiophenol (0.04 mol) and substituted benzoic acid were added to 15 g of polyphosphoric acid and refluxed for 4 hr at 220$^0$C. The reaction mixture was cooled and poured into ice-cold 10% sodium carbonate solution. The precipitates were filtered and recrystallised from methanol (90%).

**Scheme 1**



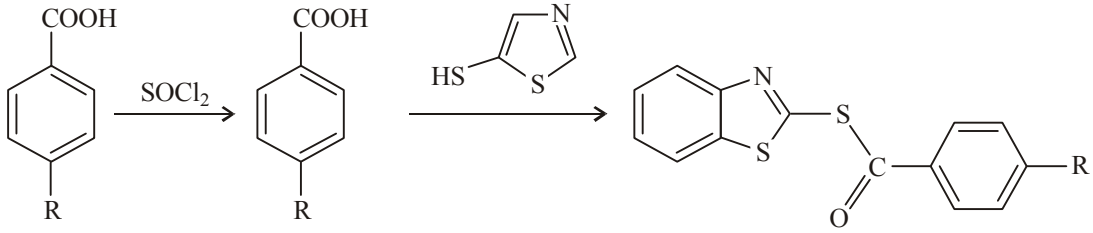

Compound **1:** $R_1$ = H, $R_2$ = H, $R_3$ = $NH_2$, $R_4$ = H
Compound **2:** $R_1$ = F, $R_2$ = H, $R_3$ = Cl, $R_4$ = H
Compound **4:** $R_1$ = H, $R_2$ = H, $R_3$ = Cl, $R_4$ = H

### General method of synthesis of substituted benzothiazol-2-yl benzothioate (Comp. 5, 6) (Scheme 2)

A quantity equivalent to 0.01 mol of substituted benzoic acid and 0.04 mol of thionyl chloride were magnetically stirred and refluxed at 70$^0$C for 1 hr. The excess of thionyl

chloride was removed from distilling with benzene to get acid chloride. The acid chloride (0.01 mol) and 0.01 mol of mercaptobenzothiazole were added in 25 mL pyridine and heated on water bath for 15 min. The reaction mixture was cooled and poured in ice cold water to get precipitate that was recrystallised from methanol (94%).

**Scheme 2**

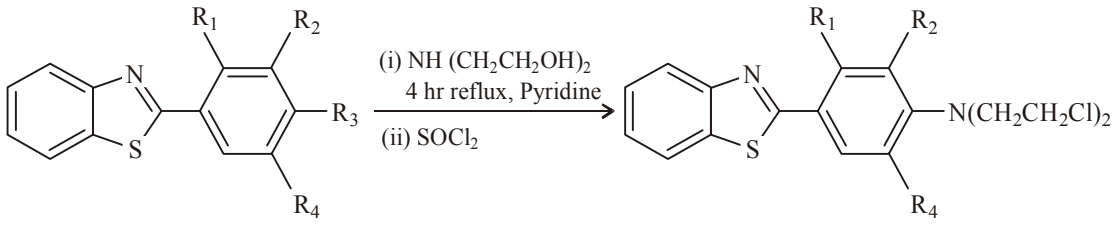



Compound **5:** R = $NO_2$
Compound **6:** R = $NH_2$

**Method of synthesis of 4-(benzo[d]thiazol-2-yl)-N, N-bis (2-chloroethyl) benzenemine (Comp. 3, 7) (Scheme 3)**

Equimolar quantities of o-aminophenol (0.04 mol) and substituted benzoic acid were added to 15 g of polyphosphoric acid and refluxed for 4 hour at $220^0$C. The reaction mixture was cooled and poured in 10% sodium carbonate solution. The precipitate was filtered and recrystallised from methanol (90%) to get the product. 2-Susbstituted phenyl benzothiazole (0.01mol) and 0.01 mol of diethanolamine were dissolved in 25 mL pyridine and refluxed for 4 hours, cooled and poured in cold water. The mixture was filtered after 1 hour and precipitates were recrystallised from methanol to get the product. The resultant product (0.01 mol) was refluxed with 0.03 mol of thionyl chloride for 4 hour. The excess of thionyl chloride was removed by distilling with benzene. After distillation, residue was collected, washed with cold water and recrystallised from ethanol (95%).

**Scheme 3**




Compound **3:** $R_1$ = H, $R_2$ = H, $R_4$ = H
Compound **7:** $R_1$ = H, $R_2$ = $NH_2$, $R_4$ = $NH_2$

**Table 1: IUPAC name and chemical structure of the synthesised compounds**

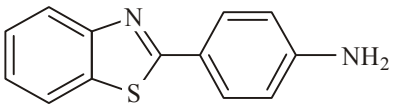
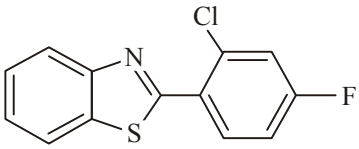
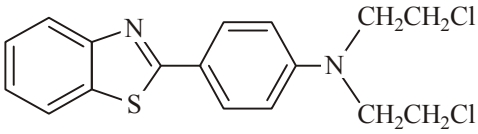
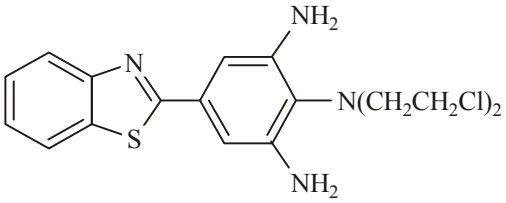
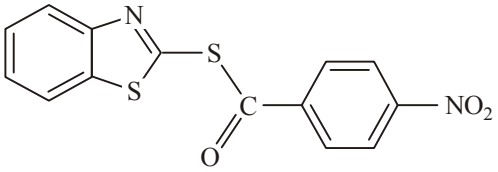
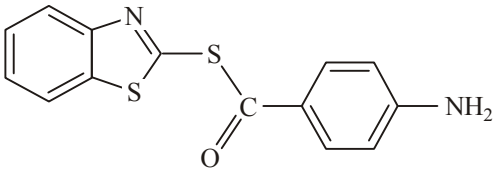
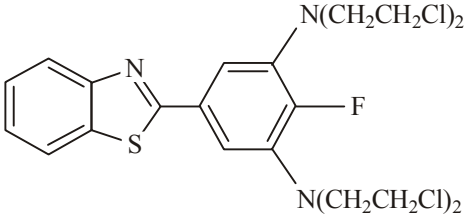
| S. No. | Name of compounds | Chemical structure |
|---|---|---|
| 1. | 4-(Benzo[d]thiazol-2-yl)benzenamine |  |
| 2. | 2-(2-Chloro-4-fluorophenyl) benzo[d]thiazole |  |
| 3. | 4-(Benzo[d]thiazol-2-yl)-N,N-bis(2-chloroethyl)benzenamine |  |
| 4. | 4-(Benzo[d]thiazol-2-yl)-2-bromo-N,N-bis(2-chloroethyl) benzenamine |  |
| 5. | S-Benzo[d]thiazol-2-yl 4-nitrobenzothioate |  |
| 6. | S-Benzo[d]thiazol-2-yl 4-aminobenzothioate |  |
| 7. | 2-(3,5-Bis(1,5-dichloropentan-3-yl)-4-fluorophenyl) benzo[d]thiazole |  |

**Table 2: Physical data of synthesized compounds**

| Compounds | M.P. (°C) | $R_f$ | % Yield | Molecular fomula | Mol. weight |
|---|---|---|---|---|---|
| ABSN **(1)** | 121-123 | 0.86 | 70.3 | $C_{13}H_{10}N_2S$ | 226 |
| CFBSN **(2)** | 107-109 | 0.87 | 63.1 | $C_{13}H_7NClFS$ | 263 |
| CEBSN **(3)** | 113-115 | 0.92 | 58.2 | $C_{17}H_{16}N_2Cl_2S$ | 351 |
| BBSN **(4)** | 137-140 | 0.52 | 39 | $C_{17}H_{18}N_4Cl_2S$ | 381 |
| BSNNC **(5)** | 105-107 | 0.62 | 72.2 | $C_{14}H_8N_2S_2O_3$ | 316 |
| BSNAC **(6)** | 179-182 | 0.50 | 38.1 | $C_{14}H_{10}N_2S_2O$ | 286 |
| BSNCF **(7)** | 113-115 | 0.89 | 69 | $C_{21}H_{22}N_3FSCl_4$ | 509 |

## Materials and methods

### Animals

Sixty adult Swiss albino mice (20-25 g) were procured from Visheshwarya Enterprises, Bangalore and used throughout the study. They were housed in cage boxes in a controlled environment (temperature 25 ± $2^0$C and dark/light cycle) with standard laboratory diet and water *ad libitum*.

### Methodology

Male Swiss albino mice were used for the antitumor studies[11-15]. All groups were treated with EAC ascetic lymphoma cell line (0.2 mL of 2 x $10^6$ cells/mice) intraperitoneally except normal group. This was taken as day zero. On first day, 5 mL/kg body weight of normal saline (0.9% NaCl w/v) was administered in group 1 (normal). Propylene glycol, 5 mL/kg body weight per day was administered in group 2 (cancer control). Benzothiazole derivatives were administered accordingly and doses given (2.5 mg/mL, 2.5 mg/mL, 1.25 mg/mL, 1.25 mg/mL, 2.5 mg/mL, 1.25 mg/mL, 0.625 mg/mL) into their respective groups for 14 days orally. After $14^{th}$ day of cancer cell line injection, animals were sacrificed for evaluation of blood and enzyme parameters like body weight of animal, life span of animal, cytological studies on cell line, differential count, packed cellular volume of ascetic fluid, RBC count, hemoglobin count and WBC count. The rest of animal groups were kept for checking the survival time of tumor bearing hosts.

**Table 3: Spectral data of synthesized compounds**

| Compds. | IR spectra data ($cm^{-1}$) | $^1H$ NMR (DMSO, ppm) | Mass | UV |
|---|---|---|---|---|
| **1** | 3133.44 (Ar C-H), 1402.1 (C = C), 1652.88 (C = N), 1558.38 (C - C), 1320.18 (C - N), 3534.31 (N - H) | 4. (m, 2H, $NH_2$), 6.5-7.2 (m, 4H, Aryl- H), 7.5-8.2 (m, 4H, 2-benzothiazole) | 226, 210, 149, 134, 76, 69,57 | 230 |
| **2** | 3152.43, 1420.15, 1642.27, 1550.17, 753.15 | 6.9-7.2 (m, 2H, Aryl-H), 7.7 (s, 1H, Aryl-H),7.2-7.5 (m, 4H, 2-benzothiazole) | 264, 210, 191, 136, 108, 82, 76, 55 | 340 |
| **3** | 2917.13, 1507.27, 1314.42, 757.01, 1473.51 | 2.9-3.1 (m, 8H,-N ($CH_2$ $CH_2Cl)_2$), 6.9-7.0 (m, 4H, Aryl-H), 7.1-7.6 (m, 4H, 2-benzothiazole) | 352, 282, 256, 210, 178, 123, 76 | 310 |
| **4** | 2924.85, 1561.27, 1333.68, 686.61, 1646.13, 600.78 | 2.5-2.6 (m, 8H,-N ($CH_2$ $CH_2Cl)_2$), 3.2 (s, 2H, -$NH_2$), 3.7 (s, 2H, -$NH_2$), 6.8 (t, 2H, Aryl-H), 6.9-7.6 (m, 4H, Aryl-H) | 380, 382 | 305 |
| **5** | 3117.72, 1419.51, 1687.66, 1607.56, 1541.9 | 7.3-7.4 (t, 2H, Aryl-H), 7.7-7.8 (d, 2H, Aryl-H), 6.8-7.2 (m, 4H, 2-benzothiazole) | 318, 280, 241, 194, 167, 151, 137, 105, 77, 65 | 259 |
| **6** | 2974.03 (Ar C-H), 1607.56, 1705.92, 1318.25, 1625.88 (N-H. | 3.7 (s, 2H, -$NH_2$), 6.7 (d, 2H, Aryl-H), 7.7 (d, 2H, Aryl-H), 6.9-7.4 (m, 4H, 2-benzothiazole). | 288, 209, 171, 137, 120, 108, 77, 69 | 290 |
| ***7*** | 2958.60, 1278.72, 1523.66, 1718.46, 1349.11. | 3.7-3.8 (m, 16H, -N ($CH_2$ $CH_2Cl)_2$), 6.8-7.0 (t, 2H, Aryl-H), 7.6-7.8 (m, 4H, 2-benzothiazole) | 511, 509 | 292 |

**Effect of drug on survival time**

Animals were inoculated with 2 x $10^6$ cells/mice on day '0' and treatment with drug started 24 hr after inoculation. The control group was treated with same volume of 0.9 % NaCl solution. All the treatment was given for 14 days. The median survival time (MST) of each group, consisting of five mice was noted. The antitumour efficacy of drug was compared with that of standard drug, vincristine using 33 mg/kg. The MST can be calculated by using following formula (Table 4) -

Increase in life span = T - C/C +100 …(1)

Where T = Number of days the treated animal survived and

C = Number of days control animal survived

**Table 4: Effect of benzothiazole derivative on survival time on EAC bearing mic**e

| Experimental group | Median survival (days) | Increase of life span (%) |
|---|---|---|
| Normal saline (5 mL/kg body weight) | - | - |
| EAC cell (control) + propylene glycol (5 mL/kg body weight) | 21.13 ± 0.37 | |
| EAC cell + Comp **(1)** (25 mg/kg) | 27.5 ± 0.94 | 30.24 |
| EAC cell + Comp **(2)** (50 mg/kg) | 25.5 ± 0.95 | 20.68 |
| EAC cell + Comp **(3)** (50 mg/kg) | 25 ± 0.81 | 18.31 |
| EAC cell + Comp **(4)** (50 mg/kg) | 26.5 ± 0.72 | 27.78 |
| EAC cell + Comp **(5)** (100 mg/kg) | 25.5 ± 0.76 | 20.68 |
| EAC cell + Comp **(6)** (100 mg/kg) | 26 ± 0.46 | 23.04 |
| EAC cell + Comp **(7)** (100 mg/kg) | 26.5 ± 0.72 | 25.14 |
| EAC cell + Vincristine Std. (8.25 mg/kg) | 30.74 ± 0.42 | 45.48 |

Values are mean ± SEM (n = 5),
EAC control group was compared with normal group,
$P < 0.001$, Experimental group was compared with EAC control

**Table 5: Effect of benzothiazole derivatives on tumor volume, packed cell volume, viable and non-viable tumor cell count**

| Parameters | EAC cell control | Vincristine Std. | EAC cell + Comp. (1) | EAC Cell + Comp. (2) | EAC cell + Comp. (3) | EAC cell + Comp. (4) | EAC cell + Comp. (5) | EAC cell + Comp. (6) | EACcell + Comp. (7) |
|---|---|---|---|---|---|---|---|---|---|
| Body weight (g) | 27.2 ± 0.86 | 23.9 ± 0.02* | 24.3 ± 0.98 | 25.2 ± 0.15 | 25.3 ± 0.12 | 25.4 ± 0.098* | 24.8 ± 0.144 | 24.8 ± 0.144 | 25 ± 0.028 |
| Tumor vol. (mL) | 4.48 ± 0.07* | 2.45 ± 0.13 | 2.75 ± 0.34 | 3.23 ± 0.28 | 3.23 ± 0.034* | 2.92 ± 0.45 | 3.33 ± 0.34 | 3.1 ± 0.016* | 3.22 ± 0.80 |
| Packed cell volume | 1.78 ± 0.58 | 1.15 ± 0.03* | 1.32 ± 0.080 | 1.55 ± 0.32 | 1.57 ± 0.05* | 1.42 ± 0.80 | 1.54 ± 0.150 | 1.54 ± 0.80 | 1.51 ± 0.052 |
| Viable tumor cell count x 10 cell/mL | 11.19 ± 0.18 | 6.72 ± 0.015* | 7.1 ± 0.16 | 8.35 ± 0.016* | 8.34 ± 0.085* | 7.22 ± 0.14 | 8.36 ± 0.085 | 7.27 ± 0.86 | 8.24 ± 0.13 |
| Non-viable tumor cell count x $10^7$ cell/mL | 0.31 ± 0.4 | 1.23 ± 0.81 | 1.16 ± 0.057 | 0.66 ± 0.033* | 0.63 ± 0.33 | 1.12 ± 0.818 | 0.60 ± 0.23 | 1.04 ± 0.66 | 0.71 ± 0.33 |

Values are mean ± SEM (n = 3),
EAC control group was compared with normal group,
P < 0.0 01, Experimental groups were compared with EAC control.,
*P < 0.5, Experimental groups were compared with EAC control

## Effect of drug on hematological parameters

In order to detect influence of drug on the hematological status of EAC bearing mice, a comparison was made among ten groups; each mouse on 15$^{th}$ day after inoculation. The groups comprised of tumor bearing mice 1. Blood was drawn from each mouse by syringe with blood anticoagulant (EDTA) and white blood cells count (WBC), red blood cells (RBC) and hemoglobin were estimated (Table 6).

**Table 6: Effect on haematological parameters of EAC cell lines**

| Parameters | Hemoglobin | Total RBC (Cells/mL x $10^9$) | Total WBC (cells/mL x $10^6$) |
|---|---|---|---|
| Normal saline (5 mL/kg) | 12.14 ± 0.12 | 6.98 ± 0.08 | 7.71 ± 0.05 |
| EAC Cell (control) +Vehicle | 9.56 ± 0.31 | 3.60 ± 0.12 | 20.12 ± 1.67 |
| EAC Cell + Std. | 11.12 ± 0.5 | 6.48 ± 0.86 | 8.80 ± 0.12 |
| EAC Cell + Comp. **(1)** | 10.66 ± 0.020 | 6.23 ± 0.056 | 9.76 ± 0.041 |
| EAC Cell + Comp. **(2)** | 9.83 ± 0.19 | 4.61 ± 0.034* | 12.8 ± 0.019 |
| EAC Cell + Comp. **(3)** | 9.63 ± 0.13 | 3.86 ± 0.63 | 12.98 ± 0.067* |
| EAC Cell + Comp. **(4)** | 10.96 ± 0.02* | 6.11 ± 0.5 | 9.36 ± 0.57 |
| EAC Cell + Comp. **(5)** | 9.73 ± 0.014* | 4.51 ± 0.34 | 13.75 ± 0.13 |
| EAC Cell+ Comp. **(6)** | 10.16 ± 0.6 | 5.66 ± 0.058* | 9.15 ± 0.75 |
| EAC Cell + Comp. **(7)** | 10.16 ± 0.19 | 5.40 ± 0.064* | 10.16 ± 0.057 |

Values are mean ± SEM (n = 3),

EAC control group was compared with normal group,

$P < 0.001$, Experimental groups were compared with EAC control.,

*$P < 0.5$, Experimental groups were compared with EAC control

### Stastical analysis

All data were analyzed by using one way analysis of variance (ANOVA) and results are expressed as mean ± SEM

## RESULTS AND DISCUSSION

The present investigation indicates that benzothiazole derivatives showed significant anti-tumor activity in EAC bearing mice. The effect of benzothiazole derivatives on tumor volume, viable and non-viable cell counts, and survival time was measured. (Table 5). Administration of benzothiazole derivatives reduces the tumor volume, packed cell volume, viable cell count and non-viable cell count, when compared to EAC control mice.

The hemoglobin contents in the EAC control mice were compared with experimental group and shown increase in percentage of hemoglobin in benzothiazole derivatives treated EAC bearing mice as compared to EAC control mice and moderate changes in RBC count were also observed in the benzothiazole derivatives treated mice, which showed increase in percentage in benzothiazole derivatives bearing EAC cell lines as compared to EAC control mice. The total WBC counts were significantly higher in the EAC treated mice, when compared with normal mice. Whereas, the percentage of WBC count is significantly reduced in benzothiazole derivatives treated EAC bearing mice as compared to EAC control mice.

The differential count, the percentage of neutrophil was increased in benzothiazole derivatives bearing EAC cell lines as compared to EAC control mice while the lymphocytes count was decreased in benzothiazole derivatives bearing EAC cell lines, when compared with EAC control mice.

**Table7: Effect of benzothiazole derivatives on differential count on EAC bearing mice**

| Treatment | Neutrophil | Monocyte | Lymphocyte | Eosinophill |
|---|---|---|---|---|
| Normal | 75.30 ± 1.53 | 1.50 ± 0.01* | 23.21 ± 1.5 | 0.6 ± 0.08* |
| EAC cell + Control | 28.23 ± 1.32 | 0.80 ± 0.03 | 69.03 ± 0.91 | 1.5 ± 1.08 |
| EAC cell + Comp. **(1)** | 74.6 ± 0.64 | 2.4 ± 0.043* | 22.26 ± 0.53 | 0.7 ± 0.01* |
| EACcell + Comp. **(2)** | 50.81 ± 0.23 | 0.6 ± 0.032* | 47.48 ± 0.51 | 1.1 ± 0.9 |
| EAC cell + Comp. **(3)** | 50.03 ± 0.060 | 0.4 ± 0.021 | 48.66 ± 0.026 | 0.9 ± 0.1 |
| EAC cell + Comp. **(4)** | 81.2 ± 0.92 | 0.6 ± 0.28 | 17.6 ± 0.92* | 0.6 ± 0.02* |
| EACcell + Comp. **(5)** | 71.4 ± 0.83 | 0.6 ± 0.016* | 27.1 ± 0.164 | 1 ± 0.9 |
| EAC cell + Comp. **(6)** | 69.35 ± 0.154 | 1.1 ± 0.36 | 28.85 ± 0.164 | 0.7 ± 0.04* |
| EAC cell + Comp. **(7)** | 62.98 ± 0.097 | 0.8 ± 0.16 | 35.5 ± 0.0858 | 0.7 ± 0.6* |

Values are mean ± SEM (n = 3).
EAC control group was compared with normal group,
$P < 0.001$, Experimental groups were compared with EAC control,
$P < 0.5$, Experimental groups were compared with EAC control

From results it is clear that compounds with more than one halogen showed cytotoxicity towards cancer cell lines. Amino substituted derivatives are more cytotoxic as compared to nitrosubtitued derivatives. The cytotoxicity of compounds with -N $(CH_2CH_2Cl)_2$

was more. Fluorinated analog also showed more cytotoxicity toward cancer cell lines. Compounds having -$NH_2$ at para position on the phenyl ring attached to benzothiazole shows more cytotoxicity towards cancer cell lines.

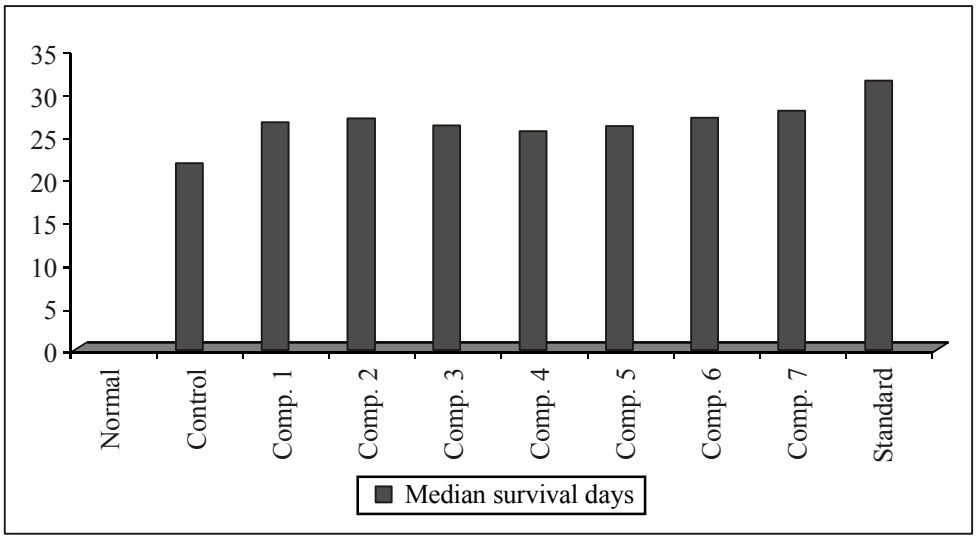



**Fig. 1: Effect of benzothiazole derivatives on median survival time EAC bearing mic**

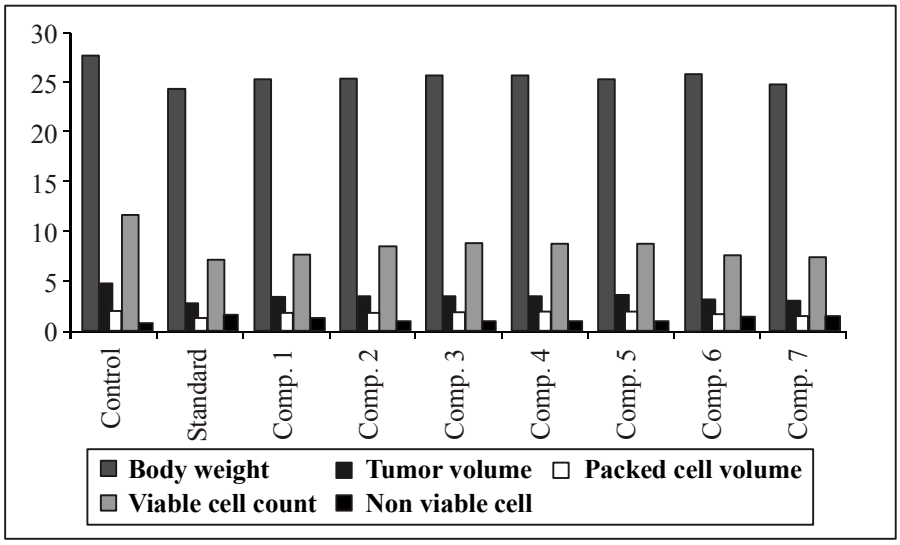



**Fig. 2: Effect of benzothiazole derivatives on tumor volume, packed cell volume, viable and non-viable counts on EAC bearing mice**

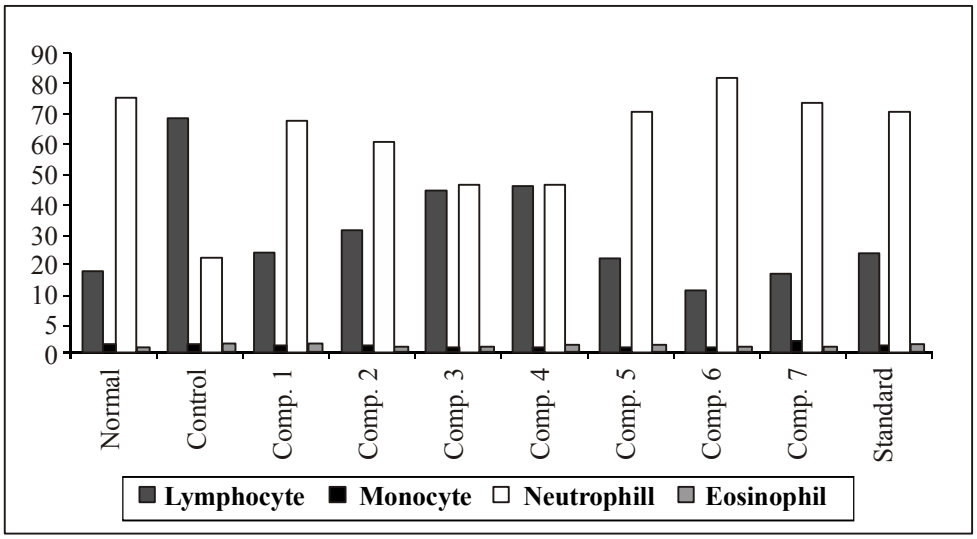



**Fig. 3: Effect of benzothiazole derivatives on differential count of EAC bearing mice**

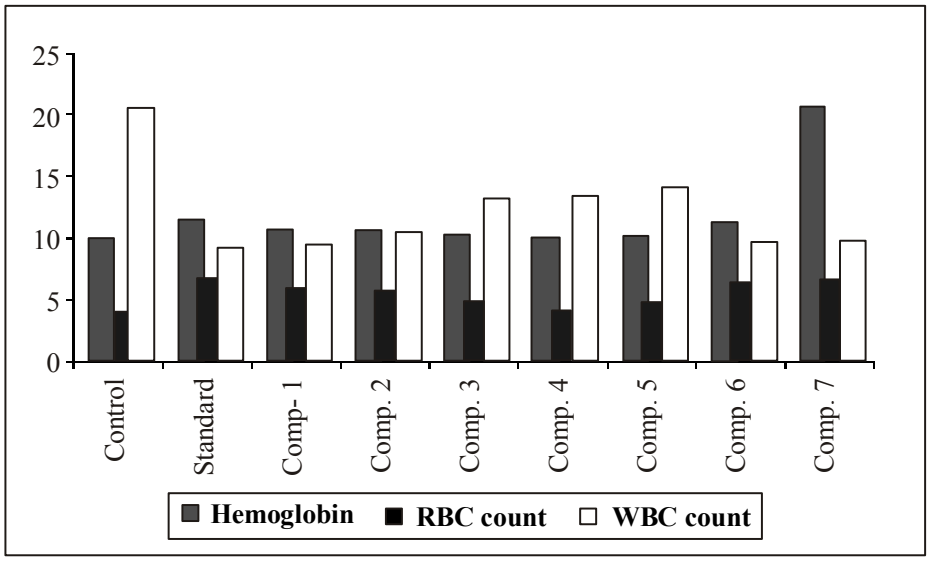



**Fig. 4: Effect of benzothiazole derivatives on hematological parameters of EAC bearing mice**

## REFERENCES

1. N. Siddiqui and A. Rana, Ind. J. Pharm. Sci., **69**, 10 (2007).

2. L. Recane, Ind. J. Het. Chem., **55**, 2085(2001).

3. S. R. Pattan and S. N. Narendra Babu., Ind. J. Het. Chem., **11**, 333 (2002).

4. K. P. Bhusar, Ind J. Het. Chem., **10**, 231 (2001).

5. V. B. Vibhute, Ind. J. Het. Chem., **11**, 79 (2001).

6. M. F. G. Steven, J. Med. Chem., **45**, 744 (2002).

7. Y. Sugno, Biol. Med. Chem. Lett., **15**, 3328 (2005)

8. S. T. Huang and I. Hsei, Biol. Med. Chem., 146, **106** (2006).

9. J. Patockova, M. Krsiat, P. Marhol, E. Tumova, K. Kavitha and S. Manoharan, Ind. J. Pharm., **38(3),** 185 (2006).

10. B. Rajkapoor, Ind. J. Pharm**., 36(1),** 38 (2004).

11. B. M. Nicol, Ind. J. Pharm., **38(4),** 260 (2006).

12. M. Gupta, U. K. Mazumdar, R. S. Kumar and T. Shivakumar, Acta Pharm. Sini., **25(8),** 1070 (2004).

13. J. A. Khanam and S. P. Bag, Ind. J. Pharm**., 29(3),** 157 (1997).

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