www.sphinxsai.com

International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol.2, No.1, pp 681-689, Jan-Mar 2010

Synthesis and Anticancer Activity of Some Novel 2-Substituted Benzothiazole Derivatives

*[1]Devmurari V.P., [1]Pandey Shivanand, [1]Goyani M.B., [2]Nandanwar R.R., [1]Jivani N.P., [2]Perumal P.

[1]Smt R.B.Patel Mahila Pharmacy College, Atkot,Ta: Jasadan,India.

[2]J.K.K. Nataraja College Of Pharmacy Komarapalayam,Tamilnadu,India.

*Email:viraldev1985@gmail.com

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-mercaptobenzothiazole 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 comp have 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 have 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 potential to exhibit anticancer activity [3, 8-13]. So, it was decided to synthesise some novel substituted benzothiazole derivative to evaluate their anticancer activity.

EXPERIMENTAL

The melting range of the synthesized compounds was performed by LAB INDIA visual melting range apparatus. The UV-visible studies were performed by instrument ELICO SL164 double beam spectrophotometer. The IR spectrum studies of the synthesized compounds were prepared by pressed-pellet technique. IR spectra were recorded in KBr disc on a FTIR 8300, KBr press (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 provided by Merk followed by visualization under UV light. The solvent

system used for thin layer chromatography was acetonitrile: methanol. (40:40:20).

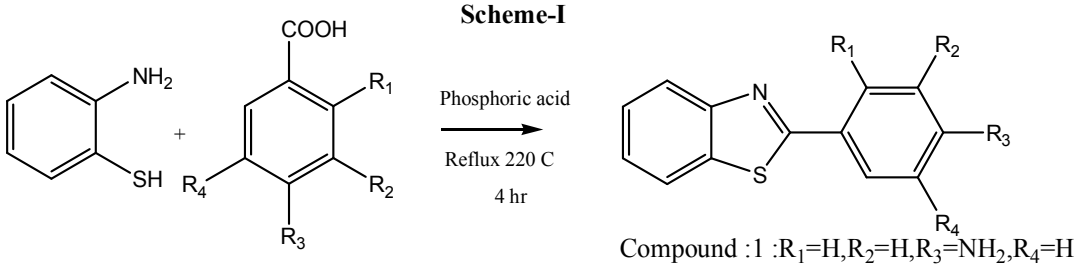
General method of synthesis of 2-substitutedphenyl benzothiazole (Comp: - 1, 2, 4) (Scheme-I)

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

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

A quantity equivalent to 0.01 mol of substituted benzoic acid and 0.04 mol of thionyl chloride were magnetically stirred and refluxed at 70^0C for 1hr. The excess of thionyl chloride was removed from distilling with benzene to get acid chloride. The acid chloride (0.01mol) and 0.01mol of mercaptobenzothiazole were added in 25ml pyridine and heated on water bath for 15 min.[4,5] The reaction mixture was cooled and poured in ice cold water to get precipitate that was recrystallized from methanol (94%).

Scheme-I

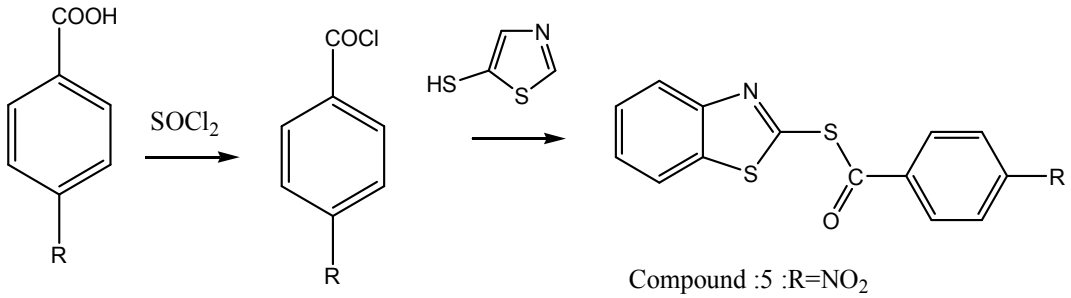


Compound :1 :R_1=H,R_2=H,R_3=NH_2,R_4=H

Compound :2 :R_1=F,R_2=,R_3= Cl,R_4=H

Compound :4 :R_1=H,R_2=H,R_3=Cl,R_4=H

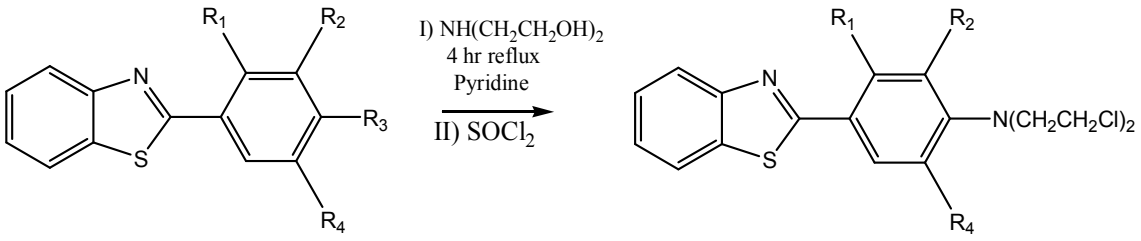
Scheme-II



Compound :5 :R=NO_2

Compound :6 :R= NH_2

Scheme-III



Compound :3 :R_1=H ,R_2=H, R_4=H

Compound :7 :R_1=H ,R_2=NH_2 ,R_4=NH_2

Method of synthesis of 4-(benzothiazol-2-yl)-N, N-bis (2-chloroethyl) benzenamine ((Comp: - 3, 7) (Scheme-III)

Equimolar quantities of o-aminophenol (0.04mol) and substituted benzoic acid were added to 15g 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 recrystallized 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 precipitate recrystallized from methanol to get the product. The resultant product (0.01mol) was refluxed with 0.03mol of thionyl chloride for 4 hour. The excess of thionyl chloride was removed by distilling with benzene.[6,7] After distillation, residue was collected, washed with cold water and recrystallized from ethanol (95%). List of all the synthesized compounds with their chemical structure and IUPAC name is shown in Table-1. Physical data and Spectral data of all the synthesised compounds is shown in Table-2 and Table-3 respectively.

MATERIAL AND METHOD

Animals: Sixty Adult Swiss albino mice (20-25gm) were procured from visheshwarya enterprises, Bangalore and used throughout the study. They were housed in cage boxes in a controlled environment (temperature 25 $\pm 2^0$c and dark/light cycle) with standard laboratory diet and water ad libitum.

Methodology: Male Swiss albino mice were used for the antitumor studies. [11-13] All groups were treated with EAC ascetic lymphoma cell line (0.2ml of $2x10^6$cells/mice) intraperitoneally except normal group. This was taken as day zero. On first day 5ml/kg body weight of normal saline (0.9% Nacl w/v) was administered in group 1(normal). Propylene glycol, 5ml/kg body weight per day was administered in group 2(cancer control). Benzothiazole derivatives were administered accordingly doses given above (2.5mg/ml, 2.5mg/ml, 1.25mg/ml, 1.25mg/ml, 2.5mg/ml, 1.25mg/ml, 0.625mg/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, WBC count. The rest of animal groups were kept for checking the survival time of tumor bearing hosts.

Effect of drug on survival time: Animals were inoculated with $2x10^6$ 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 33mg/kg. The MST can be calculated by using following calculation. (Table-4 and Figure-1.)

Increase in life span = T-C/C +100

Where T=number of days the treated animal survived

C=number of days control animal survived

Effect of drug on haematological parameters: In order to detect influence of drug on the haematological 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. Tumor bearing mice treated with compound 7. Control mice 3 and normal 4. Blood was drawn from each mouse by syringe with blood anticoagulant (EDTA) and white blood cells count (WBC), red blood cells (RBC) and haemoglobin were estimated. (Table-6 and Figure-4)

Statistical analysis: All data were analyzed by using one way analysis of variance (ANOVA) and results are expressed as mean $\pm$ SEM

RESULT AND DISCUSSION

The present investigation indicates that benzothiazole derivative showed significant anti-tumor activity in EAC bearing mice. The effect of benzothiazole derivatives on tumor volume, viable and non-viable cell count, and survival time was measured. (Table-5 and Figure-2) 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 content in the EAC control mice were compared with experimental group, shown increased in percentage of hemoglobin in benzothiazole derivatives of 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 of 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. (Table-7 and Figure-3)

From result it shows 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 comp with -N $(CH_2CH_2Cl)_2$ showed more toxicity. 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.

Table-1. IUPAC name and Chemical Structure of the synthesised compounds

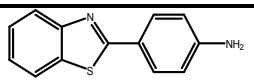
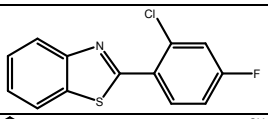
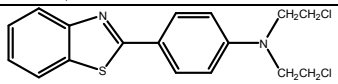
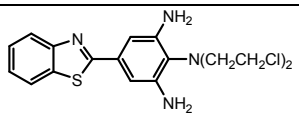
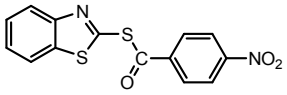
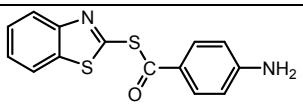
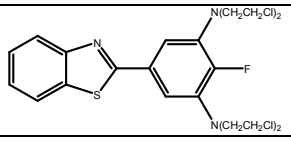
Sr.No.	Name of Compounds	Chemical structure
1.	4-(benzothiazol-2-yl)benzenamine	
2.	2-(2-chloro-4-fluorophenyl) benzothiazole	
3.	4-(benzothiazol-2-yl)-N,N-bis(2-chloroethyl)benzenamine	
4.	4-(benzothiazol-2-yl)-2-bromo-N,N-bis(2-chloroethyl)benzenamine	
5.	S-benzothiazol-2-yl 4-nitrobenzothioate	
6.	S-benzothiazol-2-yl 4-aminobenzothioate	
7.	2-(3,5-bis(1,5-dichloropentan-3-yl)-4-fluorophenyl) benzothiazole	

Table-2. Physical data of synthesized compounds

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

Table-3. Spectral data of synthesized compounds

Compounds	IR spectra data	^{1}H NMR (DMSO, ppm)	Mass
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
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
3	2917.13,1507.27,1314.42 ,757.01,1473.51	2.9-3.1(m, 8H,-N($CH_2CH_2Cl)_2$), 6.9-7.0 (m, 4H,Aryl-H),7.1-7.6 (m, 4H, 2-benzothiazole)	352, 282, 256, 210, 178, 123, 76
4	2924.85 , 1561.27, 1333.68 , 686.61, 1646.13, 600.78 .	2.5-2.6 (m, 8H,-N($CH_2CH_2Cl)_2$), 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
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
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
7	2958.60, 1278.72 , 1523.66 , 1718.46, 1349.11.	3.7-3.8 (m, 16H, -N($CH_2CH_2Cl)_2$), 6.8-7.0 (t, 2H, Aryl-H), 7.6-7.8(m, 4H, 2-benzothiazole)	511,509

Table-4: Effect of benzothiazole derivative on survival time on EAC bearing mice.

5	**Median survival (days)**	***Increase of life span (%)***
Normal saline(5ml/kg body weight)	-	-
EAC cell (control) +propylene glycol (5ml/kg body weight)	21.13±0.37	
EAC cell + Comp-1 (25mg/kg)	27.5±0.94	*30.24*
EAC cell + Comp-2 (50mg/kg))	25.5±0.95	*20.68*
EAC cell + Comp-3 (50mg/kg)	25±0.81	*18.31*
EAC cell + Comp-4 (50mg/kg)	26.5±0.72	*27.78*
EAC cell + Comp-5 (100mg/kg)	25.5±0.76	*20.68*
EAC cell + Comp-6 (100mg/kg)	26±0.46	*23.04*
EACcell + Comp-7 (100mg/kg)	26.5±0.72	*25.14*
EACcell + vincristine std.(8.25mg/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 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(gm)	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 countx 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-vible tumoe cell count x 107 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*

Table-6. Effect on haematological parameters of EAC cell lines

Parameters	**Hemoglobin**	**Total RBC (Cells/mlx109)**	**Total WBC (cells/ml x106)**
Normal Saline (5ml/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*

Table-7.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

FIGURE-1. EFFECT OF BENZOTHIAZOLE DERIVATIVE ON MEDIAN SURVIVAL TIME OF EAC BEARING MICE

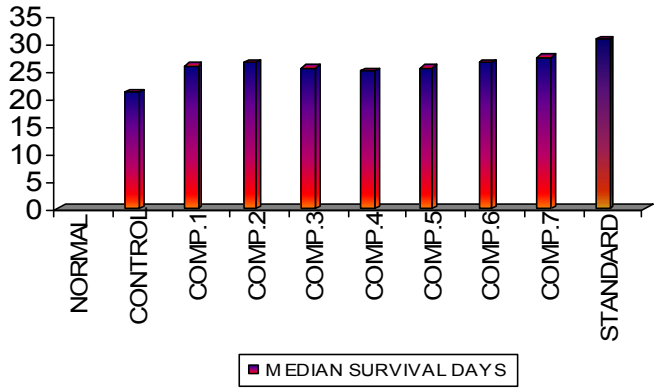


FIGURE-2. EFFECT OF BENZOTHIAZOLE DERIVATIVES ON TUMOR VOLUME, PACKED CELL VOLUME,VIABLE AND NON-VIABLE CELL COUNT ON EAC BERAING MICE

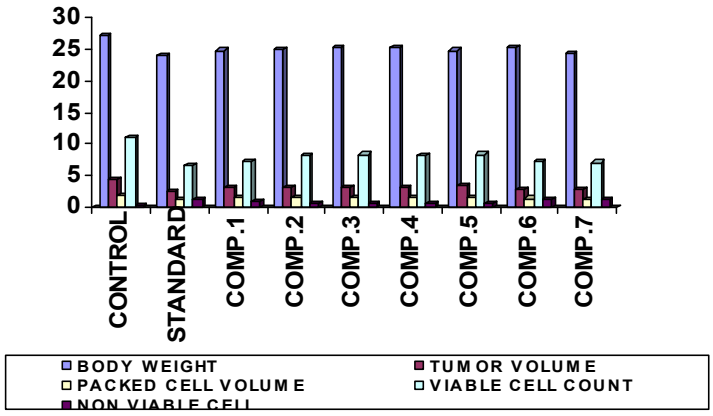


FIGURE-3. EFFECT OF BENZOTHIAZOLE DERIVATIVES ON DIFFERNTIAL COUNT OF EAC BEARING MICE

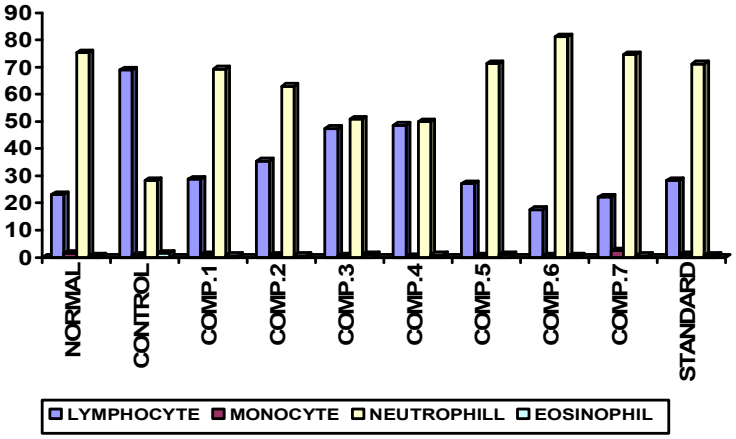
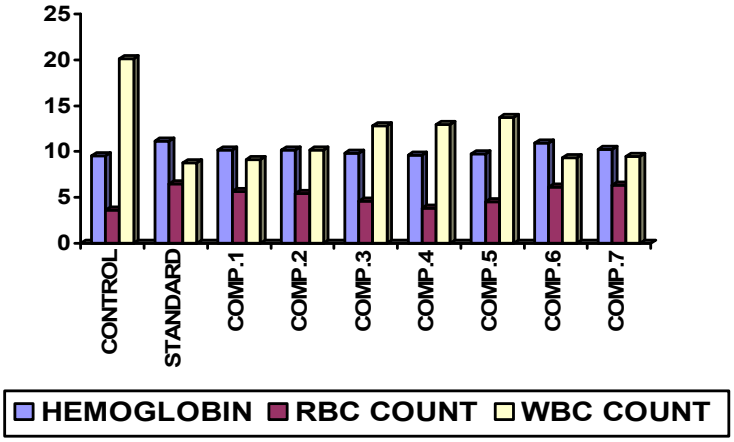


FIGURE-4. EFFECT OF BENZOTHIAZOLE DERIVATIVES ON HEMATOLOGICAL PARAMETERS OF EAC BEARING MICE



REFERENCES

1. *Siddiqui N., Rana A., Benzothiazole, a new profile of biological activities, Ind. J of Pharm. Sci., 2007, 69, 10-17.*
2. *Recane L., Antimicrobial and anti HIV agent of benzothiazole derivatives, Indian J. of Het. Chem., 2001, 55, 2085.*
3. *Pattan S.R, Narendra babu S.N., Synthesis and antifungal activity of 2-amino [5'-(4-sulphonyl benzylidene)-2, 4- thiazolidene Dione] -7-(substituted)-6-fluro benzothiazoles, Ind J of Het. Chem., 2002, 11,333-334.*
4. *Bhusari K.P., Synthesis and antimicrobial activity of some 2-substituted Aminobenzothiazole, Ind. J of Het. Chem., 2001, 10, 231-232.*
5. *Vibhute V.B., Synthesis and antibacterial activity of some new 2-(substituted phenyl) sulphonamido)-6-substituted benzothiazoles, Ind. J of Het. Chem., 2001 11,79-80.*
6. *Malcolm F.G.S., Synthesis and pharmaceutical properties of Antitumour benzothiazole amino acid prodrugs, J of Med Chem., 2002, 45, 744-747.*
7. *Yuichi Sugno., Synthesis and biological evaluation of benzothiazole derivatives as potent Antitumour agent; Bio. and Med. Chem. Let., 2005,15, 3328-3332.*
8. *Huang S.T., IJen H., Synthesis and anticancer evaluation of bis (benzimidazoles), bis (benzoxazoles), and benzothiazoles, Bio. and Med. Chem., 2006, 146, 106-6119.*
9. *Patockova J., Krsiat M., Marhol P., Tumova E., 2003. Kavitha K., Manoharan S, Anticarcinogenic and antilipid peroxidafive effect of Tephrosig purpurea (Linn). Pers, in 7, 12 dimethsl ben2 (a) anthracene (OMBA) induced hamster beceal pouch carcinoma. Ind. J. Pharm., 2003, 38(3), 185-189.*
10. *Rajkapoor B., Antitumor activity of Indigotera aspalathoides on Ehrlich ascites carcinoma in mice. Indian J. Pharmacology, 2004, 36(1), 38-40.*
11. *Nicol B.M., The effect of cyclophosphamide alone and in combination with ascorbic acid against*

ascites Dalton's lymphoma., Indian J. Pharm., 2006, 38(4), 260-265.

12. *Gupta M., Mazumdar U.K., Kumar R.S, Shivakumar T., Antitumor activity and antioxidant role of Baechinia raseemose against Ehrlich ascites carcinoma in Swiss albino mice. Acta Pharmacological Sinica., 2004, 25(8), 1070-1076.*
13. *Khanam J.A., Bag S.P, Antineoplastic activity of copper benzohydroxamic and complex against Ehrlich ascites carcinoma (EAC) in mice, Ind J. Pharm., 1997, 29(3). 157-16.*

*****