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Research Article

Synthesis Of Bis - [2-(Chloroethyl) Amino] Acetamido - 4 - Substituted Phenyl Thiazole Derivatives As Possible Antioxidant And Alkylating Anticancer Agents

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Abstract

A series of new thiazole derivatives containing nitrogen mustard have been prepared by the cyclization with various substituted acetophenone with thiourea in presence of iodine to form 2-amino-4-phenyl thiazole. The 2-amino-4-phenyl thiazole acetylation with chloroacetylchloride was used to produce the corresponding 2-chloroacetamido-4-phenyl thiazoles. The resultant chloroacetyl derivatives were subjected to a nucleophilic substitution reaction with diethanolamine by heating under reflux in dry pyridine. The compounds thus obtained were subjected to chlorination reaction using phosphorous oxychloride to get title compounds. Compounds prepared were recrystallized from dimethyl sulphoxide. All the synthesized compounds were screened for their *in-vitro* cytotoxic activity by MTT assay, *in-vivo* anticancer activity against Dalton's ascetic lymphoma (DAL) in Swiss albino mice and *in-vitro* anti-oxidant activity by 2'2- diphenyl-1-picryl hydrazyl and hydrogen peroxide scavenging methods.

Keywords: Thiazole derivatives, nitrogen mustard, acetophenone, 2-amino-4-phenyl thiazole, MTT assay

Introduction

Cancer is the root cause of several chronic and progressive diseases that adversely affect a number of organs including the nervous system, vascular system and whole body. Cancer is a major and grooming public health problem throughout the world. More than 70% of all cancer occurs in developing countries. Based on the projection, death due to cancer will continue to raise upto 11.4 million by 2030. The resource available for prevention, diagnosis and treatment of cancer are limited or nonexistent.

Several physiological activities of various thiazole derivatives, have proved the efficiency and efficacy in combating various diseases and is found to have good antibacterial[1] and antifungal activities, and it has also been seen that the thiazole analogues incorporated with different nuclei show variety of pharmacological profile such as local anaesthetic[2], antidiabeties[3], anti-inflammatory[4] antioxidant and anticancer[5] etc. Based on the above reports various Bis-2-[(chloroethyl) amino] acetamido-4-substituted phenyl thiazole derivatives based on figure 1 were synthesized.

Materials And Methods

Apparatus:

All the Melting points reported were determined in open capillary tubes and Theil's melting point apparatus and are uncorrected. The reactions were monitored by TLC (Precoated-merck) using methanol: ethylacetate (1:1) and detected by UV and also using iodine as visua-

lizing agent. The IR Spectra of the synthesized compounds were recorded on a Fourier Transform IR spectrometer (Shimadzu 8400S) using KBr pellet method and the values of Vmax are reported in cm^{-1}. ^1H NMR spectra (DMSO-d_6) were obtained on DMM X-200 MHz spectrophotometer using tetramethylsilane (TMS) as internal standard (chemical shift are expressed in δ ppm) (As shown in Table 1).

Chemicals and Reagents:

The chemicals used in the present project work were of AR grade and LR grade, purchase from SD-fine, Loba Chemie, Qualigens, Sigma, Ranchem, and Merck India.

Mechanism and Reactions:

Synthesis of 2-Amino- 4- substituted phenyl thiazole[6]- Thiourea (30.4 gm, 0.4mole) and Iodine (50.8, 0.2 mole) were triturated and mixed with acetophenone (24gm, 0.2mole). The mixture was heated on a water bath with occasional stirring for 8 hrs. The obtained solid was triturated with diethyl ether to remove unreacted acetophenone, washed with aqueous sodium thiosulphate to remove excess iodine and then with water. The crude product was dissolved in hot water, filtered to remove the sulphone and 2-amino-4-phenyl-thiazole was precipitated by the addition of ammonia and water, crystallized from ethanol to give white crystal.

IR spectrum (cm^{-1}): 3443(OH), 3292(NH_2), 3109(aryl C-H), 1629(C=N), 1527(C=C aromatic), and 715(C-S-C). ^1H NMR (DMSO-d_6): 9.4(s, 2H, NH_2), 7.3-8.2 (m, 4H, Ar), 3.9 (s, 1H, Thiazole) and 2.5 (s, 2H, CH_2Cl).

Synthesis of 2- Chloroacetamido- 4 - substituted phenyl thiazole[6]- The solution of 2 –amino-4 phenyl thiazole (3.7gm, 0.02mol) in dry benzene (60ml) was cooled to 0°C to 5°C. Chloroacetylchloride (5ml, 0.04) dissolved in dry benzene (20ml) was slowly added to the solution with vigorous stirring. When addition was complete the reaction mixture was refluxed for 3hr and benzene was removed in vacuum. The residue was washed with 5% $NaHCO_3$ and subsequently with water. The crude product was dried and crystallized from ethanol to give colorless crystals.

I.R. Spectrum (cm^{-1}): 3433(OH), 3198(NH), 3082(C-H, Ar), 3002(-CH_2), 1668(C=O), 1568(C=N), 1528(C=C), 8O6(C-S-C) and 731(C-Cl). ^1H NMR (DMSO- d_6): 9.4 (s, 1H, NHCO), 6.7-6.9 (m, 4H, Ar), 5.1 (s, 1H, Thiazole) and 3.6 (s, 2H, CH_2Cl).

Synthesis of bis [2-(Hydroxyl ethyl)-amino] acetamido -4- substituted phenyl thiazole[7]: A mixture of chloroacetyl derivative (0.01mole) and diethanolamine (0.012 mole) in sufficient dry pyridine was heated under reflux for 3hr. Then pyridine was poured into little crushed ice containing few drops of hydrochloric acid with stirring. It was kept aside overnight and the resulting product was filtered and washed with small portion of cold water and dried. It was recrytallized from appropriate solvent to get pure compound.

I. R.Spectrum (cm^{-1}): 3429 (OH), 3292(NH), 3120(C-H, Ar), 2985(-CH_2-), 1633(C=O), 1531(C=N), and 825 (C-S-C). ^1H NMR (DMSO-d_6): 9.8(s, 1H, NH), 6.7-7.8(m, 4H, Ar), 5.1 (s, 1H, Thiazole), 6.7 (br, s, 2H, $CH_2CH_2OH)_2$, 3.6 (t, 4H, -$NCH_2CH_2OH)_2$, 2.5 (t, 4H, -$NCH_2CH_2OH)_2$.

Synthesis of bis 2-[(chloroethyl) amino] acetamido - 4 - substituted phenyl thiazole[7]: A mixture of Bis [2-(chloroethyl) amino] derivatives and $POCl_3$ (20ml) was gently refluxed for 3hr. The excess $POCl_3$ was removed under vacuum, and then poured into cold water to obtain solid. The product was recrystallized from suitable solvent.

I.R.Spectrum (cm^{-1}): 3257(OH), 3207(NH), 3068(Ar, CH), 2906(-CH_2-), 1637(C=O), 1566(C=N), 1529(C=C, Ar), 785(C-S-C), and 725(C-Cl). ^1H NMR (DMSO-d_6): 8.7 (s, 1H, -NHCO), 7.1-7.7(m, 4H, Ar), 6.6(s, 1H, CH thiazole), 5.1(s, 1H, Thiazole), 3.6(t, 4H, $NCH_2CH_2Cl)_2$ and 2.6(t, 4H, $NCH_2CH_2Cl)_2$ (as shown in figure 1, figure 2 and Table 1).

BIOLOGICAL ACTIVITIES

Antioxidant activity:

The antioxidant activity of the sample was assessed on the basis of radical scavenging effect of the stable DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical and H_2O_2 scavenging method.

DPPH method[8]: Taken 5ml of DPPH solution and 0.25 ml of each of the test sample was added separately in the test tubes. The tubes were incubated at 37°C for 30 min. and absorbance of each solution was measured at 517 nm against the corresponding test blanks. The remaining DPPH was calculated

IC_{50} value is the concentration of the sample required to scavenge 50% DPPH free radical.

H_2O_2 scavenging method[9]: Taken 1 ml of each test sample and then added 2 ml H_2O_2 in PBS (phosphate buffer saline pH 7.4) in the test tubes separately The absorbance of hydrogen peroxide at 230 nm was determined after ten minutes against a blank solution containing phosphate buffer saline without hydrogen peroxide.

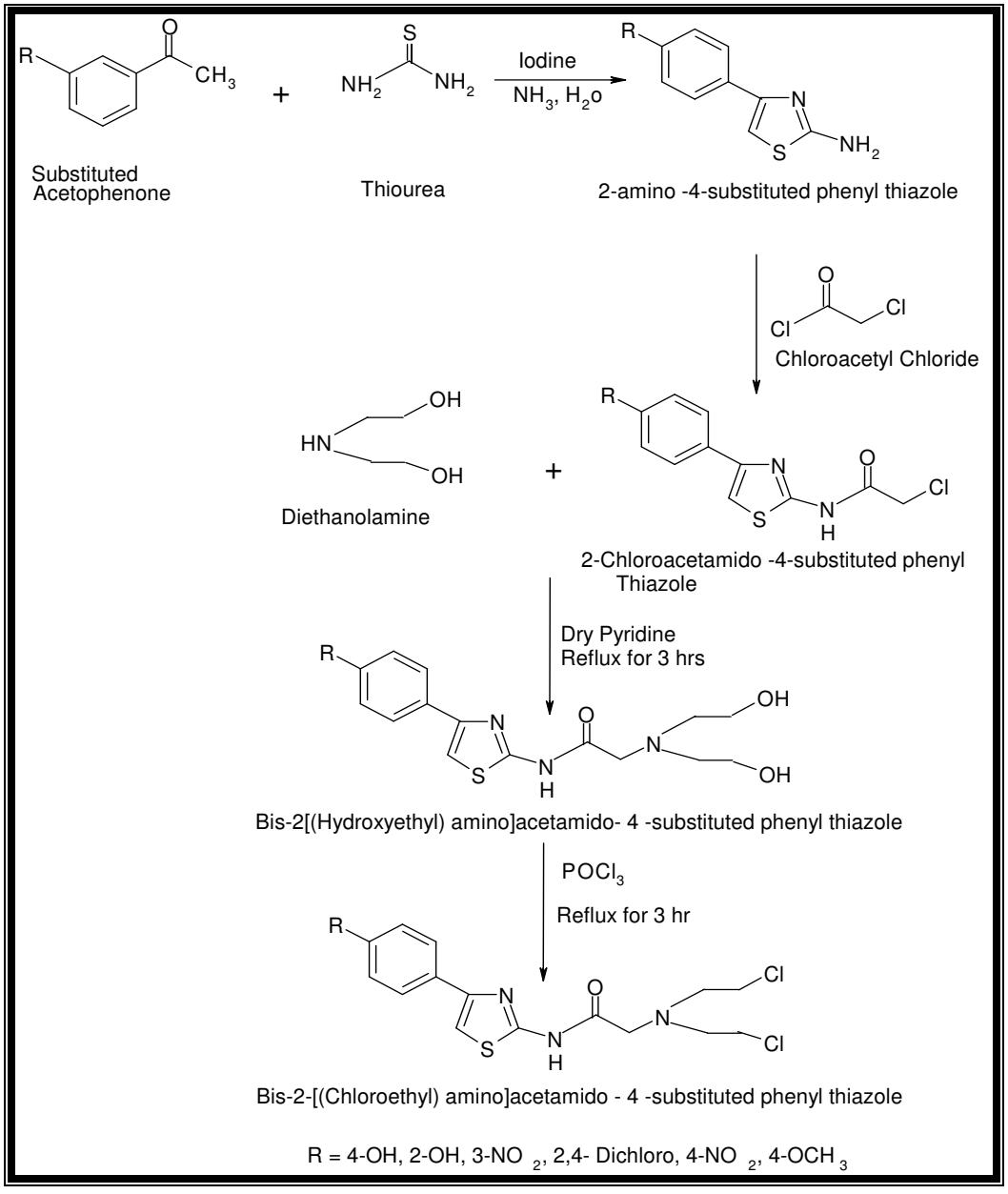

Figure 1: Scheme for synthesis of bis - [2-(Chloroethyl) Amino] Acetamido - 4 - Substituted Phenyl Thiazole derivatives

***In- vitro* cytotoxic activity[10]:** The cells at approximately 80% confluence were selected, i.e. logarithmically growing cells. Both cell lines were trypsinized from preculture bottles before adding to the 96-well micro plate. The cell suspensions ($3x10^4$cells/ml) for HepG2(human liver cancer cell line) were prepared. A 100 µl aliquot of the cell suspension was gently introduced into each well of the test plate. The cells were then cultured in a CO_2 (5% in air) incubator over night. After incubation, culture medium was discarded. 100 µl of the test chemical dissolved in culture medium was added into each well and then cultured for a further 48 hr. After the 48-hr incubation, 90 ml of culture medium containing the test chemical was discarded and then 90 ml of fresh culture medium was added. 90 µl of the medium was discarded and then 90 ml of medium containing MTT at the concentration of 0.55 mg/ml was added into each well. The plate was incubated further for 4 hr. The medium containing MTT (microculture tetrazolium) was discarded by inverting the plates and then each well was washed with 200 ml of PBS. The test plate was inverted on paper towels to absorb the remaining drops of water. Two hundred µl of acid-isopropanol was added to each well. To extract and solubilise the formazan, the test plate was agitated by microplate shaker for 10 min. OD_{590} (optical density at λmax 590) was measured by an automatic microplate reader.

***In-vivo* anticancer screening[11]:** Animals were divided into nine groups consisting of 6 animals each. Tumor were induced in all group of animals by injecting 0.3 ml of $1x10^6$ cell mL^{-1} of Dalton's Ascitic lymphoma (DAL) to the peritoneal cavity of mice. This was taken at day 0. Treatment was started 24 hr after inoculation. The Control group was treated with same volume of 0.9% sodium chloride solution. The Standards group was treated with standard drug 5- Fluorouracil (20mg/kg b w *p.o.*).All other groups were treated with different synthesized derivatives of thiazole at 35 mg/kg b w *p.o.* All treatments were carried out for 9 days.

Tumor growth response[12]: Antitumor effect of the drug was assessed by observation of average body weight analysis, and calculation of mean survival time.

TABLE -1: Analytical data of bis-2-[(chloroethyl) amino] acetamido-4-substituted phenyl thiazole derivatives

Compd. No.	Substituent R	Molecular Formula	Mol. Wt.	Melting Point (˚C)	Yield (%)
A	H	$C_{15}H_{17}N_3OCl_2S$	357	101-104	60
B	4-OH	$C_{15}H_{17}N_3O_2Cl_2S$	373	172-174	65
C	2-OH	$C_{15}H_{17}N_3O_2Cl_2S$	373	167-170	62
D	3-NO_2	$C_{15}H_{16}N_4O_3Cl_2S$	402	120 -122	60
E	2,4- dichloro	$C_{15}H_{15}N_3OCl_4S$	425	156-158	45
F	4-NO_2	$C_{15}H_{16}N_4O_3Cl_2S$	402	125-126	40
G	4-OCH_3	$C_{16}H_{19}N_3O_2Cl_2S$	387	147-150	35

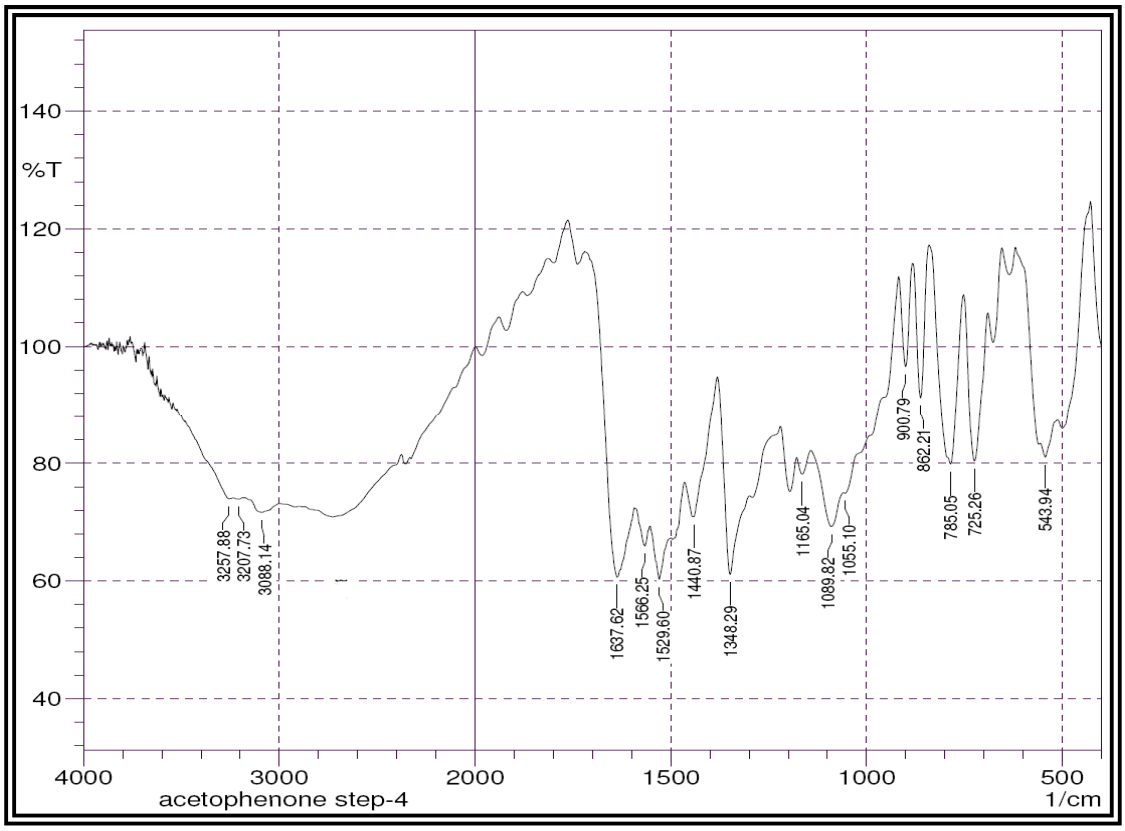

Figure 2: FTIR Spectra of Compoud B

Mean survival time: Mean survival time of treated groups was compared with control group using the following equation.

$$\text{ILS (\%)} = \frac{\text{MST of treated group}}{\text{MST of control group}} \times 100$$

Body weight analysis: Body weight analysis of treated groups was compared with control group using the following equation.

$$\text{\% Decrease in body weight} = \frac{\text{(Gain in body weight - (Gain in body weight of control) of treated group)}}{\text{Gain in body weight of control}} \times 100$$

RESULTS AND DISCUSSION

***In-vitro* antioxidant activity:**

All the above synthesized compounds were tested for their antioxidant activity, using *in-vitro* DPPH and H_2O_2 methods. Among the seven compounds tested, compound-B, containing 4-hydroxy derivatives, exhibited potent antioxidant activity in the H_2O_2 scavenging method. The IC_{50} value of the compound was found to be 280±0.88 µg/ml. The compound C was moderately active and compound A was found to be weakly active and other compounds were inactive.

In the DPPH method, the compound B and C (IC_{50} = 120±1.7, 165±0.88) showed moderate activity. The compounds A and D (IC_{50} = 305±0.57, 450±1.45) were found to be weakly active and other compounds were inactive. However, the standard, ascorbic acid showed very low IC_{50} value (IC_{50} = 13.33±0.88) indicating their potent nature (As shown in Table 2).

TABLE-2: *In-vitro* antioxidant activity of compounds (A-I) by DPPH and H_2O_2 methods

Compounds	Substituent R	DPPH Method IC_{50} ± SEM (µg/ml)*	H_2O_2 Method IC_{50}±SEM (µg/ml)*
A	H	305 ± 0.57	490 ± 1.4
B	4-OH	120 ± 1.7	280 ± 0.88
C	2-OH	165 ± 0.88	380 ± 1.2
D	3-NO_2	450 ± 1.45	750 ± 1.7
F	2,4-dichloro	> 500	832 ± 2.3
G	4-NO_2	>500	>1000
H	4-OCH_3	>500	>1000
I	Ascorbic acid	13.33 ± 0.88	200 ± 0.8

**Average of three determinations*

TABLE-3: In-vitro cytotoxic activity of compound A-G by MTT assay

Compounds	Mol. Formula	IC_{50} (mg/L)
A	$C_{15}H_{17}N_3OCl_2S$	0.83
B	$C_{15}H_{17}N_3O_2Cl_2S$	0.13
C	$C_{15}H_{17}N_3O_2Cl_2S$	0.15
D	$C_{15}H_{16}N_4O_3Cl_2S$	1.95
E	$C_{15}H_{15}N_3OCl_4S$	15.52
F	$C_{15}H_{16}N_4O_3Cl_2S$	15.72
G	$C_{16}H_{19}N_3O_2Cl_2S$	16.25

TABLE-4: *In-vivo* anticancer activity effect of the synthesized thiazole derivatives on the survival and body weight analysis of tumor bearing mice

Groups	Dose (mg/kg b w)	MST (days)	% Increase in Life span	% decrease in body weight
Saline (Control group A)	2ml/kg	16.83 ± 0.79	100	-
5-FU (standard group B)	25	32.66 ± 0.76	194.05	12.0
Compound A	35	12.16 ± 1.1	72.25	6.76
Compound B	35	23.00 ± 0.6	136.74	10.7
Compound C	35	20.33 ± 0.8	120.79	9.16
Compound D	35	17.0 ± 0.57	101.01	7.68
Compound E	35	15.33 ± 0.5	91.08	4.80
Compound F	35	12.05 ± 1.2	71.59	1.58
Compound G	35	11.45 ± 1.5	68.03	1.10

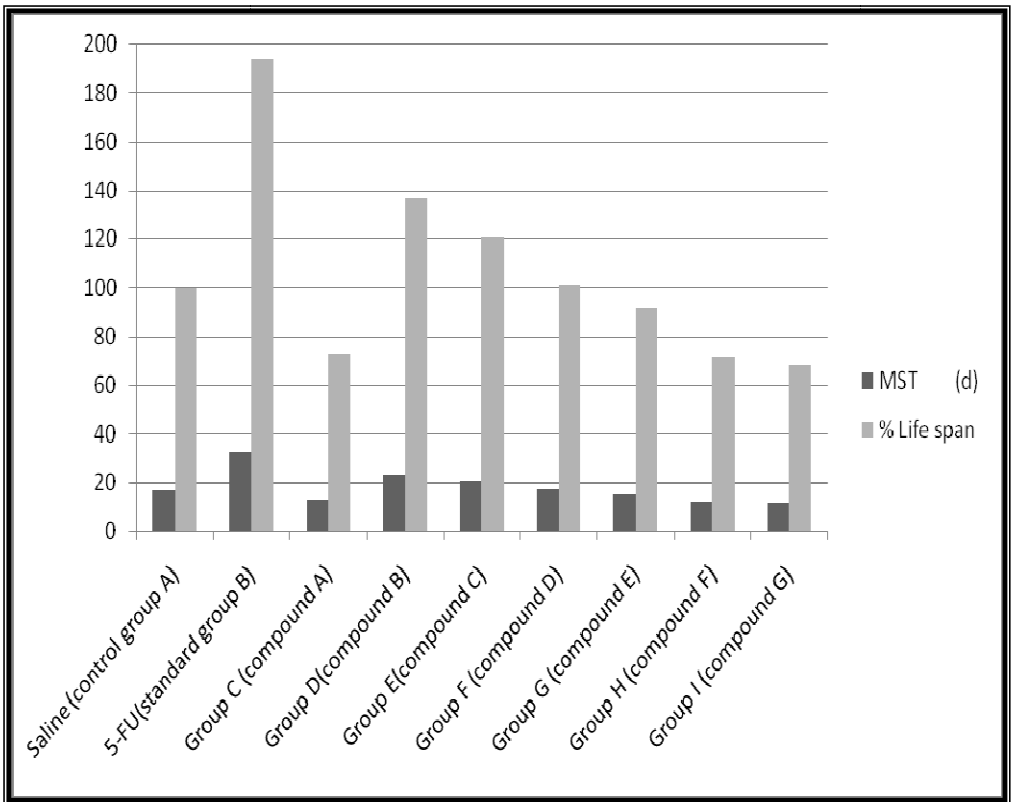

Figure 3: MST and Percentage Life span

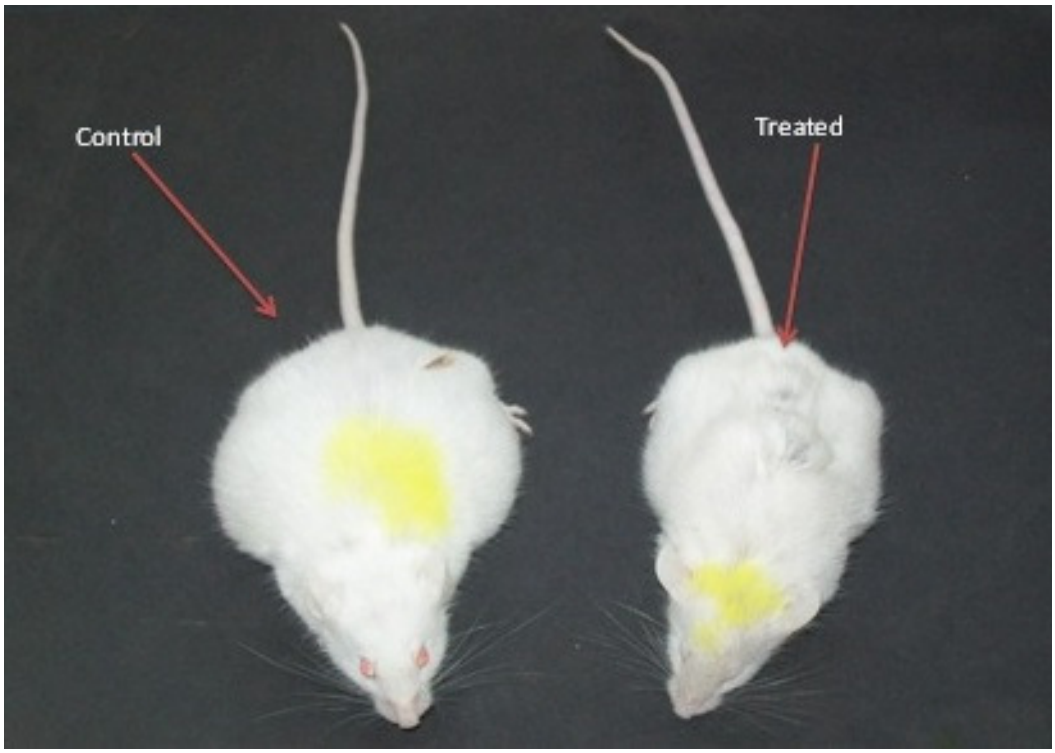

Figure 4:- Tumor bearing mice control and treated group's compounds B and C

***In- Vitro* Cytotoxic activity:** All the synthesized compounds were screened for in-vitro cytotoxic activity using MTT assay method. Among the seven compounds tested compounds B and C, containing 4-hydroxyl and 2-hydroxy derivative on phenyl had *in-vitro* cytotoxic activity significant activity, compounds without substitution and 3-nitro derivatives exhibited moderate activity and other compounds did not show any activity (as shown in Table 3).

***In-Vivo* anticancer screening:** *In-vivo* anticancer activity were screened by tumor induced by injecting 0.3 ml of $1x10^6$ cell mL^{-1} of Dalton's Ascitic lymphoma (DAL) to the peritoneal cavity of mice. The effect of all the synthesized compounds on survival time and body weight analysis on tumor bearing mice showed MST for the control group to be 16.83 days, while it was compound B (35mg/kg/d, *p.o*.) showed MTS of 23 days. Compound C (35mg/kg/d, p.o.) showed the MST of 20 days, and the percentage increasing in life span of compounds B and C was 136.74% and 120.79%. Tumors bearing mice treated with the standard drug, 5-FU (20mg/kg/d *p.o.*) showed a MST of 36.66 days and the percentage increasing life span of 194%. 5-FU showed a decrease in body weight by 12% analysis and compound B and C showed a decrease in body weight by 10.7 and 9.16% respectively. 4-hydroxy and 2-hydroxy compounds showed significant activity, 3-nitro derivatives showed moderate activity and compounds without substitution showed less activity and other compounds did not show any activity (as shown in Table 4, Figure 3 and Figure 4).

Conclusion

A series of titled compounds, i.e. [A –G] have been synthesized using appropriate synthetic procedure. The yield of the synthesized compounds were found to be in range from 35% - 65%. Structure of synthesized compounds were characterized and confirmed with the help of analytical data's such as IR and ^1H NMR. Out of seven synthesized compounds only one compounds i.e. hydroxyl group on phenyl at 4th position (Compound B) exhibited moderate antioxidant activity, hydroxyl group at 2nd position (Compound C) showed mild activity, no substitution (Compound A) and nitro group on phenyl at 3rd position (Compound D) showed less activity. However none of the compounds showed better antioxidant activity than the standard drug. All the synthesized compounds were screened for *in-vitro* cytotoxic activity, out of seven synthesized compounds only two compounds Compound B and Compound C exhibited significant cytotoxic activity, Compound A showed moderate activity and Compound D showed mild activity. All the synthesized compounds were screened for *in-vivo* anticancer activity. Out of seven synthesized compounds Compound B and Compound C exhibited significant anticancer activity, Compound D showed less activity. Remaining compounds E, F and G did not show any activity.

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Conflict of Interest: None

REFERENCES

1. S.R. Pattan, M.Shamrez Ali, J.S. Pattan, S.S. Purohit, V.V.K. Reddy and B.R. Nataraj, "Reaction of substituted 2-acetamidothiazole derivatives and their antimicrobial activity", *Indian J. Chem*., 45B, 1929 (2006).
2. Ram Lakhan and Om Prakash Singh, "Local Anesthetic activity and synthesis of 2-(N-Substituted or N, N-Disubstituted aminoacetamido)-4-aryl Thiazoles", *Agric. Biol. Chem*. 48(6), 1441, (1984).
3. Narsingh Sachan, Shivajiro S. Kadam, Vithal M. Kulkarni, *Indian J. Heterocyclic Chem.*, 17, 57 (2007).
4. T. Karabasanagouda, Airody Vasudeva Adhikari, Ramgopal Dhanwad and G. Parameshwarappa, "Synthesis of some new 2-(4-alkylthiophenoxy)-4-substituted-1,3-thiazoles as possible anti-inflammatory and antimicrobial agents", *Indian J. Chem*.47B, 144 (2009).
5. T.F. Abbs Fen Reji, S. K. C. Devi, K. K. Thomas, K.G. Sreejalekshmi, S.L. Manju, M. Francis,

S.K. Philip, A. Bharathan and K.N. Rajasekharan, "Synthesis and cytotoxicity studies of thiazole analogs of the anticancer marine alkaloid dendrodoine", *Indian J. Chem.*,47B,1145 (2008).

6. Hui-Ling Liu, Zongcheng Li, Thorleif Anthonsen, "Synthesis and fungicidal activity of 2-Imino-3-(4-arylthiazole-2-yl)-thiazolidine-4-ones and their 5-Arylidine Derivatives", *Molecules*, 5, 1055 (2000).
7. V. Murugan, K. M. Geetha, M. S. Sandhyavali M S, and S. Badami, "Synthesis and Antioxidant activity of S-[bis-2-(Chloroethyl)-amino]-4-substituted-6-phenyl Pyrimidine-2-yl-ethanethioate derivative with Nitrogen Mustards", *Asian J. Chem.*, 20, 5166 (2008).
8. Anni Shirwaikar, H. N. Aswath Ram, P. Mohaptra, "Antioxidant and Antiulcer activity of aqueous extract of a polyharbal formulation", *Indian J. of Experimental Biology*, 44(6), 474 (2006).
9. G. K. Jayaprakasha, L. J. Rao, K. K. Sakariah, "Antioxidant activity of flavidin in different in-vitro model systems", *Bioorganic and Medicinal Chemistry*,12, 5141 (2004).
10. M.J. Edmondson, S. L. Armstrong, A. Martinez, "A rapid and simple MTT-based spectrophotometer assay for the determining drug sensitivity in monolayer culture", *Tissue culture method*,11, 15 (1988).
11. Rathina Sabapathy Thirumurugan, S. Kavimani, and Radhey Shyam Srivastava, "Antitumor Activity of Rhinacanthone against Dalton's Asitic Lymphoma", *Biol. Pharma. Bull.*, 23(12)1438 (2000).
12. S. Kavimani, K.T. Manisenthilkumar, R. Ilango, and G. Krishnamoorthy, "Effect of the Methanolic Extract of Glinus Lotoides on Dalton's Ascitic Lymphoma", *Biol. Pharm. Bull.*,22(11),1251 (1999).