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Synthesis of 2-[(Bis-(2-chloroethyl)amino)acetamido]-5-substituted-1,3,4-thiadiazole as Possible Alkylating Anticancer Agents

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A few series of 2-[(bis-(2-chloroethyl)amino)acetamido]-5-substituted-1,3,4-thiadiazole have been synthesized by chlorination of 2-[(bis-(2-hydroxyethyl)amino)acetamido]-5-substituted-1,3,4-thiadiazole with phosphorous oxychloride and phosphorous pentachloride. The synthesized compounds containing nitrogen mustards were screened for their anticancerous activity by short term *in vitro* and *in vivo* anticancer activity by body weight analysis, mean survival time and percentage increase in life span method using Swiss albino mice bearing Dalton's Lymphoma ascites (DLA) 1×10^6 cells/mL. The structures of the synthesized compounds were confirmed by spectral analysis (IR, NMR and mass). Investigation of anticancer activity was done by using DLA cell line. Only compound E showed significant activity in the antitumour screening when compared with the control, *i.e.*, mice treated with CMC.

Key Words: Synthesis, Anticancer activity, Substituted 1,3,4-thiadiazole, Dalton's Lymphoma ascites cell line.

INTRODUCTION

1,3,4-Thiadiazole is a versatile lead molecule for their wide variety of biological activities. A few of them, which are worthy of mention, are diuretic[1], CNS depressants[2], hypoglycemic[3], antimicrobial[4], anthelmintics[5], antiviral[6] and anticancer[7–9] activities. The objective of the study was to synthesize series of 2-[(bis-(2-chloroethyl)amino)acetamido]-5-substituted-1,3,4-thiadiazoles **(Scheme-1)** and screened for their possible anticancer activity against DLA cells.

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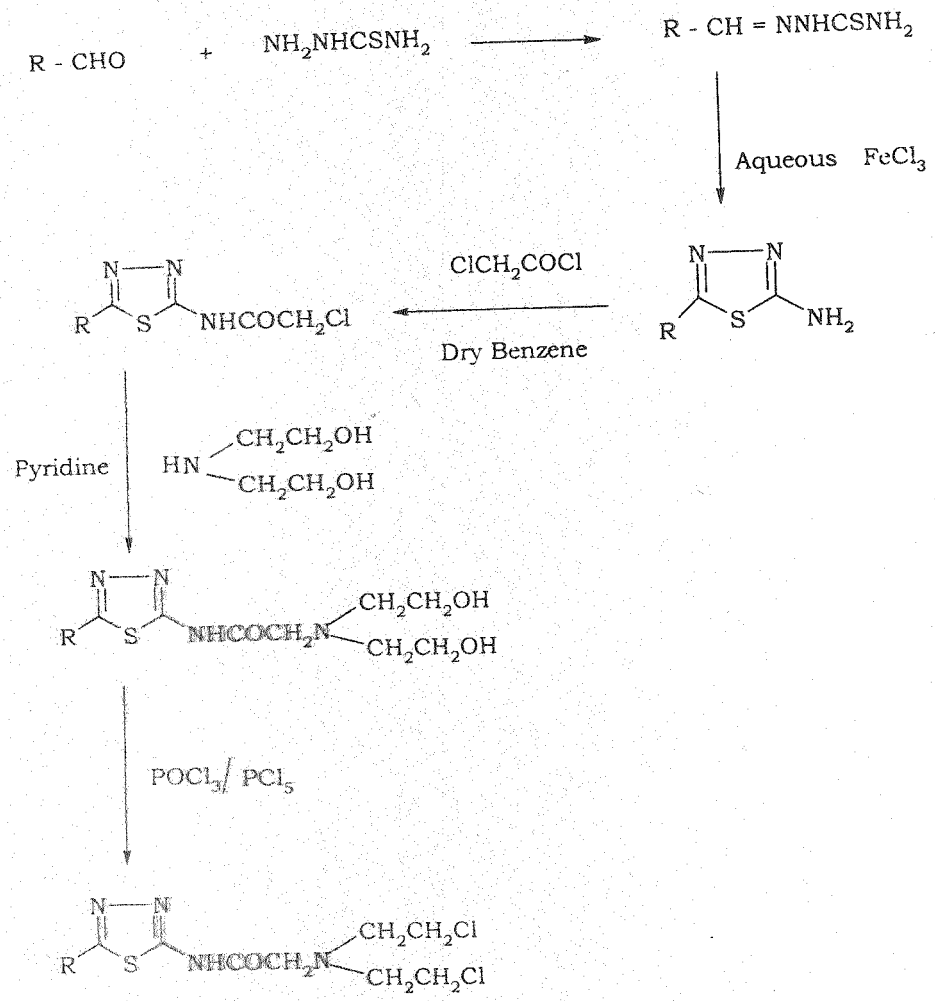


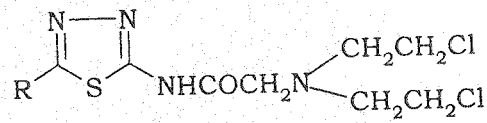
R = C_6H_5, C_6H_4OH, C_6H_4Cl, $C_6H_4NO_2$, $C_6H_4N(CH_3)_2$ and C_4H_3S

(Scheme-1)

EXPERIMENTAL

Aldehydes reacts with thiosemicarbazide to form the corresponding aldehyde thiosemicarbazones followed by oxidative cyclization using ferric chloride as oxiding agent to form 2-amino-5-aryl-1,3,4-thiadiazole. This was then reacted with chloroacetyl chloride in presence of dry benzene to form 2-amino-5-substituted-1,3,4-thiadiazoles followed by refluxing with diethanolamine in pyridine to form 2-[(bis-(2-hydroxyethyl)amino)acetamido)]-5-substituted-1,3,4-thiadiazole. The later was treated with phosphorus oxychloride in presence of phosphorus pentachloride to form 2-[(bis-(2-chloroethyl)amino)acetamido)]-5-substituted-1,3,4-thiadiazole (Table-1).

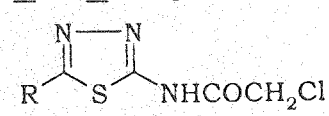
TABLE-1
PHYSICAL PROPERTIES OF 2-[(BIS-(2-CHLOROETHYL)AMINO)ACETAMIDO]-5-SUBSTITUTED-1,3,4-THIADIAZOLE DERIVATIVE



Compd. No.	R	m.w.	m.p. (°C)	Yield (%)	R_f value
A	Phenyl	358	240–243	69	0.77
B	2-Hydroxyphenyl	374	225–228	57	0.65
C	4-Dimethylaminophenyl	401	170–172	61	0.93
D	4-Hydroxyophenyl	374	215–218	74	0.84
E	4-Chlorophenyl	392	280–285	79	0.73
F	3-Nitrophenyl	389	228–230	72	0.63
G	Thiophene	364	195–200	71	0.68

Melting points were determined by Veego VMP-1 melting point apparatus and Labinda digital melting point apparatus in °C and are uncorrected. The purity was checked by TLC using silica gel-G as stationary phase and ethyl acetate : methanol as mobile phase. The structures of the synthesized compound were elucidated by using Perkin-Elmer Infrared-283 spectrophotometer in KBr phase; ^{1}H NMR spectra were taken on AMX-400 MHX spectrophotometer and mass spectra were recorded on Shimadzu 2010A LC-MS system.

Synthesis of thiosemicarbazones (I), 2-amino-5-substituted-1,3,4-thiadiazole (II) and 2-chloroacetamido-5-substituted-1,3,4-thiadiazoles (III)[10]: 2-Amino-5-aryl-1,3,4-thiadiazole (II, 0.01 mol) and chloroacetyl chloride (0.012 mol) were heated under reflux in dry benzene (20 mL) for 3 h on a steam bath. Benzene was distilled as far as possible and cooled. The product resulted was filtered and washed with small portions of cold water and recrystallized from suitable solvents to get a crystalline solid. IR (KBr, cm^{-1}): 3172 ν(N—H), 3062 ν(aryl C—H str.), 1616 ν(C=O), 1586 ν(C=N), 737 ν(C—S—C) and 705 ν(C—Cl). ^{1}H NMR (DMSO, δ, ppm): 1.3 (s, 1H, NHCO), 1.8 (s, 2H, CH_2Cl) and 7.3 (m, 3H, S—CH—CH—CH, thiophene, compound G).



Synthesis of 2-[(Bis-(2-hydroxyethyl)amino)acetamido)]-5-substituted-1,3,4-thiadiazole[11] (IV): Compound **III** (0.01 mol) and diethanolamine (0.015 mol) in pyridine (20 mL) was refluxed for 3 h over a gentle flame. The excess pyridine was distilled off as far as possible and the residue was poured into a little crushed ice containing few drops of hydrochloric acid with stirring. It was kept aside for overnight and the product resulted was filtered and washed with small portions of cold water and dried. It was recrystallized from appropriate

solvent to get pure compound. IR (KBr, cm^{-1}): 3283 ν(O═H), 3155 ν(N—H), 1616 ν(C═O), 1579 ν(C═N) and 738 ν(C—S—C). ^1H NMR (DMSO, in δ, ppm): 1.3 (s, 1H, NHCO), 1.7 (s, 2H, CH_2N), 3.35 [(t, 4H, CH_2—N—$(CH_2CH_2OH)_2$], 3.4 [(t, 4H, CH_2—N—$(CH_2CH_2OH)_2$], 5.6 (br, s, 4H, D_2O exchangeable 2 × OH) and 7.2 (m, 3H, S—CH—CH—CH, thiophene, compound G).

Synthesis of 2-[(Bis-(2-chloroethyl)amino)acetamido)]-5-substituted-1,3,4-thiadiazole[11] (V): Phosphorous oxychloride (20 mL) and a pinch of phosphorous pentachloride was added dropwise to 2-[(bis-(2-chlorohydroxyethyl)-amino)acetamido]-5-substituted-1,3,4-thiadiazole (0.01 mol) while being cooled in ice and the mixture was allowed to warm slowly to room temperature and then heated under reflux for 1 h. The excess phosphorous oxychloride was evaporated in vacuum and the viscous residue decomposed by addition of crushed ice. The product was filtered, washed with cold water, dried and finally purified by re-crystallized from aqueous ethanol. IR (KRr, cm^{-1}): 3104 ν(N—H), 7111 ν(C═O), 1620 ν(C═N), 775 ν(C—S—C) and 745 ν(C—Cl). ^1H NMR (DMSO, δ, ppm): 1.3 (s, 1H, NHCO), 1.7 (s, 2H, CH_2N), 3.32 [(t, 4H, N—$(CH_2CH_2Cl)_2$], 3.73 [(t, 4H, N—$(CH_2CH_2Cl)_2$] and 7.2 (m, 3H, S—CH—CH—CH, thiophene, compound G). Ms (m/z %): 366 (M + 2, 33%), 296 (18%), 250 (12%), 237 (68%) and 177 (47%).

Anticancer Screening

In vitro short term anticancer activity was checked by using DLA cells[12]. The cells (1×10^6 cells/mL) were exposed to different concentrations (125–1000 μg/mL) of test sample for 3 h at 37°C. At the end of 3 h the cell viability was determined by tryphan blue exclusion method.

From this, the percentage cytotoxicity can be calculated using the following formula:

$$\text{Percentage cytotoxicity} = \frac{100 - (\text{Total cells} - \text{Dead cells})}{\text{Total cells}} \times 100$$

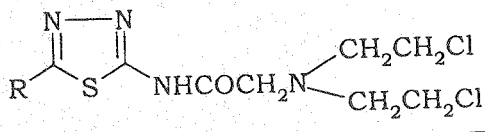
The percentage cytotoxicity was recorded in Table-2.

Acute toxicity and gross behavioural studies[13]: The acute oral toxicity study for the test compounds was carried out by following the OECD guidelines No. 420. In brief, Swiss albino female mice weighing 25–30 g were used for the evaluation. Each group consisting of 3 female mice (overnight fasted) was kept in the colony cage at 25 ± 2°C with 55% relative humidity and 12 h light/dark cycle was maintained. A specified fixed dose of 1000 mg/kg was selected and administered orally as a single dose as fine suspension prepared in 0.3% w/v carboxyl methyl cellulose (CMC).

The acute toxic symptoms and the behavioural changes produced by the test compounds were observed continuously for 4 h and at 8th, 12th, 24th h and onset of toxic symptoms and gross behavioural changes were also recorded.

For the delayed onset of toxic signs and symptoms, the mice from all the test compounds treated groups were monitored for 14 d. Further, no mortality rate was observed and hence all the mice were used for *in vivo* anticancer screening.

TABLE-2
SHORT TERM *IN VITRO* ANTITUMOUR ACTIVITY IN DLA CELLS
(1×10^6 cells/mL)



Compd. No.	R	% Growth inhibition of drug (various concentrations on DLA cells in μg/mL)				CTC_{50} (μg/mL)
		1000	500	250	125	
A	Phenyl	81.32	72.56	63.28	42.85	170.0
B	2-Hydroxyphenyl	85.71	65.00	50.23	33.03	250.0
C	4-Dimethylaminophenyl	78.94	69.85	59.18	21.07	212.0
D	4-Hydroxyphenyl	78.94	64.28	57.14	43.25	187.5
E	4-Chlorophenyl	92.85	91.04	88.31	44.12	138.0
F	3-Nitroophenyl	78.57	64.28	59.85	47.21	150.0
G	T hiophene	92.85	78.94	62.28	43.62	148.5

Dalton's Lymphoma ascites tumour model[14]: Healthy adult Swiss mice weighing 20–30 g were used. The antitumour activity of the test compounds was determined by an ascites tumour model in mice (Kuttan *et al.*, 1990). DLA cells were propagated in Swiss albino mice by injecting 1×10^6 cells intraperitoneally. The cells were aspirated aseptically from the developed tumour during the log phase of the 11th day of tumour transplantation by withdrawing the fluid from intraperitoneal cavity.

The ascitic fluid was washed 3 times with phosphate buffer saline (PBS) by centrifugation at 300–400 rpm. The supernatant liquid was discarded and cells were diluted with normal saline and the tumour cell count was done using tryphan blue dye exclusion methods using a haemocytometer. The cell suspension was diluted to get 1×10^6 cells in 0.1 mL of PBS. The tumor cells were injected into the peritoneal cavity of all the animals and treatment was started 24 h after the tumour inoculation (once daily) for 10 d as described below.

The mice were divided into 6 groups with five animals in each group as follows:

Group (I): Served as solvent control and received 0.3% CMC suspension.

Group (II): Served as positive control and treated with cyclophosphamide[11] (27.3 mg/kg body weight).

Groups (III–VI): Served as test groups and were treated with test compounds (A, E, F and G) as a single dose (100 mg/kg body weight) by oral route, once daily for 10 d.

During the course of anticancer study, the animals were subjected to the following screening methods:

Body weight analysis[15]: The body weight of all mice was weighed daily, after tumour inoculation. Average gain in body weight was determined and recorded in Table-3 and % decrease in body weight was calculated by the formula:

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

TABLE-3
EFFECT OF TEST COMPOUNDS ON BODY WEIGHT OF MICE, INOCULATED WITH DLA CELLS (1×10^6 cells/mL)

Group	Treatment	Dose (mg/kg)	Body weight			Decrease body weight from solvent control	% Decrease in body weight
			11th d	20th d	30th d		
I	CMC	100	26.21	30.11	33.88	—	—
II	Cyclophosphamide	27.3	26.83	30.66	25.64	8.24	24.32
III	Compound A	100	29.30	33.49	29.60	4.28	12.63
IV	Compound E	100	27.16	30.70	26.34	7.54	22.25
V	Compound F	100	28.52	34.85	30.71	3.71	10.95
VI	Compound G	100	30.50	32.88	28.28	5.60	16.52

Drugs treated with 100 mg/kg were compared with control.

Determination of mean survival time (MST)[16]: The surviving times of DLA tumor-bearing mice were noted and mean survival time (MST) was calculated. The mean survival time was recorded in Table-4.

Determination of percentage increase in life span (% ILS): From the result of mean survival time, percentage increase in life span was calculated by the following formula and recorded in Table-4.

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

TABLE-4
EFFECT OF TEST COMPOUNDS ON MEAN SURVIVAL TIME AND % INCREASE IN LIFE SPAN OF MICE INOCULATED WITH DLA CELLS (1×10^6 cells/ mL)

Group	R	Dose (mg/kg)	Mean survival time in days	% ILS
CMC	—	100	21	—
Cyclophosphamide	—	100	29 ± 0.11‡	38.09
Compound A	Phenyl	100	22 ± 0.26	4.76
Compound E	4-Chlorophenyl	100	26 ± 0.33‡	23.80
Compound F	3-Nitrophenyl	100	24 ± 0.71†	14.28
Compound G	Thiophene	100	25 ± 0.13†	19.04

Data expressed as mean ± SEM of five animals.
Drugs treated with 100 mg/kg were compared with control.
Statistical analysis were performed by student 'T' test.
‡$p < 0.001$, †$p < 0.01$, *$p < 0.05$.

RESULTS AND DISCUSSION

In comparison with cyclophosphamide, employed as the reference standard in this investigation, the compound E considerably favoured the percentage decrease in body weight of the carcinoma induced mice, increase in the MST and also good % ILS when compared with the control, *i.e.*, mice treated with CMC.

Conclusion

All of the test compounds are safe up to a dose of 1000 mg/kg. It could be concluded from the present investigation that compound E (2-[(bis-(2-chloro-ethyl)amino)acetamido]-5-(4-chlorophenyl)-1,3,4-thiadiazole is the most potent antitumour compound by both *in vitro* and *in vivo* screening and the remaining compounds showed moderate activity.

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