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Synthesis, Anticancer activity of some 1- (Bis N, N-(ChloroEthyl) - Amino Acetyl)-3, 5- Disubstituted 1, 2-Pyrazolines

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ABSTRACT: Interesting pharmacological properties exhibited by 1, 2 pyrazolines prompted us to develop a simple method to synthesize a few nitrogen mustard containing the above heterocyclic nucleus to evaluate for their possible anticancer activity. The title compounds were synthesized according to the standard procedures. The synthesized compounds were screened for their anticancerous activity by short term in vitro antitumor activity and in vivo anticancer activity by body weight analysis, Mean survival time and percentage increase in life span methods in Swiss albino mice bearing DLA $1x10^6$ cells/ml. The structure of the synthesized compounds was confirmed by spectral analysis (IR, NMR and Mass). Investigation of anticancer activity was done by using Daltons Lymphoma Ascites (DLA) cell line. It has been concluded that 1- (Bis-N, N-(chloroethyl) - amino acetyl)-3,5-disubstituted-1,2-pyrazoline showed potent anticancer activity which have undergone *invivo* screening.

Key words: Synthesis, Anticancer activity, 1, 2-Pyrazolines, Daltons Lymphoma Ascites (DLA) cell line.

INTRODUCTION

Alkylating agents have been found no doubt as potent anticancer agents. Nitrogen mustards are still playing a role in the chemotherapy of cancer in spite of newer chemotherapeutic agents. The capacity of these drugs to interfere with DNA integrity and function in rapidly proliferating tissues provides the basis of their therapeutic application. 1, 2-Pyrazolines is a versatile lead molecule for wide variety of biological activities. A few of them, which are worthy of mention are antitumor[1], antiangiogenic[2], antiproliferative[3], cytotoxic and DNA binding[4], antiviral[5], antitubercular[6], antibacterial[6] and antifungal[6] activities. The objective of the study was to synthesize series of 1- (Bis-N, N-(chloroethyl) - amino acetyl)-3,5-disubstituted-1, 2-Pyrazoline **(scheme I)** and the synthesized compounds were screened for anticancer activity against DLA cell line.

SYNTHESIS

The substituted acetophenone react with substituted benzaldehyde in cold condition, to get chalcone which was further heated to reflux with hydrazine hydride in ethanol to form substituted pyrazoline. Then it was reacted with chloroacetyl chloride in presence of dry benzene to form chloroacetyl derivative. The chloroacetyl derivatives was refluxed with diethanolamine in pyridine to get 1- (Bis-N, N-(hydroxy ethyl) - amino acetyl) -3,5-disubstituted-1, 2-Pyrazolines. The later was treated with phosphorus oxychloride in presence of phosphorus pentachloride to form 1- (Bis-N, N-(chloroethyl) - amino acetyl)-3,5-disubstituted-1, 2-Pyrazolines (Table **1**).

ANTICANCER SCREENING

Short term study for *in vitro* anti tumor activity[7,8]

Daltons Lymphoma Ascites (DLA) cells, Drug dilutions, Phosphate buffer saline solutions, Haemocytometer and Tryphan blue solution (0.4%). Cells were collected, counted and adjusted to $1x\ 10^6$ cells/ml. The drug dilutions were made with phosphate buffer saline and the drug dilutions were further adjusted to required concentrations. The drug dilutions were then added to the DLA cells and incubated at 37 ^{0}C for 3 hours. At the end of 3 hours, tryphan blue dye exclusion test was performed and percentage viability was calculated. From this, percentage cytotoxicity was calculated and recorded in Table **2.**

In Vivo Anticancer Screening[9]

Healthy adult Swiss mice weighing 20 - 30 g were obtained from J.S.S. College of Pharmacy Animal House, Ootacamund. The animal house was well ventilated and animals had $\pm$ 12 hour day and night schedule with temperature between 11-20˚C. The animals were housed in large spacious hygienic cages during the

course of experimental period. The animals were fed with rat pellet feed and water supplied by M/s Hindustan Lever Limited, Bangalore, India. The experiments were performed as per the recommendations of CPCSEA, Chennai. DLA (Dalton's Lymphoma Ascites tumor cells) were supplied by the department of Biotechnology, J.S.S. College of pharmacy, Ootacamund.

SCHEME

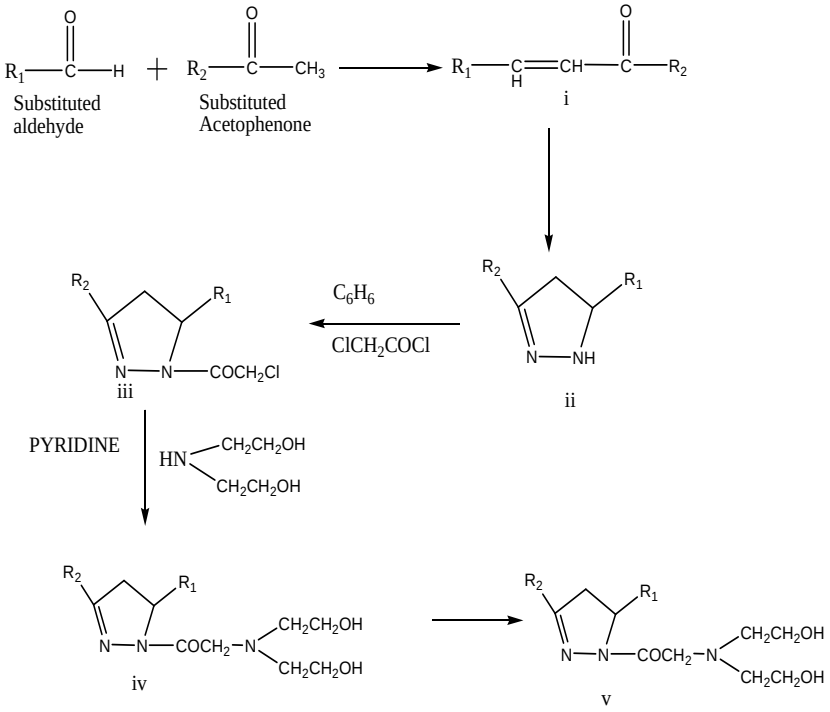

Table: 1 Physical properties of 1- (Bis-N, N-(chloroethyl) - amino acetyl)-3,5-substituted-1, 2-Pyrazoline

Compd. No.	Substituent		Molecular Formula	Molecular weight	Melting Range [^{0}C]		R_f Value
	R^1	R^2					
A	C_6H_5	C_6H_5	$C_{21}H_{23}N_3OCl_2$	403	265-268	65	0.73
B	C_6H_4Cl	C_6H_5	$C_{21}H_{22}N_3OCl_3$	437	215-218	52	0.54
C	$C_6H_4NO_2$	C_6H_5	$C_{21}H_{22}N_4O_3Cl_2$	448	190-192	57	0.86
D	C_4H_3O	C_6H_5	$C_{19}H_{21}N_3O_2Cl_2$	393	220-213	72	0.49
E	C_6H_4OH	C_6H_5	$C_{21}H_{23}N_3O_2Cl_2$	419	230-232	75	0.55
F	C_5H_4N	C_6H_5	$C_{20}H_{22}N_4OCl_2$	405	210-212	68	0.68

***TLC mobile phase : Methanol : Pet .ether (9: 1) * Solvent for Recrystallization: Ethanol**

Table: 2 Short term *in vitro* antitumor activity in DLA cells ($1x10^6$)

Compd No.	Substituent		% Growth Inhibition of Drug Various Concentration on DLA cells in µg/ml				CTC_{50} µg/ml
	R^1	R^2	1000	500	250	125	
A	C_6H_5	C_6H_5	81.32	72.56	63.28	42.85	170
B	C_6H_4Cl	C_6H_5	85.71	65.00	50.23	33.03	250
C	$C_6H_4NO_2$	C_6H_5	78.94	69.85	59.18	21.07	212
D	C_4H_3O	C_6H_5	78.94	64.28	57.14	43.25	187.5
E	C_6H_4OH	C_6H_5	92.85	91.04	88.31	44.12	138
F	C_5H_4N	C_6H_5	78.57	64.28	59.85	47.21	150

Table: 3 Effect of Test compounds on body weight of mice, inoculated with DLA cells $1x10^6$

Group	Treatment	Dose (mg/kg)	Body weight			Decrease in body weight from 11^{th} day to 20^{th} day	% Decrease in body weight
			0^{th} day	11^{th} day	20^{th} day		
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	29.3	33.49	29.60	4.28	12.63
IV	Compound D	100	27.16	30.70	26.34	3.71	10.95
V	Compound E	100	28.52	34.85	30.71	7.54	22.25
VI	Compound F	100	30.5	32.88	28.28	5.6	16.52

Data expressed as mean ± SEM of five animals
Drugs treated with100mg/kg were compared with control
#Carboxy methyl cellulose

Dalton's Lymphoma Ascites tumor model

The antitumor activity of the test compounds was determined by an ascites tumor model in mice by Kuttan, et al., (1990). Dalton's Lymphoma Ascites cells were propagated in Swiss albino mice by injecting $1x10^6$ cells intraperitoneally. The cells were aspirated aseptically from the developed tumor during the log phase of the 11^{th} day of tumor transplantation by withdrawing the fluid from intraperitoneal cavity. The ascitic fluid was washed 3 times with PBS (Phosphate buffer saline) by centrifugation at 300-400 rpm. The supernatant liquid was discarded and cells were diluted with normal saline and the tumor cell count was done using tryphan blue dye exclusion methods using a haemocytometer. The cell suspension was diluted to get $1x10^6$ 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 hours after the tumor inoculation (once daily) for 10 days as described below. The mice were divided into VI 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[10,11] (27.3 mg/kg body weight)

Group- (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 days. During the course of anticancer study, the animals were subjected to the following screening methods:

Body weight analysis[12]

All the mice were weighed daily, after tumor inoculation. Average gain in body weight was determined and recorded in Table **3** and % decrease in body weight was calculated.

Determination of Mean survival time (MST) [13]

The surviving time of DLA tumor-bearing mice was 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) [13]

Using mean survival time percentage increase in life span was calculated and recorded in Table **4**.

Table: 4 Effect of test compounds on mean survival time and % increase in life span of mice inoculated with DLA cells

Group	Dose (mg/kg)	Mean Survival Time in days	%ILS
CMC	100	21	------
Cyclophosphamide	100	29 ± 0.11	38.09
Compound A	100	22 ± 0.26	4.76
Compound D	100	25 ± 0.13	19.04
Compound E	100	26 ± 0.33	23.80
Compound F	100	24 ± 0.71	14.28

Data expressed as mean ± SEM of five animals

Drugs treated with 100mg/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 favored the percentage decrease in body weight of the carcinoma induced mice. Compounds D and E showed significant increase in the MST and also good % ILS when compared with the control, i.e., mice treated with CMC.

CONCLUSION

All of the present test compounds are safe up to a dose of 100mg/kg (Body weight on oral administration in experimental analysis). It could be concluded from the present investigation that 1- (Bis-N, N-(hydroxyethyl) - amino acetyl)-3-phenyl-5-(2-hydroxy phenyl)-1, 2-Pyrazoline is the most potent antitumor compound by both *in vitro* and *in vivo* screening. Similarly 1,2-pyrazoline nitrogen mustards with a phenyl and hydroxyphenyl group at second and fifth position is also found to be effective in inhibition of cancer growth.

EXPERIMENTAL DESIGN

Melting point were determined by Veego VMP-1 melting point apparatus and Labinda digital melting point apparatus in ^{0} C and are uncorrected. The purity was checked by TLC using silica gel G as stationary phase. The structures of the synthesized compound were elucidated by using Perkin Elmer Infrared – 283 spectrophotometer in KBR phase. ^{1}H – NMR spectra was taken on their AMX – 400 MHX spectrophotometer. Mass spectra were recorded on shimadzu 2010A LC–MS system.

Experimental Procedure

The requisite starting compounds 1, 2 and 3 were prepared by following the methods reported in the literature[14]

Synthesis of 1- (Bis-N, N-(hydroxyethyl) - amino acetyl)-3, 5-disubstituted-1, 2-Pyrazoline[15]

Compound 3 (0.01 mole) and diethanolamine (0.012 mole) in pyridine (20 ml) was refluxed for 3 hours 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.

The I*R* spectrum (KBr, in cm^{-1}) of the test compound A showed absorption band at: 3446 (OH), 1654 (C=O), 1584 (C=N) and 1019 (CH_2).

The ^{1}H-NMR spectrum (DMSO, in δ, ppm) of the compound A exhibited characteristic proton peaks at: 2.50 (s, 2H, C<u>H</u>$_2$N), 3.34 (s, 2H, C<u>H</u>$_2$), 4.46 (t, 4H, OH-C<u>H</u>$_2$.CH$_2$-N-CH$_2$C<u>H</u>$_2$OH), 5.06 (t, 4H, OH-CH$_2$.C<u>H</u>$_2$-N-C<u>H</u>$_2$CH$_2$OH), 6.04 (brs, 2H, D_2O exchangeable 2xOH), 7.29 (m, 4H, Ar-Phenyl) and 7.88 (m, 5H, Ar-Phenyl).

Synthesis of 1- (Bis-N, N-(chloroethyl) - amino acetyl)-3, 5-substituted-1, 2-Pyrazoline[15]

Phosphorous oxychloride (20 ml) and few drops of phosphorous pentachloride was added dropwise to 1-(Bis-N, N-(hydroxyethyl) - amino acetyl)-3,5-substituted-1, 2-Pyrazoline (0.01 mole) while being cooled in ice and the mixture was allowed to warm slowly to room temperature and then heated under reflux for one hour. 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 and dried. It was finally purified by recrystallization from a suitable solvent.

The I*R* spectrum (KBr, in cm^{-1}) of the test compound A showed absorption band at: 1667 (C=O), 1595 (C=N) and 828 (C-Cl).

The ^{1}H-NMR spectrum (DMSO, in δ, ppm) of the compound A exhibited characteristic proton peaks at: 2.33 (s, 2H, CH$_2$N), 3.40 (s, 2H, C<u>H</u>$_2$), 4.36 (t, 4H, Cl-C<u>H</u>$_2$.CH$_2$-N-CH$_2$C<u>H</u>$_2$Cl), 5.16 (t, 4H, Cl-CH$_2$.C<u>H</u>$_2$-N-C<u>H2</u>CH2Cl), 6.04 (brs, 2H, D_2O exchangeable 2xOH), 7.29 (m, 4H, Ar-Phenyl) and 7.88 (m, 5H, Ar-Phenyl).

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