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Synthesis of Quinazolinone Derivatives with Nitrogen Mustard as Possible Anticancer Agents

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A few series of 1-sustituted phenyl 2-[(*bis*-(2-chloroethyl)amino]-methyl-7-nitro-4(1*H*)-quinazolinone have been synthesized by chlorination of 1-sustituted phenyl 2-[(*bis*-(2-hydroxyethyl)amino]methyl-7-nitro-4(1*H*)-quinazolinone with phosphorous oxychloride and phosphorous penta chloride. All the 7 compounds were incorporated with nitrogen mustard moiety. 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 1×10^6 cells/mL. The structures of the synthesized compounds was confirmed by spectral analysis. Investigation of anticancer activity was done by using Daltons Lymphoma Ascites (DLA) cell line.

Key Words: Synthesis, Anticancer activity, Quinazolinone, Daltons Lymphoma Ascites (DLA) cell line.

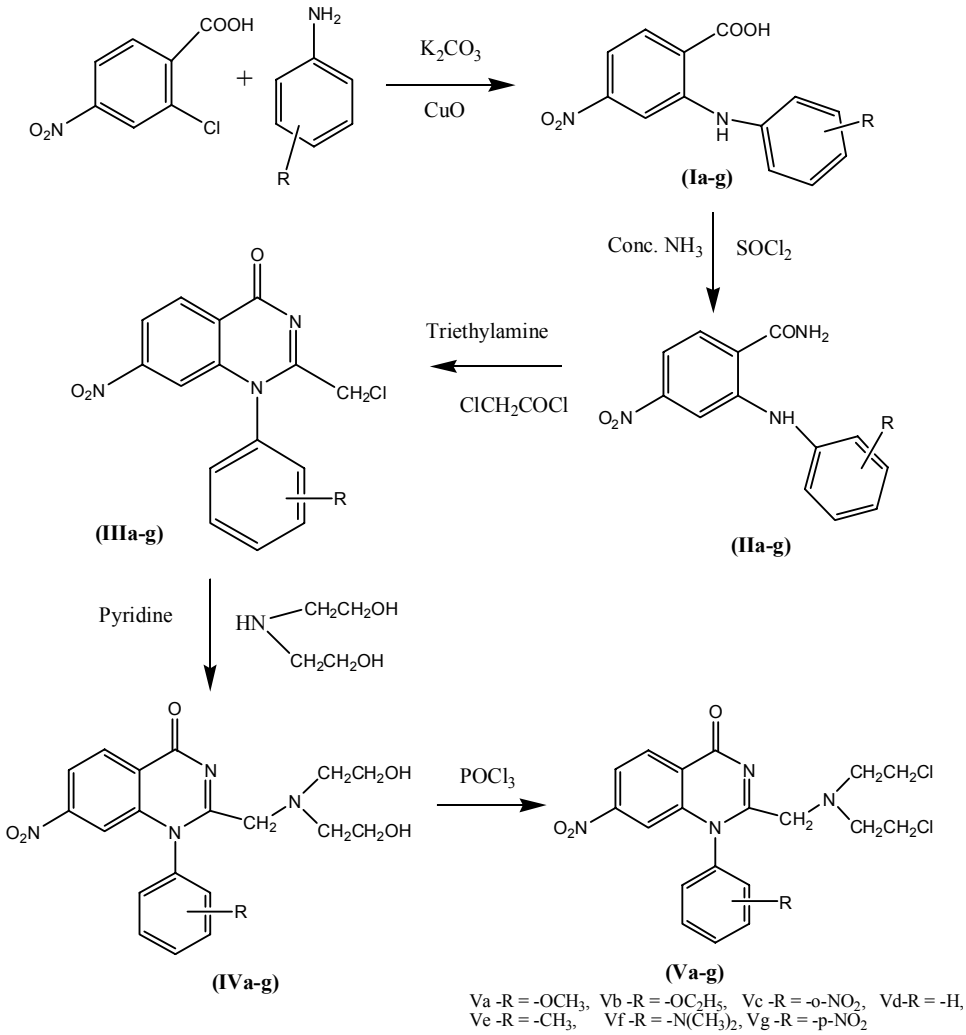
INTRODUCTION

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.

Quinazolinone is a versatile lead molecule for wide variety of biological activities. A few of them, which are worthy of mention, are antimicrobial, CNS acting agent[1], antiinflammatory[2], antiviral[3] and anticancer[4] activities. The objective of the study is to synthesize series of 1-subtituted phenyl 2-[(*bis*-(2-chloroethyl)amino]methyl-7-nitro-4(1*H*)-quinazolinone (**Scheme-I**) and the synthesized compounds were screened for anticancer activity against Daltons Lymphoma Ascites (DLA) cell line.

EXPERIMENTAL

Melting point was determined by Veego VMP-1 melting point apparatus and Labinda digital melting point apparatus in ºC and are uncorrected. The purity was checked by TLC using silica gel G as stationary phase. The structure of the synthesized



Scheme-I

compound was 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.

Synthetic procedure: The requisite starting compounds **Ia-g** was prepared by following the methods reported in the literature[5].

Synthesis of N-substituted phenyl anthranilamides (IIa-g): Compound **I** (0.28 mol) and thionyl chloride (0.03 mol) was refluxed gently for 0.5 h. Excess of thionyl chloride was distilled off. The residue of acid chloride was treated with concentrated ammonia solution (25 mL) and warmed. The residue obtained was cooled and dried. The compound was recrystallized from aqueous ethanol.

The IR spectrum (KBr, ν_{max}, cm^{-1}) of the test compound **IIa** showed absorption band at 3379 (NH_2), 3197 (NH), 1703 (C=O), 1603 (C=C) and 1537 (C-NO_2).

Synthesis of 1-substituted phenyl-2-chloromethyl-4 (1*H*)-7-nitro-quinazolinones (IIIa-g): A mixture of compound **II** (0.018 mol), chloroacetylchloride (0.02 mol), triethylamine (2 mL) in methanol (15 mL) was stirred for 1 h in ice bath. The mixture was then refluxed for 3 h and cooled, the crystals obtained were recrysallized from ethanol.

The IR spectrum (KBr, ν_{max}, cm^{-1}) of the test compound **IIIa** showed absorption band at 1690 (C=O), 1654 (C=N), 1610 (C=C), 1537 (C-NO_2) and 716 (C-Cl).

Synthesis of 1-subtituted phenyl-2-[(*bis*-(2-hydroxylethyl)amino]methyl-7-nitro-4(1*H*)-quinazolinone derivative (IVa-g): 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 spectrum (KBr, ν_{max}, cm^{-1}) of the test compound **IVa** showed absorption band at 3419 (OH), 1694 (C=O), 1654 (C=N), 1600 (C=C) and 1528 (C-NO_2).

^{1}H NMR spectrum (DMSO, δ ppm) of the compound **IVa** exhibited characteristic proton peaks at: 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, 4H, 2-OH) and 7.2 (m, 3H, S-CH-CH-CH, thiophene).

Synthesis 1-subtituted phenyl-2-[(*bis*-(2-chloroxylethyl)amino]methyl-7-nitro-4(1*H*)-quinazolinone derivative[6] (Va-g): Phosphorous oxychloride (20 mL) and few drops of phosphorous pentachloride was added drop wise to 2-[(*bis*-(2-hydroxyethyl)amino]methyl-7-nitro-4(1*H*)-quinazolinone (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 and dried. It was finally purified by recrystallization from a suitable solvent. The physical characteristics of the compounds are mentioned in Table-1.

IR spectrum (KBr, ν_{max}, cm^{-1}) of the test compound **Va** showed absorption band at 1682 (C=O), 1637 (C=N), 1597 (C=C), 1524 (C-NO_2) and 739 (C-Cl).

^{1}H NMR spectrum (DMSO, δ ppm) of the compound **Va** exhibited characteristic proton peaks at 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).

Short term study for *in vitro* antitumor activity[7] Daltons Lymphoma Ascites (DLA) cells were collected, counted and adjusted to 1×10^6 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 ºC for 3 h. At the end of 3 h, tryphan blue dye exclusion test was performed and percentage viability was calculated.

TABLE-1
PHYSICAL PROPERTIES OF SYNTHESIZED COMPOUNDS

Compd. No.	R	m.w.	m.p. (ºC)	Yield (%)	R_f value
Va	OCH_3	450	260-262	71	0.4
Vb	OC_2H_5	464	212-214	62	0.8
Vc	*o*-NO_2	465	210-211	42	0.5
Vd	H	420	240-241	60	0.7
Ve	CH_3	434	220-222	61	0.4
Vf	$N(CH_3)_2$	463	242-243	55	0.6
Vg	*p*-NO_2	465	262-264	48	0.7

***In vivo* anticancer screening[8]:** Healthy adult Swiss mice (20-30 g) was well ventilated and animals had + 12 h 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. The experiments were performed as per the recommendations of CPCSEA, Chennai.

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.*[11,12]. Dalton's Lymphoma Ascites cells were propagated in Swiss albino mice by injecting 1×10^6 cells intraperitoneally. The cells were aspirated aseptically from the developed tumor during the log phase of the 11th day of tumor transplantation by withdrawing the fluid from intraperitoneal cavity.

The ascitic fluid was washed 3 times with 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 1×10^6 cells in 0.1 mL of phosphate buffer saline. The tumor cells were injected into the peritoneal cavity of all the animals and treatment was started 24 h after the tumor inoculation (once daily) for 10 d as described below.

The mice were divided into VI groups with 5 animals in each group as follows: Group-(I): Solvent control and received 0.3 % CMC suspension. Group-(II): Positive control and treated with cyclophosphamide[9] (27.3 mg/kg body wt.). Group- (III-VI): Test groups and were treated with test compounds (**Va** to **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: Determination of body weight analysis[10], mean survival time (MST) and percentage increase in life span (% ILS)[11].

All the mice were weighed daily, after tumor inoculation. Average gain in body weight was determined and recorded in Table-2 and % decrease in body weight was calculated. The surviving time of DLA tumour-bearing mice was noted and mean survival time (MST) was calculated. Using mean survival time percentage increase in life span was calculated and recorded in Table-3.

TABLE-2
EFFECT OF TEST COMPOUNDS ON BODY WEIGHT OF MICE, INOCULATED WITH DLA CELLS 1×10^6

Group	Treatment	Dose (mg/kg)	Body weight			Decrease in body wt from 11th day to 20th day	% Decrease in body weight
			0th day	11th day	20th day		
I	Carboxy methyl cellulose	10 mL/kg	26.60	33.80	37.66	–	–
II	Cyclophosphamide	27.3	21.40	36.20	33.60	5.29	16.29
III	**Va**	50.0	20.50	35.50	33.50	3.67	10.33
IV	**Vb**	50.0	19.50	34.50	33.25	1.25	3.62
V	**Vc**	50.0	20.06	35.66	33.24	3.16	8.86
VI	**Vd**	50.0	31.16	36.33	34.66	4.5	12.38
VI	**Ve**	50.0	21.50	36.50	34.50	20	5.47

Data expressed as mean ± SEM of five animals.
Drugs treated with100mg/kg were compared with control.

TABLE-3
EFFECT OF TEST COMPOUNDS ON MEAN SURVIVAL TIME AND % INCREASE IN LIFE SPAN OF MICE INOCULATED WITH DLA CELLS (1×10^6)

Group	Dose (mg/kg)	Mean survival time (d)	% ILS
Carboxy methyl cellulose	10mL/kg	18.0 ± 0.70	–
Cyclophosphamide	27.3	28.2 ± 0.73	56.66
Va	50	25.6 ± 0.33	42.59
Vd	50	20.1 ± 0.42	13.88
Ve	50	24.5 ± 0.34	36.11
Vf	50	26.1 ± 0.42	45.33
Vg	50	21.3 ± 0.36	18.51

Data expressed as mean ± SEM of five animals.
Drugs treated with 100 mg/kg were compared with control.

RESULTS AND DISCUSSION

In comparison with reference standard in this investigation, the compound **Vf** considerably favoured the percentage decrease in body weight of the carcinoma-induced mice. Compounds **Va** and **Vg** showed significant increase in the mean survival time (MST) and also good % ILS when compared with the control, *i.e.*, mice treated with carboxy methyl cellulose (CMC).

All of the present test compounds are safe up to a dose of 100 mg/kg Body weight on oral administration. The synthesized compounds have most potent anti-tumor activity by both *in vitro* and *in vivo* screening. Similarly quinazolinone nitrogen mustards with a nitro and chloro group is also found to be effective in inhibition of cancer growth.

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