Asian Journal of Chemistry Vol. 18, No. 2 (2006), 900–906

Synthesis of 2-[{Bis-(2-chloroethyl)amino}methyl]-6,8-dinitro-1-(4-substituted phenyl)-1H-quinazolin-4-one Derivatives as Possible Antineoplastic Agents

V. MURUGAN*, MANIJRI KULKARNI, R.M. ANAND, E.P. KUMAR†, B. SURESH† and V.M. REDDY‡

Department of Pharmaceutical Chemistry, J.S.S. College of Pharmacy
Rocklands, Post Box No. 20, Ootacumand-643 001, India
Tel: (91)(944)3045718; (91)(423)2445718; E-mail: murugan62@yahoo.com

Synthesis of six 2-{[bis-(2-chloroethyl)amino]methyl}-6,8-dinitro-1-(4-substituted)-1H-quinazoline-4-one derivatives is reported. The compounds contain nitro groups at positions 6 and 8 of quinazolinone moiety and a phenyl group at position 1. All the six 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. Out of six compounds studied, compound **IIIc** (nitro-derivatives) showed significant anticancer activity.

Key Words: **2-{[bis-(2-chloroethyl)amino]methyl}-6,8-dinitro-1-(4-substituted phenyl)-1H-quinazoline-4-one derivatives, Antineoplastic.**

INTRODUCTION

Alkylating agents have been found as potent anticancer agents. Nitrogen mustards are still playing a major 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 for their therapeutic application.

Quinazolinone derivatives exhibit a wide range of biological properties such as antibacterial[1], anthelmintic[2], CNS depressant[3], antitubular[4], analgesic[5] and fungicidal activities[6]. Recently, anticancer and cytotoxic activities of 2-substituted quinazoline-4(3H)-ones were reported[7]. In addition, some 2-alkyl-3-aryl quinazoline-4(3H)-ones were prepared and screened for their *in vitro* cytotoxic activity and *in vivo* anticancer activity[8].

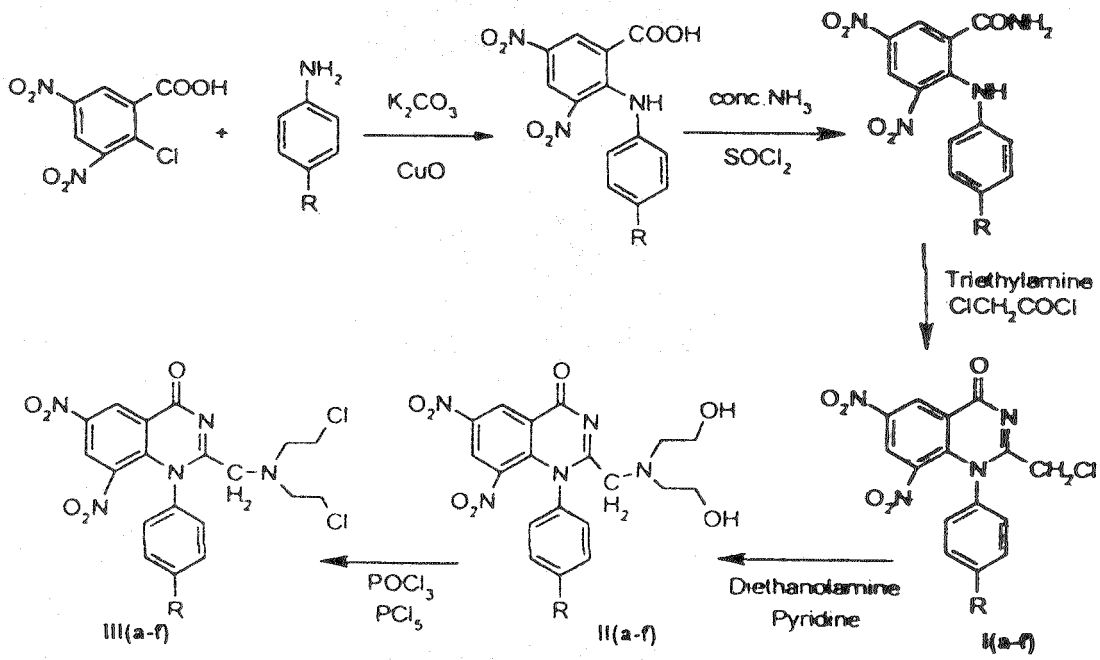
In continuation of these works, 1,6,8-trisubstituted quinazolinones with a nitrogen mustard moiety connected through a methylene group at position 2 have been synthesized in order to get better anticancer activity. All the synthesized compounds were screened for their anticancer activity.

Synthesis

Here, we describe the synthesis of six derivatives of 2-{[bis-(2-chloroethyl)amino]methyl}-6,8-dinitro-1-(4-substituted phenyl)-1H-quinazoline-

†Department of Pharmacology, J.S.S. College of Pharmacy, Rocklands, P.B. 20, Ootacamund-643 001, India.
‡Department of Pharmaceutical Chemistry, Kakathiya University, Warangal-506 009, India.

4-one (**Scheme-1**). 2-Chloro-6,8-dinitrobenzoic acid and substituted aniline were refluxed with anhydrous potassium carbonate and copper oxide to form N-substituted phenyl anthranilic acid. It was then reacted with thionyl chloride and concentrated ammonia to form N-substituted phenyl anthranilamide. This was cyclized with triethylamine in the presence of chloroacetyl chloride, which was further refluxed with diethanolamine in pyridine to form 2-[{bis-(2-hydroxyethyl)amino}methyl]-6,8-dinitro-1-(4-substituted phenyl)-1H-quinazolin-4-one derivative. The latter was treated with phosphorous oxychloride in presence of phosphorous pentachloride to form 2-[{bis-(2-chloroethyl)amino}methyl]-6,8-dinitro-1-(4-substituted phenyl)-1H-quinazoline-4-one derivative (Table-1).



Scheme-1

Anticancer Screening

Short-term *in vitro* Antitumour Activity in DLA Cells[9]

Requirements: Dalton's lymphoma ascites (DLA) cells, drug dilutions, phosphate buffer saline solutions, haemocytometer and tryphan blue (0.4%).

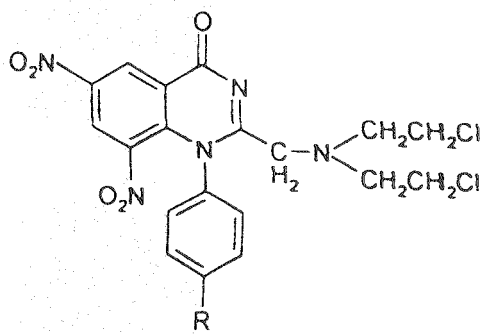
Method: The DLA cells were collected, counted and adjusted to 1×10^6 cells/mL. The drug dilutions were made with phosphate buffer saline and were further adjusted to concentrations ranging from 31.25–500 µg/mL. The drug dilutions were then added to the DLA cells and incubated at 37°C for 3 h. At the end of 3 h, tryphan blue dye exclusion test was performed, cells were counted using Olympus 1×70 inverted microscope, using haemocytometer.

The percentage cytotoxicity was calculated using the formula:

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

Perusal of Table-2 reveals that compound **IIIb** followed by compounds **IIIc** and **IIId** had shown very good cytotoxic activity with their CTC_{50} values 51, 61 and 65 µg/mL respectively. The compounds that are cytotoxic at low dose level are effective as cytotoxic agents.

TABLE-1
[{BIS-(2-CHLOROETHYL) AMINO} METHYL]-6, 8-DINITRO-1-(4-SUBSTITUTED PHENYL)-1H-QUINAZOLINE-4-ONE DERIVATIVES



Compound	Substituent (R)	m.w.	Yield (%)	R_f Value	m.p. (°C)	TLC mobile phase* (ratio)
IIIa	—H	466	76	0.45	260–262	1 : 2
IIIb	—Cl	500	80	0.67	220–223	1 : 2
IIIc	$-NO_2$	512	65	0.34	210–213	1 : 4
IIId	$-OCH_3$	496	74	0.24	200–202	1 : 3
IIIe	$-OC_2H_5$	510	71	0.34	205–207	2 : 5
IIIf	$-CH_3$	480	54	0.82	206–209	1 : 4

*Mobile phase used: ethyl acetate : methanol

TABLE-2
SHORT TERM *IN VITRO* ANTITUMOUR SCREENING IN DLA CELLS (1×10^6)

Compd.	Substituent (R)	% Cytotoxicity of drug at various concentrations on DLA cells (μg/mL)					[a]CTC_{50} (μg/mL)
		500	250	125	62.5	31.25	
IIIa	—H	65.33	55.13	48.33	45.16	38.51	201
IIIb	—Cl	79.14	71.12	68.20	62.70	45.32	51
IIIc	$-NO_2$	90.01	81.12	75.31	52.12	48.13	61
IIId	$-OCH_3$	76.10	65.20	53.40	49.13	41.02	65
IIIe	$-OC_2H_5$	59.10	50.03	41.20	38.41	31.10	250
IIIf	$-CH_3$	49.20	45.00	34.10	28.51	21.11	560

[a]Cytotoxic concentration.

Acute Toxicity and Gross Behavioural Studies[10]

The acute oral toxicity study for the test compounds was carried out by following the OECD guidelines No. 420. 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 carboxy methylcellulose (CMC). The acute

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

TABLE-3
EFFECT OF TEST COMPOUNDS ON % DECREASE IN BODY WEIGHT, MEAN SURVIVAL TIME (MST) AND % INCREASE IN LIFE SPAN (% ILS) OF MICE INOCULATED WITH DLA CELLS

Group	Compound	Dose	% Decrease in body weight	MST[a] + SEM[b]	% ILC[c]
I	Carboxy methyl cellulose	100	—	24 ± 0.36	—
II	Cyclophosphamide	27.3	16.32*	29 ± 0.36†	20.83
III	**IIIa**	100	13.18*	24.3 ± 0.36	1.25
IV	**IIIb**	100	13.88*	28 ± 0.36†	16.66
V	**IIIc**	100	11.21*	28 ± 0.36†	4.16
VI	**IIId**	100	12.10*	25 ± 0.36	2.08
VII	**IIIe**	100	7.63*	24.5 ± 0.76	

Statistics: t-test; † = $p < 0.01$, * = $p < 0.05$.
[a]Mean survival Time, [b]Standard error mean, [c]Percentage increase in life span.

The experimental studies revealed that all the categories of synthesized quinazolinone derivatives are quite safe up to 1000 mg/kg and no death of animals was recorded. Further, no significant gross behavioural changes were observed in experimental animals except in the compound **IIId**, which showed depression on the first day and recovered on second day.

In vivo Anticancer Screening[11, 12]

Synthesized quinazolinones were evaluated for their anticancerous activity by body weight analysis, mean survival time and percentage increase in life span at a dose of 100 mg/kg body weight in Swiss albino mice inoculated with DLA cells (1×10^6).

EXPERIMENTAL

Animals: Swiss albino mice, 7–9 weeks of age, weighing 20–30 g of either sex were used. They were housed in polypropylene cages and were given standard mouse pellet and water *ad libitum*.

Tumour cells: Dalton's lymphoma ascites cells were supplied by the Department of Pharmaceutical Biotechnology, J.S.S. College of Pharmacy, Ootacamund. Tumer cells were maintained and propagated intra-peritoneally by serial transplantation in adult Swiss albino mice.

Preparation of drug solution: The synthesized compounds **(IIIa–e)** were made into a suspension using 0.3% w/v carboxymethyl cellulose to get final concentration of 100 mg/mL.

Experimental design: The antitumour activity of the test compounds was determined by an ascites tumour model in mice. 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 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 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 method using a haemocytometer. The cell suspension was diluted to get 1×10^6 cells in 0.1 mL of phosphate buffer saline. The tumour 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 days as described below.

Control group mice bearing DLA administered with 0.3% carboxymethyl cellulose suspension.

Standard group mice bearing DLA treated with cyclophosphamide, 27.3 mg/kg body weight, once daily.

Number of animal in each group: five.

Route of Administration: oral.

Mode of treatment: Treatment started 24 h after inoculation of the tumour. Mice were treated with test compounds (**IIIa–e**) as a single dose 100 mg/kg body weight by oral route, once daily for 10 days.

Body weight analysis: After tumor inoculation, all the mice were weighed daily up to 10 days. Average gain in body weight was determined. By treating the mice with test compounds, decrease in body weight was calculated by the formula:

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

Mean survival time: The survival times of DLA tumour-bearing mice were noted and mean survival time (MST) was calculated.

Percentage increase in life span (% ILS): Using mean survival time, percentage increase in life span was calculated by the formula:

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

Chemistry

The melting points were recorded on the conventional melting point apparatus. Purity of the compounds was checked by TLC on ready made precoated TLC plates having silica gel F28 as adsorbent using methanol and ethyl acetate as mobile phase. IR spectra were recorded in KBr on a Perkin-Elmer Infrared-283 spectrophotometer as nujol and are expressed in cm^{-1}. ^1H NMR spectra were obtained on the AMX-400 liquid state spectrometer at 440 MHz in DMSO. Mass spectra were measured with an FAB mass spectrometer (LSIMS).

Synthesis of N-substituted phenyl anthranilic acid (General procedure)[13]: A mixture of 2-chloro-3,5-dinitrobenzoic acid (12.3 g, 0.05 M), aniline/substituted aniline (5 g, 0.05 M), anhydrous potassium carbonate (7 g) and copper oxide (0.5

g), was refluxed for 2 h using an oil bath. The excess of aniline was removed by distillation. Decolorizing carbon and water were added to the residual solution.

The mixture was boiled and filtered by suction. The filtrate was added with stirring to dilute HCl solution (10 mL). The precipitated product was filtered with suction when cold. The product was dried in air and recrystallized from acetone.

Synthesis of N-substituted phenyl anthranilamides (General procedure)[14]: A mixture of N-substituted phenyl anthranilic acid (0.28 M) and thionyl chloride (3.6 g, 0.03 M) was refluxed gently for 30 min. 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 acetone.

Synthesis of 2-chloromethyl-6,8-dinitro-1-substituted phenyl-4-(1H)-quinazolinones (General procedure)[15]: A mixture of N-phenyl anthranilamide (0.018 M), chloroacetyl chloride (2.3 g, 0.02 M), 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 recrystallized from acetone. IR (cm^{-1}): 1644 ν(C═O), 1610 ν(C═N), 1515 ν(C═C, aromatic), 1458 ν(C—NO_2), 726 ν(C—Cl). MS for compound **Ic**: m/z = 438 (M^+).

Synthesis of 2-[{bis-(2-hydroxyethyl)amino}methyl]-6,8-dinitro-1-(4-substituted phenyl)-1H-quinazoline-4-ones (General procedure)[16]: A mixture of 1-substituted-phenyl-2-chloromethyl-6,8-dinitro-4-(1H)-quinazolinones (0.1 M) and diethanolamine (0.15 M) in pyridine (20 mL) was refluxed for 3 h over a gentle flame. Pyridine was distilled off as far as possible and the residue was poured into a little crushed ice containing a few drops of dilute HCl with stirring. It was kept aside for overnight and the product resulted was filtered and washed with small portions of cold water. It was then recrystallized from a suitable solvent to get a pure compound. IR (cm^{-1}): 3310 ν(OH), 1644 ν(C═O), 1610 ν(C═N), 1592 ν(C═C), 1514 ν(C—NO_2). ^{1}H NMR: 7.02–9.15 δ (m, 6H, of Ar—H), 3.63 δ [m, 4H, of $(CH_2OH)_2$], 2.55 δ [br, s, 4H, of $(C—N—CH_2—CH_2)_2$], 2.4 δ (s, 2H, of $C—CH_2$) and 2.00 δ [s, 2H, of $CH_2OH)_2$]. MS for compound **IIc**: m/z = 475 (M + 1).

Synthesis of 2-[{Bis-(2-chloroethyl)amino}methyl]-6,8-dinitro-1-(4-substituted phenyl)-1H-quinazolin-4-ones (General procedure)[17]: Phosphorous oxychloride (5 mL) and a pinch of phosphorous pentachloride were added to 2-[{bis-(2-hydroxyethyl)amino}methyl]-1-substituted-6,8-dinitro quinazolinone (26 mM), while being cooled in ice and the mixture was allowed to warm slowly to room temperature. It was then heated under reflux for 1 h. Excess of phosphorous oxychloride was evaporated in vacuum and the viscous residue decomposed by addition of crushed ice. The product was then filtered and was finally purified by recrystallization using a suitable solvent. IR (cm^{-1}): 1644 ν(C═O), 1609 ν(C═N), 1543 ν(C═C, aromatic), 1462 ν(C—NO_2), 720 ν(C—Cl). ^{1}H NMR: 7.02–9.15 δ (m, 6H, of Ar—H), 3.44 δ [br, m, of 4H, $(CH_2CH_2Cl)_2$], 2.64 δ [br, m, 4H, of $(CH_2CH_2Cl)_2$] and 2.4 δ (s, 2H, of $C—CH_2$). MS for compound **IIIc**: m/z = 512 (M^+).

RESULTS AND DISCUSSION

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

Conclusion

We hereby conclude that the 1,6,8-trisubstituted quinazolinones with a nitrogen mustard moiety connected through a methylene group at position 2 are effective in mice bearing Dalton's lymphoma ascites. Further, it is concluded from the present investigation that the quinazolinon-2-methyl nitrogen mustard with either a nitro or a chloro group at *para-phenyl* position is a most potent anticancer compound, which can be further developed.

ACKNOWLEDGEMENT

The authors wish to place on record their heartfelt thanks to His Holiness Jagadguru Sri Shivarathri Deshikendra Mahaswamigalavaru of Suttur Mutt, Mysore.

REFERENCES

1. V.K. Srivastava, S. Singh, A. Gulati and K. Shankar, *Indian J. Chem.*, **26B**, 652 (1987).
2. D.P. Gupta, S. Ahmad, A. Kumar and K. Shankar, *Indian J. Chem.*, **27B**, 1060 (1988).
3. M.R. Chaurasia and A.K. Sharma, *J. Indian Chem. Soc.*, **62**, 308 (1985).
4. V. Joshi and R.P. Chaudari, *Indian J. Chem.*, **26B**, 602 (1987).
5. V.J. Ram, R.C. Srimal, D.S. Kushwaha and L.J. Mishra, *J. Prakt. Chem.*, **332**, 629 (1990).
6. N. Tiwari, B. Chaturvedi and Nizamuddin, *Indian J. Chem.*, **28B**, 200 (1989).
7. V. Murugan, C.C. Thomas, G.V.S. Rama Sarma, E.P. Kumar and B. Suresh, *Indian J. Pharm. Sci.*, **65**, 386 (2003).
8. V. Murugan, Apsara, E.P. Kumar, B. Suresh and V. Malla Reddy, *Indian J. Heterocycl. Chem.*, **14**, 67 (2004).
9. R. Kuttan, P. Bhanumathy, K. Nirmala and M.C. George, *Cancer Lett.*, **29**, 197 (1985).
10. U.K. Sheth, N.K. Dadkar and U.G. Kamat, Selected Topics in Experimental Pharmacology, 1st Edn., p. 124 (1972).
11. M.N. Gosh, Fundamentals of Experimental Pharmacology, 2nd Edn., Scientific Book Agency, Calcutta, p. 155 (1984).
12. V. Ramnath and R. Kuttan, *Amala Res. Bull.*, **20**, 3 (2000).
13. A.R. Sallmann, *Am. J. Med.*, **80**, 29 (1986).
14. P. Lee, J.A. Anderson, J. Miller, J. Webb and W. Buchman, *J. Rheumatol.*, **3**, 283 (1976).
15. J. Zuckner, *Am. J. Med.*, **80**, 39 (1986).
16. J.R. Caldwell, *Am. J. Med.*, **80**, 43 (1986).
17. M.S. Tute, Comprehensive Medicinal Chemistry, Vol. 4, Pergamon Press, New York, p. 99 (1990).

(*Received*: 7 March 2005; *Accepted*: 12 December 2005) AJC-4506