RESEARCH PAPER

Accepted 14 June 2003
Revised 2 May 2003
Received 18 April 2001
Indian J. Pharm. Sci., 2003, 65(4): 386-389

# Synthesis of 2-Substituted Quinazolin-4(3H)-ones as a New Class of Anticancer Agents

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**Anticancer and cytotoxic activities of four title compounds were determined. Among the compounds tested, 6,8-dibromo-2-[2-(styryl)]-4-quinazoline-3-(4-benzhydrazides), (IIIb) and 6,8-dibromo-2-[(phenylbutadienyl)ethylene]-4-quinazoline-3-(4-benzhydrazides) (IIId) showed significant activity. The compounds 6,8-dibromo-2-[2-(thiophene)-ethylene]-4-quinazoline-3-(4-benzhydrazides) (IIIa) and 6,8-dibromo-2-[(3-nitrostyryl)ethylene]-4-quinazoline-3-(4-benzhydrazides), (IIIc) were exhibited moderate activity. All the title compounds were characterized by analytical and spectral data.**

Different types of quinazolines exhibit a wide spectrum of biological activities including anthelmintic[1] antiinflammatory[2] anticonvulsant[3] hypoglycemic[4] antiperkinsonian[5] antitubercular[6] antibacterial[7] and anticancer[8]. Among the quinazolines, the 2-styrylquinazolin-4-(3H)-ones were reported as antimitotic anticancer agents, which inhibit tubulin polymerization[9]. Based on these findings, we attempted to synthesize the title compounds presuming that the incorporation of styryl moiety in 6,8-dibromo quinazolinone may impart prominent anticancer activity. 2-methyl–3,6,8-trisbstituted quinazolinone (I) prepared from 6,8-dibromo-3,1,4-benzoxazinone[11] was treated with various aromatic aldehydes in presence of glacial acetic acid to get different 2-styrylquinazoline-4-(3H)-ones (II), which were further treated with phenyl hydrazine to afford its 6,8-dibromo-2 substituted styryl-4-quinazoline-3(4-benzphenylhydrazides) (III, Scheme I) .

## MATERIALS AND METHODS

Melting points of the compounds were determined in the open capillary using melting point apparatus and are uncorrected. All the reactions were monitored by TLC using chloroform and methanol (1:1) as solvent on silica gel G plates and iodine as visualizing agent. The IR spectra of the compounds were recorded on a Perkin-Elmer FTIR spectrometer-1600 (KBr) and expressed in $cm^{-1}$ , while PMR were recorded on a Perkin-Elmer EM-390-90 MHz spectrometer using TMS as internal standard.

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### Preparation of 2-methyl-3, 6, 8-trisubstituted quinazolinone (I):

A mixture of 6, 8-dibromo-2-methyl-3,1-benzoxazin-4-one[12] (0.1 mol) and ethyl 4-amino benzoate (0.1 mol) was heated on an oil bath at 130-140° for 25 min. A jelly like mass, thus separated on cooling was treated ethanol resulting in the separation of a solid mass. This crude product was filtered, dried and recrystallized from ethanol. IR: 1710 (CO-N), 1685 ($COOC_2H_5$), 1664 (C=N) and 680 (C-Br) $cm^{-1}$.

### 2-Styryl/Substituted quinazoline-4-(3H)ones (II):

A mixture of quinazolinone (0.1 mol) and various aromatic aldehydes (0.12 mol) in 50ml of glacial acetic acid was stirred and refluxed for 12 h, cooled to 25°, ice water was poured into the solution, filtered and dried to get the final product, which was recrystallized from ethanol. IR: 3090 (Olefinic CH=CH str), 2950 (-Et str of ester), 1710 (CO-N str), 1680 (CO-OEt str), 1600 (C=N str), 1590-1575 (several bands, Aromatic C=C str), 1455 (-Et bend of ester), 1300(C-O-Et str of ester), 970 (Olefinic CH=CH bend), 845,760,740 (Aromatic CH bend) and 690 (C-Br) $cm^{-1}$. $^1H$-NMR ($CDCl_3$) δ ppm: 8.35 to 6.90 (m, 11 H, Ar-H), 6.53 (d, 1H, Olefinic-H, Ph-CH=C-), 5.10 (d, 1H, Olefinic-H,

quinazolinonyl-CH=C-), 4.45 (q, 2H, Methylene gr of-OEt), 1.80 (t, 3H, methyl group of –OEt).

**6, 8-dibromo-2-substituted styryl-4-quinazolone-3-(4-benzphenyl hydrazides) (III):**

A mixture of appropriate ester (0.0025 mol) in alcoholic KOH and 0.005 mol of phenyl hydrazine were refluxed on a water bath for 16-18 h the excess solvent was neutralized with dilute hydrochloric acid and the solids which separated were recrystallized from ethanol. IR: 3320 (NH str), 3075 (Olefinic CH=CH str), 1700 (CO-N str), 1675 (CO-NH str), 1620 (NH bend), 1600 (C=N str), 1598 to 1580 (Aromatic C=C str), 955 (Olefinic –CH=CH-bending), 849, 750 and 730 (Ar-CH bend) and 680 (C-Br) $cm^{-1}$. $^1H$-NMR ($CDCl_3$) δ ppm: 7.10 to 8.50 (m, 16H, Aromatic-H including CO-NH), 6.54 (d, 1H, Olefinic-H, Ph-CH=C-), 5.14 (d, 1H, Olefinic-H, quinazolinonyl CH=C-) and 4.15 (bs, 1H, Ph-NH-).

***In vitro* short term antitumor study[12]:**

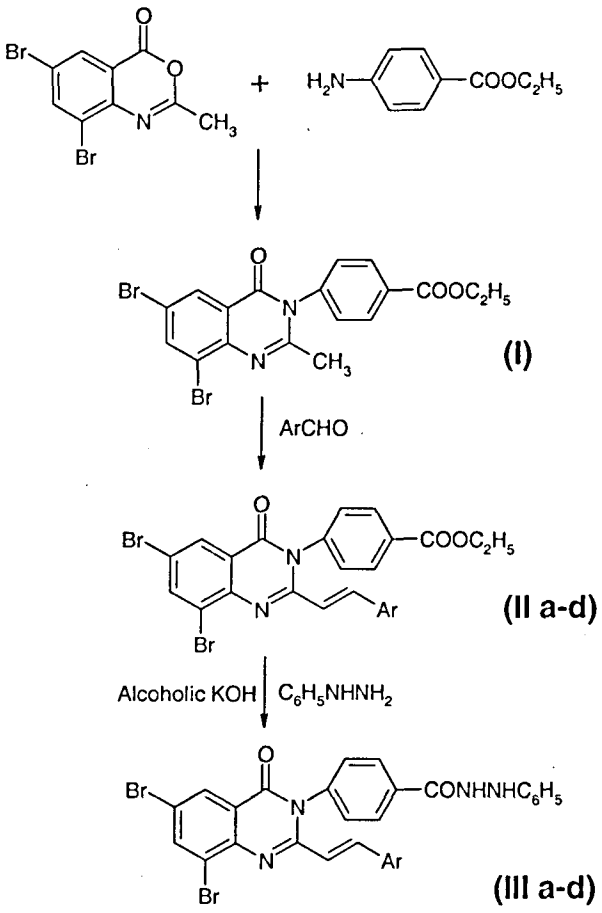
The synthesized compounds were added to the DLA cells and incubated at 37° for 3 h. At the end of 2 h, trypan blue dye exclusion test was performed and the percentage viability was calculated using the formula, % Viability=Total cells–Dead cells/Total cellsx100.

***In vivo* antitumor study[13]:**

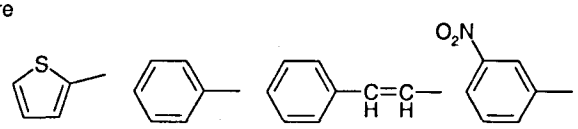
*In vivo* antitumor studies were performed in Swiss albino mice using DLA cells as cancer cell lines. All the test compounds were prepared as a fine suspension in 0.3%w/v CMC and administered orally at 50 mg/kg and 100 mg/kg doses. The DLA cells were propagated in the interaperitoneal cavity of the mice by injecting $1x10^6$ cells/ml. Treatment was started 24 h after tumor inoculation once daily for 10 d. The body weight of the individual mice were recorded at daily intervals and also increase in life span of mice was calculated from the formula, %ILS=T-C/Cx100, where, T is the number of days where the treated animals had survived and C is the number of days the controlled animals had survived. All the animal experiment protocols have met with the approval of the Institutional Animal Ethics Committee.

## RESULTS AND DISCUSSION

The results of *in vitro* short-term antitumor study showed that compound IIIa was cytotoxic at 250 μg/ml concentration and compound IIIb was cytotoxic below 250 μg/ml concentration, whereas, compound IIId was toxic below the concentration of 125 μg/ml. Compound IIIc showed cytotoxic activity at 250 μg/ml. Compound IIId was most toxic




Where

Ar = 


(IIIa) (IIIb) (IIIc) (IIId)

**Scheme 1:**

with 88.5% inhibition at concentration of 125 μg/ml. Hence, from the data it can be concluded that compound IIId was most toxic and had very good cytotoxic properties. In the order, next come IIIb followed by compound IIIc.

*In vivo* antitumor studies were carried out on DLA cells using Swiss albino mice as experimental animals. Increased in body weight and mean survival time (MST and percentage increase in life span, %ILS) of the experimental animals were taken as main parameters in the study. It was found that all the test compounds considerably opposed the average increase in the body weight of the carcinoma induced mice, which was comparable to the reference standard methotrexate, however, at hundred fold higher con-

centrations. Particularly, compounds IIId and IIIc were found to be superior in their action. A drastic increase in the body weight was noted in DLA tumor bearing mice. On treatment with IIId, a significant reduction in body weight was observed when compared with the positive control group on the day 15.

Treatment revealed that the MST as a maximum of 23 d for compound IIId in comparison with the untreated group. The % ILS of the treated groups (administered with a dose of 50 mg/kg) was 67%, for compound IIId and showed significant antitumor property. The above result indicated that compound IIId excellent in its antitumor activity, while com-

TABLE 1: PHYSICAL AND *IN VITRO* ANTICANCER ACTIVITY DATA OF 2-STYRYL QUINAZOLIN-4(3H)-ONES USING DLA CELLS.

| Compound No. | Substituents (Ar) | mp (°) | Concentration (μg/ml) | Mean total cells | Mean dead cells | % Viability |
|---|---|---|---|---|---|---|
| IIIa | $C_4H_3S$ | 218 | 500 | 50 | 41 | 18.0 |
| | | | 250 | 55 | 28 | 49.1 |
| | | | 125 | 51 | 17 | 66.7 |
| IIIb | $C_6H_5$ | 210 | 500 | 57 | 42 | 26.3 |
| | | | 250 | 50 | 35 | 30.0 |
| | | | 125 | 47 | 16 | 67.3 |
| IIIc | $C_8H_7$ | 229 | 500 | 49 | 42 | 14.3 |
| | | | 250 | 48 | 25 | 47.9 |
| | | | 125 | 54 | 19 | 64.8 |
| IIId | $C_6H_4O_2N$ | 220 | 500 | 42 | 37 | 11.9 |
| | | | 250 | 42 | 46 | 11.5 |
| | | | 125 | 54 | 45 | 16.7 |

All the title compounds were tested for their cytotoxic activity by *in vitro* short term anticancer study using DLA cells.

TABLE 2: EFFECT OF TEST COMPOUNDS ON MICE INOCULATED WITH DLA CELLS.

| Group | Drug and dose (mg/kg) | Increase in body weight | % increase in body weight | MST (d) | %ILS |
|---|---|---|---|---|---|
| I | CMC | 10.8±5.08 | 77.3 | 13.8±0.58 | - |
| II | Methotrexate (1.3) | 2.2±4.37** | 16.9 | 21.6±0.97** | 56.5 |
| III | IIIa (50) | 7.4±4.37** | 59.2 | 20.6±0.50** | 49.3 |
| IV | IIIb (50) | 4.0±3.85** | 26.8 | 21.2±1.62** | 53.6 |
| V | IIIc (50) | 5.4±5.66** | 33.6 | 19.2±0.58** | 39.1 |
| VI | IIId (50) | 3.4±2.57** | 21.4 | 23.0±0.94** | 66.7 |
| VII | IIIa (100) | 7.2±6.00** | 45.8 | 15.0±0.00** | 8.7 |
| VIII | IIIb (100) | 3.8±7.36** | 37.1 | 19.4±0.39** | 40.6 |
| IX | IIIc (100) | 8.0±2.80** | 51.1 | 19.8±0.80** | 43.5 |
| X | IIId (100) | 1.8±2.93** | 13.4 | 19.4±0.50** | 40.6 |

Values are Mean±SEM of five animals; **p<0.01 versus control group. The *in vivo* anticancer activity of the synthesized compounds have been evaluated against Dalton's lymphoma ascites cells in Swiss albino mice by injecting $1x10^6$ cells in the intraperitoneal cavity.

pound IIIb is next in the order of potency with 21 d MST and 53.6 % ILS. Compound IIIa comes next in the order followed by IIIc.

Even certain other parameters like the significance of difference between survival time of control and groups treated with reference standard and the test compounds were also taken into consideration to evaluate the anticancer profile of the test compounds and these data were also in agreement with the data given in Table 2.

A regular rapid increase in ascites tumor volume was noted in tumor bearing mice (increase in body weight). Ascites fluid is a direct nutritional source for tumor cells and the faster increase in ascites fluid with tumor growth would possibly be a mean to meet more nutritional requirement of tumor cells. The test compounds increase the MST and lowered the ascites fluid volume (decrease in body weight) to a considerable extent.


## ACKNOWLEDGMENTS

The authors wish to thank His Holiness Jagadguru Sri Sri Shivarathri Deshikendra Mahaswamigalavaru, Sri Suttur Mutt, Mysore, for providing facilities for research work.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