# SYNTHESIS OF SOME NEW 7-NITRO-2-PHENYL-3-(3'-ARYLCARBAMIDO-1'-CARBONYLALKYL)-4(3H)-QUINAZOLONES AS POTENTIAL ANTIVIRAL AGENTS

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ABSTRACT

A new series of 4(3H)-quinazolone derivatives, 7-nitro-2-phenyl-3-(3'-arylcarbamido-1'-carbonylalkyl)-4(3H)-quinazolones, have been synthesized and their antiviral activity *in vivo* and *in vitro* studied against RDV and SRV. Most of them have been found antiviral both *in vivo* as well as *in vitro*.

## INTRODUCTION

OWING to the high antiviral activity associated with several 6-nitro-2(1H)-quinazolinone and 4(3H)-quinazolinone analogs[1-6] and urea derivatives[7-12], some 7-nitro-2-phenyl-3-carboxyalkyl-4(3H)-quinazolinones II) and 7-nitro-2-phenyl-3-(3'-arylcarbamido-1-carbonylalkyl-4(3H)-quinazolinones(IV) have been synthesized and the relationship between chemical structures and their virucidal activity was studied.

The structure of the compounds II and IV were confirmed by their sharp melting points, analytical and infrared data. Infrared spectra were recorded in Perkin Elmer model 157 and 177 spectrophotometer in KBr pellets; strong peaks were observed at 3460 $cm^{-1}$ and 3260 $cm^{-1}$ (—NH), 1750 $cm^{-1}$ (C—O ring), 1625 (C = O amide), 1605 $cm^{-1}$ (C ≡ N), 1520 and 1320 $cm^{-1}$ ($NO_2$). Purity of the compounds was checked through TLC in methanol benzene solutions.

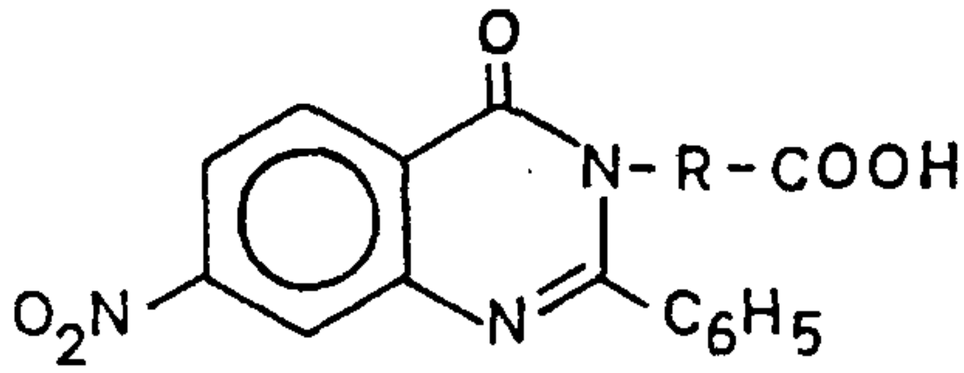
## EXPERIMENTAL

*7-Nitro-2-phenyl-1,3-benzoxazin-4-one(I)*:

It was synthesized by condensation of 4-nitro anthranilic acid and benzoyl chloride in pyridine, adopting the reported method[13].

TABLE 1

*7-Nitro-2-phenyl-3-carboxyalkyl-4(3H)-quinazolinones(II)*




II

| Sl. No. | R | Yield | m.p. °C | Antiviral activity: Per cent decrease in virus infectivity | | | |
|---|---|---|---|---|---|---|---|
| | | | | RDV | | SRV | |
| | | | | Conc. of compounds (mg/ml) | | | |
| | | | | Toxic to 50% CAM culture | Used for activity | *In vivo* | *In vitro* | *In vivo* |
| *1. | $CH_2$ | 75 | 250–1 | 0.30 | 0.15 | 30 | 46 | 25 |
| 2. | $CH—(CH_3)$ | 80 | 248 | 0.30 | 0.15 | 20 | 61 | 45 |
| 3. | $(CH_2)_3$ | 70 | 220 | 0.25 | 0.12 | 25 | 75 | 85 |
| 4. | $—CHCH_2.CH(CH_3)_2$ | 78 | 250 | 0.30 | 0.15 | 20 | 69 | 73 |

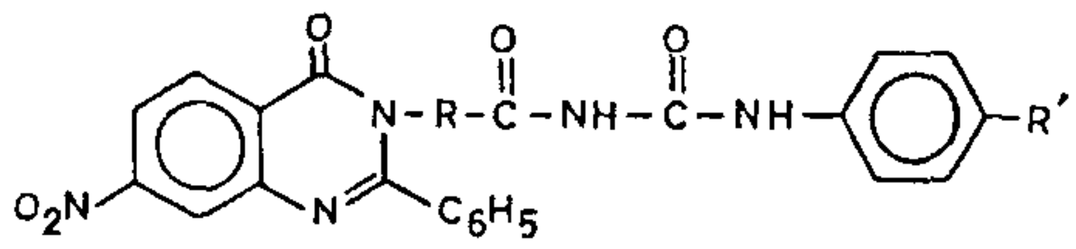
*Infectivity assayed in the leaves of *C. tetragonoloba* plants.

Calculated and analytical values of elemental analyses of C, H and N were found less than ± 0.4 ranges. M.Ps were determined in open capillary tubes and are uncorrected.

Antiviral activities are the average of five replications.

TABLE 2

*7-Nitro-2-phenyl-3-(3'-aryl-carbamido-1'-carbonylalkyl)-4(3H)-quinazolinones (IV)*



IV

| Sl. No. | R | R' | m.p. °C | % N analyses | | Antiviral activity: Per cent decrease in virus infectivity | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | RDV | | | SRV* | |
| | | | | | | Conc. of compound (mg/ml) | | | | |
| | | | | Found | Calcd. | Toxic to 50% CAM culture | Used for activity | *In vivo* | *In vitro* | *In vivo* |
| *1. | $CH_2$ | H | 167 | 15.73 | 15.80 | 0.25 | 0.12 | Nil | 66 | 15 |
| 2. | $CH_2$ | $CH_3$ | 138–9 | 15.50 | 15.31 | 0.25 | 0.12 | 20 | 73 | 17 |
| 3. | $CH_2$ | Cl | 158 | 15.71 | 14.65 | 0.25 | 0.12 | 30 | 79 | 48 |
| 4. | $CH(CH_3)$ | H | 173 | 15.11 | 15.31 | 0.27 | 0.13 | 49 | 64 | 67 |
| 5. | $CH(CH_3)$ | $CH_3$ | 125 | 14.53 | 14.86 | 0.25 | 0.12 | 30 | 59 | 36 |
| 6. | $CH(CH_3)$ | Cl | 231 | 14.01 | 14.24 | 0.30 | 0.15 | 10 | 75 | 61 |
| 7. | $CH_2CH_2CH_2$ | H | 246 | 15.00 | 14.86 | 0.30 | 0.15 | 40 | 68 | 27 |
| 8. | $CH_2CH_2CH_2$ | $CH_3$ | 193 | 14.40 | 14.43 | 0.25 | 0.12 | Nil | 61 | 29 |
| 9. | $CH_2CH_2CH_2$ | Cl | 168 | 13.67 | 13.95 | 0.25 | 0.12 | 20 | 62 | 45 |
| 10. | $CHCH_2CH(CH_3)_2$ | H | 185 | 14.11 | 14.02 | 0.25 | 0.12 | 50 | 45 | 59 |
| 11. | $CHCH_2CH(CH_3)_2$ | $CH_3$ | 170 | 13.44 | 13.64 | 0.27 | 0.13 | 30 | 14 | 72 |
| 12. | $CHCH_2CH(CH_3)_2$ | Cl | 172 | 13.01 | 13.12 | 0.27 | 0.13 | Nil | 9 | 33 |

* Infectivity assayed in the leaves of *Cyamopsis tetragonoloba* plants.

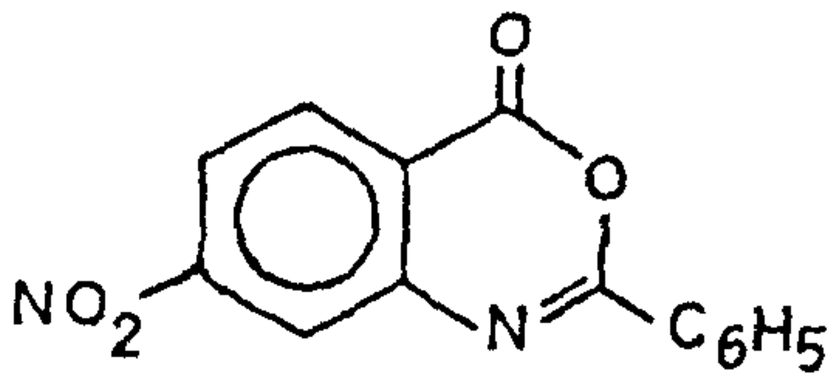
Yield varied from 60–75%.

Melting points were determined in open capillary tubes and are uncorrected.

Calculated and analytical values of elemental analyses of C and H were found to agree within ± 0.3% range.

*7-Nitro-2-phenyl-3-carboxyalkyl-4(3H)-quinazolinones(II)*:

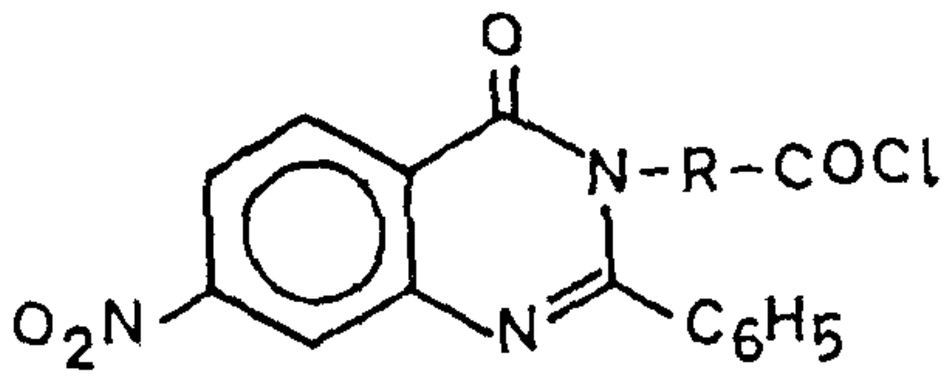
7-Nitro-2-phenyl-1,3-benzoxazin-4-one (0.005 mole) and appropriate aminoacid (0.006 mole) were refluxed with 30 ml of pyridine and water (15 ml) for 5 hr. The excess of the solvent was distilled off under vacuuo and the residual mass was then digested with 200 ml of N—HCl for 1 hr on a steam bath. The crude product separated, was filtered, dried and recrystallized from ethanol (table 1).



I

*7-Nitro-2-phenyl-3-acylchloride-4(3H)-quinazolinones(III)*:

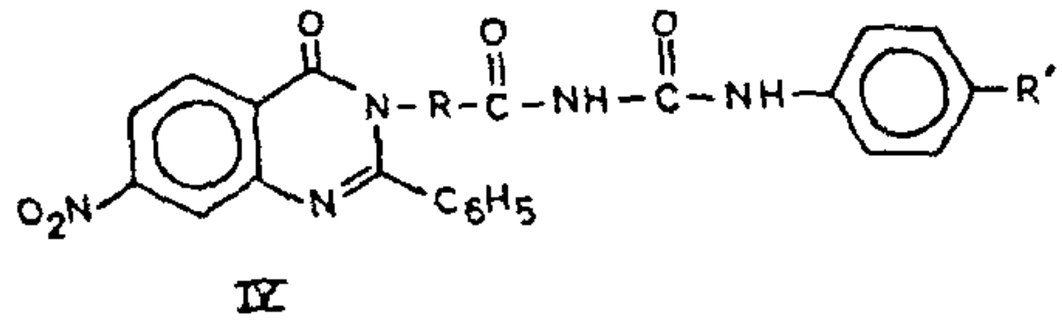
Synthesized by refluxing II (0.005 mole) with thionyl chloride (0.01 mole) in anhydrous benzene for 30 min, on a steam bath. The excess of thionyl chloride and benzene were distilled off under reduced pressure and the acid chloride was used for the next reaction without further purification.



III

*7-Nitro-2-phenyl-3-(3'-arylcarbamido-1'-carbonylalkyl)-4(3H)-quinazolinones(IV)*:

An equimolar quantities of the above synthesized compound (III) and 4-substituted phenyl ureas[14] were refluxed with anhydrous benzene for 5 hr. The excess of the solvent was distilled off under vacuuo and the solid product thus left was treated with 10% sodium bicarbonate solution, filtered and washed with and water dried. It was then recrystallized from ethylacetate (table 2).



IV

*Antiviral activity*

Antiviral activity against ranikhet disease virus (RDV)[15] was assayed in a stationary culture of the chick embryonal chorioallantoic membrane (CAM), when chick embryos were given the test compounds 6 hr before the virus challenge. Solutions of the test compounds were prepared by dissolving 25 mg of the compounds in 1 ml ethanol. The volume was then made upto 10 ml with 9 ml PBS. One-tenth ml of the test solutions was transferred into the allantoic cavity of a chick embryo (2.5 mg/embryo) 6 hr before inoculation with the virus (0.064 HA units/ml). In control sets, an equal number of embryos were inoculated with a corresponding PBS—ethanol mixture[16].

The decrease in virus multiplication in the treated sets was calculated from haemagglutination (HA) titres of allantoic fluid collected after 48 hr incubation at 37° C. The inhibition effect, upon virus multiplication was obtained by substracting this titre from that of the corresponding control.

The solutions of the compounds used for antiviral activity against sunnhemp rosette virus were prepared by dissolving 2.5 mg of the compound in 1 ml of ethanol. The volume was made up to 10 ml with distilled water. For *in vitro* experiments, virus (1/50) and compounds (5 mg $ml^{-1}$). The volume was made up and compounds (5 mg $ml^{-1}$) were mixed (1:1) and incubated for 30 min at 22–25° C. The mixture was then inoculated on the leaves of the test plants. The leaves of the control plants were rubbed with virus solutions in which alcoholic distilled water was added instead of compounds. For *in vivo* experiments, test solutions were applied on the upper surface of leaves of *C. tetragonoloba* plants, 24 hr prior to virus challenge. The leaves of control plants were treated in the same way with a mixture of 1 ml of ethanol and 9 ml of distilled water. The per cent inhibition was calculated by the formula $[(C-T)/C] \times 100$, where $C$ is the number of local lesions on control leaves and $T$ on the treated leaves. The data were analysed statistically[17,18].

## Results and Discussion

When the RDV infectivity was assayed in chick embryos, none of the title compounds exhibited appreciable decrease in virus titre. Compound no. 1 showed 30% inhibitory effect (table 1).

These compounds exhibited significant antiviral effect both *in vitro* and *in vivo* against sunnhemp rosette virus. The inhibition ranged from 46–75% *in vitro* and 45–85% *in vivo* (table 1).

It may, therefore, be concluded that the inhibitory effect of these compounds considerably increased with the increase of alkyl chain at position 3—of quinazolinone nucleus. Maximum inhibition was observed with a compound containing 3-C alkyl chain.

7-Nitro-2-phenyl-3-(3′-aryl carbamido-1′-carbonylalkyl)-4(3H)-quinazolinones (table 2) did not show high antiviral activity against RDV. Screening the results against RDV *in vivo* showed that the introduction of an unsubstituted phenyl group at position 3′—in carbamido substituted quinazolinones, enhances the antiviral efficacy. Maximum activity (50%) was noted with the compound containing 4-C alkyl chain and unsubstituted phenyl group (table 2).

From the results of the antiviral activity against SRV, it was observed that all the compounds, except compounds no. 11 and 12 (table 2) exhibited pronounced inhibitory action, *in vitro*. When administered *in vivo*, significant decrease in virus infectivity was observed with compound nos. 3, 4, 6, 10 and 11.

Thus, it may be concluded that 1 or 2-C alkyl chain in position 3—of quinazolinone and an electron attracting moiety at *p*-position of the phenyl carbamido group, enhances its inhibitory action.

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1. Yamamoto, M., Ishizumi, K., Morooka, S., Mori, K. and Noguchi, H., *Ger offen.*, 1973, 2242375 (*Chem. Abstr.*, 1973, **78**, 159660).
2. Yamamoto, M., Morooka, S., Koshiba, M., Komatsu, T., Noguchi, H., Inaba, S. and Yamamoto, H., U.S., 1972, 4146717 (*Chem. Abstr.*, 1979, **91**, 20538).
3. Verma, R. S., Bahadur, S. and Agnihotri, A. K., *J. Chem. Engg. Data*, 1981, **26**, 103.
4. Misra, V. S. and Dhar, S., *J. Indian Chem. Soc.*, 1978, **55**, 172.
5. Abdulla, R. F., *Eur. Pat.*, 1980, 10426 (*Chem. Abstr.*, 1981, **94**, 30789).
6. Singh, P., *J. Indian Chem. Soc.*, 1978, **55**, 801.
7. Weinstein, L., Chang, T. and Hudson, J. B., *Antibiot. Chemother.*, 1957, **7**, 443.
8. Rada, B., Blaskovic, D., Gut., J. and Sorm, F., *Acta Virol.*, 1963, **7**, 152.
9. Sidwell, R. W., Arnett, G. and Dixon, G. J., *Appl. Microbiol.*, 1966, **14**, 405.
10. Shindarov, L., Galabov, A., Neykova, N., Simov, D., Davidkov, K. and Kalcheva, V., *Acta Virol.*, 1971, **15**, 404.
11. Paget, C. J., Ashbrook, C. W., Stone, R. L. and Delong, D. C., *J. Med. Chem.*, 1969, **12**, 1097.
12. Takahashi, H., Yashima, I., Okamoto, S. and Ochiai, Y., *Japan kokai*, 1976, 7622823 (*Chem. Abstr.*, 1976, **85**, 15348).
13. Sammour, A., Fahmy, A. F. M. and Mahmoud, M., *Indian J. Chem.*, 1973, **11**, 222.
14. Schruber, R. S., *Organic Synthesis*, John Wiley and Sons Inc., New York, 1951, **31**, 8.
15. Babbar, O. P. and Dhar, M. M., *J. Sci. Ind. Res.*, 1956, **C15**, 249.
16. Mukerji, D. D., Shukla, S. K., Verma, H. N. and Awasthi, L. P., *Acta Pharm. Jugosl.*, 1981, **31**, 151.
17. Snedecor, G. W., *Statistical Methods*, Allied Pacific Private Ltd., Bombay, 1961.
18. Bahadur, S., Singh, S. P., Verma, H. N. and Awasthi, L. P., *Biologia Plantarum*, 1980, **22**, 241.