SYNTHESIS OF α-[METHYL-PHTHALIMIDO]-α′-[SUBSTITUTED STYRYL]-CYCLOHEXANONE THIOSEMICARBAZONES AS POTENTIAL ANTIVIRAL AGENTS

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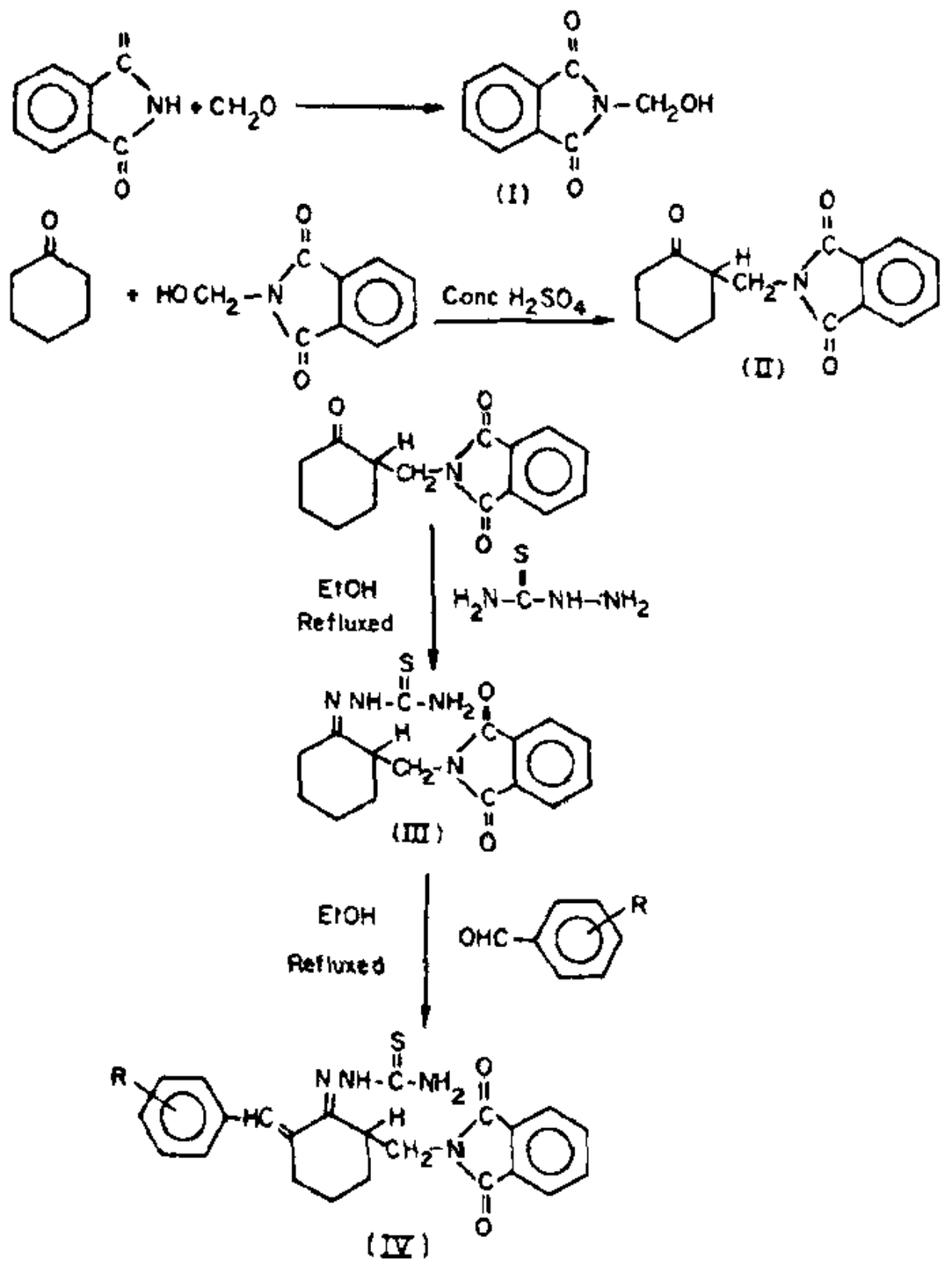
ABSTRACT

α-(Methyl-phthalimido)-α'-(substituted styryl)-cyclohexanone thiosemicarbazones (IV) were prepared by the reaction of α-(methyl-phthalimido)-cyclohexanone thiosemicarbazones (III) with aromatic aldehydes in ethanol. Four synthesised compounds were screened against Ranikhet disease virus both *in vitro* and *in vivo* for their antiviral activity at a concentration of 1 mg/ml/culture and 2 mg embryo respectively and were found to inhibit 15–51 % *in vitro* activity. No significant activity *in vivo* was observed.

INTRODUCTION

ONE of the areas, where most encouraging antiviral activity has been encountered is that of thiosemicarbazones[1–8]. The most effective and potent compound of this series is N-methyl isatin-β-thiosemicarbazone[9, 10]. In view of aforesaid structural analogy, a few new thiosemicarbazone derivatives have been synthesised with the expectation that the presence of thiosemicarbazone moiety in this class of compounds might render improved therapeutic results.

The synthesis was accomplished along the following route.



EXPERIMENTAL PROCEDURE

The melting points were determined in open capillaries in conc. H_2SO_4 melting point bath and were therefore uncorrected. IR spectra were recorded on Perkin–Elmer spectrophotometer using KBr. PMR spectra were recorded on Perkin–Elmer spectrometer using TMS as internal reference (chemical shift in δ, ppm). Purity of the compounds was checked on silica gel TLC plates and the spots were located by iodine vapours. All new compounds gave satisfactory N and S analysis.

N-(Methylphthalimido)-cyclohexanone (II)

N-Hydroxymethylphthalimide[11] (0.01 mole) and cyclohexanone (0.01 mole) were dissolved in the minimum quantity of conc. H_2SO_4 (30 ml) and the solution was cooled to 0°. The resultant reaction mixture was stirred for one hour at room temperature and left overnight. The mixture was poured into ice cooled water. A dark brown solid separated which was filtered and washed repeatedly with water to remove any sulphonated product. The crude compound thus obtained, was recrystallised from methanol as light brown needles, m.p. 115°, Anal. for $C_{15}H_{16}NO_3$; N, calcd. 5.42 %, Found N, 5.32 %; IR (KBr): 1750 cm^{-1} (C=O in ketone), 1670 cm^{-1} (C=O in amide), 2800, 2900 cm^{-1} (CH_2). PMR ($CDCl_3$): δ7.5 (Ar-H), δ1.2 (CH_2-cyclic) and δ5.5 (CH_2N).

α-(Methylphthalimido)-cyclohexanonethiosemicarbazone (III)

A mixture of α-(methyl-phthalimido)-cyclohexanone and thiosemicarbazide (equimolar amounts) in ethanol (50 ml) was refluxed for two hours on a steam-bath. The contents were cooled and the ethanol was distilled off. The solid obtained, was washed with water

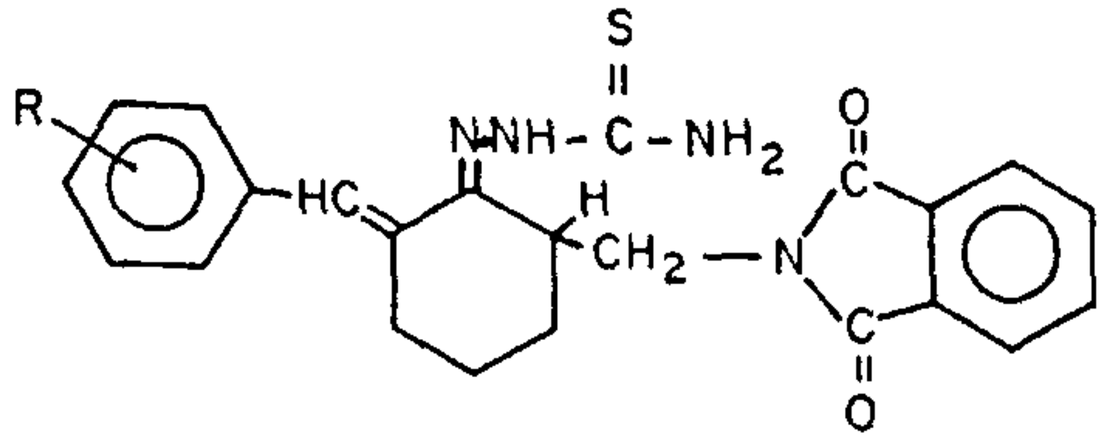
and recrystallised from methanol as white needles, m.p. 180°. Anal. for $C_{16}H_{18}N_4O_2S$, N, Calcd. 16.93%, N, Found 16.75%.

The complete absence of characteristic IR spectrum at 1750 cm^{-1} supported the formation of these compounds.

α-(Methylphthalimido)-α'-(substituted-styryl)-cyclohexanone thiosemicarbazone (IV)

α-(Methylphthalimido)-cyclohexanone thiosemicarbazone (0.001 mole) was dissolved in boiling ethanol (25 mole). To this solution, was added a solution of an aromatic aldehyde (0.001 mole) in ethanol (25 ml) with constant shaking. When the addition was complete, the reaction mixture was heated under reflux for two hours on a water-bath. Excess of ethanol was distilled off and the pasty mass obtained, was triturated with petroleum ether (b.p. 60–80°). The crude solid was dried at 100° in a vacuum desiccator and recrystallised from acetone-methanol (1 : 1). IR (KBr): 1670 cm^{-1} (C(=O) in amide), 1640 cm^{-1} (C=N), 1190 cm^{-1} (C=S), 3300 cm^{-1} (N–H), 1650 cm^{-1} (N–H bending).

Table 1 *α-(Methyl-phthalimido)-α'-(substituted-styryl)-cyclohexanone-thiosemicarbazones*



Comp. No.	R	M.P. °C	Molecular formula
1.	H	191	$C_{23}H_{22}N_4O_2S$
*2.	2-OH	174	$C_{23}H_{22}N_4O_3S$
3.	4-OCH_3	120	$C_{24}H_{24}N_4O_3S$
4.	2-OH,5-OCH_3	182	$C_{24}H_{24}N_4O_4S$
5.	2-F	130	$C_{23}H_{21}N_4O_2SF$
6.	4-$(CH_3)_2N$	155	$C_{25}H_{27}N_5O_2S$
7.	4-Cl	142	$C_{23}H_{21}N_4O_2SCl$
8.	1-CH=CH–	210	$C_{25}H_{24}N_4O_2S$

The yield ranged from 35 to 47 percent.
*PMR[$CFCl_3$ + DMSO(d_6)]: δ7.8 (aromatic H), δ5.15 (phenolic H), δ0.7 (CH_2 in alicyclic system), δ5.5 (–CH_2 in side-chain), δ6.5 (HC=CH).

BIO-ASSAY

The four compounds were subjected for antiviral activity against Ranikhet disease virus (RDV) both *in vitro* and *in vivo* using chorio–allantoic membrane culture (CAM) and chick embryo system.

a. METHODS AND MATERIALS (*IN VITRO*)

The method of Babbar and Dhar was followed[12–14].

The soluble compounds were dissolved in the nutrient fluid and the insoluble compounds were suspended in it in the presence of Tween®80 and the pH adjusted to 7.2 before sterilization. The solutions were then sterilized by autoclaving at 15 lbs pressure for 15 min. Two fold serial dilutions were then made and 1 ml of each dilution added to each of the test tubes containing the CAM culture.

The dilution of a compound causing toxic symptoms in 50% of the CAM culture was taken as the end point. The highest non-toxic dose as reported in table 2 was given to each culture along with the virus (0.064 HA units). Virus multiplication was measured by haemagglutination (HA) titre (mean of log_2) of the culture collected after 48 hr of incubation at 37°C. Inhibition in virus multiplication (log_2 d value) was obtained by subtracting this titre from that of the control. The mean difference (d) of 2 log_2 HA units is significant at 1% level.

b. METHODS AND MATERIALS (*IN VIVO*)

For activity each test compound (2 mg/embryo) was given just after virus infection (0.1 ml of 0.064 HA units/ml stock of RDV) in the allantoic cavity of 11 days old chick embryos and incubated for 48 hr. The presence of virus particles was calculated by fall in haemagglutination titre of the allantoic fluid.

c. RESULT AND DISCUSSION

Four compounds of this series were selected for screening against Ranikhet disease virus. The results show the percentage antiviral activity as compared to that of normal control in CAM culture and chick embryo system. It can be seen from the results recorded in table 2 that in the case of *in vitro* screening the maximum activity (51%) was exhibited by α-(methyl-phthalimido)-α'-(fluoro styryl) cyclohexanone–thiosemicarbazone. The activity decreased when 2-fluoro-group was replaced by 2-hydroxy, 4-dimethyl amino-group, and H. Hence, it could be concluded that the presence of 2-fluoro-group was desirable for high activity among these compounds. All

Table 2 *Antiviral activity against Ranikhet disease virus*

Comp. No.	R	*In CAM Culture* Dose mg/ml culture	% activity	*In chick embryo system* Dose mg/ embryo	% activity
1.	H	1	15	2	Inactive
2.	2-OH	1	33	2	Inactive
5.	2-F	1	51	2	Inactive
6.	4-$(CH_3)_2N$–	1	30	2	Inactive

the four compounds were found to be inactive in chick embryo system.

ACKNOWLEDGEMENT

The authors express their sincere thanks to Dr V. S. Misra, Head, Chemistry Department, Lucknow University, Lucknow for his interest and to Dr L. M. Singh and Dr B. Chaudhary, Department of Virology, CDRI, Lucknow, for antiviral screening facilities. One of the authors (Saksena Y.) is also grateful to CSIR, New Delhi for the award of a fellowship.

13 March 1984; Revised 21 September 1984

1. Hamre, D., Bernstein, J. and Donovick, R., *Proc. Soc. Exp. Biol. Med.*, 1950, **73**, 275.
2. Thompson, R. L., Price, M. L. and Minton, S. A., *Proc. Soc. Exp. Biol. Med.*, 1951, **78**, 11.
3. Varma, R. S. and Nobles, W. L., *J. Pharm. Sci.*, 1967, **56**, 775.
4. Rao, A. R., McFadzean, J. A. and Squires, S., *Ann. N.Y. Acad. Sci.*, 1965, **130**, 118.
5. Jones, D. H., Slack, R., Sqires, R. and Wooldridge, K. R. H., *J. Med. Chem.*, 1965, **8**, 676.
6. Varma, R. S. and Nobles, W. L., *J. Med. Chem.*, 1969, **10**, 972.
7. Lothar, H., Mariton, T., Emil, T. and Klaus, W., *Ger, (East)*. 121, 938, *Chem. Abstr.*, 1977, **87**, 53384f.
8. Memorandum on control of small pox outbreaks, Ministry of Health and Scottish Home and Health Department, H.M.S.O., *Brit. Med. J.*, 1962, **1**, 1317.
9. Andreant, A., Bonazzi, D., Cavrini, V., Gatti, R., Giovannitti, G., Franchi, L. and Nanetti, A., *Franco Ed. Sci.*, 1977, **32**, 703, *Chem. Abstr.*, 1978, **88**, 157635.
10. Miroslaw, K., Michael, K. and Magdalena, M., *Acta Pol. Pharm.*, 1977, **34**, 237, *Chem. Abstr.*, 1978, **88**, 32470m.
11. Buc, S. R., *J. Amer. Chem. Soc.*, 1947, **69**, 254.
12. Babbar, O. P. and Dhar, M. M., *J. Sci. Ind. Res.*, (New Delhi), 1956, **15C**, 249.
13. Babbar, O. P., *J. Sci. Ind. Res.*, 1961, **20c**, 216.
14. Babbar, O. P., *J. Sci. Ind. Res.*, 1961, **20c**, 232.

ANNOUNCEMENT

R. D. BIRLA NATIONAL AWARD

Rupees One lakh national award instituted by the Rameshwardas Birla Smarak Kosh, has been awarded to Prof. G. N. Ramachandran, F.R.S., Bangalore, for his outstanding research in medical and related fields.

Prof. Ramachandran is one of the distinguished research scientists of the country, internationally recognised for his work on the structure of the connective tissue protein, collagen which is of fundamental consequence to molecular biology and molecular medicine. His ideas on immunology and the importance of Vitamin 'C' are of great help to further the researches in the field of health.

Prof. Ramachandran was the editor of *Current Science* for seven years from 1951–1957 and he spared no pains to maintain its scientific standard.