Arch. Pharm. (Weinheim) *314*, 918–922 (1981)

Potential Biologically Active Agents, XXXII[1]

Synthesis and Antiviral Activity of Some 3-(Arylthiosemicarbazono)-2-indolinones

Rajendra S. Varma* and Pradeep K. Garg,

Department of Chemistry

and Hirday N. Verma and Lalji P. Awasthi

Plant Virus Laboratory, Department of Botany, Lucknow University, Lucknow - 226007, India

Eingegangen am 20. Januar 1981

A series of 3-(arylthiosemicarbazono)-2-indolinones **1**, 1-methyl-3-(arylthiosemicarbazono)-2-indolinones **2** and 1-(aminomethyl)-3-(arylthiosemicarbazono)-2-indolinones **3** have been synthesised. All compounds were screened for their antiviral activity against Sunnhemp rosette virus (SRV) *in vitro* as well as *in vivo*. Twelve compounds show significant antiviral activity.

Potentiell biologisch aktive Verbindungen, 32. Mitt.[1]: Synthese und antivirale Wirksamkeit einiger substituierter 3-Arylthiosemicarbazono-2-indolinone

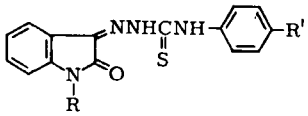
Es wird über die Synthese von 3-Arylthiosemicarbazono-2-indolinonen **1**, 1-Methyl-3-arylthiosemicarbazono-2-indolinonen **2** und 1-Aminomethyl-3-arylthiosemicarbazono-2-indolinonen **3** berichtet. Die synthetisierten Verbindungen sind gegen *Sunnhemp rosette virus* (SRV) mit *Chenopodium amaranticolor* als Wirtspflanze getestet worden. Zwölf Verbindungen zeigen starke Virushemmung.

Isatin derivatives have generally been associated with antiviral[2], antibacterial[3], anthelmintic[4] and herbicidal[5] properties. In addition to this, cysticidal[6] and hypotensive[7] responses have also been reported in certain isatin derivatives.

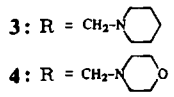
The biological activities of thiosemicarbazides and thiosemicarbazones are well established[8]. *Thompson* et al.[9] have suggested that their activity is due to the presence of a cyclic component and a $=N-NH-C(S)-NH_2$ group. In the present paper, we report the synthesis of arylthiosemicarbazides incorporated isatins.

Condensation of isatin with arylthiosemicarbazides[10] in equimolar quantities in ethanol gave **1**. Compounds **1** were also prepared[11] by refluxing 3-arylimino-2-indolinones[12] with arylthiosemicarbazides. The conformity of the products prepared by both methods was checked by Tlc, m.p., mixed m.p. and superimposable IR. Treatment of isatin with dimethylsulphate in ethanolic potassium hydroxide yielded 1-methylisatin, which was reacted with arylthiosemicarbazides to yield **2**. *Mannich* condensation of **1** with secondary amines furnished **3**. All the synthesised compounds gave satisfactory elemental analysis. Further support for their structures was derived from IR spectral data.

0365-6233/81/1111-0918 $ 02.50/0



1: R = H
2: R = CH_3



Authors are thankful to the Head of Chemistry Department for providing laboratory facilities and to the U.G.C., New Delhi for the financial assistance. Thanks are also due to Dr. *R.S. Kapil* for his interest in the work.

Experimental

MP: open capillary tubes, uncorr. *IR spectra:* Perkin-Elmer 137 and 177 spectrophotometers (KBr).

3-Arylthiosemicarbazono-2-indolinones **1** *(Table 1)*

Method A: A mixture of 4.41 g (0.03 mol) of isatin and 0.03 mol of arylthiosemicarbazide in 25 ml of ethanol, containing 2 drops of glacial acetic acid was refluxed for 2 h. The reaction mixture was allowed to cool to room temp. The solid mass separated was recrystallised from ethylacetate.
Method B: A solution of 4 mmol. of isatin in dry benzene was refluxed with 4 mmol. of arylthiosemicarbazide in a "Dean Stark's apparatus" for 4 h. The reaction mixture was cooled and the solid product thus separated was washed with pet. ether (b.p. 60–80°) and dried in air.

Table 1: *3-Arylthiosemicarbazono-2-indolinones* **1**

Compound No.	R'	Yield %	mp °C	Molecular formula (Mol. Wt.)	Calcd. Found N
1a	H	60	234–235	$C_{15}H_{12}N_4OS$ (296)	18.8 18.7
1b	Cl	60	241–242	$C_{15}H_{11}ClN_4OS$ (330.5)	16.9 16.9
1c	Br	70	242–243	$C_{15}H_{11}BrN_4OS$ (375)	14.9 14.8
1d	Me	60	238–239	$C_{16}H_{14}N_4OS$ (310)	18.1 17.8
1e	OMe	65	242–243	$C_{16}H_{14}N_4O_2S$ (326)	17.2 17.0

IR (KBr): 3200, 3300 cm^{-1} (NH); 1690–1700 cm^{-1} (C=O); 1160–70 cm^{-1} (C=S). 1590–1600 cm^{-1} (C=N).

1-Methyl-3-arylthiosemicarbazono-2-indolinones **2** *(Table 2)*

2 were prepared by heating 3.22 g (0.02 mol) of 1-methylisatin with 0.02 mol of arylthiosemicarbazide in 25 ml of ethanol containing 3 drops of glacial acetic acid for 4 h under reflux. At the end of this period, the mixture was cooled and the solid product was recrystallised from ethylacetate.

Table 2: *1-Methyl-3-arylthiosemicarbazono-2-indolinones* **2**

Compound No.	R′	Yield %	mp °C	Molecular formula (Mol. Wt.)	Calcd.	Found
2a	H	60	226–227	$C_{16}H_{14}N_4OS$ (310)	C: 61.9 H: 4.51	61.8 4.85
2b	Cl	65	235–236	$C_{16}H_{13}ClN_4OS$ (344.5)	N: 16.3	15.9
2c	Br	68	242–243	$C_{16}H_{13}BrN_4OS$ (389)	N: 14.4	13.9
2d	Me	69	218–219	$C_{17}H_{16}N_4OS$ (324)	N: 17.3	16.9
2e	OMe	62	200–201	$C_{17}H_{16}N_4O_2S$ (340)	N: 16.5	16.6

Table 3: *1-Aminomethyl-3-arylthiosemicarbazono-2-indolinones* **3, 4**

Compound No.	R′	Yield %	mp °C	Molecular formula	Elemental analysis Calcd.	Found
3a	H	60	160–161	$C_{21}H_{23}N_5OS$ (393)	N: 17.8	17.4
3b	Cl	55	184–185	$C_{21}H_{22}ClN_5OS$ (427.5)	N: 16.4	15.9
3c	Br	60	170–171	$C_{21}H_{22}BrN_5OS$ (472)	C: 53.4 H: 4.66	53.2 4.55
3d	OMe	62	168–169	$C_{22}H_{25}N_5O_2S$ (423)	N: 16.6	16.1
4a	H	65	186–187	$C_{20}H_{21}N_5O_2S$ (395)	N: 17.7	17.5
4b	Cl	65	181–182	$C_{20}H_{20}ClN_5O_2S$ (429.5)	C: 55.6 H: 4.65	55.4 5.01
4c	Br	72	185–186	$C_{20}H_{20}BrN_5O_2S$ (474)	N: 14.8	15.1
4d	Me	63	161–162	$C_{21}H_{23}N_5O_2S$ (409)	C: 61.6 H: 5.62	61.9 5.80
4e	OMe	65	187–188	$C_{21}H_{23}N_5O_3S$ (425)	N: 16.5	16.1

IR (KBr): **3c** and **4c**, 3200 (N–H), 2920 (–CH), 1690 (C=O), 1590 (C=N), 1160–70 cm^{-1} (C=S).

1-Aminomethyl-3-arylthiosemicarbazono-2-indolinones **3** *(Table 3)*

0.01 mol **1** was suspended in 15 ml of warm ethanol. To this suspension 1.5 ml of 37 % formalin and 0.01 mol of amine were added with vigorous stirring. This mixture was then heated on a water bath for 10 min and allowed to stand at room temp. overnight. The product separated was washed with cold methanol und dried well in air. Finally, it was recrystallized from carbon tetrachloride pet. ether (bp. 60–80°).

Antiviral Activity

The culture of *Sunnhemp rosette virus (SRV)*[13] was maintained in *Crotalaria juncea* plants. The virus inoculum was prepared by grinding fresh leaves showing severe disease symptoms in a sterilized pestle and mortar with an equal amount (W/V) of distilled water. The pulp obtained was squeezed through two folds of cheese cloth. The solution was partially clarified by centrifugation at 5000 g for 15 min. The supernatant thus obtained was diluted to 1/100 with sterile water and used as inoculum.
The solutions of the test compounds were prepared by dissolving 5 mg compound in 0.5 ml N,N-dimethylformamide then 4.5 ml ethanol was added. The mixture was diluted with 5 ml sterile water so as to make its final vol. 10 ml. This solution is called test solution.
For evaluating the *in-vitro* antiviral activity of the compounds, 1 ml of the test solution was mixed separately with one ml of virus inoculum, incubated at room temp. for 15 min and then inoculated on six leaves of *C. amaranticolor* plants. An equal number of leaves rubbed with a mixture of virus and solvent (1:1) served as controls. The treatments were distributed on Latin square pattern.

Table 4: *Antiviral activity of* **1, 2, 3** and **4** *against Sunnhemp rosette virus in Chenopodium amaranticolor leaves*

	% inhibition	
Compound No.	Mixture of virus and chemical incubated *in-vitro* and then applied	Applied 24 h prior to virus challenge
1a	42.65[b]	24.26
1b	31.29[b]	15.92
1c	80.44[a]	72.96[a]
1d	75.68[a]	76.25[a]
1e	41.67[a]	83.14[a]
2a	22.28	45.22[b]
2b	35.04[b]	49.45[a]
2c	27.21	81.25[a]
2d	72.62[a]	28.55
2e	82.65[a]	76.15[a]
3a	−11.44	25.22[b]
3b	0.42	10.25
3c	77.55[a]	59.26[a]
3d	37.92[b]	55.42[a]
4a	14.46	15.46
4b	67.34[a]	75.91[a]
4c	35.04[b]	48.25[b]
4d	27.04	24.41
4e	27.21	45.25[b]

Data significant, a = at 1 % level, b = at 5 % level.

For *in-vivo* activity, test solutions were rubbed separately on to the upper surface of leaves of *C. amaranticolor* plants, 24 h prior to virus challenge. An equal number of identical leaves rubbed with distilled water, instead of chemical solution, served as control.

Local lesions were counted 6–8 d after virus inoculation and the percentage inhibition of virus activity was calculated by the formula $\frac{C-T}{C} \times 100$, where C is the number of local lesions on controls and T on treated leaves.

All the experiments were performed in an insect free glass house. At least 3–5 *C.amaranticolor* plants of same height and vigour with 4–6 equal sized leaves were used for each treatment. Before virus inoculation, leaves were washed with distilled water, blotted dry and sprinkled with 600 mesh carborundum powder.

The data were analysed statistically for the significance of results[14].

It is evident from the results presented in Table 4 that many of the compounds which have shown high antiviral activity *in-vitro* were active *in-vivo* as well. Since they have shown activity both *in-vitro* as well as *in-vivo*, it is most probable that they affect the virus particles directly either by forming an inhibitor virus complex or by competition for receptor sites or they may act indirectly through the host by altering host cell physiology[15–19].

The antiviral activity exhibited by these compounds varied depending on the substitution pattern of the 3-aryl group.

References

1 XXXI: R.S. Varma and R.K. Pandey, Indian J. Chem., in press.
2 T.S. Osdane in Medicinal Chemistry, 3rd Ed., p. 662, A. Burger Ed., Wiley Interscience, New York 1970.
3 R.S. Varma and W.L. Nobles, J. Pharm. Sci. *64,* 881 (1975).
4 R. Cavier, R. Royer, R. Rips and L. Rene, Eur. J. Med. Chem. Chim. Ther. *4,* 21 (1969).
5 E. Hambsch, German Patent 10.13.456 (1957); C.A. *54,* 16733 (1960).
6 R.S. Varma and I.A. Khan, Indian J. Med. Res. *67,* 315 (1978).
7 John Wyeth and Brothers Ltd., Brit. 1.240.648 (1971); C.A. *75,* 118342 (1971).
8 D.Sc. Thesis, *R.S. Varma,* Lucknow University 1974.
9 R.L. Thompson, S.A. Minton Jr., J.A. Officer and G.H. Hitchings, J. Immunol. *70,* 229 (1953).
10 V.S. Misra and R.S. Varma, J. Indian Chem. Soc. *39,* 553 (1962).
11 R.S. Varma and P.K. Garg, J. Indian Chem. Soc., in press.
12 R.S. Varma and I.A. Khan, Indian J. Chem. *16,* 1113 (1978).
13 H.N. Verma and L.P. Awasthi, Phytopathol. Z. *92,* 83 (1978).
14 G.W. Snedecor in Statistical Methods, Allied Pacific Private Limited, Bombay 1961.
15 F.C. Bawden, Adv. Virus Res. *2,* 31 (1954).
16 H.N. Verma and L.P. Awasthi, Geobios *5,* 207 (1978).
17 H.N. Verma, L.P. Awasthi, S.P. Singh and S. Bahadur, Geobios *6,* 86 (1979).
18 S. Bahadur, S.P. Singh, H.N. Verma and L.P. Awasthi, Biol. Plant. *22,* 241 (1980).
19 S. Bahadur, S.P. Singh, H.N. Verma and L.P. Awasthi, Indian J. Microbiol. *20,* 58 (1980).

[Ph 368]