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Synthesis of Some New Substituted Thiosemicarbazides as Potential Antiviral Agents

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The substituted thiosemicarbazides **1–15**, synthesized by the condensation of substituted 5-chloro-diphenylamine-2-carboxylic acid hydrazides with appropriate arylisothiocyanates, have been tested for their antiviral activity against the plant virus, sunnhemp rosette virus, *in vitro* as well as *in vivo* and against the animal virus, ranikhet disease virus, in a stationary culture of chorioallantoic membranes of chick embryo. The majority of these compounds showed significant antiviral activity against both viruses. The structure-activity relationships have further been studied and are discussed.

Synthese einiger neuer substituierter Thiosemicarbazide als potentielle antivirale Agentien

Die substituierten Thiosemicarbazide **1–15**, synthetisiert durch Kondensation substituierter 5-Chlor-diphenylamin-2-carbonsäurehydrazide mit geeigneten Arylisothiocyanaten, wurden auf ihre Wirksamkeit gegen ein pflanzenpathogenes Virus, Sunnhemp-rosette-Virus, sowohl *in vitro* als auch *in vivo* und gegen ein tierpathogenes Virus, Ranikhet-disease-Virus, in einer stationären Kultur der Chorionallantoismembran des Hühnerembryos geprüft. Die meisten dieser Verbindungen zeigen signifikante antivirale Wirksamkeit gegen beide Viren. Die Struktur-Aktivitätsbeziehungen wurden studiert und diskutiert.

Diphenylamine-2-carboxylic acid derivatives have been reported to exhibit diverse biological properties such as anthelminthic[1], antibacterial[2,3]; antifungal[4] and antiviral[5,6]. Further, a large number of thiosemicarbazides have also been found to be remarkably antibacterially[7,8], antifungally[9] and antivirally[10,11] active. Our interest was thus arisen to synthesize the title compounds having both the diphenylamine-2-carboxylic acid nucleus and the thiosemicarbazide group and to see if these would be effective as antiviral agents.

Substituted 5-chlorodiphenylamine-2-carboxylic acids, prepared by the *Ullmann* reaction of 2,4-dichlorobenzoic acid with substituted aromatic amines in presence of anhydrous K_2CO_3, Cu powder and amyl alcohol, were converted into methylesters by refluxing with thionyl chloride and absol. methanol. The methyl esters on reaction with

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hydrazine hydrate in absol. ethanol gave the corresponding hydrazides which were converted into the substituted thiosemicarbazides **1–15** on reaction with different aryl isothiocyanates in dry benzene. The structures of the compounds were established on the basis of their elementary analyses and spectral data (IR and PMR).

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Experimental

MP: in H_2SO_4 bath in open capillary tubes, uncorr. *IR spectra*: Perkin-Elmer 157 Spectrophotometer in KBr. *PMR spectrum*: in $CDCl_3$ at A-90 MHz spectrometer.

Thiosemicarbazides **1–15**

A mixture of 5 mmol substituted 5-chlorodiphenylamine-2-carboxylic acid hydrazide[2,3] and of 5 mmol of an appropriate aryl isothiocyanate in 20 ml dry benzene was refluxed on a steam bath for 2–3 h. The solid, obtained on concentrating and cooling the reaction mixture, was washed with little benzene and recrystallized from ethanol or ethylacetate. The thiosemicarbazides thus synthesised have been listed in table 1. IR (KBr): 3240–3200 (NH), 3170–3090 (NH amide), 2975–2925 (CH), 1655–1638 (CO), 1100–1085 cm^{-1} (CS). PMR ($CDCl_3$): δ (ppm) = **6**: 2.11 (s, CH_3), 6.64 (d, J = 9.0 Hz, 1 H aromat.), 6.65–6.90 (m, 2 H aromat.), 7.0–7.32 (m, 7 H aromat.), 7.39 (d, J = 9.0 Hz, 1 H aromat.), 8.55 (br s, NH).

Biological Activity

Antiviral activity against plant virus

The compounds recorded in table 1 were tested for their antiviral activity against sunnhemp rosette virus (SRV) *in vitro* as well as *in vivo*. The virus inoculum was prepared by grinding fresh leaves of *Crotolaria juncea* L. showing severe disease symptoms with an equal amount of distilled water (w/v) in a sterilized pestle and mortar. The pulp was squeezed through two folds of muslin cloth and the juice was centrifuged at 3000 g for 15 min. The supernatant, thus obtained, was diluted to 1:100 with distilled water and used as inoculum. Plants of *Cyamopsis tetragonoloba* (L) taub. were used as assay hosts.

The solutions of the compounds were prepared by dissolving 5 mg of the compound in 1 ml ethanol and the total vol. was filled upto 10 ml with distilled water. For *in vitro* experiments, solution of the compound and virus inoculum were mixed (1:1) and the mixture was incubated at room temp. for 30 min and then applied on the upper surface of leaves of test plants. The leaves of control plants were rubbed with virus and distilled water (1 ml ethanol + 9 ml distilled water) instead of compound. For *in vivo* experiments solutions of the chemical compound were rubbed on the upper surface of leaves of test plants, 24 h prior to the virus challenge. Controls consisted of leaves rubbed with distilled water.

All the experiments were done in an insect free glass house at about 20–30° under natural light conditions. Carborundum powder (600 mesh) was evenly dusted over the leaf surface before inoculation. Local lesions were counted after 4–6 days of virus inoculation and the percent inhibition was calculated by the formula $\frac{C-T}{C} \times 100$, where C is the number of local lesions on control leaves and T on the treated leaves. Data were analysed statistically for the significance of results[12].

Antiviral activity against animal virus

The fifteen thiosemicarbazides recorded in table 1 were also screened for antiviral activity against ranikhet disease virus (RDV) in a stationary culture of minced chorioallantoic membranes (CAM) of chick ambryo. The strain of RDV employed was the same as employed by *Babbar* and *Dhar*[13]. CAM of 10 days old chick embryo (W and H) were used in all the experiments. Portions of CAM, which remained attached to the shell on opening the egg, were washed with glucose nutrient medium. The CAM were then cut into 2 to 3 sq. mm pieces and again washed thoroughly with nutrient medium to remove yolk and albumin completely. Soluble compounds were dissolved in the nutrient fluid and the insoluble compounds in the same fluid in presence of Tween 80. The pH of the medium was adjusted to 7.2 before sterilization. The solutions were then sterilized by autoclaving at 15 lb for 15 min. Twofold serial dilutions were then made and 1.0 ml of each dilution was added to each of the six tubes containing CAM culture. The dilution of the compound producing toxic symptoms to 50 percent of CAM culture was taken as the end point. The highest non-toxic doses were given to each culture along with the virus.

One ml of CAM suspension in nutrient fluid was inoculated in each tube (20 × 160 mm) and was kept at room temp. for 30–60 min so that the CAM pieces could stick to the glass surface. The nutrient fluid was then decanted and one ml of the fresh nutrient medium (with or without the compound) enclosing 0.064HA units/ml of the virus, was added to the tubes and incubated at 37° for 48 h. The virus multiplication was measured by the haemagglutination (HA) titre of the culture, collected after incubation. Inhibition in virus multiplication was obtained by substracting this titre from that of control. Average of six replications was noted.

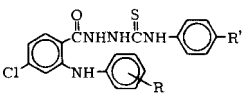

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Table 1: *Compounds* **1–15**

Compound Number	R	R'	Yield %	M.P. °C	Molecular formula	% N	
						Calcd.	Found
1	H	H	65	161	$C_{20}H_{17}ClN_4OS$	14.1	13.9
2	H	CH_3	70	189–190	$C_{21}H_{19}ClN_4OS$	13.6	13.4
3	H	Cl	80	162	$C_{20}H_{16}Cl_2N_4OS$	13.0	12.7
4	2-CH_3	H	60	175	$C_{21}H_{19}ClN_4OS$	13.6	13.3
5	2-CH_3	CH_3	75	178	$C_{22}H_{21}ClN_4OS$	13.2	13.0
6	2-CH_3	Cl	72	166	$C_{21}H_{18}Cl_2N_4OS$	12.6	12.3
7	3-CH_3	H	70	153	$C_{21}H_{19}ClN_4OS$	13.6	13.9
8	3-CH_3	CH_3	70	165–166	$C_{22}H_{21}ClN_4OS$	13.2	13.0
9	3-CH_3	Cl	85	165	$C_{21}H_{18}Cl_2N_4OS$	12.6	12.7
10	4-CH_3	H	68	146	$C_{21}H_{19}ClN_4OS$	13.6	13.4
11	4-CH_3	CH_3	80	184–185	$C_{22}H_{21}ClN_4OS$	13.2	13.1
12	4-CH_3	Cl	70	180	$C_{21}H_{18}Cl_2N_4OS$	12.6	12.5
13	2-OCH_3	H	65	168	$C_{21}H_{19}ClN_4O_2S$	13.1	12.9
14	2-OCH_3	CH_3	68	179–180	$C_{22}H_{21}ClN_4O_2S$	12.7	12.5
15	2-OCH_3	Cl	75	181	$C_{21}H_{18}Cl_2N_4O_2S$	12.2	11.9

Table 2: *Antiviral activity of compounds* **1–15** *against sunnhemp rosette virus (SRV) and ranikhet disease virus (RDV)*

Compound Number	Antiviral activity against				
	SRV		RDV		
	Percent inhibition		Conc. of compound mg/ml		Percent inhibition
	in vitro	*in vivo*	Toxic to 50 % CAM culture	Used for activity	
1	23	34[a]	0.5	0.25	90
2	33[a]	32[a]	0.3	0.15	90
3	72[a]	40[a]	0.5	0.25	90
4	28	62[a]	0.5	0.25	30
5	60[a]	91[a]	0.5	0.25	75
6	10	26	0.5	0.25	90
7	55[a]	84[a]	0.5	0.25	70
8	89[a]	89[a]	0.5	0.25	35
9	81[a]	74[a]	0.6	0.3	90
10	85[a]	25	0.6	0.3	85
11	67[a]	22	0.5	0.25	86
12	76[a]	63[a]	0.5	0.25	80
13	34[a]	64[a]	0.3	0.15	80
14	36[a]	35[a]	0.4	0.2	60
15	31[a]	11	0.5	0.25	70

a = Results significant at 1 % level.

Results and Discussion

Table 2 summarizes the results of antiviral activity of thiosemicarbazides **1–15** against SRV as well as RDV. The results of activity against SRV reveal that all compounds except **1, 4** and **6** caused significant inhibition of the virus *in vitro* and except for compounds **6, 10, 11** and **15** they were all active *in vivo* as well. Compound **8** exhibited maximal inhibition *in vitro* (89 %) and compound **5** *in vivo* (91 %). In some, activity *in vivo* was increased while in others it was decreased when compared with that *in vitro*.

From the results of antiviral activity *in vitro* it appears that among the compounds having no substituent in the aryl thiosemicarbazides moiety, the one with an unsubstituted 5-chlorodiphenylamine moiety is less active than those which have substitution of either a methyl or methoxy group in that part of the molecule, 4′-methyl substituted compound being most active. *In vivo*, however the 4′-methyl substituted compound becomes completely inactive and the 3′-methyl substituted exhibits maximal activity, 2′-methyl and 2′-methoxy substituted compounds being almost equally active. Among those which have substitution of a methyl group in the nucleus of the aryl thiosemicarbazide moiety, the

compound with methyl substituent at 2'-, 3'- or 4'- *in vitro* and 2'- or 3'- *in vivo* is comparatively more active than those, which have no substituent in the 5-chlorodiphenylamine part of the molecule. The compound with methyl substituent at position 3'- is most active *in vitro* and 2'- methyl *in vivo*. The activity is almost retained both *in vitro* and *in vivo* when a methoxy group is present at position 2'-. With a chlorine substituent in the thiosemicarbazide part of the molecule, and a 2'-methyl or methoxy group, the compound is much less active, while the compound becomes more potent antiviral agent when methyl is either at 3'- or 4'- both *in vitro* and *in vivo*, 3'-methyl substituted being most active.

The results of the activity against RDV indicate that all compounds, except **4** and **8** which exhibited much less activity, caused significant inhibition of the virus. One finds that thiosemicarbazides with unsubstituted 5-chlorodiphenylamine moiety seem to exhibit maximal inhibition, compound **2** being least toxic as well. The derivatives with 2'-, 3'-, 4'-methyl and 2'-methoxy groups, by and large shows less activity. But the two, 2'- and 3'-methyl substituted ones carrying a chloro substituent in the thiosemicarbazide portion, exhibit the same percentage of inhibition as compounds **1, 2,** and **3**.

It can, therefore, be concluded that the unsubstituted 5-chlorodiphenylamine moiety in the thiosemicarbazides renders them more active against RDV, while the reverse holds true when tested against SRV.

References

1 P. Ramanujam and J.G. Asthana, Indian J. Pharm. Sci. *35*, 177 (1973).
2 S. Bahadur, S.P. Singh and R.S. Varma, Indian J. Pharm. Sci. *41*, 36 (1979).
3 S. Bahadur, S.P. Singh and R.S. Varma, Indian J. Chem. *19B*, 826 (1980).
4 P. Ramanujam and J.G. Asthana, Planta Med. *25*, 43 (1974).
5 H.N. Verma, L.P. Awasthi, S.P. Singh and S. Bahadur, Geobios **6**, 86 (1979).
6 S. Bahadur, S.P. Singh, H.N. Verma and L.P. Awasthi, Biol. Plant. *22*, 241 (1980).
7 R.P. Bhamaria, R.A. Bellare and C.V. Deliwala, Indian J. Exp. Biol. *6*, 62 (1968).
8 S.P. Singh and S.K. Shukla, J. Antibact. Antifung. Agents (in press) (1982).
9 A.K. Sen Gupta and H.K. Misra, J. Antibact. Antifung. Agents *8*, 17 (1980).
10 A. Krbavcic, M. Plut, A. Pollak, M. Tilser, M. Likar and P. Schauer, J. Med. Chem. *9*, 430 (1966).
11 S. P. Singh, H. N. Verma and L. P. Awasthi, Acta Virol. Engl. Ed. (in press).
12 G.W. Snedecor in Statistical Methods, Allied Pacific Private Ltd., Bombay 1966.
13 O. P. Babbar and M. M. Dhar, J. Sci. Ind. Res. Sect. *C 16*, 160 (1957).

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