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Synthesis of Some New Formazans as Potential Antiviral Agents

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Fifteen new formazans were synthesized by the condensation of the 4-nitrophenylhydrazone of 3-nitro-anisaldehyde with the appropriate phenyldiazonium salts. Their *in vivo* antiviral activity was evaluated against Ranikhet disease virus (RDV) in chick embryos. Out of the compounds screened, 1-[4′-carboxyphenyl]-3-[3-nitro-4-methoxyphenyl]-5-[4-nitrophenyl]formazan **(8)** showed maximum (62.5 %) protection against RDV. The structure activity relationship has further been studied and is discussed.

Synthese einiger neuen Formazane als potentielle antivirale Agentien

Fünfzehn neue Formazane wurden durch Kondensation des 4-Nitrophenylhydrazons des 3-Nitroanisaldehydes mit Phenyldiazoniumsalzen synthetisiert und ihre in-vivo antivirale Wirksamkeit gegen Ranikhet-disease-Virus (RDV) in Hühnerembryonen geprüft. Unter diesen Verbindungen, wurde die stärkste signifikante Schutzwirkung (bis zu 62.5 %) mit 1-[4′-Carboxyphenyl]-3-[3′-nitro-4′-methoxyphenyl]-5-[4′-nitrophenyl] formazan **(8)** erzielt. Die Struktur-Aktivitätsbeziehungen werden studiert und diskutiert.

The biological utility of formazans for staining tissues and visualization of reducing enzymes in normal neoplastic tissues[1,2], are known for a long time. Their antibacterial[3] activity has come to light when *Kuhn* und *Jerchel* screened them against various strains of bacteria. *Libman* et al.[4] reported the antiviral activity of p-aminophenyl-2,5-diphenyltetrazolium chloride against influenza A and Nigg mouse pneumonitic virus. Recently *Misra* et al.[5] have synthesized a number of formazans and their tetrazolium salts and observed some of them to be active against RDV. In view of these observations of a broad spectrum of antiviral properties associated with formazan derivatives, it was thought of interest to synthesize some new formazan derivatives as potential antiviral agents.

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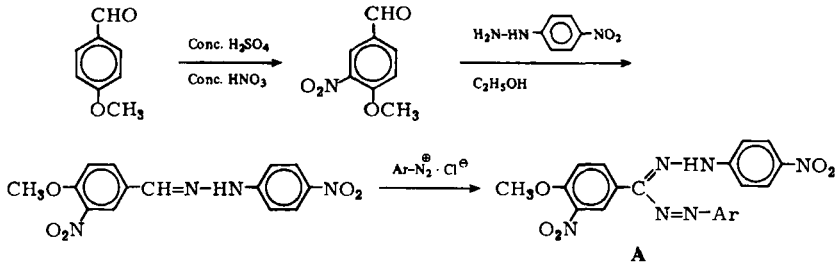

A

The 1-substituted aryl-3-(3′-nitro-4′-methoxyphenyl)-5-(4′-nitrophenyl)-formazans **A** were synthesized by condensation of the 4-nitrophenyl hydrazone derivative of 3-nitro-4-methoxy-benzaldehyde[6] with appropriate aryl diazonium salts. The formazans thus synthesized were characterized by their sharp melting point, elementary and I.R. analysis.

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Experimental

MP: in H_2SO_4 bath in open capillary tubes, uncorr. *IR Spectra:* Perkin-Elmer 137 Spectrophotometer. *TLC:* silica gel G coated glass plates of 2 mm thickness.

3-Nitro-4′-methoxybenzaldehyde

It was synthesized by the method of *Einhorn* and *Grabfield*[7]. 13.6 ml 4-methoxybenzaldehyde were gradually added with stirring to the well cooled (0 °C) nitrating mixture containing 4.2 ml conc. nitric acid and 106.5 ml conc. sulphuric acid. The temp. was kept at 0 °C during the addition. The reaction mixture was then left for 1 h at room temp. and was decomposed by pouring in ice-cold water. The decomposed product was washed with cold water, dried and finally recrystallised from ethanol as yellow needles which melted at 82 °C (Lit. m.p. 83.5[6]), yield 85 %.

1-Aryl-3-(3′-nitro-4-methoxy phenyl)-5-(4′-nitro-phenyl)-formazans

0.015 mole of an appropriate amine were dissolved in 3 ml glacial CH_3COOH and stirred with 2 ml conc. HCl at 0 °C. A solution of 1 g $NaNO_2$ in 4 ml H_2O was then added, dropwise with constant shaking and maintaining the temp. between 0–5 °C. The diazotised product was added gradually with stirring to a solution of 0.01 mole the p-nitrophenylhydrazone derivative of 3-nitro-4-methoxy-benzaldehyde in 20 ml pyridine and the temp. was kept below 10 °C. The reaction product was allowed to stand at room temp. over night and then was decomposed by pouring in 200 ml ice-cold water. The separated solid was washed with cold water and finally with methanol. The crude product was recrystallised. The formazans thus prepared have been listed in table 1.

Pharmacological activity

The formazans **1–15** were evaluated for their antiviral activity against Ranikhet disease virus in vivo in 10 to 12 days old chick embryos (W.L.H.). The strain of RDV was the same as employed by *Babbar* and *Dhar*[8].

Table 1: *1-Aryl-3-(3'-nitro-4'-methoxyphenyl)-5-(4'-nitrophenyl)formazans* **1–15**

Compound no.	Ar	Molecular formula	m.p. °C	% N. Analysis	
				Calcd.	Found
1a	C_6H_5-	$C_{20}H_{16}N_6O_5$	161	20.0	19.9
2a	p-Cl-C_6H_4-	$C_{20}H_{15}N_6O_5Cl$	110	18.5	18.5
3b	p-Br-C_6H_4-	$C_{20}H_{15}N_6O_5Br$	148	16.8	16.8
4b	p-I-C_6H_4-	$C_{20}H_{15}N_6O_5I$	102	15.4	15.3
5a	p-NO_2-C_6H_4-	$C_{20}H_{15}N_7O_7$	82	21.1	21.2
6a	o-COOH-C_6H_4-	$C_{21}H_{16}N_6O_7$	208	18.1	18.2
7a	m-COOH-C_6H_4-	$C_{21}H_{16}N_6O_7$	165	18.1	17.9
8a	p-COOH-C_6H_4-	$C_{21}H_{16}N_6O_7$	170	18.1	18.0
9b	p-$COOCH_3$-C_6H_4	$C_{22}H_{18}N_6O_7$	115	17.6	17.5
10b	p-$COOC_2H_5$-C_6H_4	$C_{23}H_{20}N_6O_7$	80	17.1	17.3
11b	p-$COOC_3H_7$-C_6H_4	$C_{24}H_{22}N_6O_7$	90	16.6	16.5
12b	p-$COOC_4H_9$-C_6H_4	$C_{25}H_{24}N_6O_7$	73	16.2	16.4
13a	o-OCH_3-C_6H_4-	$C_{21}H_{18}N_6O_6$	222	18.7	18.6
14a	m-OCH_3-C_6H_4-	$C_{21}H_{18}N_6O_6$	94	18.7	18.5
15a	p-OCH_3-C_6H_4-	$C_{21}H_{18}N_6O_6$	73	18.7	18.7

[a] recrystallised from benzene, [b] recrystallised from CCl_4, Yield Varied from 60–75 %.

The compounds were dissolved in 1 ml ethanol and then diluted with glass distilled water so as to make final conc. of the test solutions 2 mg/ml.

0.25 ml of the solution, in each case, were inoculated (0.5 mg/Embryo) in the allantoic cavity of chick embryo, 6 h prior to virus challenge. The virus (0.064 HA units/ml) was inoculated by the same route.

By exactly the same procedure the control embryos were inoculated with 0.25 ml/embryo of buffer (PBS) solution mixed with 1 ml of ethanol. The inoculated embryos were incubated at 37 °C for 48 h.

The decrease in virus multiplication in treated sets was calculated by haemagglutination (HA) titre of the allantoic fluid collected 48 h after incubation. Inhibition in virus multiplication was determined by substracting this titre from that of the control test (treated with PBS). The results presented (Table 2) represent the average of 5 replicates.

Table *2: Antiviral activity of 1-Aryl-3-(3'-Nitro-4'-methoxyphenyl)-5-(4'-nitrophenyl)-formazans against Ranikhet disease virus*

Compound Number	Conc. compound used (mg/egg)	% inhibition	Compound Number	Conc. compound used (mg/egg)	% inhibition
1	0.25	Nil	9	0.25	24.00
2	0.25	25.00	10	0.25	12.00
3	0.5	50.00	11	0.25	25.00
4	0.25	Nil	12	0.25	Nil
5	0.25	12.00	13	0.5	38.00
6	0.5	50.00	14	0.5	38.00
7	0.25	12.00	15	0.25	Nil
8	0.5	62.5			

Structure-activity Relationships

The study of structure activity relationships of formazans reveals that the structure variations in the aryl moiety have profound effect on the activity of the compounds (Table 2).

Among the *p*-halogen substituents, *p*-bromo substituent showed 50 % protection against RDV as compared to the unsubstituted ring, whereas a NO_2 group at *p*-position was found to be inactive. The introduction of a carboxyl group at *o*- or *p*-position gives rise to significant antiviral activity (50 and 62.5 % resp.), while the *m*-substituent exhibited only little activity. When the COOH group was esterified the activity of the compounds decreases considerably.

The protection against virus infection was also observed in *o* and *m* methoxy substituted compounds whereas the *p*-derivative was found to be inactive.

In conclusion, the investigation of structure activity relationships showed that a free (ionic)carboxyl group enhances the antiviral activity whereas esters exhibit no marked antiviral activity. Maximum protection was observed with a *p*-carboxyl group.

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