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Selective inhibition of virus multiplication by new acylated 1,2,4-triazole derivatives

Stephen Horváth[1,*], Tamás Somorai[2] and Géza Szilágyi[3]

[1]*Department of Virology,* [2]*Research Secretariat and* [3]*Synthetical Major Department, Institute for Drug Research, P.O. Box 82, H-1325 Budapest, Hungary*

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Summary

Six out of 99 new acylated 1,2,4-triazole derivatives specifically inhibited rubella virus replication in RK 13 cell cultures. These are the following: 3-methylthio-5-(2-chlorobenzamido)-1H-1,2,4-triazole; 3-methylthio-5-(2-bromobenzamido)-1H-1,2,4-triazole; 3-methylthio-5-(2-methylbenzamido)-1H-1,2,4-triazole; 3-methylthio-5-(2-nitrobenzamido)-1H-1,2,4-triazole; 3-methylthio-5-(2-methylthiobenzamido)-1H-1,2,4-triazole and 3-ethylthio-5-(2-methylbenzamido)-1H-1,2,4-triazole. The compounds did not directly interfere with the infectivity of the rubella virus particles and the antiviral effect was demonstrable only within cells infected with rubella virus. The active compounds did not inhibit the replication of herpes simplex virus type 1, influenza virus and adenovirus in cell culture systems. Structure-activity relationships are discussed.

antivirals; rubella virus; cell culture

Introduction

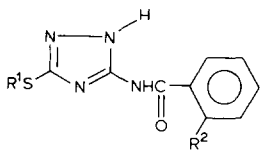
Among the few triazole compounds [1,2,11,12] shown to possess antiviral activity, the nucleoside derivative 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (Virazole, ribavirin, Viramid) is the most prominent. This compound has been reported to inhibit the replication of a wide variety of DNA- and RNA-containing viruses in both cell culture and animal model systems. It was found to be particularly effective against myxo- and paramyxovirus infections [3–5,13–18]. In the present study we have investigated the antiviral effects in cell culture of a series of newly synthesized 1,2,4-triazole derivatives.

* To whom correspondence should be addressed. Telephone: 690011.

Materials and Methods

Compounds

3-Alkylthio-5-substituted benzamido-1,2,4-triazoles were synthesized from 3-alkylthio-5-amino-1H-1,2,4-triazoles by substituted benzoylchlorides. The 1-acylated compounds obtained could be subjected to thermal rearrangement in order to produce the end products.



As a common structural feature, the most active compounds contain a 2-substituted benzamide moiety in position 5 of the triazole ring. All N^1-substituted derivatives and the compounds substituted by an amino, sulphone or sulphoxide group in position 3 were inactive. The introduction of more than one substituent outside in position 2 of the benzene ring as well as the presence of a side chain between the benzene ring and carbonyl group yielded inactive compounds.

The synthesis of the new acylated 1,2,4-triazole derivatives will be published elsewhere [10]. The triazole derivatives which proved active against rubella virus are listed in Table 1.

TABLE 1

Chemical composition of triazole derivatives active against rubella virus

Compound	R^1	R^2	Molecular formula	Name (molecular weight)
4c	Me[a]	Cl	$C_{10}H_9ClN_4OS$	3-methylthio-5-(2-chlorobenzamido)-1H-1,2,4-triazole (268.7)
4i	Me	Br	$C_{10}H_9BrN_4OS$	3-methylthio-5-(2-bromobenzamido)-1H-1,2,4-triazole (313.2)
4m	Me	Me	$C_{11}H_{12}N_4OS$	3-methylthio-5-(2-methylbenzamido)-1H-1,2,4-triazole (248.3)
4r	Me	NO_2	$C_{10}H_9N_5O_3S$	3-methylthio-5-(2-nitrobenzamido)-1H-1,2,4-triazole (279.2)
4t	Me	MeS	$C_{11}H_{12}N_4OS_2$	3-methylthio-5-(2-methylthiobenzamido)-1H-1,2,4-triazole (280.3)
4u	Et	Me	$C_{12}H_{14}N_4OS$	3-ethylthio-5-(2-methylbenzamido)-1H-1,2,4-triazole (262.3)

[a] Me, methyl; Et, ethyl.

All compounds were of the purest grade available. Stock solutions were freshly prepared at a concentration of 6.32 mg/ml and sterilized in ampoules at 100°C for 10 min. Solvents used are listed in Table 2 and all the compounds were soluble in these.

Cell cultures

RK 13, HeLa and Hep-2 cell lines were used. The RK 13 cell line was kindly supplied by Dr. J.M. Best (Department of Virology, St. Thomas' Hospital Medical School, London); HeLa and Hep-2 cell lines were provided by Professor K.R. Dumbell (Department of Virology, St. Mary's Hospital Medical School, London).

The cells were grown at 37°C in Eagle's minimal essential medium prepared in Earle's balanced salt solution containing 10% (v/v) inactivated calf serum, 10 U/ml penicillin and 10 μg/ml streptomycin [6]. After 24 h cultivation the cells were used in antiviral experiments during which they were maintained at 35°C in the same chemically defined medium containing 2% (v/v) calf serum.

Viruses

The antiviral experiments were carried out with rubella virus (Judith strain); herpes simplex virus type 1 (HSV-1), strain 10711; adenovirus type 5, strain H1526 and influenza virus, strain AO PR8. The rubella and HSV-1 strains were kindly supplied by Dr. Marguerite S. Pereira (Central Public Health Laboratory, Colindale) and the adenovirus strain was provided by Dr. H.G. Pereira (National Institute for Medical Research, Mill Hill, London). Rubella virus was cultivated in RK 13, HSV-1 in HeLa and adenovirus in Hep-2 cell cultures. Influenza virus was cultivated in embryonated eggs and titrated in roller tubes containing chorioallantoic membrane suspensions [7].

Parameters of cell growth

Cultures containing actively dividing cells were used to measure the cytotoxicity (CT) of triazole derivatives. The 50% (CT_{50}) and zero (CT_0) cytotoxicities of compounds were determined graphically and the values are expressed in log μM units. This procedure is described in detail elsewhere [8].

Assay of antiviral activity

All the experiments were carried out in two parts. In the first part, the cell cultures were inoculated with 0.1 ml of each virus dilution and the infected cultures were incubated at 35°C in the presence of various concentrations of the triazole derivatives. On the 6th day (or 9th day for rubella virus) the same cultures were used for the second part of the experiment in which the culture medium was replaced by fresh maintenance medium without compound. In both parts of the experiment the 50% cell cultures infective titres ($CCID_{50}$) were calculated on the basis of the virus-induced cytopathic effect observed in each group of virus-infected cell cultures. In the second part of the experiment the $CCID_{50}$ values were determined on the 12th day (or 18th day for rubella virus). Influenza virus titres were calculated on the bases of the results of hemagglutination tests. Assay techniques and determination of parameters which characterize the potency and specificity of the compounds are described in detail elsewhere [7,9].

Effects of compound 4m on the infectivity of rubella virus particles and on the replication of rubella virus in cell cultures

(a) A concentrated stock of rubella virus particles was added to maintenance medium containing 2% calf serum together with compound *4m* at a final concentration of 2.21 log μM. The drug–virus suspension was incubated at 35°C and virus infectivity titrations were carried out in RK 13 cell cultures.

(b) RK 13 cells in growth medium containing 10% calf serum were pretreated with compound *4m* at a concentration of 2.21 log μM. After 3 days the cell cultures were washed twice with fresh medium. Rubella virus infectivity was then determined in both pretreated and untreated cell cultures.

(c) Quantitative assays of rubella virus infectivity were carried out in groups of cell cultures maintained in the presence of 2% calf serum. After the cell cultures had been infected with various dilutions of rubella virus, compound *4m* was added at either 0, 1 or 24 h after infection to give a final concentration of 2.21 log μM.

Results

Determination of CT of triazole derivatives

The values of CT_{50}, CT_0 and slopes for the newly synthesized compounds are presented in Table 2. The compounds including the inactive ones showed a similar toxicity for RK 13 and HeLa cells. Very small differences in CT were noted among the different compounds.

Measurement of the antiviral activity of triazole derivatives

The antiviral activity of the new triazole derivatives was determined using rubella, influenza, adenovirus or HSV-1, as the virus challenge, according to the method described elsewhere [9].

The parameters used for the characterization of the potency and the specificity of the compounds active against rubella virus are presented in Table 3.

The reduction of rubella virus yield in antiviral experiments at CT_0 concentrations was relatively high, with D values between 3 and 6.8 logs; i.e. the triazole derivatives inhibited rubella virus multiplication specifically. The TI and 'shadow' showed moderate inhibition of rubella virus replication at compound concentrations lower than CT_0. Taking into account all parameters (D, TI, DS, I_0, 'shadow') the most active compounds were the following: *4i, 4m* and *4t*. Compound *4m* was chosen for additional studies.

None of the newly synthesized triazole derivatives had any significant effect against the replication of HSV-1, influenza or adenovirus.

Effects of the compound 4m on the infectivity of rubella virus particles and on the replication of rubella virus in cell cultures

(a) Compound *4m* did not specifically inactivate rubella virions. There was a loss of infectivity of both treated and control virus particles, which could be attributed to thermal inactivation during the incubation period at 35°C.

TABLE 2

Cytotoxicity of triazole derivatives in RK 13 and HeLa cell cultures

Compound	Solvent	RK 13		HeLa		Slope (log CT_{50}–log CT_0)	
		CT_{50}[a]	CT_0[b]	CT_{50}	CT_0	RK 13	HeLa
4c	25% Ethanol 25% Dimethyl formamide	2.47	1.77	2.27	1.47	0.7	0.8
4i	25% Ethanol 15% Dimethyl formamide	2.41	1.91	2.61	2.11	0.5	0.5
4m	50% Dimethyl formamide	2.51	1.71	2.61	2.21	0.8	0.4
4r	50% Dimethyl formamide	2.35	1.75	2.15	1.55	0.6	0.6
4t	70% Ethanol 10% Dimethyl sulfoxide	2.25	1.75	2.45	1.85	0.5	0.6
4u	40% Ethanol 5% Dimethyl sulfoxide	2.18	1.48	2.28	1.48	0.7	0.8

[a] CT_{50}, concentration (log μM) causing 50% reduction in cell growth.
[b] CT_0, concentration (log μM) causing 0% reduction in cell growth.

TABLE 3

Characterization of the antiviral effects shown by various triazole derivatives against rubella virus replication in RK 13 cells

Compound	D at CT_0	I_0 (log μM)	TI	DS at CT_0	Area of 'shadow' (%)
4c	≥5.3	0.37	1.4	3.0	60
4i	≥6.8	0.71	1.2	4.8	60
4m	≥6.8	0.41	1.3	4.2	70
4r	3.0	0.95	0.8	1.0	80
4t	≥5.8	0.15	1.6	5.0	65
4u	≥6.0	0.08	1.4	2.0	60

D, decrease in virus infectivity titer (log units) at CT_0 compared to the control level. I_0, maximum concentration of compound which did not affect viral multiplication. TI, therapeutic index (log CT_0 – log I_0). DS, decrease in the virus infectivity 'shadow' titer (log units) at CT_0 compared to the control level. 'Shadow' is the area between the dose–response curve obtained from the first and second parts of the antiviral assays expressed in percent [9].

(b) The virus titer yielded by cell cultures pretreated with compound *4m* was $5.5 \times 10^4/0.1$ ml as compared to $3.2 \times 10^5/0.1$ ml for the virus titer from control cell cultures. This difference is not significant.

(c) Decrease in virus titers obtained after rubella virus-infected RK 13 cells had been exposed to compound *4m* was higher than 10^5, irrespective of the time compound *4m* had been added to the infected cell cultures (0, 1 or 24 h).

Discussion

The nucleoside, 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (ribavirin) is a well-established antiviral compound with a remarkably broad-spectrum activity against DNA- and RNA-containing viruses [15,18,19]. The growth of rubella virus was also inhibited by ribavirin but only at its CT_{50} concentration. The inhibitory effect was characterized by a D value of 6, a TI of zero and an 80% 'shadow' [9].

Only small differences exist between the structures of those newly synthesized triazole derivatives that show antiviral activity. More drastic changes in structures produced inactive compounds, and only 6 out of the 99 derivatives showed any antiviral activity.

Analysis of the structure-activity relationship of the active triazole derivatives has revealed the following:

(a) Influence of R^1: to endow antiviral activity, R^1 should be a methyl, ethyl or propyl group. The antiviral activity decreased with increasing length of the side chain in position 3; methylthio≈ethylthio>propylthio>>butylthio compounds. Triazoles with longer side chains at C-3 are inactive.

(b) Influence of R^2: a substituent in the *ortho* position is required for antiviral activity; the most active antiviral compounds have a chloro, bromo, methyl or methylthio group at this position. Other substitutions, i.e. methoxy, alkoxycarbonyl or more than one substituent at the phenyl ring lead to a loss of antiviral activity.

(c): Ring N^1-substitution by an acyl, alkyl or phenyl group results in inactive compounds.

(d): Compounds bearing an amino, sulphone or sulphoxide group in position 3 and unsubstituted compounds are inactive.

The D values at the CT_0 concentration of the active compounds are as high as could be expected for a highly effective antiviral agent. However, the 'shadow' and TI are lower than the optimal value [9].

The active triazole derivatives do not affect the infectivity of the rubella virions and the antiviral effect is directed only against infected cells. Compound *4m* added to the cell cultures as late as 24 h after infection still inhibits rubella virus replication very effectively. Of the four virus strains used in the present study (HSV-1, influenza, adeno, rubella) only rubella virus was inhibited in its multiplication cycle.

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