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ANTIVIRAL ACTIVITY OF SUBSTITUTED 6-METHYLMERCAPTOPYRAZOLO(3,4-d)PYRIMIDINES AND THEIR RIBOSIDES

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Summary. — The antiviral activities of pyrazolo(3,4-d)pyrimidines and their nucleosides, divided into 7 groups based on the substituent in position 4 (mercapto-, methylmercapto-, amino-, hydrazine-, dialkyl amino-, oxy-, and methoxyderivatives), were studied against herpes simplex virus type 1 (HSV 1) and vaccinia virus. Numerous compounds inhibited the virus yields by 1-4 log CPD_{50}. Most antiviral compounds inhibited HSV 1 more than vaccinia virus. Only methylmercapto- derivatives inhibited both viruses approximately to the same degree.

Key words: pyrazolo(3,4-d)pyrimidines; pyrazolo(3,4-d)pyrimidine nucleosides; antiviral activity; herpes simplex virus; vaccinia virus

Introduction

Among pyrazolopyrimidine analogues of purines and their nucleosides, compounds exerting biological activities are known (Montgomery, 1970; Preobrazhenskaya, 1973; Panzica *et al.*, 1978). Previously we found pyrazolo (3,4-d) pyrimidines showing an antiviral activity (Bektemirov *et al.*, 1978). We are reporting the results of investigations on the antiviral activity of 4- and 3,4-substituted 6-mercapto- or 6-methylmercaptopyrazolo(3,4-d)pyrimidines and their ribosides against herpes simplex virus type 1 (HSV 1) and vaccinia virus.

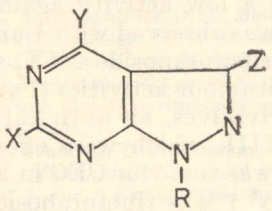
Materials and Methods

Viruses. HSV 1 strain VR-3 and vaccinia virus strain L-IVP were used at a multiplicity of 0.1 CPD_{50} per cell. The viruses were propagated and assayed as described (Bektemirov *et al.*, 1979).

Test compounds. The derivatives used (Table 1) were prepared as described (Korbukh *et al.*, 1979; Bulychev *et al.*, 1980). Their antiviral effect was assayed in chick embryo cell (CEC) monolayer cultures obtained by the routine method (Soloviev and Bektemirov, 1963, pp. 151—153) and grown in 100-ml Povitskaya bottles. The compounds were added 1 hr after inoculation of the cultures. In control untreated cultures HSV 1 and vaccinia virus multiplied to a titre of 10^6 CPD_{50}/ml. The antiviral activity of the compounds was determined as described (Bektemirov *et al.*, 1979). Most compounds were tested starting with a concentration of 250 μg/ml. If toxic for the CEC, the concentration was successively decreased in 2-fold steps.

Table 1. Antiviral activities of substituted pyrazolo(3,4-d)pyrimidines

Compound No.	X	Y	Z	R[1])	Antiviral activity[2]) HSV 1	Vaccinia virus
I	SH	SH	H	H	0	0
II	SH	SH	CN	H	10^1	0
III[3])	SH	SH	$C(=NOH)NH_2$	H	0	0
IV	SCH_3	SH	$C(=S)NH_2$	Rib	0	0
V[5])	SCH_3	SCH_3	H	H	0	10^2
VI	SCH_3	SCH_3	H	Rib	10^3	10^2
VII[4])	SCH_3	SCH_3	CN	H	0	10^2
VIII	SCH_3	SCH_3	CN	Rib	10^1	0
IX[5])	SCH_3	SCH_3	$C(=S)NH_2$	H	10^1	10^1
X	SCH_3	SCH_3	$C(=S)NH_2$	Rib	10^3	10^2
XI	SCH_3	SCH_3	$C(=NOH)NH_2$	H	10^3	10^3
XII	SCH_3	SCH_3	$C(=NOH)NH_2$	Rib	0	0
XIII	SCH_3	SCH_3	$C(=NH)OCH_3$	Rib	0	10^1
XIV	SCH_3	NH_2	H	Rib	0	10^1
XV[3])	SCH_3	NH_2	$C(=S)NH_2$	H	10^2	10^2
XVI	SCH_3	NH_2	$C(=S)NH_2$	Rib	10^4	10^2
XVII	SCH_3	NH_2	$(C=NH)NH_2$	Rib	0	0
XVIII	H	NH_2	H	H	10^1	0
XIX[3])	H	NH_2	H	Rib	0	0
XX	SCH_3	$NHNH_2$	H	H	10^2	0
XXI	SCH_3	$NHNH_2$	H	Rib	10^3	10^1
XXII[4])	SCH_3	$NHNH_2$	$C(=NNH_2)NH_2$	H	0	10^1
XXIII	SCH_3	$NHNH_2$	$C(=NNH_2)NH_2$	Rib	10^2	10^1
XXIV[4])	SCH_3	N	CN	Rib	0	10^2
XXV	SCH_3	N	CN	Rib	$10^{2.5}$	10^1
XXVI	SCH_3	OH	H	H	10^2	0
XXVII	SCH_3	OH	H	Rib	0	10^1
XXVIII	SCH_3	OSH_3	$C(=NH)OCH_3$	Rib	10^2	10^1



[1]) Rib = β-D-ribofuranoside.

[2]) Antiviral activity expressed by decrease in titre (CPD_{50}) evaluated 24 hr after inoculation. 0 means no titre decrease, i. e. the compound was inactive.
The test compounds were used in a concentration of 250 μg/ml with the following exceptions:

[3]) used in a concentration of 125 μg/ml (250 μg/ml was toxic);

[4]) used in a concentration of 62.5 μg/ml (250 and 125 μg/ml were toxic);

[5]) used in a concentration of 32 μg/ml (250, 125 and 62.5 μg/ml were toxic).

Results and Discussion

The compounds tested could be divided into 7 groups based on the substituent in position 4: mercapto-, methylmercapto-, amino-, hydrazine-, dialkyl amino-, oxy, and methoxy- derivatives (see Table 1).

Table 2. Dose-dependence of the antiviral activity of the test compounds

Compound No.	Virus	Decrease in virus titre (log CPD_{50}) produced by the compounds at concentrations of (μg/ml)				
		250	125	62.5	30	15
VI	HSV 1	3	1	1	1	1
	Vaccinia	2	1	1	1	1
X	HSV 1	3	1	1	1	1
	Vaccinia	2	1	1	1	1
XI	HSV 1	3	2	1	1	1
	Vaccinia	3	1	1	1	1
XVI	HSV 1	4	2	2	1	1
XXI	HSV 1	3	1	1	1	1

In untreated control cultures, HSV 1 and vaccinia virus multiplied to a titre of 10^6 CPD_{50}/ml.

No compounds with antiviral activity were found among mercapto-derivatives (I-IV). Only compound II showed a weak innibition of HSV 1 reproduction.

Of 4-methylmercapto- derivatives, compounds VI, X and XI showed a marked and compounds V, VII, VIII, IX and XIII a weak antiviral activity. A typical feature of this group of substances was their approximately equal inhibiting activity against HSV 1 and vaccinia virus. In this respect they were similar to 5-iododeoxyuridine. In compounds V and VI and IX and X, the nucleosides were more active than the corresponding heterocyclic bases, whereas for the pair XI and XII the reverse was true.

The highest antiviral activity was shown by 4-aminoderivatives with a thiocarbamoyl group in position 3; nucleoside XVI was more active than the heterocyclic base XV. Nucleoside XIV exerted a poor antiviral effect. Its position isomer 2-(β-D-ribofuranosyl)-4-amino-6-methylmercaptopyrazolo (3,4-d)pyrimidine, showed a low activity against HSV 1. No antiviral activity against either virus was observed with 4-aminopyrazolo (3,4-d)pyrimidine XVIII and its 1-β-D-ribofuranoside XIX, which are known to possess marked cytotoxic and antitumour activities (Panzica *et al.*, 1978).

Among 4-hydrazine derivatives, an antiviral activity was found in ribofuranosides XXI and XXIII, which were effective predominantly against HSV 1. Compound XXII was toxic for CEC in a concentration of 125 μg/ml.

Also active against HSV 1 was ribofuranoside XXV with N-morpholine group in position 4. When the morpholine cycle was substituted with piperidine (compound XXIV), the activity against HSV 1 disappeared but the compound became active against vaccinia virus with a simultaneous significant increase in its toxic effect for CEC.

Or 4-oxy- derivatives, compound XXVI was active against HSV 1. The corresponding nucleoside XXVII was less active against vaccinia virus. Compound XXVIII with a methoxy group in position 4 showed an insignificant antiviral activity against both viruses.

To evaluate the possibility of clinical utilization, the compounds showing the highest antiviral activity in our experiments were tested in lower concentrations (Table 2). A decrease in the concentration resulted in a marked

decline of antiviral activity. Compound XVI in a dose of 125 μg/ml reduced the virus yield by 2 log CPD_{50}, and ribofuranoside XXI in the same concentration reduced it by 1 log CPD_{50}. The antiviral activity of even the most active compounds was thus manifested only in high concentrations which fact significantly limits the possibilities of their clinical application.

Summarizing the results of the investigation on the biological activities of the compounds tested, we may conclude that antiviral activity was observed quite frequently. According to Dobrynin *et al.* (1980), compounds of this series show a cytotoxic activity with the same frequency. The interesting feature of these compounds is their different cytotoxic and antiviral activity. As a rule, antiviral activity was shown by compounds with a relatively low toxicity for tumour cells.

A comparison of the antiviral activities of nucleosides and the corresponding heterocyclic bases showed that most nucleosides were more active (e. g., compounds V and VI; IX and X; XV and XVI; XVII and XVIII). Reverse relationships were less frequent (compounds VII and VIII; XI and XII; XXVI and XXVII).

The most probable cause of the observed enhancement of the antiviral activity of nucleosides is their lower toxicity for CEC as compared with the corresponding heterocyclic bases. The nucleosides could thus be used in higher concentrations. Lowering of the concentrations of the test compounds decreased their antiviral activity. The results suggest that further search for antiviral preparations among substituted pyrazolo(3,4-d)pyrimidines and their nucleosides appears promising.

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