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ANTIHERPES ACTIVITY OF SOME NOVEL ANALOGUES OF (*E*)-5-(2-BROMOVINYL)-2′-DEOXYURIDINE (*E*-BrVUdR) IN TWO DIFFERENT CELL LINES

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Summary. — In a series of 5-vinyl-2′-deoxyuridine (VUdR) analogues (5-(2-X-vinyl)-UdRs) the (*E*)-5-(2-bromovinyl)-UdR (*E*-BrVUdR) proved the most potent inhibitor of plaque formation of two herpes simplex virus type 1 (HSV-1) strains in human embryonic lung fibroblast (HELF) and African green monkey kidney (Vero) cell cultures. The (*Z*)-5-(2-bromovinyl)-UdR (*Z*-BrVUdR) isomer and the 5-(2,2-dibromovinyl)-UdR (Br_2VUdR) analogue were 10—20 times less efficient, whereas the (*E*)-5-(2-cyanovinyl)-UdR (CNVUdR) and the (*E*)-5-(2-carboxyvinyl)-UdR (COOHVUdR) derivative were only marginally active (10^3—10^4 times less than *E*-BrVUdR). The antiherpes potential of the 5-(2-X-vinyl)-UdRs was compared with that of 5-iodo-, 5-fluoro-, 5-formyl- and 5-ethyl-UdR (IUdR, FUdR, fUdR, EUdR) as well as of 9-(2-hydroxyethoxymethyl)guanine (acyclovir, ACV), 2′-fluoro-5-iodo-1-β-D-arabinofuranosyl(ara)-cytosine (FIAC), 2′-fluoro-5-methylarauracil (FMAU), arabinosyl-thymine (araT) and (*E*)-5-(2-bromovinyl)- and 5-vinyl-araU (BrVaraU, VaraU). In HELF cells the following order of decreasing activity against HSV-1-77 was found: *E*-BrVUdR > > BrVaraU > VaraU > FIAC > FMAU = VUdR = = *Z*-BrVUdR = ACV = araT = FUdR > Br_2VUdR > > IUdR > fUdR > EUdR > CNVUdR > COOHVUdR. The inhibition of HSV-1 replication by most of the investigated compounds was somewhat weaker in the plaque inhibition assay on Vero than on HELF cells, but in the case of the 5-X-araU reference compounds the activity was strongly reduced in Vero cells. In HELF cells the order of decreasing potential against HSV-2 strain 42/78 (HSV-2-42/78) was: FIAC = FMAU > > araT > IUdR = VUdR > ACV = FUdR = > fUdR = EUdR = VaraU > *E*-BrVUdR > *Z*-BrVUdR > Br_2VUdR > > BrVaraU; CNVUdR and COOHVUdR were nearly inactive.

Key words: herpes simplex virus; antiviral activity; (E)-5-(2-bromovinyl)-2′-deoxyuridine; (Z)-5-(2-bromovinyl)-2′-deoxyuridine; 5-(2,2-dibromovinyl)-2′-deoxyuridine

Introduction

Among a great number of 5-substituted derivatives of 2′-deoxyuridine (UdR) (De Clercq, 1980; Reefschläger *et al.*, 1982a) and 2′-deoxycytidine (CdR) (De Clercq *et al.*, 1982), of 1-β-D-arabinofuranosyluracil (araU) (Sakata *et al.*, 1980; Machida *et al.*, 1981; Reefschläger *et al.*, 1983*a*) and in comparison with 2′-fluoro-2′-deoxyarabinofuranosylpyrimidines (Watanabe *et al.*, 1979), as well as with acyclic nucleoside analogues (Elion *et al.*, 1977; De Clercq, 1982; J. Reefschläger, in preparation), (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (*E*-BrVUdR) is one of the outstanding selective and most potent inhibitors of herpes simplex virus type 1 (HSV-1). 10—30 nM *E*-BrVUdR inhibit HSV-1 replication in vitro (ID_{50}) whereas herpes simplex virus type 2 (HSV-2) is only affected at concentration orders of a magnitude higher (8—25 μM) (De Clercq *et al.*, 1980*b*; Reefschläger *et al.*, 1982*b*). The inetficiency of *E*-BrVUdR against HSV-2 was also demonstrated in vivo in hairless mice (De Clercq and Zhang, 1982).

Various attempts to improve the anti-HSV-2 qualities of 5-(2-X-vinyl)--UdR nucleosides by further modifications of the vinyl group (De Clercq *et al.* 1980*a*, 1981; Coe *et al.*, 1982, Reefschläger *et al.*, 1983*b*), of the base (De Clercq *et al.*, 1982) and of the sugar component (Sakata *et al.*, 1980; Machida *et al.*, 1981; Busson *et al.*, 1981; Reefschläger *et al.*, 1983a; Cheng *et al.*, 1981*b*) yielded new potent and selective inhibitors of HSV-1 but did not succeed in narrowing the "gap" between their anti-HSV-1 and their anti--HSV-2 activities.

In order to gain more insight into steric and electronic demands on the 5-(2-X-vinyl) substituent for activity against HSV-1 and HSV-2, a series of new analogues of *E*-BrVUdR was synthesized, i.e. (*Z*)-5-(2-bromovinyl)--UdR (*Z*-BrVUdR), 5-(2,2-dibromovinyl)-UdR (Br_2VUdR), (*E*)-5-(2-cyanovinyl)-UdR (CNVUdR) and (*E*)-5-(2-carboxyvinyl)-UdR (COOHVUdR), and assayed for plaque inhibition of two HSV-1 and two HSV-2 strains in human embryonic lung fibroblasts (HELF) and in African green monkey kidney (Vero) cell cultures. The new derivatives were compared with some known 5-substituted UdR analogues, e.g. 5-iodo-, 5-fluoro-, 5-formyl- and 5-ethyl--UdR (IUdR, FUdR, fUdR, EUdR) as well as with acyclovir (ACV), 2′--fluoro-5-iodoaracytosine (FIAC), 2′-fluoro-5-methylarauracil (FMAU), arabinosylthymine (araT), 5-vinyl-arauracil (VaraU) and (*E*)-5-(bromovinyl)--arauracil (BrVaraU). The synthesis of *Z*-BrVUdR and antiviral properties of *Z*-BrVUdR and of CNVUdR were described recently (Jones *et al.*, 1981; De Clercq *et al.*, 1981).

Materials and Methods

Chemicals. IUdR and FUdR were from Serva (Heidelberg, F.R.G.); ACV was kindly provided by Burroughs Wellcome Co. (Research Triangle Park, N.C.). FIAC and FMAU were a kind gift of J. J. Fox (Sloan Kettering Institute, New York, N. Y.). EUdR was prepared by the method of Niedballa and Vorbrüggen (1974), araT as described by Nakayama *et. al.* (1979), and *E*-BrVUdR, VUdR, fUdR as well as BrVaraU and VaraU were prepared as described previously (Reefschläger *et al.*, 1982*a*; Langen and Bärwolff, 1975; Langen *et al.*, 1976; Bärwolff

and Langen, 1978; Reefschläger *et al.*, 1983*a*). The preparation of *Z*-BrVUdR, Br_2VUdR, CNVUdR and COOHVUdR will be described elsewhere (D. Bärwolff, in preparation).

Cells and viruses. The origin of HELF and of Vero cells, details of their cultivation and media as well as the source of the HSV-1 and HSV-2 strains and the plaque inhibition assays on Vero or HELF cells have been described previously (Reefschläger *et al.*, 1982*a*, *b*; 1983a). The 50% inhibition (ID_{50}) values are the mean of 2–6 plaque inhibition assays with three concentrations each within the inhibition interval of the compounds and with triplicate cultures. The mean inhibition values for these three concentrations were plotted on a decimal scale against the logarithm of the concentration, and the concentration inhibiting plaque formation by 50% was determined graphically.

Results

Inhibition of HSV-1 in human embryonic lung fibroblast (HELF) cell cultures

The results of plaque inhibition assays using HSV-1-77 (a recent clinical isolate) and HSV-1-V3 (a laboratory strain) with some analogues of *E*-BrVUdR (Fig. 1) compared with those of the known 5-substituted UdR derivatives IUdR, FUdR, fUdR, and EUdR as well as of the potent and selective antiherpes agents ACV, FIAC, FMAU, araT, BrVaraU and VaraU are shcwn in Table 1. Of all investigated compounds *E*-BrVUdR was the most effective against both HSV-1 strains. The parent compound VUdR as well as *Z*-BrVUdR and Br_2VUdR were about 10 to 20 times weaker

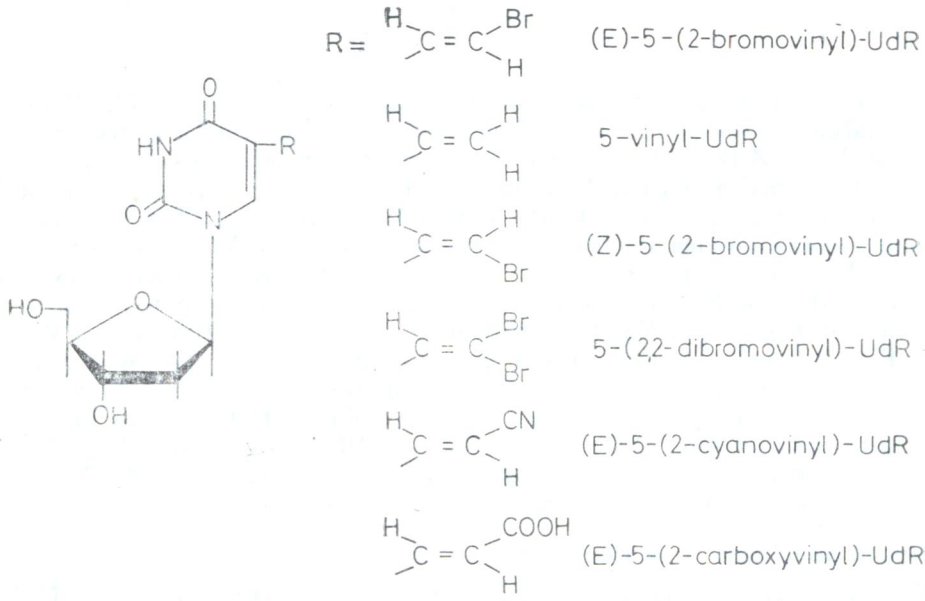

Fig. 1.
Structural formulae of (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (*E*-BrVUdR) analogues

Table 1. Inhibitory effects of 5-substituted UdR analogues and of some reference compounds on plaque formation by two HSV-1 strains in HELF cell cultures

5-Substituent	ID_{50} (μM)*		Ratio $\frac{ID_{50}(HSV\text{-}1\text{-}V3)}{ID_{50}(HSV\text{-}1\text{-}77)}$
	HSV-1-77	HSV-1-V3	
5-X-UdR analogues:			
(*E*)—CH=CH—Br	0.026	0.05	2
—CH=CH_2	0.26	0.5	2
(*Z*)—CH=CH—Br	0.26	0.5	2
—F	0.3	—	—
—CH=CBr_2	0.48	0.75	2
—I	0.8	—	—
—CHO	6.8	—	—
—CH_2—CH_3	10	11	1
(*E*)—CH=CH—CN	95	>1000	>11
(*E*)—CH=CH—COOH	300	780	3
Reference compounds:			
Acyclovir (ACV)	0.24	6.50	27
2′-Fluoro-5-iodoaracytosine (FIAC)	0.14	0.12	1
2′-Fluoro-5-methylarauracil (FMAU)	0.26	0.09	<1
1-β-D-Arabinofuranosylthymine (araT)	0.24	3.1	13
(*E*)-5-(2-Bromovinyl)-ara U (BrVaraU)	0.045	0.55	12
5-Vinyl-araU (VaraU)	0.08	0.37	5

* ID_{50} = concentration required to reduce the number of plaques by 50% as compared with untreated infected cells

inhibitors of both HSV-1 strains, whereas CNVUdR and COOHVUdR were only marginally active (10^3—10^4 times less than *E*-BrVUdR). However, the new analogue *Z*-BrVUdR, though surpassed by the arabincsyl nucleosides BrVaraU, VaraU and FIAC, was against HSV-1-77 as active as FUdR and the prominent compounds VUdR, ACV, FMAU and araT. The activity of all arabinosyl reference nucleosides as well as of ACV and VUdR was superior to Br_2VUdR while the potential of IUdR, fUdR and EUdR was 2—38-fold less than that of *Z*-BrVUdR and Br_2VUdR. In comparison to these two derivatives, the HSV-1-V3 strain was more sensitive to *E*-BrVUdR, FMAU and FIAC, showing similar results with VUdR, BrVaraU, however it was less inhibited by araT and ACV. Comparing the sensitivity of HSV-1-V3 and HSV-1-77 to the compounds (Table 1, column 4) the ratios indicate that with the exception of CNVUdR all 5-X-UdR analogues as well as FIAC and FMAU are similarly effective against both strains whereas the 5-X-araU derivatives and ACV are 5—27 times less inhibitory to HSV-1-V3.

Inhibition of HSV-1 in Vero cells

The sensitivity of HSV-1-77 and HSV-1-V3 towards the *E*-BrVUdR analogues was also tested in a plaque inhibition assay on Vero cells (Table 2). Again, *E*-BrVUdR exhibited the lowest ID_{50} value for both strains, followed

Table 2. Inhibitory effects of 5-substituted UdR analogues and of some reference compounds on plaque formation by two HSV-1 strains in Vero cell cultures

5-Substituent	HSV-1-77		HSV-1-V3		Ratio $\frac{ID_{50}(HSV\text{-}1\text{-}V3)}{ID_{50}(HSV\text{-}1\text{-}77)}$
	ID_{50} (μM)*	Ratio $\frac{ID_{50}(Vero)}{ID_{50}(HELF)^{a)}}$	ID_{50} (μM)*	Ratio $\frac{ID_{50}(Vero)}{ID_{50}(HELF)^{a)}}$	
(*E*)—CH=CH—Br	0.065	3	0.23	5	4
—CH=CH_2	0.13	<1	0.48	1	4
(*Z*)—CH=CH—Br	0.95	4	4.8	10	5
—CH=CBr_2	0.95	2	3.3	4	3
—I	—	—	4.6	—	—
—CHO	—	—	3.6	—	—
(*E*)—CH=CH—CN	>1000	11	>1000	1	1
(*E*)—CH=CH—COOH	245	1	>1000	>1	>4
Reference compounds:					
ACV	0.41	2	15	2	37
FIAC	0.12	1	0.25	2	2
FMAU	0.65	3	0.85	9	1
araT	4.2	18	57	18	14
BrVaraU	3.8	84	>1000	>1818	>263
VaraU	3.2	40	1000	2702	313

* For explanation see legend to Table 1.

[a] Calculated ID_{50} (HELF) values from Table 1.

Table 3. Inhibitory effects of 5-substituted UdR analogues and of some reference compounds on plaque formation of HSV-2

5-Substituent	ID_{50} (μM)		Ratio $\frac{ID_{50}(HSV\text{-}2\text{-}42/78)^{**}}{ID_{50}(HSV\text{-}1\text{-}77)}$
	HSV-2-42/78 HELF	HSV-2-US Vero	
—CH=CH_2	2.2	—	9
—I	2.2	1.2	3
—F	3.7*	—	12
—CHO	6.0	—	1
—CH_2—CH_3	7.0	—	1
(*E*)—CH=CH—Br	27*	3.5	1039
(*Z*)—CH=CH—Br	65*	16	250
—CH=CBr_2	140*	31	292
(*E*)—CH=CH—CN	>500	>1000	>5
(*E*)—CH=CH—COOH	>500	>1000	>2
Reference compounds:			
ACV	3.4*	5.5	14
FIAC	0.37	0.25	3
FMAU	0.48	0.50	2
araT	1.4	—	6
BrVaraU	290	—	6444
VaraU	9	—	113

* ID_{50} values for an another strain HSV-2-82 in HELF cell cultures are: 1.4, 2.3, 13, 21 and 68 μM for FUdR, ACV, *E*-BrVUdR, *Z*-BrVUdR, and Br_2VUdR respectively.
** See Table 1.

by VUdR, and *Z*-BrVUdR and Br_2VUdR were 15—20 times less active As above, CNVUdR and COOHVUdR were nearly inactive. Considering the activity of the reference compounds against both HSV-1 strains, IUdR and fUdR were as active as *Z*-BrVUdR and Br_2VUdR against HSV-1-V3 while the other showed superior activity. Obviously the ID_{50} values of the 5-X--araUs and of ACV for HSV-1-V3 were higher. The almost identical sensitivity of HSV-1-V3 and HSV-1-77 to 5-X-UdR analogues, FIAC and FMAU on the one hand, and the great differences in sensitivity to 5-X-araU derivatives and ACV on the other, was striking (Table 2). In Vero cells the latter compounds exhibit low, if any, activity against HSV-1-V3. Comparing the potential of all investigated analogues against the HSV-1 strains evaluated in Vero or in HELF cells (Table 2, columns 3 and 5) the results point to slight differences only among the ID_{50} values of all derivatives with the exception of the 5-X-araUs which anti-HSV-1 potential was strongly reduced or even lacking in Vero cells (Reefschläger *et al.*, 1983*a*).

Inhibition of HSV-2 in HELF and Vero cell cultures

The inhibition of HSV-2-42/78 (a recent clinical isolate) in HELF cell cultures and of HSV-2-US (a laboratory strain) in Vero cells by the *E*-BrVUdR analogues was evaluated in a plaque inhibition assay in comparison to the

reference compounds tested against HSV-1 (Table 3). None of the novel analogues exhibited better inhibition results against the two HSV-2 strains than *E*-BrVUdR or the parent compound VUdR, which along with IUdR is the most potent of the 5-substituted UdRs. CNVUdR and COOHVUdR were inactive. Of all investigated inhibitors the reference compounds FIAC, FMAU and araT emerged as the best inhibitors of HSV-2 replication, while ACV was less active and comparable with VUdR, IUdR and FUdR, but somewhat more efficient than fUdR, EUdR and VaraU. The reference arabinosyl nucleoside BrVaraU only exhibited a higher ID_{50} value than all active 5-(2-X-vinyl)-UdR analogues.

Discussion

Our results show that the substitution of an H atom on C-2 of the vinyl group in 5-vinyl-2′-deoxyuridine (VUdR) by bromine in the *E* configuration leads to a compound — (*E*)-5-(2-bromovinyl)-UdR (*E*-BrVUdR) — with superior inhibitor activity towards HSV-1 (Table 1 and 2) but reduced activity against HSV-2 (Table 3). When the bromine substituent occupies the *Z* position or when both C-2 hydrogen atoms are replaced by bromine, the resulting compounds — (*Z*)-5-(2-bromovinyl)-UdR (*Z*-BrVUdR) and 5-(2,2-dibromovinyl)-UdR (Br_2VUdR) — display considerably lower activity against both HSV types. In the case of the *E* and *Z* isomers of 5-(2-fluorovinyl)-UdR (FVUdR) these differences are even more pronounced (Reefschläger *et al.*, 1983*b*). Apparently, not only the halogen atom in *E* position but also the hydrogen atom in *Z* position is necessary for high anti-HSV-1 activity. Indeed, *Z*-BrVUdR and Br_2VUdR do not differ appreciably in their activity against HSV-1 and HSV-2 (Table 1, 2 and 3); i.e. the introduction of a second (the "E") bromine substituent into *Z*-BrVUdR hardly modifies the antiherpetic properties of the compound. The importance of the C-2 H in *Z* position is further emphasized by the total lack of activity of *Z*-FVUdR and (*Z*)-5-(2-carboxy-2-fluorovinyl)-UdR, in contrast to *E*--FVUdR (Reefschläger et al., 1983*b*) and (*E*)-5-(2-carboxyvinyl) -UdR (COOHVUdR) (Tables 1 and 2). The replacement of the bromine in *E*--BrVUdR by carboxy- or cyano-residues leads to very weak inhibitors (COOHVUdR and (*E*)-5-(2-cyanovinyl)-UdR (CNVUdR) of HSV-1 which are devoid of an anti-HSV-2 effect. The reduction in anti-HSV-1 activity by a chlorine substitution of a vinyl C-1 H atom in VUdR (De Clercq, 1980), and the relatively low activity of some polyhalogenated 5-alkenyl-UdRs (Coe *et al.*, 1982) also demonstrate the detrimental effect of replacing vinylic H atoms by halogens.

In plaque inhibition assays of two HSV-1 strains in HELF cell cultures VUdR as well as *Z*-BrVUdR and Br_2VUdR were 10 to 20-fold less active than *E*-BrVUdR, comparable with FUdR, but more effective than IUdR and 20—40 times stronger than fUdR and EUdR. We confirm the data of Jones *et al.* (1981) concerning the anti-HSV-1 activity of *Z*-BrVUdR, though they reported a greater difference between the ID_{50} values of the two isomers

(taking into account 0.5% *E* isomer in *Z*-BrVUdR). Possibly our preparation contains a higher percentage of *E* isomer. The higher affinity of the HSV-1-induced thymidine kinase (TK) to the *E* isomer, as compared to the *Z* isomer and, therefore, the better phosphorylation of the former, is held responsible for their different antiherpetic potencies (Cheng *et al.*, 1981*a*) The (*E*)-5-(2-X-vinyl) group does not only bestow excellent anti-HSV-1 properties to deoxyuridine nucleosides (X = chloro, bromo, iodo, methyl, trifluoromethyl) (De Clercq *et al.*, 1980*a*, 1981; Cheng *et al.*, 1980; Reefschläger *et al.*, 1982*a*) but also to deoxycytosines (X = bromo, iodo) (De Clercq *et al.*, 1980a, 1982) and to arabinosyl uracil nucleosides (X = chloro, bromo) (Machida *et al.*, 1981; Busson *et al.*, 1981; Reefschläger *et al.*, 1983*a*). Compared to a series of known antiherpetics *Z*-BrVUdR in its action against HSV-1-77 is comparable to ACV, FMAU and araT, and is surpassed by BrVaraU, VaraU and FIAC only, while Br_2VUdR is inferior to these reference compounds. Against HSV-1-V3 the following order of decreasing activity emerged: FMAU = FIAC > VaraU = *Z*-BrVUdR = BrVaraU = = Br_2VUdR > araT > ACV. In HELF cells this strain was remarkably less sensitive to 5-X-araUs and especially to ACV than HSV-1-77 (Table 1, columns 3 and 4).

In the plaque inhibition assay in Vero cells (Table 2) *E*-BrVUdR again proved the most effective compound against HSV-1, and the new compounds were about as active here as in HELF cells. ACV, FIAC and FMAU were more potent than the new analogues *Z*-BrVUdR and Br_2VUdR towards HSV-1-77. The same order was observed with HSV-1-V3, with the exception of its relative insensitivity to ACV which was also noted in HELF cells. However, the reference compounds araT, VaraU, and BrVaraU were surprisingly ineffective in Vero cells (Table 2, column 4 and 5; Reefschläger *et al.*, 1983*a*). The inactivity of BrVaraU towards HSV-1 in Vero cells and in another African green monkey kidney cell line (BS-C-1) was confirmed by De Clercq (1982).

As shown in Table 3 the anti-HSV-2 potentials of the new compounds neither exceeded those of VUdR and of *E*-BrVUdR, nor those of the standard 5-X-UdRs IUdR, FUdR, fUdR and EUdR. Besides IUdR, VUdR remains the 5-X-UdR derivative most effective against HSV-2 (De Clercq, 1980). The activity declines in the order VUdR > *E*-BrVUdR > *Z*-BrVUdR > > Br_2VUdR and is much lower than that of the established antiherpetics, the activity of which rises in the order BrVaraU ≪ VaraU < ACV < araT < < FMAU = FIAC. The antiviral activity ratios in column 4 express the preference of the 5-(2-X-vinyl)-UdR analogues for HSV-1 whereas other 5-substituted UdR analogues, VUdR and araT, as well as ACV and the 2′-fluorinated arabinosyl nucleosides FMAU and FIAC hardly discriminate between HSV-1 and HSV-2. We would like to point out that the replacement of deoxyribose by arabinose further enhances the gap between anti-HSV-1 and anti-HSV-2 activities of 5-vinyl and 5-bromovinyl uracil nucleosides (Table 3, column 4; and Reefschläger *et al.*, 1983*a*). The low anti-HSV-2 activities of *E*- and *Z*-BrVUdR may be explained by the low affinity of the HSV-2-induced TdR/dTMP kinase towards these substrates (Cheng *et al.*,

1981*a*). However, not the first but the second phosphorylation step is critical (Descamps and De Clercq, 1981; Fyfe, 1982) while the DNA polymerases of HSV-1 and HSV-2 exhibit similar affinities to BrVUTP (Ruth and Cheng, 1981). The antiherpetic acitivities of some other 5-(2-X-vinyl)-UdR analogues which are presently under investigation, e.g. *E*- and *Z*-FVUdR, (*E*)- and (*Z*)-5-(2,2-dihalogenovinyl)-UdRs, (*Z*)-5-(2-carboxy-2-flucrovinyl)-UdR and (*E*)-5-(2-ethoxyvinyl)-UdR support the conclusion that potent inhibitors of HSV-2 are not accessible via substitution of the 5-vinyl C-2 H atoms of pyrimidine nucleosides.

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