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### Antiherpetic activity and mode of action of 2-thio-5-alkyl-pyrimidine nucleoside analogues

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Twenty 2-thio-5-alkyl-pyrimidine nucleoside analogues were synthesized and examined for inhibitory activity against herpes simplex virus (HSV) type 1, ACV resistant TK$^+$ HSV-1 (HSV-ACV-R), varicella-zoster virus (VZV), human cytomegalovirus (HCMV) and GCV resistant HCMV (HCMV-GCV-R) replication in vitro. We purified TKs of HSV-1, HSV-ACV-R and VZV by thymidine agarose affinity gel method and searched inhibitory effects of these compounds against TK activity. As a result, 2-thio-5-propyl-uracil deoxyriboside (TN51) and 2-thio-5-propyl-cytosine deoxyriboside (TN54) showed potent anti-HSVactivity in MRC-5 cells. TN54 was inhibitory against HSV-ACV-R too. 2-thio-5-bromobinyl-uracil arabinoside (TN65) and 2-thio-5-styrene-uracil arabinoside (TN67) showed potent anti-VZV activity. However, none of the compounds inhibited HCMV and HCMV-GCV-R replications. We found that, TN51 inhibited HSV-TK and VZV-TK activites strongly compare with ACV, and TN54 inhibited HSV-TK and HSV-ACV-R-TK activities. The cytotoxicity of the 2-thio-5-alkyl-pyrimidine nucleoside analogues was estimitated by MTT method and all TN51, TN54, TN65 and TN-67 were not cytotoxic to MRC-5 and RPMI8226 (Human B cell line) cells at 100 μ g/ml.

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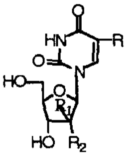
Synthesis, Antiviral Evaluation And Structure Activity Relationships Of Novel 5-Ethyl Analogs Of 2'-Deoxyuridine, 1-β-D-Arabinofuranosyl Uracil And Uridine, R. Kumar*, M. Nath, R. Blush and S.K. Sharma. Department of Medical Microbiology and Immunology, Faculty of Medicine, University of Alberta, Edmonton, AB, Canada T6G 2H7.

Pyrimidine nucleoside analogs that possess a 2-carbon substituent at C-5 position, such as 5-ethyl-2'-deoxyuridine, 5-(2-chloroethyl)-2'-deoxyuridine, *E*-(5)-(2-bromovinyl)-2'-deoxyuridine and *E*-(5)-(2-bromovinyl)-1-β-D-arabinofuranosyl uracil, have exhibited potent and selective antiviral activities against herpes simplex virus type-1 (HSV-1), type-2 (HSV-2), Epstein Barr virus (EBV) and Varicella zoster virus (VZV). A number of C-5 ethyl substituents were therefore investigated to determine their usefulness as antiviral pharmacophores. The new class of 5-(1-cyanamido-2-haloethyl)-(**4**-**6**, X = Cl, Br, I) were synthesized by the regiospecific addition of halogenocyanamides (X-NHCN) to the 5-vinyl substituent of the respective 5-vinyl-2'-deoxyuridine (**1**), 2'-arabinouridine (**2**) and uridine (**3**). Reaction of **1** with sodium azide and ceric ammonium nitrate in acetonitrile-methanol or water afforded the 5-(1-methoxy-2-azidoethyl)-(**7**) and 5-(1-hydroxy-2-azidoethyl)-products (**8**). Of the newly synthesized compounds, 5-(1-cyanamido-2-bromoethyl)-2'-deoxyuridine (**4**, X = Br) exhibited most potent *in vitro* activity against HSV-1, HSV-2 and VZV. Compound **4** (X = Br) was 16, 25 and 15 times less active than the reference drug acyclovir against HSV-1, HSV-2 and VZV, respectively. In contrast, the corresponding 5-[1-methoxy (or hydroxy)-2-azidoethyl]-2'-deoxyuridines (**7** and **8**) were inactive against herpes viruses. However, the host cell cytotoxicity against uninfected human foreskin fibroblast (HFF) cells of compounds within these new class of compounds was found to be minimum ($IC_{50}$ = >100 μg/ml). The structure activity relationships of these nucleosides will be discussed.



**1**, R = $CH{=}CH_2$, $R_1 = R_2 = H$
**2**, R = $CH{=}CH_2$, $R_1 = OH$, $R_2 = H$
**3**, R = $CH{=}CH_2$, $R_1 = R_2 = OH$
**4**, R = $CH(NHCN)\text{-}CH_2X$, $R_1 = R_2 = H$
**5**, R = $CH(NHCN)\text{-}CH_2X$, $R_1 = OH$, $R_2 = H$
**6**, R = $CH(NHCN)\text{-}CH_2X$, $R_1 = R_2 = OH$
**7**, R = $CH(OCH_3)\text{-}CH_2N_3$, $R_1 = R_2 = H$
**8**, R = $CH(OH)\text{-}CH_2N_3$, $R_1 = R_2 = H$

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### Design, synthesis and evaluation of new antiviral nucleosides.

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We have recently reported the discovery of an entirely new category of potent antiviral agents based on novel deoxynucleoside analogues with an unusual fluorescent bicyclic base bearing a long alkyl side-chain, with an optimum length of C8-C10 for antiviral activity. This structural feature has a significant impact on the physical properties of these compounds; their lipophilicity is rather high, whilst their water solubility is very low. This could mean a poor bio-availability and, thus, could limit the use of these nucleosides as potential drugs. Therefore, we prepared new analogues that contain ether and glycol type side-chains, designed to enhance water solubility. The total length of the side-chain was constrained to 10 atoms, previously determined to be the optimum length for activity. The synthesis of this class of compounds involves the Pd-catalysed coupling of terminal alkynes with 5-iodo 2'-deoxyuridine. The 5-alkynyl nucleosides obtained are then treated with copper (I) iodide, leading to the desired targets. The alkynes used in the coupling step, were prepared by a Williamson type reaction between the appropriate halide and alcohol, in the presence of sodium hydride. UV studies demonstrated a dramatic increase in the solubility of these compounds, proving that this approach was essentially correct. Antiviral data are awaited.

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THE ANTIVIRAL ACTIVITY EXERTED BY VACCINIA VIRUS ON THE GROWTH OF HERPES SIMPLEX VIRUS IN BS-C-1 CELLS. Ehud Katz and Kazem Keywan. Department of Virology, Hebrew University - Hadassah Medical School, Jerusalem, Israel.

The growth of herpes simplex virus type 2 (HSV-2) in BS-C-1 cells, was inhibited following super-infection with vaccinia virus. Since this inhibition was not affected by the presence of isatin-beta-thiosemicarbazone, a specific inhibitor of vaccinia virus "late" functions, it is suggested that either a structural component of vaccinia virion or an "early" function taking place during vaccinia virus growth, is involved. Ultraviolet irradiation of vaccinia virus, that completely inactivated the infectivity of the virus, resulted in partial loss of the capability to inhibit the growth of HSV-2; at least sixteenfold more irradiated virus were needed for obtaining a comparable level of inhibition to that caused by infectious virus. Electron microscopy showed that the irradiated vaccinia virus adsorbed and penetrated into the HSV-infected cells but then remained morphologically intact within the cells for at least 22 hours. When the steps during the growth of HSV, affected by the irradiated vaccinia virus, were followed, it was found that while the synthesis of HSV DNA was only partially decreased, the synthesis of HSV proteins, observed by both polyacrylamide gel electrophoresis and immunofluorescent staining, was very strongly inhibited. This may be the reason for the failure to detect virus particles in the cells either by electron microscopy of sections of the infected cells or by purification of virions following sucrose gradient centrifugation.