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5-Bromo (or Chloro)-6-azido-5,6-dihydro-2′-deoxyuridine and -thymidine Derivatives with Potent Antiviral Activity

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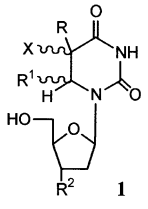
Abstract—Synthesis, antiviral, and cytotoxic activities of 5-bromo (or chloro)-6-azido-5,6-dihydro-2′-deoxyuridine (**4**,**5**) and -thymidine (**6**,**7**) are reported. Compounds **4** and **5** exhibited a broad spectrum of antiherpes activity against (HSV-1, HSV-2, HCMV, and VZV).

Pathogens of the herpes virus family, such as Herpes simplex virus type-1 (HSV-1), type-2 (HSV-2), human cytomegalovirus (HCMV), Epstein–Barr virus (EBV), and Varicella zoster virus (VZV), are ubiquitous viruses that cause mild to severe illnesses in the immunocompetent host.[1] Herpes viruses are opportunistic pathogens in individuals with AIDS and in patients whose immune system has been suppressed for transplant purposes.[2] Most individuals with AIDS are infected by one or more herpes viruses.[3,4] Acyclovir and its prodrug, are widely used drugs for the treatment of herpes viruses.[5] However, acyclovir is somewhat less active against VZV and not effective against HCMV infections. Ganciclovir is an effective agent for HCMV, however dose related toxicity is associated with its use. In addition, both acyclovir and ganciclovir resistant virus strains have been increasingly realized in the clinic.[5]



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1a, R = H, X = F, R^1 = OH, R^2 = OH
1b, R = H, X = F, R^1 = OAc, R^2 = OH
1c, R = Me, X = Br, R^1 = alkoxy, R^2 = OH
1d, R = Et, X = halogen, R^1 = alkoxy or azido, R^2 = OH
1e, R = Me, X = halogen, R^1 = alkoxy or azido, R^2 = N_3
1f, R = Me, X = halogen, R^1 = alkoxy or azido, R^2 = F

5,6-Dihydropyrimidine nucleosides have attracted attention as potential antiviral and antitumor agents.[6,7] Physiological dihydro nucleosides play an important role in nucleic acid metabolism and appear frequently in the sequence of tRNA.[8] Previous studies have shown that 5,6-dihydro analogues of thymidine (**1c**) can act as competitive substrates, to thymidine, for thymidine kinase.[9,10] 5-Fluoro-6-hydroxy (or acetoxy)-5,6-dihydro-2′-deoxyuridine diastereomers (**1a**,**b**) have been investigated as prodrugs to 5-fluoro-2′-deoxyuridine.[6] In earlier studies, we described the synthesis and in vitro antiviral activity of a series of 5-halo-6-alkoxy (or azido)-5,6-dihydro derivatives (**1d–f**) of antiviral pyrimidine nucleosides as potential prodrugs.[11–16] It was observed that the groups at C-5 and C-6 positions in the 5,6-dihydro derivatives created a potentially interesting enhancement of lipophilicity with respect to that of the parent nucleosides. It was also found that 5,6-dihydropyrimidine nucleosides (**1d–f**) serve as slow releasers (prodrugs) of the parent nucleosides in vivo and were stable to glycosidic bond cleavage. These beneficial properties of 5,6-dihydropyrimidine nucleosides (**1d–f**) encouraged us to further investigate 5,6-dihydro derivatives of 2′-deoxyuridine and -thymidine to study their biological activity.

In an earlier study, we reported the synthesis, antiviral and cytotoxic properties of 5-[1-azido (hydroxy or alkoxy)-2-haloethyl]-2′-deoxyuridines.[17–20] We observed that among azido, hydroxyl or alkoxyl groups, an azido substituent was a determinant of antiviral and a potential determinant of cytotoxic activities. It was therefore of interest to explore the effect of azido substituent attached to the C-6 position of 2′-deoxyuridine and thymidine on biological properties. In this communication, we report the synthesis, antiviral and cytotoxic activities of the 5-bromo (or chloro)-6-azido-5,6-dihydro derivatives of 2′-deoxyuridine and -thymidine (**4–7**).

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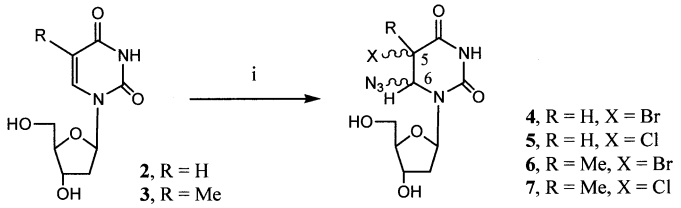

Scheme 1. Reagents and conditions: (i) *N*-bromosuccinimide, NaN_3, DME, 0 °C, 30 min (**4**,**6**); *N*-chlorosuccinimide, NaN_3, DME, 0 °C, 12 h (**5**,**7**).

The target 5-bromo (or chloro)-6-azido-5,6-dihydro-2′-deoxyuridine (**4**,**5**) and -thymidine (**6**,**7**) were synthesized[21] in a single step, by a simple and convenient route, by reaction of 2′-deoxyuridine (**2**) or thymidine (**3**) with *N*-bromosuccinimide or *N*-chlorosuccinimide and sodium azide in 1,2-dimethoxyethane (DME) in 40–58% yields as illustrated in Scheme 1. The 5-halo-6-azido-5,6-dihydro derivatives **4**–**7** most likely arise via the initial formation of a 5,6-halonium ion intermediate which is susceptible to regiospecific nucleophilic attack by azide anion, at the sterically less hindered C-6 position. Another possible explanation for regioselectivity in the formation of **4** and **5** is that the halonium ion is open with the help of the N-atom pair of electrons and then the azide attacks to the imine. The target compounds **4**–**7** were characterized by ^{1}H NMR, ^{13}C NMR, and elemental analyses.[22]

The compounds **4**,**5** and **6**,**7** are a mixture of diastereomers,[22] and differ in configuration at C-5 and/or C-6 positions. These diastereomers could not be separated by flash silica column chromatography, or the multiple development TLC technique. In an earlier study we reported that (+) and (−) diastereomers of 5-bromo-6-methoxy-5,6-dihydro derivatives of 5-ethyl-2′-deoxyuridine exhibited similar anti-HSV-1 and HSV-2 activity.[11] Similar observations were also made by Fouque and Teoule where (+) and (−) diastereomeric forms of 5-bromo-6-alkoxy-5,6-dihydro analogues of thymidine did not exhibit any difference in their biological activities.[10]

The antiviral activity of 5-halo-6-azido-5,6-dihydro-2′-deoxyuridine (**4**,**5**) and -thymidine (**6**,**7**) was measured against herpes viruses (HSV-1, HSV-2, HCMV, VZV, and EBV) by the National Institutes of Health, USA as described earlier.[19] The results are summarized in Table 1. The 5-bromo-6-azido (**4**) and 5-chloro-6-azido (**5**) derivatives of 2′-deoxyuridine were found to exhibit potent in vitro antiviral activity against several herpes viruses. In these compounds (**4**,**5**), the nature of the halogen at C-5 was a determinant of HSV-1, HSV-2, HCMV and VZV antiviral activities, where the relative activity order was [Cl > Br (HSV-1, HSV-2, and VZV), Br > Cl (HCMV)]. The anti-HSV-1 and HSV-2 activity exhibited by the 5-chloro-6-azido (**5**) analogue was nine and four times higher, respectively, to that of the reference drug, acyclovir. In contrast, the 5-bromo-6-azido (**6**) and 5-chloro-6-azido (**7**) derivatives of -thymidine were both inactive against HSV-1 and HSV-2. These test results indicate that a methyl substituent at the C-5 position of 5-halo-6-azido-5,6-dihydro-2′-deoxyuridine is detrimental to anti-HSV-1 and HSV-2 activity. Although 5-chloro-6-azido (**6**) and 5-bromo-6-azido (**7**) 5,6-dihydro derivatives of thymidine were both inactive against HSV-1 and HSV-2, the 5-bromo-6-azido (**6**) analogue exhibited marked antiviral activity against HCMV and VZV that was approximately 1/20 and 1/4, respectively, of that exhibited by the reference drugs ganciclovir and acyclovir. These studies suggest that 5-halo-6-azido derivatives of 2′-deoxyuridine (**4**,**5**) can undergo phosphorylation by herpes virus-encoded thymidine kinase or UL97 and/or inhibit viral DNA polymerase to exhibit potent antiviral activity.

The 5-halo-6-azido derivatives of 2′-deoxyuridine (**4**,**5**) and thymidine (**6**,**7**) were evaluated by the National Cancer Institute, USA antiviral evaluation branch in an in vitro anti-HIV screen using HIV-1 infected CD4 lymphocytes (CEM cell line).[25] The results indicated that compounds **4**–**7** were inactive against HIV-1. However, they were cytotoxic to uninfected host cells at higher concentrations (> 100 μM).

Table 1. In vitro antiviral activities of 5-halo-6-azido derivatives of 2′-deoxyuridine (**4**,**5**) and thymidine (**6**,**7**)[23]

Compd	R	X	EC_{50} (μM)[23,24]					IC_{50} (μM)	
			HSV-1 (E-377)	HSV-2 (MS)	HCMV (AD-169)	VZV (Ellen)	EBV (P3HR-1)	Cyto-toxicity	Cell proliferation
4	H	Br	8.28	3.42	11.4	46.0	91.42	127	7.4
5	H	Cl	0.33	1.6	28.9	9.8	ND[b]	> 327	54.6
6	Me	Br	> 274	> 274	15.0	16.0	103.8	73.0	142.7
7	Me	Cl	> 313	> 313	> 50	> 12.5	ND	113	134.8
CDU[a]			15.0	ND	ND	ND	ND	ND	ND
Acyclovir			3.08	6.16	ND	3.96	8.81	> 440	ND
Ganciclovir			ND	ND	0.78	ND	ND	> 390	ND

[a]5-Chloro-2′-deoxyuridine.
[b]Not determined.

The anticancer activities of compounds **4**–**7** were determined by the National Cancer Institute, USA, using an in vitro assay.[26] These tests were designed to evaluate selectivity against individual tumor cell lines or tumor types by revealing differential growth inhibitions. The data are presented in Table 2. Although compounds **4** and **5** showed selectivity for a number of tumor cell lines (GI_{50}), the 5-halo-6-azido compounds (**4**–**7**) were not cytotoxic (TGI and $LC_{50} > 100$ μM) except **5**, which had a TGI value of > 36.3 μM for non small cell lung cancer (NCI-H522).

Table 2. In vitro evaluation and selectivities of 5-halo-6-azido derivatives of 2′-deoxyuridine (**4**,**5**) and thymidine (**6**,**7**) against various tumor cell lines[26,27]

No.	R	X	Tumor (cell line selectivity)	GI_{50} (μM)
4	H	Br	Leukemia (CCRF-CEM)	38
			Leukemia (RPMI-8226)	19.6
			Small cell lung cancer (DMS114)	90.9
			Melanoma (MALME-3M)	15.5
			Melanoma (M19-MEL)	91.5
			Melanoma (SK-MEL-5)	42.2
			Renal cancer (CAKI-1)	66.3
5	H	Cl	Leukemia (CCRF-CEM)	26.3
			Leukemia (RPMI-8226)	72.4
			Non small cell lung cancer (NCI-H522)	5.37
			CNS cancer (SF-268)	93.3
			Melanoma (MALME-3M)	13.1
			Melanoma (M19-MEL)	52.4
			Melanoma (SK-MEL-5)	12.3
6	Me	Br	No selectivity	
7	Me	Cl	No selectivity	

In summary, the 5-halo-6-azido-5,6-dihydro derivatives, which prefer a hydrogen atom at the C-5 position for maximum activity, exhibited antiviral activity against HSV-1, HSV-2, HCMV and VZV. The 5-chloro-6-azido derivative of 2′-deoxyuridine (**5**), which possesses low host cell cytotoxicity ($IC_{50} = > 327$ μM; Table 1), exhibited broad spectrum antiviral activity against herpes viruses HSV-1, HSV-2, HCMV, and VZV. These promising results support the introduction of an azido moiety at C-6 position of the pyrimidine base. The antiviral activity of compound **5** is apparently intrinsic, and not due to its putative conversion into 5-chloro-2′-deoxyuridine analogue like other 5,6-dihydro pyrimidine nucleosides.[6,11–16] This is because (i) compound **5** was resistant to 5,6 double bond generation by reaction with glutathione, a tissue nucleophile at 37 °C and (ii) the 5-chloro-2′-deoxyuridine analogue exhibits moderate anti herpes activity. Although compound **5** was much less active against HCMV, it was nine and four times more potent against HSV-1 and HSV-2, respectively, and nearly as potent as ACV against VZV. An in vitro phosphorolysis study in the presence of *Escherichia coli* thymidine phosphorylase for 30 min at 37 °C indicated that the compound **5** is resistant to glycosidic bond cleavage. Compound **5** could serve as a useful lead compound for the development of an improved antiviral drug. Further in vitro and in vivo work, and structure–activity relationship studies of this class of compounds are currently underway.

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21. General procedure for the synthesis of nucleosides derivatives **4**–**7**. A mixture of **2** or **3** (0.1 mmol), *N*-bromosuccinimide (0.1 mmol) or *N*-chlorosuccinimide (0.2 mmol), sodium azide (0.4 mmol) in water (0.1 mL) and DME (10 mL) was stirred at 0 °C for 30 min to 12 h. Removal of the solvent in vacuo and purification of the crude product by silica gel

column chromatography with $CHCl_3$-MeOH (92:8, v/v) as eluent afforded products **4**–**7**. **CAUTION**: Halogenated solvents such as dichloromethane must not be used in these reactions, since its reaction with sodium azide may produce potentially explosive polyazidomethane.

22. Selective data for compound **4**: (mixture of two diastereomers in a ratio of 1:3) 300 MHz δ_H (CD_3OD) 2.04–2.15 and 2.16–2.34 (2 m, 1H each, H-2′), 3.70–3.80 (m, 2H, H-5′), 3.88–3.98 (m, 1H, H-4′), 4.34–4.42 (m, 1H, H-3′), 4.52 and 4.66 (2 d, $J_{5,6}=3.0$ Hz, 1H total, H-5), 5.93 and 5.96 (2 d, $J_{5,6}=3.0$ Hz, 1H total, H-6), 6.16 and 6.32 (2 t, 1H total, H-1′), δ_C (CD_3OD) 39.38 and 40.48 (C-5), 40.31 and 39.49 (C-2′), 63.26 and 62.86 (C-5′), 70.29 and 70.56 (C-6), 72.72 and 72.90 (C-3′), 85.18 and 86.11 (C-1′), 88.15 and 88.63 (C-4′), 151.76 (C-4), 166.95 and 167.04 (C-2). Anal. calcd for $C_9H_{12}BrN_5O_5$: C, 30.87, H, 3.45, N, 20.0. Found: C, 30.58, H, 3.49, N, 19.89. **5**: (mixture of two diastereomers in a ratio of 1:3) δ_H (CD_3OD) 2.04–2.34 (m, 2H, H-2′), 3.72–3.86 (m, 2H, H-5′), 3.88–3.98 (m, 1H, H-4′), 4.35–4.41 (m, 1H, H-3′), 4.44 and 4.58 (2 d, $J_{5,6}=3.0$ Hz, 1H total, H-5), 5.95 and 5.97 (2 d, $J_{5,6}=3.0$ Hz, 1H total, H-6), 6.18 and 6.34 (2 t, 1H total, H-1′). Anal. calcd for $C_9H_{12}ClN_5O_5$: C, 35.36, H, 3.95, N, 22.91. Found: C, 34.98, H, 3.86, N, 22.50. **6**: (mixture of three diastereomers in a ratio of 4:1:1) δ_H (CD_3OD) 1.96, 1.98 and 2.0 (3 s, 3H total, CH_3), 2.06–2.40 (m, 2H, H-2′), 3.70–3.88 (m, 2H, H-5′), 3.90–4.0 (m, 1H, H-4′), 4.40–4.48 (m, 1H, H-3′), 5.97, 5.99 and 6.06 (3 s, 1H total, H-6), 6.06, 6.18 and 6.30 (3 t, 1H total, H-1′). Anal. calcd for $C_{10}H_{14}BrN_5O_5$:C, 32.98, H, 3.87, N, 19.23. Found: C, 33.06, H, 3.92, N, 19.24. **7**: (mixture of three diastereomers in a ratio of 2:4:1) δ_H (CD_3OD) 1.78, 1.80 and 1.82 (3 s, 3H total, CH_3), 2.04–2.32 (m, 2H, H-2′), 3.70–3.85 (m, 2H, H-5′), 3.88–3.98 (m, 1H, H-4′), 4.38–4.46 (m, 1H, H-3′), 5.92, 5.94 and 6.0 (3 s, 1H total, H-6), 6.15, 6.18, and 6.30 (3 t, 1H total, H-1′). Anal. calcd for $C_{10}H_{14}ClN_5O_{5.1/2H_2}O$): C, 36.53, H, 4.59, N, 21.30. Found: C, 36.19, H, 4.79, N, 20.96.

23. IC_{50} is the drug concentration (μM) required to reduce the uptake of neutral red stain by uninfected cell monolayers to 50% of untreated, uninfected controls; EC_{50} is the drug concentration (μM) required to reduce the viral cytopathic effect (CPE) in infected cell monolayers to 50% of untreated, infected controls.

24. Mean of two to four assays, standard deviations were within 10% of the mean.

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27. Cell line selectivity is defined as an increased sensitivity to the test compounds of tumors from one or more neoplastic diseases relative to other cell lines which encompassed a panel of leukemia, non small cell lung, small cell lung, colon, CNS, melanoma, ovarian and renal cancers, respectively, ~54 tumor cell lines originating from the eight types of tumors listed were used in this assay, the reference drugs 5-fluorouracil and 5-fluoro-2′-deoxyuridine showed selectivity for one or more cell line in each of the right tumor types. The GI_{50}, TGI, and LC_{50} values are the test compound concentrations (μM) that results in a 50, 0 and −50% growth of cells, respectively, relative to the growth of the tumors in the absence of the test compounds, No selectivity means that the GI_{50}, TGI, and LC_{50} values were >100 μM for all tumor cell lines for all tumor types.