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Synthesis and Enzymatic Transformations of 5-Halo-6-Methoxy-5,6-Dihydro Derivatives of 5-[1-Methoxy-2-halo (or 2,2-dihalo)ethyl]-2′-deoxyuridines as Potential Herpes Simplex Virus Inhibitors

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The 5-halo-6-methoxy-5,6-dihydro derivatives of 5-[1-methoxy-2-halo(or 2,2-dihalo)ethyl]-2′-deoxyuridines (3–12) were synthesized and investigated as potential anti-herpes agents. These 5,6-dihydro derivatives were designed to act as potential prodrugs to 5-[1-methoxy-2-halo(or 2,2-dihalo)ethyl]-2′-deoxyuridines (2a–e), with enhanced metabolic stability, and ready conversion to the parent molecules. These 5,6-disubstituted-5,6-dihydro analogs are stable to *E. coli* thymidine phosphorylase, and undergo regeneration of the 5,6-olefinic bond to provide parent moieties (2a–e), upon incubation with glutathione at 37°C. The compounds (3–12) themselves were found to be non-inhibitory against herpes simplex virus type-1 (HSV-1), likely due in part to their inability to undergo conversion to parent compounds in cell culture medium.

Keywords: Pyrimidine nucleosides; 5-Substituted-2′-deoxyuridines; 5,6-Dihydro derivatives; Inhibitors; Drug design

INTRODUCTION

Pathogens of the herpes virus family are ubiquitous viruses that cause mild to severe illnesses in immunocompetent hosts. They include: herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2), Epstein Barr virus (EBV), Varicella zoster virus (VZV), human cytomegalo virus (HCMV), human herpes viruses types 6,7, and 8 (HHV-6, HHV-7 and HHV-8). Herpes viruses' are serious and often life threatening opportunistic pathogens in immuno-compromised individuals including solid organ or bone marrow transplant recipients and persons with acquired immunodeficiency syndrome (AIDS).[1] Most individuals with AIDS are infected by one or more herpes viruses. HSV-1 and HSV-2 infections lead to orolabial, genital and anorectal mucocutaneous disease, esophagitis, and life-threatening encephalitis [central nervous system (CNS) infection].[1] EBV is increasingly associated with diseases of epithelial cells and lymphocytes, such as nasopharyngeal carcinoma, Burkitt's lymphoma, Hodgkin's disease, Infectious mononucleosis and EBV-associated lymphoproliferative disease.[2,3] Complications resulting from VZV infections can be life-threatening in immunocompromised patients.[1] Shingles due to reactivation of VZV is a major presenting sign of underlying HIV infection. Shingles may become chronic and lead to hyperkeratotic, verrucous lesions and may also lead to zoster encephalitis. VZV infection of the eye can present as acute retinal necrosis or necrotizing retinitis.[1] At the present time, these viruses are not controlled by vaccination. Acyclovir (**1**) is a widely used drug for the treatment of opportunistic herpes virus infections in immunocompromised patients. However, resistance to acyclovir is increasing. Therefore, the search to find new agents for the treatment and suppression of herpes viruses in

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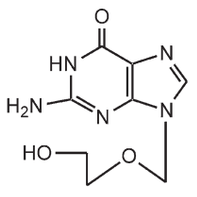
patients with AIDS has to be continued for the management of these infections.



1 (Acyclovir)

In earlier studies,[4,5] we reported the synthesis and anti-HSV-1 activity of 5-(1-methoxy)-2-iodoethyl-2′-deoxyuridine (**2a**) and 5-(1-methoxy)-2-bromoethyl-2′-deoxyuridine (**2b**). The activity of **2b** ($EC_{50} = 0.1\,\mu g/ml$) approached that of acyclovir ($EC_{50} = 0.01\,\mu g/ml$). In subsequent studies, we found that both compounds **2a** ($EC_{50} = 0.7\mu g/ml$) and **2b** ($EC_{50} = 0.19\,\mu g/ml$), exhibited potent *in vitro* activity against EBV relative to the reference drug acyclovir ($EC_{50} = 7.4\,\mu g/ml$).[6] Compounds **2a** and **2b** also effectively inhibited VZV replication *in vitro* ($EC_{50} = 2.5\,\mu g/ml$ range) at concentrations comparable to acyclovir. Like acyclovir, 5-(1-methoxy)-2-bromo-2-chloroethyl-2′-deoxyuridine (**2e**) was a potent inhibitor of HSV-1 and VZV.[7]

Previously we have investigated a variety of 5,6-dihydro derivatives of pyrimidine nucleosides.[8–11] The 5,6-dihydro diastereomers of antiviral agents were found to exhibit potent antiviral activity, with significantly enhanced lipophilicity, compared to parent nucleoside analogs. These 5,6-dihydro analogs were chemically stable under acidic conditions and possessed enhanced oral bioavailability and improved pharmacokinetic parameters compared to the parent compounds. These analogs could serve as prodrugs to antiviral nucleosides. The beneficial properties of 5,6-dihydro uracil nucleosides and our objective to extend the structure–activity studies provided the impetus to investigate 5,6-dihydro derivatives of 5-(1-methoxy-2-halo(or 2,2-dihalo)ethyl)-2′-deoxyuridines (**2a–e**) as potential anti-herpes agents. We now report the synthesis, anti-HSV-1 activity and enzymatic transformation studies of 5-halo-6-methoxy-5,6-dihydro derivatives of 5-[1-methoxy-2-halo(or 2,2-dihalo)ethyl]-2′-deoxyuridines (**3–12**) as potential prodrugs of **2a–e** that may possess improved pharmacokinetic and/or biodistribution properties.

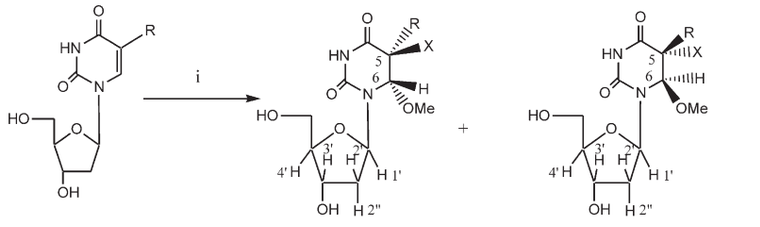
MATERIALS AND METHODS

Chemistry

Melting points were determined with a Buchi capillary apparatus and are uncorrected. Nuclear magnetic resonance spectra (^{1}HNMR, ^{13}CNMR) were determined on a Bruker AM-300 spectrometer using Me_4Si as an internal standard. The assignment of all exchangeable protons (OH, NH) was confirmed by the addition of D_2O. Preparative thin layer chromatography (PTLC) was carried out using Whatman PLK5F plates (1 mm in thickness). Silica gel column chromatography was carried out using Merck 7734 silica gel (100–200 micro particle size). Thin layer chromatograpy (TLC) was performed with Whatman MK6F silica gel microslides. HPLC analyses were performed using a Water′s System with detection at 230 nm (5,6-dihydro compound). The 5-substituted-2′-deoxyuridines **2a–e** were prepared by using our previously reported procedures.[4,5,7]

Reaction of a diastereomeric mixture (R, S), of 5-(1-methoxy-2-halo(or 2,2-dihalo)ethyl-2′-deoxyuridines (**2a–e**)[4,5,7] with N-bromosuccinimide and N-chlorosuccinimide in methanol and glacial acetic acid at 25°C produced the mixture of (+)-trans-(5S, 6S)-(**3**,**5**,**7**,**9**,**11**) and (−)-trans-(5R, 6R)-(**4**,**6**,**8**,**10**,**12**) diastereomers of 5-bromo-5-(1-methoxy-2-halo (or 2,2-dihalo)-ethyl-6-methoxy-5,6-dihydro-2′-deoxyuridines in 57–78% yields that could not be separated by column or TLC chromatography (Scheme 1). These 5,6 dihydro derivatives (**3–12**) of 5-(1-methoxy-2-halo (or 2, 2-dihaloethyl)-2′-deoxyanidines most likely arise via the initial formation of a 5, 6-halonium ion intermediate which is susceptible to regiospecific nucleophilic attack by methanol at the sterically less hindered C-6 position. The configuration of compounds (**3–12**) at the C-5 and C-6 positions was assigned by comparing the ^{1}HNMR spectral data with that of similar compounds, for which the absolute configuration is known, such as 5-bromo-6-methoxy-5, 6-dihydrothymidine,[12,13] 5-bromo-6-hydroxy-5, 6-dihydro-2′deoxyuridine[14] and 5-bromo-5-ethyl-6-methoxy-5,6-dihydro-2′-deoxyuridine diastereomers.[14] The most distinct differences in chemical shift positions in the ^{1}HNMR spectra of these diastereomers occurred at the H-1′, H-2′, and H-2″ protons in the sugar moiety and the H-6 proton of the base. Each trans compound (**3–12**) was a mixture of two diastereomers which differ in configuration (R,S) at the 1-carbon of the 5-(1-methoxy)-2-halo (or 2,2-dihalo) ethyl substituent. Attempts to separate the (+) trans-(5S,6S) and (−)-trans-(5R,6R) (**3–12**) and diastereomers of each trans compound by flash column chromatography, or multiple development TLC chromatography were unsuccessful.

For example ^{1}HNMR spectrum (CD_3OD) of compound (**7**) and (**8**) exhibited four separate sets of doublets assigned for the CHC*H*Br_2 resonances at 6.06, 6.32, 6.48 and 6.48, four separate sets of doublets assigned to the C*H*CHBr_2 resonances at 4.10, 4.21,



2a, R = $CH(OMe)CH_2I$ | **3**, R = $CH(OMe)CH_2I$, X = Br | **4**, R = $CH(OMe)CH_2I$, X = Br
2b, R = $CH(OMe)CH_2Br$ | **5**, R = $CH(OMe)CH_2Br$, X = Br | **6**, R = $CH(OMe)CH_2Br$, X = Br
2c, R = $CH(OMe)CHBr_2$ | **7**, R = $CH(OMe)CHBr_2$, X = Br | **8**, R = $CH(OMe)CHBr_2$, X = Br
2d, R = $CH(OMe)CHCl_2$ | **9**, R = $CH(OMe)CHCl_2$, X = Cl | **10**, R = $CH(OMe)CHCl_2$, X = Cl
2e, R = CH(OMe)CHBrCl | **11**, R = CH(OMe)CHBrCl, X = Cl | **12**, R = CH(OMe)CHBrCl, X = Cl

SCHEME 1 Reagents: i, N-bromosuccinimide, MeOH, glacial acetic acid, 25°C (**3**–**8**); N-chlorosuccinimide, MeOH, glacial acetic acid, 25°C (**9**–**12**).

4.28, and 4.46, and four singlets for H-6 at 5.22, 5.25, 5.30, and 5.38.

General Procedure for the Preparation of 5-halo-5-[1-methoxy-2-halo (or 2,2-dihalo)ethyl]-6-methoxy-5,6-dihydro-2′-deoxyuridines(3–12)

N-Bromosuccinimide (0.11 mmol) [or N-chlorosuccinimide (0.15 mmol)] was added slowly with stirring to a solution of (**2a**–**e**) (0.1 mmol) in methanol 10 mL and glacial acetic acid (10 μL). The reaction was allowed to proceed for 12–20 h at 25°C prior to neutralization with methanolic sodium hydroxide. Removal of the solvent in vacuo, dissolution of the residue in methanol (5 mL), adsorption on to silica gel (2 g), removal of solvent *in vacuo*; and application of this material to the top of a silica gel column followed by elution with chloroform–methanol (92:8, v/v) yielded compounds (**3**–**12**) as a mixture of the (+)-trans-(5S,6S) and (−)-trans-(5R,6R)-diastereomers in a ratio of 2:1 (**3**–**6**), 1:1 (**7**,**8**), 1:2 (**9**–**12**), determined by integration of the H-1′ resonances.

(+)-Trans-(5S,6S)-5-bromo-5-(1-methoxy-2-iodoethyl)-6-methoxy-5,6-dihydro-2′-deoxyuridine (**3**) and (−)-trans-(5R,6R)-5-bromo-5-(1-methoxy-2-iodoethyl)-6-methoxy-5,6-dihydro-2′-deoxyuridine (**4**)

This was obtained as an oil; yield (30 mg, 57%); ^{1}HNMR (CD_3OD) (each trans compound **3** and **4** is a mixture of two diastereomers). δ 2.03–2.14 (m, 2H, H-2″ of **3** and **4**), 2.20–2.28 (quintet, $J_{2',2''} = 12.9$, $J_{1',2'} = 5.8$, $J_{2',3'} = 5.8$ Hz, 1H, H-2′ of **3**), 2.30–2.24 (quintet, $J_{2',2''} = 13.8$, $J_{1',2'} = 6.6$, $J_{2',3'} = 6.6$ Hz, 1H, H-2′ of **4**), 3.33–3.85 (complex m, H-5′, $CHOCH_3$, C6–OCH_3, CH_2I), 3.84–4.12 (m, 4H, H-4′, $CHOCH_3$), 4.30–4.44 (m, 2H, H-3′), 5.21 and 5.22 (2s, 1H total, H-6 of **4**), 5.32 and 5.34 (2s, 1H total, H-6 of **3**), 5.88 and 5.94 (2t, $J_{1',2'} = J_{1',2''} = 6.0$ Hz, 1H total, H-1′ of **4**), 6.12 and 6.17 (2t, $J_{1',2'} = 5.8$, $J_{1',2''} = 7.2$ Hz, 1H total, H-1′ of 3). ^{13}CNMR (CD_3OD) δ 5.05, 5.52, 5.74 and 7.42 (CH_2I), 39.68, 40.08, 40.34, 41.23 (C-2′), 57.47, 57.86, 58.01, 59.05 and 59.18 (OCH_3), 58.32 and 61.70 (C-5 in one diastereomer), 61.84, 62.46, 62.56, and 64.66 (*C*HOMe CH_2I), 62.84, 62.96, 63.07, and 63.16 (C-5′), 63.28 and 63.40 (C-5 in one diastereomer), 72.21, 72.33, 72.39 and 73.38 (C-3′), 83.14, 83.65, 84.88, 85.35, 85.81, 85.90 and 86.23 (C-1′ and C-6), 87.21, 87.71, 88.06 and 88.35 (C-4′), 152.58 and 152.80 (C-2), 168.51 (C-4). Anal. Calcd for $C_{13}H_{20}BrIN_2O_7$: C, 29.84; H, 3.85; N, 5.35. Found: C, 30.06; H, 4.14; N, 5.25.

(+)-Trans-(5S,6S)-5-bromo-5-(1-methoxy-2-bromoethyl)-6-methoxy-5,6-dihydro-2′-deoxyuridine (**5**) and (−)-trans-(5R,6R)-5-bromo-5-(1-methoxy-2-bromoethyl)-6-methoxy-5,6-dihydro-2′-deoxyuridine (**6**)

This was obtained as an oil; yield (37 mg, 78%); ^{1}HNMR (CD_3OD) (each trans compound **5** and **6** is a mixture of two diastereomers). δ 2.04–2.16 (m, 2H, H-2″ of **5** and **6**), 2.22–2.30 (quintet, $J_{2'-2''} = 12.9$, $J_{1',2'} = 5.8$, $J_{2',3'} = 5.8$ Hz, 1H, H-2′ of **5**), 2.31–2.44 (quintet, $J_{2',2''} = 13.8$, $J_{1',2'} = 6.6$, $J_{2',3'} = 6.6$ Hz, 1H, H-2′ of **6**), 3.50–3.84 (complex m, H-5′, $CHOCH_3$, C6–OCH_3, CH_2Br), 3.85–3.92 (m, 2H, H-4′), 4.26–4.42 (m, 4H, H-3′, $CHOCH_3$), 5.10 and 5.24 (2 s, 1H total, H-6 of **6**), 5.31 and 5.33 (2 s, 1H total, H-6 of **5**), 5.90 and 5.98 (2t, $J_{1',2''} = J_{1',2''} = 6.0$ Hz, 1H total, H-1′ of **6**), 6.12 and 6.16 (2t, $J_{1',2'} = 5.8$, $J_{1',2''} = 7.2$ Hz, 1H total, H-1′ of **5**); ^{13}C NMR (CD_3OD) δ 31.36, 34.18, 34.89 and 37.07 (CH_2Br), 39.61, 39.86, 40.23 and 41.03 (C-2′), 57.60, 57.86, 59.14, 59.23 and 60.22 (OCH_3), 61.85 and 61.64 (C-5 of one diastereomer), 62.32, 62.44 and 64.42 (*C*HOMeCH_2Br), 62.90, 62.95, 63.04 and 63.14 (C-5′), 63.19 and 63.43 (C-5 in one diastereomer), 72.21, 72.27, 72.38 and 72.44 (C-3′), 82.69, 84.72, 84.79, 85.43, 85.85, 86.15, 87.03 and 87.38 (C-1′, C-6), 87.46, 87.98, 88.27 and 88.63 (C-4′), 152.45, 152.57 and 152.87 (C-2), 168.30 (C-4). Anal. Calcd. for $C_{13}H_{20}Br_2N_2O_7$: C, 32.79, H, 4.23, N, 5.88. Found: C, 32.98, H, 4.10, N, 5.58.

(+)-Trans-(5S,6S)-5-bromo-5-(1-methoxy-2,2-dibromoethyl)-6-methoxy-5,6-dihydro-2′-deoxyuridine (**7**) and (−)-trans-(5R,6R)-5-bromo-5-(1-methoxy-2,2-dibromoethyl)-6-methoxy-5,6-dihydro-2′-deoxyuridine (**8**)

This was obtained as an oil; Yield (40 mg, 72%); ^{1}HNMR (CD_3OD) (each trans compound **7** and **8** is a mixture of two diastereomers). δ 2.02–2.10 (ddd, $J_{2'-2''} = 13.8, J_{1',2''} = 6.6, J_{2'',3'} = 3.3$ Hz, 1H, H-2″ of **7**), 2.11–2.22 (ddd, $J_{2'-2''} = 14.4$, $J_{1',2''} = 7.2$, $J_{2'',3'} =$ 3.0 Hz, 1H, H-2″ of **8**), 2.25–2.40 (ddd, $J_{2',2''} = 13.8$, $J_{1',2'} = 6.6$, $J_{2',3'} = 6.6$ Hz, 1H, H-2′ of 7), 2.41–2.60 (ddd, $J_{2',2''} = 14.4$, $J_{1',2'} = 7.2$, $J_{2',3'} = 7.2$ Hz, 1H, H-2′ of **8**), 3.45–3.86 (complex m, 16H total, H-5′, CHOCH_3, C6–OCH_3), 3.88–3.92 (m, 2H, H-4′), 4.10, 4.21, 4.28 and 4.46 (4d, J = 3.5 Hz, 2H total, CHCHBr$_2$), 4.30–4.44 (m, 2H, H-3′), 5.22, 5.25, 5.30 and 5.38 (4 s, 2H total, H-6), 5.88, 5.93 (2t, $J_{1',2'} = J_{1',2''} = 7.2$ Hz, 1H total, H-1′ of **8**), 6.12 and 6.14 (2 overlapping t, $J_{1',2'} = J_{1',2''} = 6.6$ Hz, 1H total, H-1′ of **7**) 6.06, 6.32, 6.46 and 6.48 (4d, $J = 3.5$ Hz, 2H total, CHCH Br$_2$). ^{13}C NMR (CD_3OD) δ 39.52, 40.10, 40.31, 40.49 (C-2′), 43.87, 45.01, 45.26 and 47.89 (CHBr$_2$), 58.16, 58.31, 59.09 and 59.33 (OCH$_3$), 61.24, 61.96 and 62.84 (CHCHBr$_2$), 62.29 and 64.45 (C-5 in one diastereomer), 63.07, 63.20, 63.42 and 63.53 (C-5′), 70.80 and 70.89 (C-5 in one diastereomer), 72.31, 72.39, 72.60 and 72.77 (C-3′), 84.34, 85.45, 85.85, 86.08, 87.08, 87.85, 88.01, 88.74, 88.87, 89.32, 90.0, 90.16 (C-4′, C-1′, C-6), 152.39, 152.66 and 154.50 (C-2), 168.30 and 168.75 (C-4). Anal. Calcd for $C_{13}H_{19}Br_3N_2O_7$, C, 28.13, H, 3.45, N, 5.04; Found: C, 27.80, H, 3.21, N, 4.85.

(+)-Trans-(5S,6S)-5-chloro-5-(1-methoxy-2,2-dichloroethyl)-6-methoxy-5,6-dihydro-2′-deoxyuridine (**9**) and (−)-trans-(5R,6R)-5-(1-methoxy-2, 2-dichloroethyl)-6 Methoxy-5,6-dihydro-2′-deoxyuridine (**10**)

This had M.P. 142–45°C dec; yield (30 mg, 71%); ^{1}HNMR (CD_3OD) (each trans compound **9** and **10** is a mixture of two diastereomers). δ 2.10–2.18 (ddd, $J_{2'2''} = 13.8$, $J_{1',2''} = 6.0$, $J_{2'',3'} = 3.0$Hz,1H, H-2″ of **9**), 2.19–2.28 (ddd, $J_{2',2''} = 12.9, J_{1',2''} = 7.2, J_{2'',3'} = 3.1$Hz, 1H, H-2″of **10**), 2.29–2.40 (quintet, $J_{2',2''} = 13.8, J_{1',2'} =$ 6.6, $J_{2',3'} = 6.5$ Hz, 1H, H-2′of **9**), 2.42–2.55 (quintet, $J_{2'2''} = 12.9$, $J_{1',2'} = 7.2$, $J_{2',3'} = 7.2$ Hz, 1H, H-2′of **10**), 3.58–3.62 (m, 12 H, CHOCH_3, C-6, OCH_3), 3.72–3.81 (m, 4H, H-5′), 3.83–3.98 (m, 2H, H-4′), 4.22 and 4.24 (2 overlapping d, $J = 4.5$ Hz,2H total, CHCHCl$_2$), 4.35–4.48 (m, 2H, H-3′), 5.31 (s, 1H, H-6 of **10**), 5.40 (s, 1H, H-6 of **9**), 5.93 (t, $J_{1',2'} = J_{1'-2''} = 7.2$ Hz, 1H, H-1′ of **10**), 6.08 (t, $J_{1',2'} = 6.0$, $J_{1',2''} = 6.0$ Hz, 1H, H-1′ of **9**), 6.32 and 6.47 (2d, J = 4.5 Hz, 2H total, CHCHCl$_2$). ^{13}C NMR (CD_3OD) δ 39.75 and 40.65 (C-2′), 59.43 and 59.69 (OCH$_3$), 62.86 and 63.02 (C-5 of one diastereomer), 63.13 and 63.49 (CHCl$_2$), 63.22 and 63.37 (C-5′), 72.45 and 72.93 (CHCHCl$_2$), 73.19 and 73.32 (C-3′), 75.27 and 75.43 (C-5 in one diastereomer), 86.44, 87.01, 87.67, 87.76, 87.90, 88.02, 88.19 and 88.75 (C-1′, C-4′, C-6), 152.61 and 153.04 (C-2), 165.44 and 165.82 (C-4). Anal. Calcd for $C_{13}H_{19}Cl_3N_2O_7$; C, 37.01; H, 4.54; N, 6.64; Found: C, 37.04; H, 4.53; N, 6.41.

(+)-Trans-(5S,6S)-5-chloro-5-(1-methoxy-2-bromo-chloroethyl)-6-methoxy-5,6-dihydro -2′-deoxyuridine (**11**) and (−)-trans-(5R,6R)-5-chloro-5-(1-methoxy-2-bromo-2-chloroethyl)-6-methoxy-5, 6-dihydro-2′-deoxyuridine (**12**)

This was obtained as an oil; yield (35 mg, 75%); ^{1}HNMR (CD_3OD) (each trans compound **11** and **12** is a mixture of two diastereomers) δ 2.07–2.15 (ddd, $J_{2'2''} = 13.4$, $J_{1',2''} = 6.3$, $J_{2'',3'} = 3.1$Hz, 1H, H-2″ of **11**), 2.16–2.23 (ddd, $J_{2',2''} = 13.0$, $J_{1',2''} = 7.2$, $J_{2'',3'} = 3.1$ Hz, 1H, H-2″of **12**), 2.24–2.36 (quintet, $J_{2',2''} = 13.4$, $J_{1',2'} = 6.6$, $J_{2',3'} = 6.6$ Hz, 1H, H-2′of **11**), 2.40–2.51 (quintet, $J_{2'2''} = 13.0$, $J_{1',2'} = 7.2$, $J_{2',3'} =$ 7.2 Hz, 1H, H-2′of **12**), 3.52–3.70 (m, 12H, CHOCH_3, C6–OCH_3), 3.72–3.84 (m, 4H, H-5′), 3.86–3.94 (m, 2H, H-4′), 4.35 and 4.37 (2d, J = 3.4 Hz, 2H total, CHCHBrCl), 4.40–4.46 (m, 2H, H-3′), 5.28 (s,1H, H-6 of **12**), 5.38 (s, 1H, H-6 of **11**), 5.92 (t, $J_{1',2'} = J_{1'-2''} = 7.2$ Hz, 1H, H-1′ of **12**), 6.10 (t, $J_{1',2''} = 6.6$, $J_{1',2''} = 6.3$ Hz, 1H, H-1′ of **11**), 6.43 and 6.62 (2d, $J = 3.45$ Hz,2H total, CHCH BrCl). ^{13}C NMR(CD_3OD) δ 39.56 and 40.70 (C-2′), 58.28 and 58.30 (C-5 in one diastereomer), 58.73, 58.95, 59.42, 59.72 (OCH$_3$), 63.05, 63.26 (C-5′), 63.53, and 63.55 (CHC HBrCl), 72.24, 72.42, 72.84 and 73.10 (C-3′, CHCHBrCl), 75.30 and 75.32 (C-5 in one diastereomer), 86.71, 87.55, 87.61, 87.76, 87.94, 88.10, and 88.61(C-1′, C-4′, C-6), 152.51 and 153.01 (C-2), 165.51, and 165.90 (C-4). Anal Calcd for $C_{13}H_{19}BrCl_2N_2O_7$: C, 33.49, H, 4.10, N, 6.01; Found: C, 33.74, H, 4.06, N, 5.68.

In Vitro Anti-HSV-1 Assay (KOS)

African green monkey kidney (Vero) cells were grown in DMEM supplemented with 5% FBS. Cells were seeded into 24-well plates one day prior to the assay. Wild-type HSV-1, KOS, was used to infect at ~100 PFU′s per well for 1 h at 37°C. After infecting, the inoculum was replaced with serial dilutions of media containing diluted drug. All drug dilutions were done in duplicate, initially using the following concentrations: 50, 25, 10, 5 and 1 μg/mL. Once an activity range was determined, compounds were again tested at 1:2 serial dilutions to obtain a more precise EC_{50}. Controls included infected wells that were not treated with drugs, as well as infected wells treated with acyclovir at 5, 1 and 0.1 μg/mL. Plates were incubated for 48 h at 37°C. To visualize plaques, wells were fixed by incubation with methanol for 10 min at room temperature. The methanol was aspirated and replaced with 1× Giemsa stain

(Sigma) for 1 h at room temperature. Plaques were counted and compared to the number of plaques in the no-drug controls in order to calculate EC_{50}. The EC_{50} value for acyclovir was ~0.1 μg/mL.

Cell Cytotoxicity:

MTT assay: Cell viability was measured using the cell proliferation kit 1 (MTT; Boehringer Mannheim), as per manufacturer's instructions. Briefly, a 96 well plate was seeded with Vero cells at a density of 2.5×10^4 cells per well. Cells were allowed to attach for 6–8 h, and the media was replaced with media containing drugs at concentrations of 100, 50, 25, 12.5, 6.3, and 1.5 μg/mL. DMSO was also included as control. Plates were incubated for 3 days at 37°C. The color reaction involved adding 10 μl MTT reagent per well, incubating 4 h at 37°C and then adding 100 μL solubilization reagent. Absorbance was read on an ELISA plate reader at 560–650 nm following an overnight incubation at 37°C.

In Vitro Phosphorolysis Studies

In vitro phosphorolysis of 5-halo-6-methoxy-5,6-dihydro derivatives of 5-[1-methoxy-2-halo(or 2,2-dihalo)ethyl]-2′-deoxyuridines (**3**–**12**) was carried out by incubating the individual test compound with *E. coli* thymidine phosphorylase (Sigma Chemical Co.) at 37°C for 30 min. The resultant samples were analyzed by HPLC. Thymidine was used as a reference compound to confirm the phosphorolysis activity of the enzyme.

The diluted enzyme solution (130 μL) was transferred to a 1.5 mL microcentrifuge tube and 20 μL of potassium phosphate buffer was added with vortex mixing. The closed tube was then warmed to 37°C for about 2 min. The phosphorolysis reaction was initiated by adding the substrate solution (50 μL, 250 mmol) to the pre-warmed mixture. The contents of the closed tube were mixed well prior to 30 min incubation at 37°C. The reaction was terminated by removing the tube from the water bath and adding ice-cold methanol (200 μL) with thorough vortex mixing. The tube was placed in an ice bath for 10 min and then centrifuged at 12,800 rpm in an Eppendrof microcentrifuge for 3 min at 4°C. An aliquot of the clear supernatant was subjected to HPLC analysis or stored in a freezer for subsequent analysis. Each experiment was performed at least twice. HPLC analyses were performed by UV detection, at 230 nm, using water:acetonitrile (85:15, v/v) as eluent at a flow rate of 1.5–2.5 mL/min at 22°C, for the different substrates analyzed. The identity of each compounds present in the sample was determined by comparison of its retention time to that of an authentic sample.

In Vitro Regeneration of the 5,6-double Bond

Regeneration of 5-[1-methoxy-2-halo(or 2,2-dihalo) ethyl]-2′-deoxyuridines (**2a**–**e**) from the 5-halo-6-methoxy-5,6-dihydro derivatives of 5-[1-methoxy-2-halo(or 2,2-dihalo)ethyl]-2′-deoxyuridines (**3**–**12**) was determined by incubating the test compounds with glutathione (GSH) (reduced). A substrate to glutathione molar ratio of 1: 2 in phosphate buffer was incubated at 37°C for various time periods up to 24 h. The resulting sample was subjected to HPLC analysis with UV detection at both 230 nm (5,6-dihydro compound) and 265 nm (parent compounds), at using water:acetonitrile (85:15, v/v) as eluent at a flow rate of 1.5–2.5 mL/min 22°C to quantitate the amount of (**2a**–**e**) produced by 5,6-olefinic bond regeneration. The identity of each compounds present in the sample was determined by comparison of its retention time to that of an authentic sample. Retention time (min) (Rt values): **2a** (11.42); **3**,**4** (15.67–16.56); **2b** (8.98); **5**,**6** (13.15–18.30); **2c** (14.14); **7**,**8** (17.61–18.69); **2d** (12.12); **9**,**10** (18.69–19.70); **2e** (8.78); **11**,**12** (10.74–11.74).

RESULTS AND DISCUSSION

The objective of this study involved the design of 5-halo-6-methoxy-5,6-dihydro derivatives of 5-[1-methoxy-2-halo(or 2,2-dihalo)ethyl]-2′-deoxyuridines (**3**–**12**) as prodrugs of 5-[1-methoxy-2-halo(or 2,2-dihalo)ethyl]-2′-deoxyuridines (**2a**–**e**) with improved biochemical and biological characteristics for evaluation as anti-herpes agents. The efficiency of nucleoside drugs can be increased by enhancing the stability of these compounds towards phosphorolysis.[15] Thymidine phosphorylase is a specific enzyme which cleaves the base-sugar N-C bond present in pyrimidine deoxyribonucleosides.[16] To be active *in vivo*, the nucleoside drug must not undergo phosphorolysis prior to activation to the tri-phosphate nucleotide. One potential approach to prevent this undesirable phosphorolytic cleavage of pyrimidine nucleosides is reduction of the 5,6-double bond which substantially increases the stability of the glycosidic bond.[8–11] *In vitro* phosphorolysis studies which involved incubation of 5-halo-6-methoxy-5,6-dihydro derivatives of 5-[1-methoxy-2-halo(or 2,2-dihalo)ethyl]-2′-deoxyuridines (**3**–**12**) with *E. coli* thymidine phosphorylase for 30 min. at 37°C indicated that these 5,6-dihydro analogs were completely stable since no phosphorolysis occurred. In contrast, parent compounds (**2a**–**e**), and the physiological nucleoside thymidine undergo 70–90% and 90% phosphorolysis, respectively, under these experimental conditions. The decreased substrate specificity towards

phosphorylase of these dihydro derivatives should enhance their usefulness *in vivo*.

The utility of 5-halo-6-methoxy-5,6-dihydro derivatives of 5-[1-methoxy-2-halo(or 2,2-dihalo) ethyl]-2′-deoxyuridines (**3**–**12**) as potential prodrugs of (**2a**–**e**) would be dependent upon their rate of conversion to parent nucleosides. The *in vitro* incubation of the 5,6-dihydro compounds (**3**–**12**) with the model thiol, glutathione (GSH), was therefore investigated (substrate:GSH ratio = 1: 2 for times up to 24 h incubation at 37°C) to determine the ability of GSH to regenerate the 5,6-olefinic bond present in (**2a**–**e**). In mammalian tissues, the GSH concentration is in the 0.5–1 mM range, whereas the cysteine concentration is in the 0.03–0.1 mM range.[17,18] It is likely that *in vivo* dehalogenation and elimination to generate (**2a**–**e**) could occur by a chemical reaction with GSH or cysteine and/or an enzymatic reaction with a thiol-containing enzyme. Regeneration of the 5,6-olefinic bond to afford parent nucleosides (**2a**–**e**), upon incubation with GSH, was dependent upon the nature of the C-5 halo substituents in the 5-halo-6-methoxy-5,6-dihydro series (**3**–**12**), where the relative 5,6-olefinic bond regeneration order was Br (100%) > Cl (0%). These studies indicate that 5-chloro-6-methoxy-5,6-dihydro derivatives (**9**–**12**) did not undergo conversion to (**2d**) and (**2e**), even after incubation with GSH for 24 h.

To measure the ability to inhibit the virus-induced cytopathic effect in Vero cells infected with HSV-1, the 5-halo-6-methoxy-5,6-dihydro derivatives of 5-[1-methoxy-2-halo(or 2,2-dihalo)ethyl]-2′-deoxyuridines (**3**–**12**) were investigated in culture against HSV-1 using the KOS strain. The compounds **3**–**12** were tested as a mixture of two diastereomers. No significant inhibitory effect was observed up to a concentration of >25 μg/ml. The lack of anti-HSV-1 activity exhibited by the 5,6-dihydro diastereomers (**3**–**12**) is likely due, at least in part, to their inability to undergo conversion to parent nucleosides in cell culture. The culture media used contains 5% fetal bovine serum which is heat inactivated and therefore may not supply the active glutathione in relevant concentrations for the conversion. The compounds (**3**–**12**) were not studied further for their inhibitory effect against other herpes viruses *in vitro*. All of the investigated compounds exhibited no cell cytotoxicity up to a concentration of 100 μg/ml.

In conclusion, the desirable biochemical properties exhibited by the prodrugs (**3**–**12**) indicate that the 5,6-dihydro pyrimidine nucleoside prodrug concept warrants consideration in antiviral drug design as a method to prevent undesirable biotransformation, and to increase the usefulness with regeneration capabilities to active moieties *in vivo*, which would influence the plasma half-life of pyrimidine nucleosides.

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