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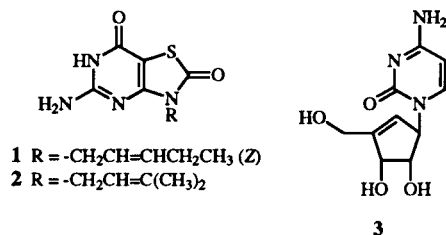
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The 1-[(*Z*)-2-penten-1-yl] and 1-(3-methyl-2-buten-1-yl) derivatives of cytosine, 5-bromocytosine, 5-fluorocytosine, and 5-iodocytosine were prepared by a reaction of the sodium salt of the requisite cytosine with (*Z*)-1-bromo-2-pentene and 4-bromo-2-methyl-2-butene, respectively. The eight alkenylcytosines **5a-5h** were evaluated in cell culture for their anti-HCMV activity. Only the derivatives of 5-bromocytosine, **5c** and **5d**, showed slight activity in this assay.

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Ganciclovir (DHPG) and foscarnet are the only drugs that are approved for the treatment of human cytomegalovirus infection, an opportunistic disease which is a serious medical challenge in AIDS patients and patients with other immunodeficiencies [1-3]. However, the toxic properties [4,5] of these agents as well as the development of strains of cytomegalovirus resistant to DHPG therapy [6,7] has motivated the search for alternative, more efficacious drugs.

We recently disclosed [8,9] that certain 3-alkenyl derivatives of the guanine congener 5-aminothiazolo[4,5-*d*]pyrimidine-2,7(3*H*,6*H*)-dione were potent *in vitro* antiherpesvirus agents. In particular, the 3-[(*Z*)-2-penten-1-yl] **1** and the 3-(3-methyl-2-buten-1-yl) **2** derivatives were found to have the better therapeutic indices of those compounds studied when targeted against human cytomegalovirus (HCMV). This rather surprising finding was followed by investigations [10,11] of other biologically significant heterocycles with identical alkenyl chains appropriately appended.



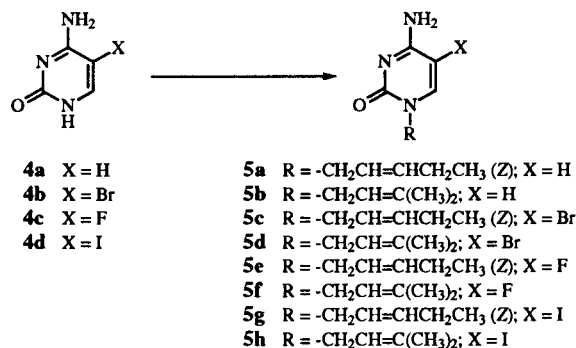
Recent studies [12,13] have provided evidence that cytosine, when substituted at N1 with an alkene bearing either a hydroxyl or a phosphonmethoxyl moiety, displays a wide spectrum of antiviral activity. Cytallene [14] and 5-fluorocytallene [15] are cytosine derivatives bearing an allenic alcohol and have shown good activity against human immunodeficiency virus. Generally however, these hydroxy- (or phosphonylmethoxy-) alkenyl- (or allenyl-) cytosines were found inactive when tested against certain herpesviruses [herpes simplex virus types

**1** and **2** (HSV-1, HSV-2) and HCMV] [13,15]. A notable exception to this generality is cyclopentenylcytosine **3** which displays a broader spectrum of antiviral activity [12]. Since we had shown [8] that alkyl and alkenyl derivatives of a guanine analogue had potent *in vitro* activity against HCMV whereas, the hydroxylated derivatives (hydroxyalkyl, ribofuranosyl) were inactive, it was of considerable interest to investigate alkenylcytosines as potential antiherpetic agents. The synthesis and *in vitro* anti-HCMV activity of 2-penten-1-yl and 3-methyl-2-buten-1-yl derivatives of cytosine and certain 5-halocytosines are the subjects of this report.

The alkenylcytosines **5a-h** (Scheme 1) were prepared in each case by treating the sodium salt of the respective cytosine **4a-d** with either (*Z*)-1-bromo-2-pentene or 4-bromo-2-methyl-2-butene. The site of alkylation was assigned as N1 based on comparisons of the uv spectra for the alkenyl derivatives with the spectra of known N1 substituted cytosine derivatives. The predominately *Z* isomer of 1-bromo-2-pentene was used for the preparation of the 2-pentenyl derivatives **5a**, **5c**, **5e** and **5g** and no attempt was made to remove small amounts of the *E* geometric isomer present in these products.

The eight alkenylcytosines were evaluated for *in vitro* activity against HCMV infected cells and for cytotoxicity to uninfected cells by methodologies described in the experimental section. The 5-bromocytosines, **5c** and **5d**,

Scheme 1



showed slight activity ( $ED_{50} = 120 \mu M$ , for both **5c** and **5d**) and the antiviral activity was well separated from cytotoxicity ( $CD_{50} = 690 \mu M$ , for both **5c** and **5d**). However, **5c** and **5d** were much less active than **1** ( $ED_{50} = 0.4 \mu M$ ) [8,9]. The other cytosine compounds of this series were either inactive or significantly less active than **5c** and **5d**.

## EXPERIMENTAL

Melting points (mp) were determined with a Thomas-Hoover Unimelt melting point apparatus and are uncorrected. Ultraviolet (uv) spectra (sh = shoulder) were recorded with a Hewlett-Packard 8452 diode array spectrophotometer. Infrared (ir) spectra were recorded in potassium bromide with a Perkin-Elmer 1420 ir spectrophotometer. Nuclear magnetic resonance ( $^1H$  nmr) spectra were recorded in dimethyl sulfoxide- $d_6$  with a Bruker AM400 wide bore spectrometer and the chemical shifts are expressed in  $\delta$  (parts per million) values relative to tetramethylsilane (internal) (key: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, and br = broad). Elemental analyses were performed by Quantitative Technologies Inc., Whitehouse, New Jersey. EM Science silica gel 60 Å (230-400 mesh) was used for all column chromatographic separations. Evaporations were carried out at a temperature  $\leq 35^\circ$  and under diminished pressure for solvents with bp  $< 80^\circ$  or at a temperature  $\leq 50^\circ$  and under high vacuum for higher boiling solvents. Cytosine and the 5-halocytosines were purchased from commercial vendors. All other chemicals used were reagent grade and were not further dried or purified unless otherwise noted.

### General Procedure for the Preparation of Alkenylcytosines **5a-h**.

A mixture of the appropriate cytosine **4a-d** (2-3 mmoles), anhydrous *N,N*-dimethylformamide (10-15 ml), and sodium hydride (1 equivalent, 80% dispersion in mineral oil) was stirred under anhydrous conditions and at ambient temperature for 0.5 hour. The appropriate alkenyl bromide [(*Z*)-1-bromo-2-pentene or 4-bromo-2-methyl-2-butene, 1.1 equivalents] was added and the mixture was heated at  $80^\circ$  for 1-1.5 hours. The mixture was evaporated under vacuum and the residue was dissolved in methanol (50 ml). Silica gel (25 g) was added and the mixture was evaporated. The dry powder was placed atop a silica gel column (5.5 x 20 cm) and the column was flash eluted with progressively increasing concentrations of methanol in dichloromethane (% methanol, volume in l): (1, 1), (2, 1), (3, 1), (5, 1), (10, 1), (20, 1). Eluate containing the homogeneous product was evaporated and the residual solid was dried under vacuum at  $90-100^\circ$  for 16-20 hours.

### 4-Amino-1-[(*Z*)-2-penten-1-yl]pyrimidin-2(1*H*)-one (**5a**).

The title compound was prepared from cytosine (**4a**) in 30% yield, mp  $202-206^\circ$ ; ir:  $\nu$  3350 and 3140 ( $NH_2$ ), 1660 ( $C=O$ ,  $C=C$ ,  $C=N$ )  $cm^{-1}$ ; uv (pH 1):  $\lambda$  max 284 nm ( $\epsilon$  13,100), 214 (11,100); (methanol):  $\lambda$  max 276 nm ( $\epsilon$  8,160), 222 sh (8,950); (pH 11):  $\lambda$  max 274 nm ( $\epsilon$  9,050), 230 (8,080);  $^1H$  nmr:  $\delta$  0.947 (t, 3 H,  $CH_3$ ), 2.10-2.17 (m, 2 H,  $CH_2$ ), 4.28 (d, 2 H,  $NCH_2$ ), 5.32-5.38 (m, 1 H,  $CH$ ), 5.51-5.58 (m, 1 H,  $CH$ ), 5.65 (d, 1 H,  $C_5H$ ), 7.00 (br s, 2 H,  $NH_2$ ), and 7.50 (d, 1 H,  $C_6H$ ).

*Anal.* Calcd. for  $C_9H_{13}N_3O \cdot 0.25H_2O$  (183.73): C, 58.83; H, 7.41; N, 22.87. Found: C, 59.02; H, 7.15; N, 22.87.

### 4-Amino-1-(3-methyl-2-buten-1-yl)pyrimidin-2(1*H*)-one (**5b**).

The title compound was prepared from cytosine (**4a**) in 44% yield, mp  $241-243^\circ$ ; ir:  $\nu$  3350 and 3140 ( $NH_2$ ), 1655 ( $C=O$ ,  $C=C$ ,  $C=N$ )  $cm^{-1}$ ; uv (pH 1):  $\lambda$  max 284 nm ( $\epsilon$  13,300), 214 (11,800); (methanol):  $\lambda$  max 276 nm ( $\epsilon$  8,500), 222 sh (9,760); (pH 11):  $\lambda$  max 274 nm ( $\epsilon$  9,210), 230 (8,400);  $^1H$  nmr:  $\delta$  1.68 (s, 3 H,  $CH_3$ ), 1.70 (s, 3 H,  $CH_3$ ), 4.21 (d, 2 H,  $NCH_2$ ), 5.17 (t, 1 H,  $CH$ ), 5.62 (d, 1 H,  $C_5H$ ), 6.96 (br s, 2 H,  $NH_2$ ), and 7.49 (d, 1 H,  $C_6H$ ).

*Anal.* Calcd. for  $C_9H_{13}N_3O$  (179.22): C, 60.32; H, 7.31; N, 23.44. Found: C, 60.18; H, 7.20; N, 23.32.

### 4-Amino-5-bromo-1-[(*Z*)-2-penten-1-yl]pyrimidin-2(1*H*)-one (**5c**).

The title compound was prepared from 5-bromocytosine (**4b**) in 29% yield, mp glass at  $130-135^\circ$ , liquefies at  $150^\circ$ ; ir:  $\nu$  3420, 3360 and 3140 ( $NH_2$ ), 1660 ( $C=O$ ,  $C=C$ ,  $C=N$ )  $cm^{-1}$ ; uv (pH 1):  $\lambda$  max 304 nm ( $\epsilon$  11,000), 218 (12,200); (methanol):  $\lambda$  max 292 nm ( $\epsilon$  6,560), 222 sh (12,100); (pH 11):  $\lambda$  max 290 nm ( $\epsilon$  7,320), 228 (9,740);  $^1H$  nmr:  $\delta$  0.947 (t, 3 H,  $CH_3$ ), 2.11-2.18 (m, 2 H,  $CH_2$ ), 4.31 (d, 2 H,  $NCH_2$ ), 5.35-5.41 (m, 1 H,  $CH$ ), 5.53-5.59 (m, 1 H,  $CH$ ), 6.85 and 7.69 (2 br s, 2 H,  $NH_2$ ), and 8.02 (s, 1 H,  $C_6H$ ).

*Anal.* Calcd. for  $C_9H_{12}BrN_3O \cdot 0.25H_2O$  (262.62): C, 41.16; H, 4.80; N, 16.00. Found: C, 41.15; H, 4.80; N, 15.84.

### 4-Amino-5-bromo-1-(3-methyl-2-buten-1-yl)pyrimidin-2(1*H*)-one (**5d**).

The title compound was prepared from 5-bromocytosine (**4b**) in 49% yield, mp  $188-191^\circ$ ; ir:  $\nu$  3405, 3360 and 3110 ( $NH_2$ ), 1660 broad ( $C=O$ ,  $C=C$ ,  $C=N$ )  $cm^{-1}$ ; uv (pH 1):  $\lambda$  max 304 nm ( $\epsilon$  11,900), 218 (13,100); (methanol):  $\lambda$  max 292 nm ( $\epsilon$  7,300), 222 sh (13,800); (pH 11):  $\lambda$  max 290 nm ( $\epsilon$  7,860), 228 (9,810);  $^1H$  nmr:  $\delta$  1.70 (s, 6 H, 2  $CH_3$ ), 4.25 (d, 2 H,  $NCH_2$ ), 5.19-5.23 (m, 1 H,  $CH$ ), 6.81 and 7.67 (2 br s, 2 H,  $NH_2$ ), and 7.99 (s, 1 H,  $C_6H$ ).

*Anal.* Calcd. for  $C_9H_{12}BrN_3O \cdot 0.5H_2O$  (267.13): C, 40.46; H, 4.91; N, 15.73; Br, 29.91. Found: C, 40.81; H, 4.90; N, 15.64; Br, 29.56.

### 4-Amino-5-fluoro-1-[(*Z*)-2-penten-1-yl]pyrimidin-2(1*H*)-one (**5e**).

The title compound was prepared from 5-fluorocytosine (**4c**) in 51% yield, mp  $176-178^\circ$ ; ir:  $\nu$  3320 and 3140 ( $NH_2$ ), 1690 ( $C=O$ ,  $C=C$ ,  $C=N$ )  $cm^{-1}$ ; uv (pH 1):  $\lambda$  max 296 nm ( $\epsilon$  12,100), 216 (11,800); (methanol):  $\lambda$  max 286 nm ( $\epsilon$  7,630), 240 (7,980), 216 sh (10,300); (pH 11):  $\lambda$  max 284 nm ( $\epsilon$  8,210), 234 (7,700);  $^1H$  nmr:  $\delta$  0.947 (t, 3 H,  $CH_3$ ), 2.10-2.18 (m, 2 H,  $CH_2$ ), 4.25 (d, 2 H,  $NCH_2$ ), 5.35-5.41 (m, 1 H,  $CH$ ), 5.52-5.59 (m, 1 H,  $CH$ ), 7.35 and 7.56 (2 br s, 2 H,  $NH_2$ ), and 7.87 (d, 1 H,  $C_6H$ ).

*Anal.* Calcd. for  $C_9H_{12}FN_3O$  (197.21): C, 54.81; H, 6.13; N, 21.31; F, 9.63. Found: C, 55.01; H, 6.07; N, 20.93; F, 9.51.

### 4-Amino-5-fluoro-1-(3-methyl-2-buten-1-yl)pyrimidin-2(1*H*)-one (**5f**).

The title compound was prepared from 5-fluorocytosine (**4c**) in 41% yield, mp  $235-239^\circ$ ; ir:  $\nu$  3310 and 3120 ( $NH_2$ ), 1680 ( $C=O$ ,  $C=C$ ,  $C=N$ )  $cm^{-1}$ ; uv (pH 1):  $\lambda$  max 296 nm ( $\epsilon$  11,800), 214 (11,800); (methanol):  $\lambda$  max 286 nm ( $\epsilon$  7,430), 240 (7,680), 216

sh (10,600); (pH 11):  $\lambda$  max 284 nm ( $\epsilon$  8,180), 232 (7,830);  $^1\text{H}$  nmr:  $\delta$  1.74 (s, 6 H, 2  $\text{CH}_3$ ), 4.24 (d, 2 H,  $\text{NCH}_2$ ), 5.23-5.27 (m, 1 H,  $\text{CH}$ ), 7.37 and 7.58 (2 br s, 2 H,  $\text{NH}_2$ ), and 7.88 (d, 1 H,  $\text{C}_6\text{H}$ ).

*Anal.* Calcd. for  $\text{C}_9\text{H}_{12}\text{FN}_3\text{O}$  (197.21): C, 54.81; H, 6.13; N, 21.31; F, 9.63. Found: C, 54.85; H, 6.06; N, 20.87; F, 9.49.

4-Amino-5-iodo-1-[(Z)-2-penten-1-yl]pyrimidin-2(1H)-one (5g).

The title compound was prepared from 5-iodocytosine (4d) in 20% yield, mp 196-198 $^\circ$ ; ir:  $\nu$  3430 and 3310 ( $\text{NH}_2$ ), 1640 ( $\text{C}=\text{O}$ ,  $\text{C}=\text{C}$ ,  $\text{C}=\text{N}$ )  $\text{cm}^{-1}$ ; uv (pH 1):  $\lambda$  max 312 nm ( $\epsilon$  9,450), 224 (14,100); (methanol):  $\lambda$  max 296 nm ( $\epsilon$  5,670), 222 (14,100); (pH 11):  $\lambda$  max 296 nm ( $\epsilon$  6,340), 230 (12,500);  $^1\text{H}$  nmr:  $\delta$  0.939 (t, 3 H,  $\text{CH}_3$ ), 2.08-2.17 (m, 2 H,  $\text{CH}_2$ ), 4.30 (d, 2 H,  $\text{NCH}_2$ ), 5.33-5.39 (m, 1 H,  $\text{CH}$ ), 5.51-5.58 (m, 1 H,  $\text{CH}$ ), 6.47 and 7.66 (2 br s, 2 H,  $\text{NH}_2$ ), and 8.00 (s, 1 H,  $\text{C}_6\text{H}$ ).

*Anal.* Calcd. for  $\text{C}_9\text{H}_{12}\text{IN}_3\text{O}$  (305.12): C, 35.43; H, 3.96; N, 13.77; I, 41.59. Found: C, 35.51; H, 3.87; N, 13.64; I, 41.40.

4-Amino-5-iodo-1-(3-methyl-2-buten-1-yl)pyrimidin-2(1H)-one (5h).

The title compound was prepared from 5-iodocytosine (4d) in 13% yield, mp 207-210 $^\circ$ ; ir:  $\nu$  3460, 3410 and 3310 ( $\text{NH}_2$ ), 1640 broad ( $\text{C}=\text{O}$ ,  $\text{C}=\text{C}$ ,  $\text{C}=\text{N}$ )  $\text{cm}^{-1}$ ; uv (pH 1):  $\lambda$  max 312 nm ( $\epsilon$  9,600), 224 (14,200); (methanol):  $\lambda$  max 298 nm ( $\epsilon$  6,400), 252 sh (6,040), 222 sh (16,900); (pH 11):  $\lambda$  max 296 nm ( $\epsilon$  6,210), 230 (12,300);  $^1\text{H}$  nmr:  $\delta$  1.68 (s, 3 H,  $\text{CH}_3$ ), 1.70 (s, 3 H,  $\text{CH}_3$ ), 4.24 (d, 2 H,  $\text{NCH}_2$ ), 5.17-5.21 (m, 1 H,  $\text{CH}$ ), 6.46 and 7.64 (2 br s, 2 H,  $\text{NH}_2$ ), and 7.99 (s, 1 H,  $\text{C}_6\text{H}$ ).

*Anal.* Calcd. for  $\text{C}_9\text{H}_{12}\text{IN}_3\text{O}$  (305.12): C, 35.43; H, 3.96; N, 13.77; I, 41.59. Found: C, 35.42; H, 3.91; N, 13.55; I, 41.55.

#### Antiviral and Cytotoxicity Assays.

Antiviral effectiveness for each compound was derived from plaque reduction experiments using the AD169 strain of HCMV in MRC-5 cells and the methodology described by Barnard *et al.* [16]. All compounds were scored for visual cytotoxicity in uninfected, stationary MRC-5 cells. For comparison, values determined (range over 19 experiments) under identical conditions for ganciclovir are  $\text{ED}_{50}$  (effective dose at 50% level) = 5-30  $\mu\text{M}$  and  $\text{CD}_{50}$  (cytotoxic dose at 50% level) = >1000  $\mu\text{M}$ .

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