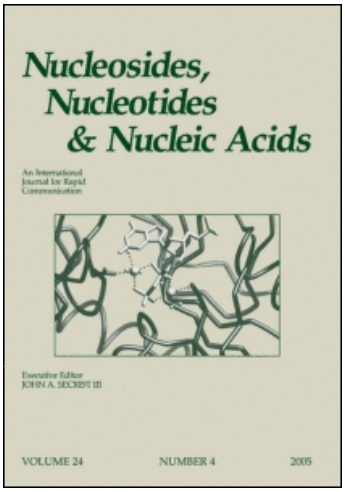

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Synthesis and Anticancer Activity of 3-(3-Oxoprop-1-enyl)-Substituted Analogues of Carbocyclic Pyrimidine Nucleosides and 2′,3′-Dideoxy Pyrimidine Nucleosides

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Abstract

Various 2′,3′-dideoxy and carbocyclic pyrimidine nucleosides, and their corresponding 3-(3-oxoprop-1-enyl) derivatives, have been synthesized and evaluated against murine L1210 and P388 leukemias and Sarcoma 180 and human CCRF-CEM lymphoblastic leukemia. Among the compounds tested, 3-(3-oxoprop-1-enyl)-3′-fluoro-3′-deoxythymidine (17), 3-(3-oxoprop-1-enyl)-3′-azido-3′-deoxythymidine (15) and 3-(3-oxoprop-1-enyl)-(±)-1-[(1α, 3β, 4α)-3-hydroxy-4-(hydroxymethyl)cyclopentyl]-5-methyl-2,4 (1H,3H)pyrimidinedione (6) were found to be the most active with ED_{50} values of 0.5, 0.2, 0.1, and 0.3 μM; 1.2, 0.5, 1.0 and 1.0 μM; and 0.8, 0.7, 1.5, and 3.0 μM, respectively. Our preliminary findings indicate that the 3-(3-oxoprop-1-enyl) derivative of carbocyclic thymidine is approximately 7 times more active than the 3-(3-oxoprop-1-enyl) derivative of carbocyclic thymine riboside against L1210 leukemia cells in vitro, with ED_{50} values of 0.8 μM and 5.5 μM, respectively. These findings suggest that the cytotoxicity of these compounds not only is dependent upon the 3-(3-oxoprop-1-enyl)-substituted group, but also may vary with the sugar moiety.

Introduction

Johnson et al.[1] recently reported the synthesis of a series of 3-(3-oxoprop-1-enyl)-substituted derivatives of pyrimidine and purine bases, and pyrimidine nucleosides. 3-(3-Oxoprop-1-enyl)thymidine, which was one of the most interesting compounds in this series, has shown significant cytotoxic activity against HeLa, L1210 leukemia, Lewis lung carcinoma, B16 melanoma, and DLD-1 human carcinoma cells in culture, as well as anticancer activities in vivo against the L1210 leukemia. 3-(3-Oxoprop-1-enyl)thymidine selectively blocks DNA synthesis in HeLa cells and inhibits the activities of thymidine kinase and DNA polymerase-α.[2] Available evidence also suggests that 3-(3-oxoprop-1-enyl)thymidine

readily undergoes addition-elimination in the presence of nucleophiles, with the thymidine moiety acting as the leaving group.[2]

Based on these concepts, a variety of 3-(3-oxoprop-1-enyl) derivatives of 2′,3′-dideoxy pyrimidine nucleosides and carbocyclic pyrimidine nucleosides were synthesized as potential anticancer agents, in the hope that these nucleoside propenals will represent a novel class of site-directed inhibitors.

In addition, 3-(3-oxoprop-1-enyl) analogues of nucleosides with known chemotherapeutic efficacy, such as carbocyclic thymidine and 3′-fluoro-3′-deoxythymidine were synthesized. The resulting products are visualized to have the potential to act in a synergistic manner to produce cytotoxicity by complementary inhibition,[3] with the propenal moiety causing damage to DNA and thereby interfering with its function as a template in replication, and the nucleoside antimetabolite inhibiting the appropriate enzyme(s) after its conversion to the respective nucleotide, which serves in the envisioned action to minimize the repair of DNA lesions.

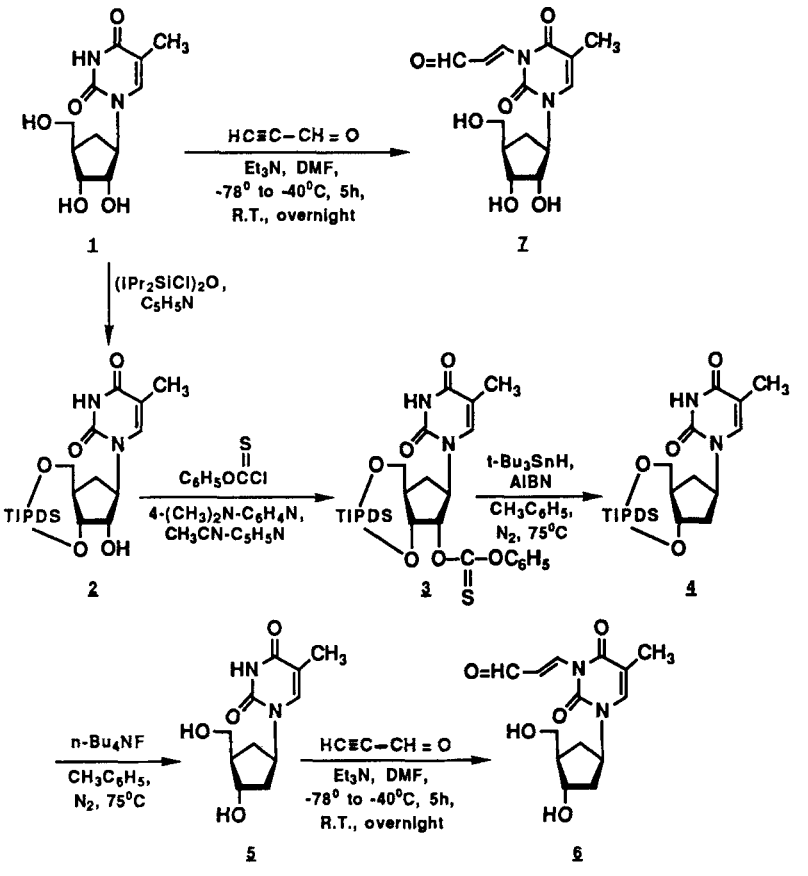
Our preliminary findings indicate that the 3-(3-oxoprop-1-enyl) derivative of carbocyclic thymidine is approximately 7 times more active than the 3-(3-oxoprop-1-enyl) derivative of carbocyclic thymine riboside against L1210 leukemia cells in vitro, with ED_{50} values of 0.8 μM and 5.5 μM, respectively. These findings suggest that the cytotoxicity of these compounds not only is dependent upon the 3-(3-oxoprop-1-enyl)-substituted group, but also may vary with the sugar moiety.

Chemistry

Treatment of (±)-1-[(1α, 2β, 3β, 4α)-2,3-dihydroxy-4-(hydroxymethyl)cyclopentyl]-5-methyl-2,4-(1H,3H)pyrimidinedione (1, carbocyclic thymine riboside) with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane in pyridine at room temperature gave the 3′,4′-O-(1,1,3,3-tetraisopropyldisilox-1,3-diyl) (TIPDS for 1,1,3,3-tetraisopropyldisilox-1,3-diyl) protected derivative 2. Reaction[4] of compound 2 with phenyl chlorothionoformate and 4-dimethylaminopyridine in dry acetonitrile under nitrogen at 40-50°C produced the respective 2′-O-phenoxythiocarbonyl ester 3. Reductive deoxygenation[4] of compound 3 in the 2′-position by tri-n-butyltin hydride, in the presence of azobis(isobutyronitrile)

(AIBN), in toluene at 75°C afforded the 2′-deoxynucleoside 4. Deblocking of 4 with tetra-n-butylammonium fluoride in toluene at 75°C under nitrogen yielded the desired (+)-1-[(1α, 3β, 4α)-3-hydroxy-4-(hydroxymethyl)cyclopentyl]-5-methyl-2,4(1H,3H)pyrimidinedione (5, carbocyclic thymidine), which was first synthesized by Shealy et al.[5] by a different methodology. Treatment[1] of compounds 1 and 5 with propargylaldehyde, in the presence of triethylamine, in DMF at -78° to -45°C for 5h, then at room temperature overnight, gave the 3-(3-oxoprop-1-enyl)-substituted derivatives of carbocyclic thymidine and carbocyclic thymine riboside, compounds 6 and 7, respectively (Scheme 1). Carbocyclic thymine riboside (1) was synthesized in our laboratory previously[6] via a ten-step route by the methodology of Shealy et al.[7] and Kam et al.[8]

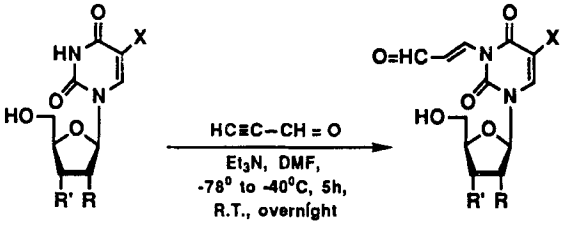
Scheme 1



TIPDS = 1,1,3,3-Tetraisopropyldisilox-1,3-diyl

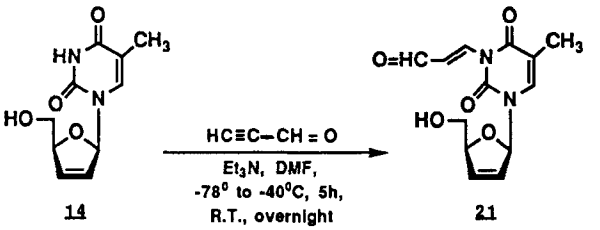
The 3-(3-oxoprop-1-enyl)-substituted nucleoside analogues, compounds 15 - 21, were synthesized by the methodology of Johnson et al.[1] The starting compounds, 3′-azido-3′-deoxythymidine (8, AZT),[9,10] 3′-azido-2′,3′-dideoxy-5-bromouridine (9),[11] 3′-fluoro-3′-deoxythymidine (10),[12,13] 2′,3′-dideoxythymidine (11),[14] 2′,3′-dideoxyuridine (12),[14] and 3′-deoxythymidin-2′-ene (14, 2′,3′-didehydro-3′-deoxythymidine, d4T)[14] were synthesized by the previously reported methodology in the literature as cited (Scheme 2).

Scheme 2



8 : X = CH_3, R = H, R′ = N_3
9 : X = Br, R = H, R′ = N_3
10 : X = CH_3, R = H, R′ = F
11 : X = CH_3, R = R′ = H
12 : X = H, R = R′ = H
13 : X = CH_3, R = H, R′ = OH

15 : X = CH_3, R = H, R′ = N_3
16 : X = Br, R = H, R′ = N_3
17 : X = CH_3, R = H, R′ = F
18 : X = CH_3, R = R′ = H
19 : X = H, R = R′ = H
20 : X = CH_3, R = H, R′ = OH



14 21

Biological Evaluation

Various 2′,3′-dideoxy and carbocyclic pyrimidine nucleosides, and their corresponding 3-(3-oxoprop-1-enyl) derivatives, were evaluated against murine L1210 and P388 leukemias and Sarcoma 180 and human CCRF-CEM lymphoblastic leukemia. The activity is expressed as the concentration (μM) required to inhibit cell replication by 50% (ED_{50}) of each of the various cell lines. The results are shown in Table 1. The 3-(3-oxoprop-1-enyl) derivatives 3-(3-oxoprop-1-enyl)-3′-azido-3′-deoxythymidine (15), 3-(3-oxoprop-1-enyl)-3′-azido-2′,3′-dideoxy-5-bromouridine (16), 3-(3-oxoprop-1-enyl)-3′-deoxythymidine (18), 3-(3-oxoprop-1-enyl)-2′,3′-dideoxyuridine (19), 3-(3-oxoprop-1-enyl)thymidine (20), and 3-(3-oxoprop-1-enyl)-3′-deoxythymidin-2′-ene (21), generally produced greater

Table 1. Comparison of the ED_{50} Values of Several Carbocyclic and 2′,3′-dideoxy Pyrimidine Nucleosides and Their Corresponding 3-(3-Oxoprop-1-enyl) Derivatives on the Replication of L1210, P388, S-180, and CCRF-CEM Cells in Vitro

	ED_{50}[a] μM			
Compd.	L1210	P388	S-180	CCRF-CEM
7	5.5	—	—	—
5	1.0	0.7	1.5	12
6	0.8	0.7	1.5	3.0
8	100	25	100	100
15	1.2	0.5	1.0	1.0
9	>100	>100	>100	>100
16	1.5	1.0	2.5	1.3
10	2.0	0.2	0.1	0.3
17	0.5	0.2	0.1	0.3
11	>500	>100	>100	>100
18	1.5	0.85	2.5	1.7
12	>100	>100	>100	>100
19	2.0	2.5	3.0	2.0
13	50	30	>100	15
20	1.0	1.0	4.0	2.5
14	150	100	100	10
21	3.5	2.0	4.5	3.5

[a]The ED_{50} values were estimated from dose-response curves compiled from at least two independent experiments and represent the drug concentration (μM) required to inhibit replication of the respective L1210, P388, S-180 and CCRF-CEM cell lines by 50% after 72h incubation.

activity, in most cases, with ED_{50} values ranging from 1.0-3.5 μM against L1210; 0.5-2.0 μM against P388; 1.0-4.5 μM against S-180; and 1.0-3.5 μM against CCRF-CEM as compared to their respective parent compounds 3′-azido-3′-deoxythymidine (8), 3′-azido-2′,3′-dideoxy-5-bromouridine (9), 3′-deoxythymidine (11), 2′,3′-dideoxyuridine (12), thymidine (13), and 3′-deoxythymidin-2′-ene (d4T, 14) with most ED_{50} values of 100 μM or more. Thymidine (13) and d4T (14) showed greater activity only in the CCRF-CEM cell line with ED_{50} values of 15 and 10 μM, respectively, but their corresponding 3-(3-oxoprop-1-enyl) derivatives were still more active than their parent compounds with ED_{50} values of 2.5 and 3.5 μM, respectively.

Of these 2′,3′-dideoxy pyrimidine nucleosides, only 3′-fluoro-3′-deoxythymidine (10) and its 3-(3-oxoprop-1-enyl) derivative (17) had similar ED_{50} values: 0.2 and 0.2 μM (P388); 0.1 and 0.1 μM (S-180); 0.3 and 0.3 μM (CCRF-CEM), except for 2.0 and 0.5 μM

Table 2. Comparison of the Cytotoxicity of Several Carbocyclic and 2′,3′-dideoxy Pyrimidine Nucleosides and their Corresponding 3-(3-Oxoprop-1-enyl) Derivatives on the Replication of L1210, P388, S-180, and CCRF-CEM Cells in Vitro[b]

	% Inhibition[c] (conc, μM)			
Compd.	L1210	P388	S-180	CCRF-CEM
7	94% (100μM)	—	—	—
5	90% (100μM)	87% (5μM)	70% (5μM)	60% (15μM)
6	96% (10μM)	90% (5μM)	74% (5μM)	65% (5μM)
8	45% (100μM)	70% (100μM)	77% (100μM)	48% (100μM)
15	97% (100μM)	100% (5μM)	100% (5μM)	100% (4μM)
9	13% (100μM)	17% (100μM)	20% (100μM)	8% (100μM)
16	96% (10μM)	100% (5μM)	80% (10μM)	100% (5μM)
10	84% (100μM)	94% (100μM)	86% (100μM)	75% (100μM)
17	100% (10μM)	100% (10μM)	100% (10μM)	100% (10μM)
11	10% (500μM)	20% (100μM)	20% (100μM)	6% (100μM)
18	97% (10μM)	100% (5μM)	88% (5μM)	100% (4μM)
12	0%(100μM)	0%(100μM)	12%(100μM)	12%(100μM)
19	98% (10μM)	100%(5μM)	100%(10μM)	100%(10μM)
13	85% (100μM)	75% (100μM)	17% (100μM)	84% (100μM)
20	96% (10μM)	100% (5μM)	63% (5μM)	100% (10μM)
14	74% (500μM)	49% (100μM)	53% (100μM)	88% (500 μM)
21	95% (100μM)	95% (5μM)	60% (6μM)	100% (10μM)

[b]Assays were carried out at least twice with the appropriate controls.
[c]% Inhibition for all cell lines was determined after 72h of incubation.

(L1210), respectively. However, a comparison of their cytotoxicity at various concentrations showed that for 3′-fluoro-3′-deoxythymidine (10), the percent of cell inhibition at 100 μM was 84% (L1210), 94% (P388), 86% (S-180), and 75% (CCRF-CEM), whereas, its 3-(3-oxoprop-1-enyl) derivative (17) had 100% inhibition of the cell replication at 10 μM in all the given cell lines. The inhibition percentage of the replication of these cell lines exerted by the compounds at various concentrations are given in Table 2.

There appears to be little variation in the ED_{50} values between the carbocyclic thymidine (5) and its 3-(3-oxoprop-1-enyl) derivative (6) in all but the CCRF-CEM cell line: 1.0 and 0.8 μM (L1210); 0.7 and 0.7 μM (P388); 1.5 and 1.5 μM (S-180); and 12 and 3.0 μM (CCRF-CEM), respectively.

Among all the tested compounds, the 3-(3-oxoprop-1-enyl) derivatives, 3-(3-oxoprop-1-enyl)-3′-fluoro-3′-deoxythymidine (17), 3-(3-oxoprop-1-enyl)-3′-azido-3′-deoxythymidine (15), and 3-(3-oxoprop-1-enyl)-(±)-1-[(1α, 3β, 4α)-3-hydroxy-4-(hydroxymethyl)cyclopentyl]- 5-methyl-2,4 (1H,3H)pyrimidinedione (6) were found to be the most active against L1210, P388, S-180 and CCRF-CEM with ED_{50} values of 0.5, 0.2, 0.1, and 0.3 μM; 1.2, 0.5, 1.0, and 1.0 μM; and 0.8, 0.7, 1.5, and 3.0 μM, respectively and merit further in vivo studies.

Experimental Section

Melting points were determined with a Thomas-Hoover Unimelt apparatus and are uncorrected. ^1H NMR spectra were recorded at 500 MHz on a Brucker WM-500 spectrometer with Me_4Si as the internal reference. The UV spectra were recorded on a Beckman-25 spectrophotometer. IR spectra were taken on the Perkin-Elmer 21 spectrophotometer. The mass spectra (at 70 eV) were provided by the Yale University Chemical Instrumentation Center. TLC was performed on EM precoated silical gel sheets containing a fluorescent indicator. Elemental analyses were carried out by the Baron Consulting Co., Orange, CT.

(±)-1-[(1α, 2β, 3β, 4α)-3,4-O-TIPDS-3-hydroxy-4-(hydroxymethyl)cyclopentyl]-5-methyl-2,4-(1H,3H)pyrimidinedione (2).

Carbocyclic thymine riboside (1, 3.78 g, 14.7 mmol) was suspended in dry pyridine (140 mL) and to the suspension 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (7.0 mL, 22 mmol) was added. The reaction mixture was stirred at room temperature for 24 h. The solvent was evaporated to dryness in vacuo to give a residue which was co-evaporated again with EtOAc and water. The organic phase was washed with cold 1 N HCl/H_2O, H_2O, saturated $NaHCO_3/H_2O$, and saturated $NaCl/H_2O$ to ~ pH 7, dried (anhydrous Na_2SO_4), and filtered. The filtrate was evaporated to dryness under reduced pressure to afford a syrup, which was then chromatographed on a silica gel column (EtOAc-hexane, 1:1, v/v). A white foamy solid (3 g) and half-solid (1.7 g) were obtained. Total yield was 4.7 g (64%). The product was recrystallized from $CHCl_3$-hexane: mp 86-88°C; on TLC it gave one spot (EtOAc-hexane, 1:1, v/v, R_f0.34); NMR δ 1.06-1.13 (m, 28H, i-pr), 1.69-1.77 (q, 1H, 5′-H_a), 1.92 (s, 3H, 5-CH_3), 2.01-2.05 (m, 1H, 5′-H_b), 2.19-2.21 (m, 1H, 4′-H), 2.92 (d, 1H, 2′-OH, D_2O exchangeable), 3.76-3.79 (dd, 1H, 6′-H_a), 3.97-4.00 (dd, 1H, 6′-H_b), 4.18-4.20 (m, 1H, 2′-H), 4.30-4.31 (m, 1H, 1′-H), 4.39-4.42 (dd, 1H, 3′-H), 6.98 (s, 1H, 6-H), 8.17 (s, 1H, 3-

NH, D_2O exchangeable); MS (EI) m/e 499 (M + 1), 455 (M-i-C_3H_7), 329 (M-1-i-C_3H_7-Th); UV (0.1 NHCl) λ_{max} 273 nm (ε 9979), λ_{min} 238 nm; UV (0.1N NaOH) λ_{max} 272 nm (ε 8012), λ_{min} 246 nm. Anal. Calcd. for $C_{23}H_{42}N_2O_6Si_2$: C, 55.42; H, 8.43; N, 5.62. Found: C, 55.72; H, 8.77; N, 5.32.

(±)-1-[(1α, 2β, 3β, 4α)-2-O-Phenoxythiocarbonyl-3,4-O-(TIPDS) 3-hydroxy-4-(hydroxymethy)-cyclopentyl]-5-methyl-2,4-(1H,3H)pyrimidinedione (3).

Compound 2 (0.2 g, 0.4 mmol) and 4-dimethylaminopyridine (0.1 g, 0.8 mmol) were dissolved in 10 mL of dry CH_3CN and 10 mL of dry pyridine. To the solution phenyl chlorothionoformate (0.2 mL, 1.2 mmol) in 5 mL of dry CH_3CN was added over a period of 10 min. The reaction mixture was stirred at 40-50°C under nitrogen for 24 h. Phenyl chlorothionoformate (0.1 mL, 0.6 mmol) was then added to the solution again. The reaction mixture continued to heat at 40-50°C with stirring for another 24 h. The solvents were evaporated in vacuo to give a dark-red residue, which was partitioned between EtOAc and water. The organic layer was separated and washed with H_2O, cold 1 N HCl/H_2O, saturated $NaHCO_3$/H_2O, and saturated NaCl/H_2O, treated with charcoal, dried (anhydrous Na_2SO_4), and filtered. The filtrate was evaporated under reduced pressure to yield a yellowish residue, which was then chromatographed on a silica gel column (hexane-EtOAc, 2:1, v/v) to afford an oil which soon crystallized: yield, 0.17 g (68%); on TLC it gave one spot (C_6H_6-EtOAc, 1:1, v/v, R_f 0.51); mp 166-167°C (gas evolution); NMR ($CDCl_3$) δ 1.01-1.16 (m, 28H, i-pr), 1.91 (s, 3H, 5-CH_3), 1.91-1.98 (m, 1H, 5′-H_a), 2.04-2.10 (m, 1H, 5′-H_b), 2.16-2.20 (m, 1H, 4′-4.65 (q, 1H, 3′-H), 5.88-5.89 (q, 1H, 2′-H), 6.91 (s, 1H, 6-H), 7.09-7.42 (m, 5H, C_6H_5), 8.08 (s, 1H, 3-NH, D_2O exchangeable); MS (FAB-thioglycerol matrix) m/e 635 (M + 1), 620 (M + 1-CH_3), 619 (M-CH_3), 575 (M-1-i-C_3H_7-CH_3), 481 [M-$C_6H_5OC(S)O$]; UV (0.1 N HCl) λ_{max} 274 nm (ε 10074), λ_{min} 259 nm; UV (0.1 N NaOH) λ_{max} 272 nm (ε 8385), λ_{min} 252 nm. Anal. Calcd. for $C_{30}H_{46}N_2O_7SSi_2$: C, 56.48; H, 7.53; N, 3.87. Found: C, 56.97; H, 7.87; N, 3.55.

(±)-1-[(1α, 3β, 4α)-3,4-O-(TIPDS)-3-hydroxy-4-(hydroxymethyl)cyclopentyl]-5-methyl-2,4-(1H,3H)pyrimidinedione (4).

Compound 3 (1.33 g, 2.09 mmol) and AIBN (0.11 g, 0.63 mmol) were dissolved in 120 mL of redistilled toluene. Under nitrogen, tri-n-butyltin hydride (2.50 mL, 9.41 mmol) was introduced and the reaction mixture was stirred at ~75°C overnight. On the second day, tri-n-butyltin hydride (2.40 mL, 8.92 mmol) and AIBN (0.11 g, 0.63 mmol) were added and the reaction

was continued at ~75°C under nitrogen. On the third day, tri-n-butyltin hydride (2.00 mL, 7.44 mmol) and AIBN (0.09 g, 0.55 mmol) were added again. The total reaction time was 70 h. The reaction mixture was then concentrated under reduced pressure to give a syrup, which was chromatographed on a silica gel column (EtOAc-hexane, 3:2, v/v) to afford 0.88 g (88%) of product: mp 152-155°C; TLC, R_f 0.57 (EtOAc-hexane, 3:2, v/v); NMR ($CDCl_3$) δ 1.04-1.13 (m, 28H, i-pr), 1.59-1.62 (m, 1H, 5′-H_a), 1.91 (s, 3H, 5-CH_3), 1.91-2.00 (m, 2H, 5′-H_b and 4′-H), 2.12-2.22 (m, 2H, 2′-H_a and 2′-H_b), 3.73 (dd, 1H, 6′-H_a), 4.00 (dd, 1H, 6′-H_b), 4.40 (q, 1H, 3′-H), 5.05-5.08 (m, 1H, 1′-H), 6.97 (s, 1H, 6-H), 8.35 (br s, 1H, 3-NH, D_2O exchangeable); MS (FAB-thioglycerol matrix) m/e 483 (M + 1), 467 (M-CH_3), 439 (M-i-C_3H_7), UV (0.1 N HCl) λ_{max} 273 nm (ε 8349), λ_{min} 237 nm; UV (0.1 N NaOH) λ_{max} 273 nm (ε 7186), λ_{min} 247 nm. Anal. Calcd. for $C_{23}H_{42}N_2O_5Si_2$: C, 57.26; H, 8.71; N, 5.80. Found: C, 57.06; H, 8.36; N, 5.78.

(±)-1-[(1α, 3β, 4α)-3-Hydroxy-4-(hydroxymethyl)cyclopentyl]- 5-methyl-2,4 (1H,3H)pyrimidinedione (5, Carbocyclic thymidine).

To a stirred solution of compound 4 (0.8 g, 0.1 mmol) in 60 mL redistilled toluene, 3.2 mL of tetra-n-butylammonium fluoride (1M solution in THF) was added. The reaction mixture was heated at ~ 75°C under nitrogen for 5 h. The solvent was removed in vacuo to produce a syrup, which was partitioned between Et_2O and H_2O. The water layer was evaporated under reduced pressure to give 2.17 g of syrup, which was then chromatographed on a silica gel column (hexane - EtOAc, 2:1, v/v). The fractions which contained the desired product (R_f 0.52, $CHCl_3$ - MeOH, 3:1, v/v) were combined and evaporated in vacuo to yield 0.4 g of residue, which was crystallized from EtOH to yield 0.37 g (74%) of product: mp 219-220°C (lit[5] mp 219-221 dec); NMR (Me_2SO-d_6) δ 1.33-1.39 (q, 1H, 5′-H_a), 1.66-1.76 (m, 4H, 5′-H_b and 5-CH_3), 1.82-1.90 (m, 2H, 2′-H_a and 4′-H), 1.99-2.05 (m, 1H, 2′-H_b), 3.35-3.40 (m, 1H, 6′-H_a), 3.42-3.49 (m, 1H, 6′-H_b), 3.96 (t, 1H, 3′-H), 4.60 (s, 1H, 6′-OH, D_2O exchangeable), 4.70 (d, 1H, 3′-OH, D_2O exchangeable), 4.92-4.96 (m, 1H, 1′-H), 7.53 (s, 1H, 6-H), 11.2 (br s, 1H, 3-NH, D_2O exchangeable); UV (0.1 N HCl) λ_{max} 273 nm (ε 10400), λ_{min} 238 nm; UV (0.1 N NaOH) λ_{max} 273 nm (ε 7473), λ_{min} 248 nm.

3-(3-Oxoprop-1-enyl)-(±)-1-[(1α, 3β, 4α)-3-hydroxy-4-(hydroxymethyl)cyclopentyl]- 5-methyl-2,4 (1H,3H)pyrimidinedione (6).

Carbocyclic thymidine (5, 0.50 g, 2.08 mmol) in anhydrous DMF (6 mL) was heated (~60°C) until dissolved, then allowed to come to room temperature. Triethylamine (0.25 g,

2.49 mmol) was added and the solution was stirred for 1 h. The solution was cooled to -78°C (acetone-dry ice bath) and propargylaldehyde (5.67 g, 0.1 mol) was added. The reaction mixture was stirred overnight, allowing the temperature to slowly come to room temperature. The solution was concentrated in vacuo at room temperature to a glass, dissolved in a minimum of MeOH, and chromatographed on a silica gel column (EtOAc-MeOH, 9:1, v/v). Fractions containing the product (R_f 0.52, EtOAc-MeOH, 9:1, v/v) were combined and concentrated (~22°C) to a small volume until formation of solid occurred. The product was filtered to yield 0.12 g (20%) of ivory solid: 172-173°C; NMR (Me_2SO-d_6) δ 1.44 (q, 1H, 5′-H_a), 1.82 (m, 1H, 5′-H_b), 1.87 (s, 3H, 5-CH_3), 1.90 (m, 1H, 4′-H), 1.96 (m, 1H, 2′-H_a), 2.10 (m, 1H, 2′-H_b), 3.40 (m, 1H, 6′-H_a), 3.49 (m, 1H, 6′-H_b), 3.99 (m, 1H, 3′-H), 4.62 (t, 1H, 6′-OH, D_2O exchangeable), 4.75 (d, 1H, 3′-OH, D_2O exchangeable), 5.00 (m, 1H, 1′-H), 7.09 (dd, 1H, -C=CH-), 7.71 (s, 1H, 6-H), 8.18 (d, 1H, N-CH=C-), 9.58 (d, 1H, -CH=O); MS (CI) m/e 295 (M + 1). Anal. Calcd. for $C_{14}H_{16}N_2O_5$: C, 57.53; H, 5.48; N, 9.59. Found: C, 57.69; H, 5.13; N, 9.27.

Compounds 7 and 15 - 21 were synthesized from the appropriate starting materials as described for the synthesis of compound 6.

3-(3-Oxoprop-1-enyl)-(±)-1-[(1α, 2β, 3β, 4α)-2,3-dihydroxy-4-(hydroxymethyl)cyclopentyl]-5-methyl-2,4 (1H,3H)pyrimidinedione (7):

Yield, 0.12 g (19%); mp 141-143°C (gas evolution); TLC, R_f 0.43 (EtOAc-MeOH, 6:1, v/v); NMR (Me_2SO-d_6) δ 1.33 (m, 1H, 5′-H_a), 1.88 (s, 3H, 5-CH_3), 1.94 (m, 1H, 5′-H_b), 2.03 (m, 1H, 4′-H), 3.39 (m, 1H, 6′-H_a), 3.43 (m, 1H, 6′-H_b), 3.73 (m, 1H, 3′-H), 4.03 (q, 1H, 2′-H), 4.60 (d, 1H, 6′-OH, D_2O exchangeable), 4.71 (m, 2H, 1′-H; and 3′-OH, D_2O exchangeable), 4.91 (d, 1H, 2′-OH, D_2O exchangeable), 7.11 (dd, 1H,-C=CH-), 7.74 (s, 1H, 6-H), 8.19 (d, 1H, N-CH=C-), 9.59 (d, 1H, -CH=O); MS (CI) m/e 311 (M + 1), 257 (M + 1-C_3H_2O). Anal. Calcd. for $C_{14}H_{18}N_2O_6$: C, 54.19; H, 5.81; N, 9.03. Found: C, 53.96; H, 6.11; N, 9.09.

3-(3-Oxoprop-1-enyl)-3′-azido-3′-deoxythymidine (15):

Yield, 0.15 g (13%); mp 107-108°C; TLC, 0.6 (EtOAc-hexane, 2:1, v/v); IR (film) 4.85 μM (azido); NMR (Me_2SO-d_6) δ 2.34-2.40 (m, 1H, 2′-H_a), 2.44-2.49 (m, 1H, 2′-H_b), 3.61-3.64 (m, 1H, 5′-H_a), 3.68-3.72 (m, 1H, 5′-H_b), 3.88 (m, 1H, 4′-H), 4.40 (q, 1H, 3′-H), 5.31 (t, 1H, 5′-OH, D_2O exchangeable), 6.11 (t, 1H, 1′-H), 7.07 (q, 1H, -C=CH-), 7.91

(s, 1H, 6-H), 8.16 (d, 1H, N-CH=C), 9.61 (d, 1H, -CH=O); MS m/e 322 (M + 1). Anal. Calcd. for $C_{13}H_{15}N_5O_5$: C, 48.60; H, 4.71; N, 21.80. Found: C, 48.61; H, 4.61; N, 21.52.

3-(3-Oxoprop-1-enyl)-3′-azido-2′,3′-dideoxy-5-bromouridine (16):

Yield, 0.25 g (22%); mp 99-104°C (dec); TLC, R_f 0.44 (EtOAc-hexane, 9:1, v/v); IR (film) 4.80 μM (azido); NMR (Me_2SO-d_6) δ 2.37-2.45 (m, 1H, 2′-H_a), 2.52-2.57 (m, 1H, 2′-H_b), 3.60-3.63 (m, 1H, 5′-H_a), 3.74-3.76 (m, 1H, 5′-H_b), 3.88-3.90 (m, 1H, 4′-H), 4.32-4.38 (m, 1H, 3′-H), 5.48 (br s, 1H, 5′-OH, D_2O exchangeable), 7.01 (q, 1H, -C=CH-), 8.10 (d, 1H, N-CH=C-), 8.59 (s, 1H, 6-H), 9.63 (d, 1H, -CH=O); MS m/e 386 (M). Anal. Calcd. for $C_{12}H_{12}N_5BrO_5$: C, 37.29; H, 3.13; N, 18.13. Found: C, 37.11; H, 3.04; N, 18.20.

3-(3-Oxoprop-1-enyl)-3′-fluoro-3′-deoxythymidine (17):

Yield, 0.29 g (24%); mp 139-140°C; TLC, R_f 0.55, (EtOAc-hexane, 9:1, v/v); NMR (Me_2SO-d_6) δ 1.88 (s, 3H, 5-CH_3), 2.25-2.39 (m, 2H, 2′-H), 3.63-3.69 (m, 2H, 5′-H), 4.19-4.26 (m, 1H, 4′-H), 5.25-5.38 (m, 2H, 3′-H; and 5′-OH, D_2O exchangeable), 6.22-6.25 (dd, 1H, 1′-H), 7.07 (q, 1H, -C=CH-), 7.87 (s, 1H, 6-H), 8.16 (d, 1H, N-CH=C-), 9.60 (d, 1H, -CH=O); MS (CI) 300 (M + 2), 299 (M + 1), 245 (M + 2-C_3H_3O). Anal. Calcd. for $C_{13}H_{15}N_2O_5F$: C, 52.35; H, 4.51; N, 9.39. Found: C, 52.52; H, 4.94; N, 9.19.

3-(3-Oxoprop-1-enyl)-3′-deoxythymidine (18):

Yield, 0.28 g (23%); mp 105-107°C; TLC, R_f 0.31 (EtOAc-hexane, 10:1, v/v); NMR (Me_2SO-d_6) δ 1.84-1.88 (m, 2H, 3′-H), 1.85 (s, 3H, 5-CH_3), 2.04-2.10 (m, 1H, 2′-H_a), 2.27-2.33 (m, 1H, 2′-H_b), 3.56 - 3.58 (m, 1H, 5′-H_a), 3.73 - 3.75 (m, 1H, 5′-H_b), 4.06 (m, 1H, 4′-H), 5.07 (t, 1H, 5′-OH, D_2O exchangeable), 5.96 - 5.98 (t, 1H, 1′-H), 7.08 (q, 1H, -C=CH-), 8.08 (s, 1H, 6-H), 8.18 (d, 1H, N-CH=C-), 9.58 (d, 1H, -CH=O); MS m/e 281 (M + 1). Anal. Calcd. for $C_{13}H_{16}N_2O_5$: C, 55.65; H, 5.74; N, 9.99. Found: C, 55.51; H, 5.53; N, 10.03.

3-(3-Oxoprop-1-enyl)-2′,3′-dideoxyuridine (19):

Yield, 0.12 g (28%); mp 119-121°C; TLC, R_f 0.44 (EtOAc-hexane, 9:1, v/v); NMR (Me_2SO-d_6) δ 1.79-1.98 (m, 2H, 3′-H), 2.06 - 2.12 (m, 1H, 2′-H_a), 2.28 - 2.36 (m, 1H, 2′-H_b), 3.53 - 3.57 (m, 1H, 5′-H_a), 3.71 - 3.75 (m, 1H, 5′-H_b), 4.08 (m, 1H, 4′-H), 5.11 (t, 1H, 5′-OH, D_2O exchangeable), 5.82 (d, 1H, 5-H), 5.95 (m, 1H, 1′-H), 7.05 (q, 1H, -C=CH-), 8.12 - 8.17 (m, 2H, N-CH=C- and 6-H), 9.59 (d, 1H, -CH=O); MS m/e 267 (M + 1). Anal. Calcd. for $C_{12}H_{14}N_2O_5$: C, 54.07; H, 5.29; N, 10.51. Found: C, 53.89; H, 5.18; N, 10.26.

3-(3-Oxoprop-1-enyl)-3′-deoxythymidin-2′-ene (21):

Yield, 0.28 g (26%), isolated as an oil; TLC, R_f 0.4 (EtOAc-hexane, 10:1, v/v); NMR (Me_2SO-d_6) δ 1.82 (s, 3H, 5-CH_3), 3.64 (d, 2H, 5′-H), 4.83 (s, 1H, 4′-H), 4.90 - 5.10 (br s, 1H, 5′-OH, D_2O exchangeable), 5.94 (m, 1H, 3′-H), 6.44 (m, 1H, 2′-H), 6.86 (s, 1H, 1′-H), 7.07 (q, 1H, -C=CH-), 7.87 (s, 1H, 6-H), 8.18 (d, 1H, N-CH=C-), 9.60 (d, 1H, -CH=O); MS m/e 279 (M + 1). Anal. Calcd. for $C_{13}H_{14}N_2O_5$: C, 56.05; H, 5.07; N, 10.06. Found: C, 55.94; H, 5.25; N, 10.25.

For comparison, 3-(3-oxoprop-1-enyl)thymidine (20) was synthesized as described by Johnson et al.[1]

Anticancer Test Procedures

Anticancer activity was assessed by growth inhibition studies using murine L1210 leukemia, P388 leukemia, Sarcoma 180, and human CCRF-CEM lymphoblastic leukemia cells as follows:

Murine L1210, P388 and S-180 cells were maintained as suspension cultures in Fischer's Medium and human CCRF-CEM cells were maintained as a suspension culture in 1640 Roswell Park Memorial Institute Medium, both supplemented with 10% horse serum and maintained at 37°C in a humidified atmosphere of 5% CO_2-95% air. Under these conditions, the generation time for L1210, P388, S-180, and CCRF-CEM cells is approximately 12, 12, 18 and 20 h, respectively. Each compound at various concentrations was added to L1210, P388, S-180, and CCRF-CEM cells (2×10^4 cells/mL) in their exponential phase of growth. The cell number of the drug-free culture (control), as well as that of the cultures supplemented with the tested compounds were determined after 24, 48, and 72 h of growth.

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