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Palladium-Catalyzed Synthesis of [*E*]-6-(2-Acylvinyl)uracils and [*E*]-6-(2-Acylvinyl)-1-[(2-hydroxyethoxy)methyl]uracils—their Antiviral and Cytotoxic Activities†

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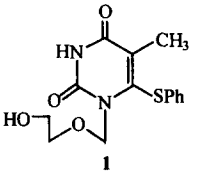
Abstract—[*E*]-6-(2-Acylvinyl)uracils and their corresponding 1-(2-hydroxyethoxy)methyl derivatives were synthesized through palladium-catalyzed reactions which involved an interesting rearrangement. Some of the acylvinyl uracils (**3**, **4**, and **5**) and the acyclonucleosides (**8** and **10**) showed pronounced activity against human T-lymphocyte Molt 4/C8 and CEM cells. However, they were less toxic to murine L1210 and FM3A cells. The compounds did not have any marked antiviral activity. © 1997 Elsevier Science Ltd.

Introduction

As a continuation of our studies[2–5] on novel 5-substituted uracils of biological significance, we have recently become interested in 6-substituted uracils.[6] Our interest was enhanced by the discovery of several 6-substituted uracils (e.g., 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine (HEPT, **1**)[7] and its derivatives) as new anti-HIV-1 agents. Another group of 6-substituted uracils, viz, 3,4-dihydro-2-alkoxy-6-benzyl-4-oxopyrimidines (DABOs, **2**),[8–10] also behave as non-nucleoside reverse transcriptase inhibitors (NNRTIs). Other investigators have also reported on the synthesis and biological activities of 6-substituted uracils and their derivatives.[11–13]



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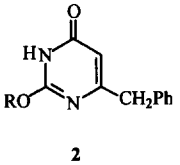

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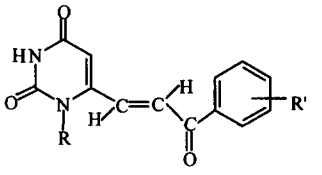
In this paper we report on the synthesis and biological activities of a number of 6-acylvinyl uracils **3–6** and their corresponding acyclonucleosides **7–10**.

The rationale for the synthesis of these compounds was based on the following: (a) the vinyl substitution at the C-6 position of the uracil ring has been shown to be important for cytotoxicity against mouse L1210 leukemia cells,[12–14] (b) an acyl vinyl group is expected to increase the biological activities due to its potential as a Michael acceptor, and (c) several acyclic nucleoside analogues are known to have significant biological activities.[7,15–20] We felt an acyl vinyl group at the C-6 position of the uracil ring with or without a hydroxyethoxymethyl group on the N-1 atom of the pyrimidine ring may provide significant cytotoxic activities to the proposed compounds.



3, R = R' = H

4, R = H, R' = CH_3 (*p*)

5, R = H, R' = OCH_3 (*p*)

6, R = H, R' = OCH_3 (*o*)

7, R = $CH_2OCH_2CH_2OH$, R' = H

8, R = $CH_2OCH_2CH_2OH$, R' = CH_3 (*p*)

9, R = $CH_2OCH_2CH_2OH$, R' = OCH_3 (*p*)

10, R = $CH_2OCH_2CH_2OH$, R' = OCH_3 (*o*)

Chemistry

Although there have been numerous papers published on the synthesis of 5-substituted uracils and their derivatives, methods for the synthesis of 6-substituted uracils and related compounds are limited. One of the earliest reports is that of Klein and Fox[12] who utilized the Wittig reaction to synthesize the 6-substituted uracils. The method has been extended recently by Otter and co-workers[13] to generate 6-vinyluridine and related compounds. Bobek and co-workers[11] have reported the synthesis of 6-ethynyluracil through both Wittig reaction and palladium-catalyzed procedure. A lithiation procedure has been extensively utilized by Miyasaka and co-workers[21–23] to synthesize a number of 6-substituted uracils, 2′-deoxyuridines and uridines. The

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†Part 24 of the series on studies on Uracil Derivatives and Analogues. For part 23 see reference 1.

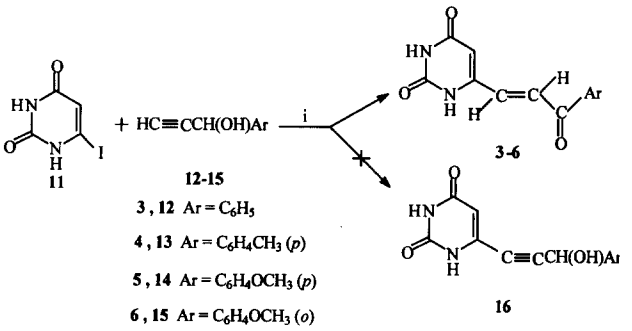
use of the Stille procedure[24] has resulted in the synthesis of a number of 6-substituted uridines.[25] A photochemical approach for the synthesis of 6-substituted uracil derivatives has also been reported.[26]

Palladium-catalyzed reactions have been extensively utilized for carboannulations[27–31] and heteroannulation processes.[32–39] Our own experience with palladium-catalyzed reactions[36–39] has prompted us to utilize these versatile reactions to synthesize the 6-substituted uracils as shown in Scheme 1.

When a mixture of 6-iodouracil **11** and 1-arylprop-2-yn-1-ols **12–15** was heated with stirring in DMF at 55 °C in the presence of bis(triphenylphosphine)palladium(II) chloride (6–7 mol%) and copper(I) iodide (12–13 mol%) as catalysts and triethylamine as base, [*E*]-6-(2-acylvinyl)uracils **3–6** were obtained in 39–60% yields. The expected 6-(3-hydroxy-3-arylprop-1-ynyl)uracils **16** were not obtained. The yields of **3–6** were not better when 6-iodo-2,4-bis(trimethylsilyloxy)pyrimidine (obtained by refluxing **11** with hexamethyldisilazane and a few drops of chlorotrimethylsilane) was used in the palladium-catalyzed coupling reactions instead of **11**. Similar results were also obtained in the reaction of N_1,N_3-6-iodouracil with the acetylenic carbinols **12–15** under the palladium-catalyzed reactions yielding the 6-(2-acylvinyl)-N_1, N_3-dimethyluracils.[40]

It should be noted, however, that the reaction of 5-iodo-N_1,N_3-dimethyluracil or 5-iodo-3′,5′-di-*O*-(*p*-toluoyl)-2′-deoxyuridine with the acetylenic carbinols under palladium-catalyzed conditions yielded the expected products(e.g., 5-(3-aryl-3-hydroxyprop-1-ynyl)-N_1,N_3-dimethyluracils or 5-(3-aryl-3-hydroxyprop-1-ynyl)-3′,5′-di-*O*-(*p*-toluoyl)-2′-deoxyuridines).[41] 6-Iodouracil **11**, however, failed to react with prop-2-yn-1-ol **17** or 3-acetoxy-3-(*p*-methoxy)phenyl-prop-1-yne **18** under various palladium-catalyzed conditions.

Also the reactions of **11** with 1-(*p*-anisyl)prop-2-yn-1-one **19** HC≡C-C(O)C_6H_4OMe-*p* or with 3-(*p*-anisyl)-3-oxo-prop-1-ene **20** failed to yield any 6-substituted uracil derivatives in contrast to the reactions of 5-iodouracil derivatives with **19** and **20**, which led to the 5-(*p*-anisoylethynyl) or [*E*]-5-(2-*p*-anisoylvinyl)uracil derivatives.[3,41] The 6-(2-acylvinyl)uracils were high melting solids (mp >280 °C), insoluble in most common organic solvents except DMSO and DMF. In the IR spectra, a strong peak at 1670 cm $^{-1}$ indicated the presence of the vinylketo group besides the carbonyl groups of the uracil ring which absorbed at 1710 and 1660 cm^{-1}. Also, in the ^{1}H NMR spectra, only a singlet due to one proton at around δ 6.12 was observed, which could be assigned to the C-5 hydrogen. No other peak between δ 5.0–6.0 was observed implying the absence of a benzylic proton in the Ar–CH–OH moiety. Also, the vinyl protons of **3–6** appeared in the aromatic region due to conjugation with the uracil ring with *J* values of 16 Hz, which confirmed the *E*-configuration of the double bond.



Scheme 1. Reaction condition: (i) $(PPh_3)_2PdCl_2$, CuI, Et_3N, DMF, 55 °C, 6 h.

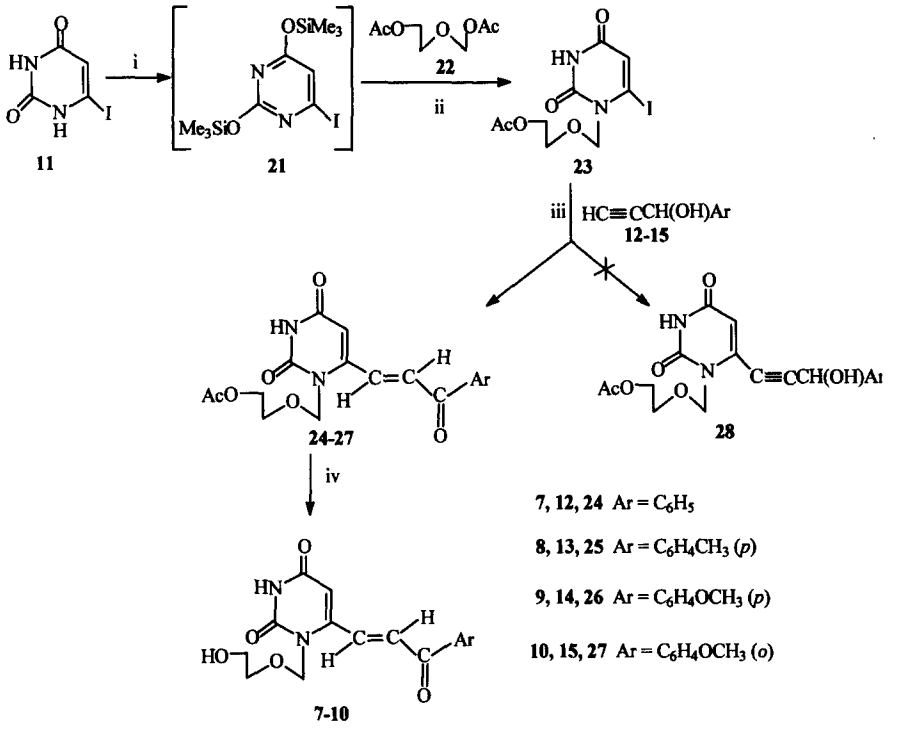
We have also synthesized the acyclonucleosides **7–10** of [*E*]-6-(2-acylvinyl)uracils **3–6** according to the Scheme 2 shown below.

The reaction of (2-acetoxyethoxy)methyl acetate **22** with 6-iodo-2,4-bis(trimethylsilyloxy)pyrimidine **21** (obtained by treatment of 6-iodouracil **11** with hexamethyldislazane and chlorotrimethylsilane) gave 1-(2-acetoxyethoxy)methyl-6-iodouracil **23** in modest yield. Compound **23** underwent the palladium-catalyzed reactions with the acetylenic carbinols **12–15** to yield the 1-(2-acetoxyethoxymethyl)-[*E*]-6-(2-acylvinyl)uracils **24–27** instead of 1-[(2- acetoxyethoxy)methyl]-6-(3-hydroxy-3-aryl-prop-1-ynyl]uracils **28**. The identity of the 6-(acylvinyl)-uracil derivatives **24–27** was established from their spectroscopic data. The formation of the vinylketones was substantiated by the presence of a peak at 1670 cm^{-1} in the IR region. In the ^{1}H NMR (taken in $CDCl_3$), the C-5 proton appeared at around δ 6.05; whereas, the two vinylic protons appeared as a singlet at around δ 7.56.[42]

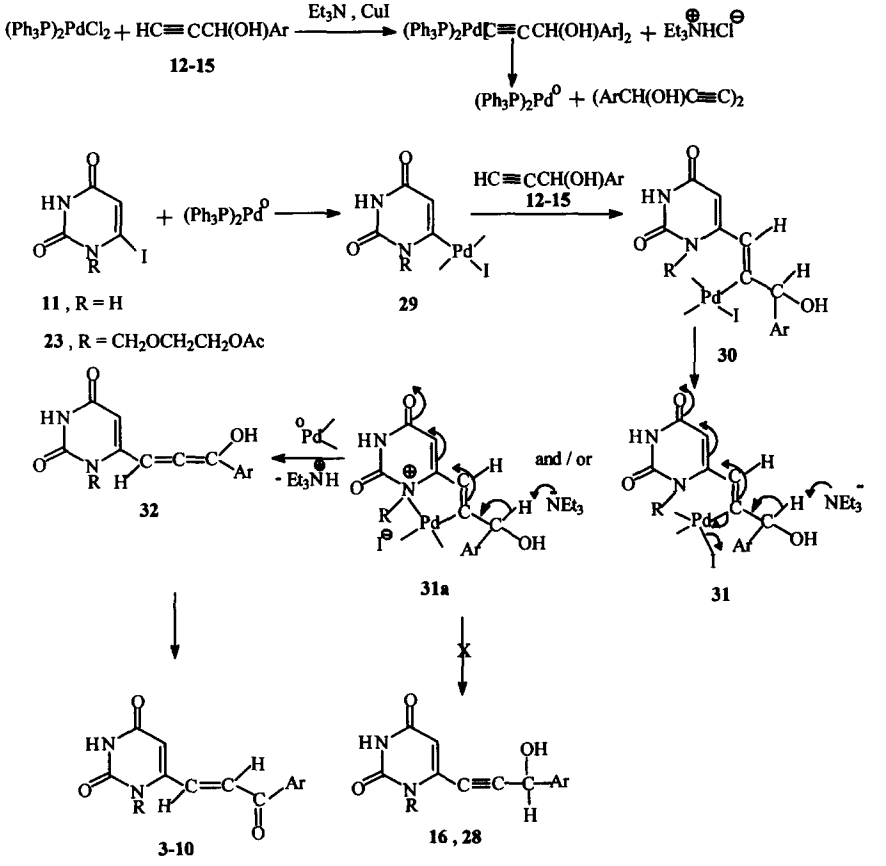
The 1-[2-(acetoxyethoxy)methyl]-[*E*]-6-(2-acylvinyl)uracils **24–27** were deacetylated by treatment with 0.1% sodium methoxide in methanol at room temperature for 4 h to the corresponding [*E*]-6-(2-acylvinyl)-1-[(2-hydroxyethoxy)methyl]uracils **7–10** in excellent yields. It should be noted that in compounds **7–10**, the two vinylic protons appeared as two doublets at around δ 7.56–7.60 and δ 8.00 with J = 16 Hz establishing the *E*-configuration of the double bond.

This unusual formation of the 6-acylvinyl uracils **3–6** and the corresponding nucleosides **7–10** from the reaction of 6-iodouracil **11** and the corresponding acyclonucleoside **23** with the acetylenic carbinols **12–15** could be explained by a mechanism as shown in Scheme 3.

The formation of $(Ph_3P)_2Pd(0)$ from $(Ph_3P)_2PdCl_2$ is well documented.[43] The palladium-catalyzed reaction of nitrogen heterocycles containing iodide on a carbon atom next to the nitrogen atom with acetylenic alcohols leading to the chalcones has been explained simply through a base-catalyzed rearrangement.[44] We believe the proximal nitrogen atom may have a role in the rearrangement process which probably takes place through an intermediate **31/31a** (Scheme 3).[45] Forma-



Scheme 2. Reaction conditions:(i) HMDS, TMSCl, bz, reflux, 4–6h;(ii) 1,2-dichloroethane, $SnCl_4$, rt, overnight; (iii) $(Pph_3)_2$, $PdCl_2$, CuI, Et_3N, DMF, 55°C, 6h, N_2; (iv) NaOMe, MeOH, rt, 4h.



Scheme 3.

Table 1. Inhibitory effects of [*E*]-6-(2-acylvinyl)uracils **3–5** and the acyclonucleosides **7–10** on the proliferation of murine leukemia (L1210), murine mammary carcinoma (FM3A), and human T-lymphocyte (Molt 4/C8, CEM) cells

	IC_{50} (μg/mL)[a]			
Compound	**L1210**	**FM3A**	**Molt 4/C8**	**CEM**
3	76.0 ± 15.2	87.6 ± 5.9	3.24 ± 0.24	2.98 ± 0.12
4	46.7 ± 9.7	76.2 ± 24.7	0.68 ± 0.02	0.61 ± 0.02
5	92.5 ± 3.6	117 ± 17	2.71 ± 0.50	1.83 ± 0.16
7	13.7 ± 2.0	58.1 ± 27.0	8.95 ± 0.88	4.51 ± 1.05
8	9.22 ± 2.69	16.4 ± 2.9	3.25 ± 0.06	3.14 ± 0.22
9	11.6 ± 5.6	44.2 ± 28.4	9.73 ± 0.76	10.3 ± 1.6
10	5.25 ± 0.51	34.8 ± 24.6	4.61 ± 0.30	3.98 ± 0.08

[a]50% inhibitory concentration, or compound concentration required to inhibit tumor cell proliferation by 50%.

tion of **32** is facilitated by electromerisation in the enone system of the uracil ring (solid arrows). Also, a palladated complex[45] **31a** may help in the ionization of C-H (dotted arrows) of the CHOH group leading to the allenol **32**. The allenol **32** then rearranges to the acylvinylketones **3–10**. The possibility of the other β-H elimination leading to the substituted acetylenic carbinols **16** and **28** is not favored.

Biological activity

[*E*]-6-(2-Acylvinyl)uracils **3–5** and [*E*]-6-(2-acylvinyl)-1-(2-hydroxyethoxy)methyl-uracils **7–10** were tested for their biological activities in various tumor cell lines and viral test systems according to previously published procedures.[46–48] As can be seen from Table 1, the [*E*]-6-(2-acylvinyl)uracils **3–5** were poorly cytostatic against murine leukemia (L1210) and murine mammary carcinoma (FM3A) cells. However, they were found to be markedly inhibitory to human T-lymphocyte (Molt 4/C8 and CEM) cell proliferation. [*E*]-6-(2-*p*-toluoylvinyl)uracil **4** was found to be the most toxic of the three compounds tested. The substitution at N_1-H of the uracil molecule in **3–6** with the 2-hydroxyethoxymethyl side chain leading to compounds **7–10** increased the cytotoxicities towards the murine cell lines, particularly L1210 cells. However, the cytotoxicities of **7–10** were lower than those of **3–5** against human T-lymphocytes.

The antiviral activities of [*E*]-6-(2-acylvinyl)uracils **3–5** and the acyclonucleosides **7–10** were also determined against HSV-1, HSV-2, vaccinia virus (VV), vesicular stomatitis virus (VSV) and HSV-1 TK^- strains in E_6SM cell cultures (Table 2). The compounds did not exhibit an appreciable antiviral selectivity, that is, they did not inhibit virus-induced cytopathogenicity at a concentration that was lower than the cytotoxic concentration (40 μg/mL for **3**, **4**, **7–10**, and 100 μg/mL for **5**). Similarly, they did not show any specific activity against other viruses: (e.g., VSV, Coxsackie virus, respiratory syncytial virus in HeLa cells and parainfluenza-3 virus, reovirus-1, sindbis virus, Coxsackie virus B4 and Punta Toro virus in Vero cell cultures [i.e., ≥40 μg/mL for **3**, **5–8**, and 100 μg/mL for **3**, **9**, and **10** (data not shown)]. Also, the compounds **4**, and **8–10** were tested for their antiviral activities against varicella-zoster virus (VZV) (strains OKA, YS and the thymidine kinase-deficient 07/1 and YS/R strains) and cytomegalovirus (CMV) (strains AD-169 and Davis) in human embryonic lung (HEL) cells (i.e., 5–10 μg/mL). Again, no specific

Table 2. Antiviral activities of [*E*]-6-(2-acylvinyl)uracils **3–6** and their acyclonucleosides **7–10** in E_6SM cell cultures

						Minimum inhibitory concn (μg/mL)	
Compound	**Minimum cytotoxic concn (μg/mL)**	**HSV-1 (KOS)**	**HSV-2 (G)**	**VV**	**VSV**	**HSV-1 TK^- (B2006)**	**HSV-1 TK (VMW1837)**
3	40	>10	>10	>10	>10	>10	>10
4	40	>10	>10	>10	>10	>10	>10
5	100	>40	>40	>40	>40	>40	>40
7	40	>10	>10	>10	>10	>10	>10
8	40	>10	>10	>10	>10	>10	>10
9	40	>10	>10	>10	>10	>10	>10
10	40	>10	>10	>10	>10	>10	>10
Brivudin	≥400	0.02	>200	2	>200	10	100
Ribavirin	>400	4	70	20	10	2	20
Ganciclovir	>100	0.0007	0.002	>100	>100	0.1	0.02
Acyclovir	400	0.02	0.04	>200	>200	40	2

[a]Required to cause a microscopically detectable alteration of normal cell morphology.
[b]Required to reduce virus-induced cytopathogenicity by 50%.

inhibitory effects on CMV or VZV were noted with any of the compounds at subtoxic concentrations (data not shown).

Conclusions

[E]-6-(2-Acylvinyl)uracils **3–5** and their acyclonucleosides **7–10** proved fairly cytostatic to human but not murine cells. No appreciable antiviral activity was noted at concentrations that were cytostatic to the host cells. Appreciable cytostatic activity, in the IC_{50} range of 1–4 μg/mL was noted with compounds **3**, **4**, **5**, **8**, and **10** against the human tumor T-lymphocyte cell lines (Molt 4/C8 and CEM). The [E]-6-(2-acylvinyl)uracils are more cytostatic than the corresponding acyclonucleosides against human T-lymphocyte cells, [E]-6-(2-*p*-toluoylvinyl)uracil **4** being the most inhibitory.

Experimental

Chemistry

Melting points were determined on a Reichert (285980) (Austria) melting point bath. UV spectra were recorded on a Hitachi 200–20 spectrometer in spectrophotometric grade ethanol (Baker). IR spectra were taken on a Perkin–Elmer 298 instrument as KBr plate or liquid films. ^{1}H NMR spectra were recorded on 100 MHz Jeol FX-100 and at 60 MHz on Varian EM 360 NMR spectrometers in solvents as indicated with tetramethylsilane as internal reference; *J* values given in Hz. Silica gel TLC was performed on 60F-254 pre-coated sheets (E. Merck) and column chromatography was done on silica gel (60–120 mesh). Elemental analyses were performed on a Perkin–Elmer 240C analyzer. 6-Iodouracil **11** was synthesized according to the procedure of Horwitz and Tomson.[49] The arylprop-2-yn-1-ols **12–15** were synthesized according to the published procedures.[50,51]

[E]-6-(2-Acylvinyl)uracils (3–6). General procedure. In a typical experiment, 6-iodouracil (**11**, 200 mg, 0.84 mmol) was dissolved in DMF (5 mL) under nitrogen atmosphere. Bis(triphenylphosphine)palladium(II) chloride (40 mg, 0.06 mmol) and copper(I) iodide (20 mg, 0.11 mmol) were then added followed by the addition of triethylamine (390 mg, 3.85 mmol). The mixture was stirred at room temperature for 10 min. 1-Arylprop-2-yn-1-ol (**12** or **13** or **14** or **15**, 1.06 mmol) was then added and the reaction mixture was heated at 55 °C for 6 h. The solvents were removed at reduced pressure, the residue was triturated with acetone (3–5 mL) and the solid was filtered and crystallized from methanol.

[E]-6-((2-Benzoylvinyl)uracil (3). 39% white solid, mp >280 °C (MeOH); IR (KBr) ν_{max} 3180(b), 1700, 1670, 1620, 1595, 1580, 1500 cm^{-1}; UV λ_{max} 321, 254 nm; ^{1}H NMR (100 MHz, DMSO-d_6) δ 6.16 (s, 1H, C5-H), 7.28 (d, 1H, *J* = 16 Hz, -CH=CH-CO), 7.52–7.84 (m, 3H, $ArH_{m,p}$), 8.08–8.24 (m, 2H, Ar-H_o), 8.25 (d, 1H, *J* = 16 Hz, -CH=CHCO), 11.12 and 11.24 (2 × bs, 2H, NH). Anal. ($C_{13}H_{10}N_2O_3$), C,H,N.

[E]-6-(2-*p*-Toluoylvinyl)uracil (4). 74%, light-yellow solid mp > 280 °C (MeOH); IR (KBr); ν_{max} 3115, 3000, 1720, 1660, 1625, 1610, 1590 cm^{-1}; UV λ_{max} 317, 248 nm; ^{1}H NMR (100 MHz, DMSO-d_6) δ 2.48 (s, 3H, $ArCH_3$), 6.12 (s, 1H, C5-H), 7.28 (d, 1H, *J* = 16Hz, -CH=CH-CO), 7.40 (d, 2H, *J* = 8 Hz, ArH_m), 8.08 (d, 2H, *J* = 8 Hz, ArH_o), 8.20 (d, 1H, *J* = 16 Hz, -CH=CH-CO), 11.16 (bs, 2H, 2 × NH); Anal. ($C_{14}H_{12}N_2O_3$), C,H,N.

[E]-6-(2-*p*-Anisoylvinyl)uracil (5). 57%, light-yellow solid mp > 280 °C (MeOH); IR (KBr) ν_{max} 3160, 3040, 1720, 1680, 1660, 1600 cm^{-1}; UV λ_{max} 332, 240 nm; ^{1}H NMR (100 MHz, DMSO-d_6) δ 3.90 (s, 3H, $ArOCH_3$), 6.12 (s, 1H, C5-H), 7.16 (d, 2H, *J* = 8 Hz, ArH_m), 7.28 (d, 1H, *J* = 16Hz, -CH=CH-CO), 8.20 (d, 2H, *J*=8Hz, ArH_o), 8.24 (d, 1H, *J* = 16 Hz, -CH=CH-CO), 11.16 (bs, 2H, 2 × NH). Anal. ($C_{14}H_{12}N_2O_4$), C,H,N.

[E]-6-(2-*o*-Anisoylvinyl)uracil (6). 35%, pale-yellow solid mp > 280 °C (MeOH); IR (KBr) ν_{max} 3140, 3100, 1725, 1660, 1640, 1590 cm^{-1}; UV λ_{max} 318, 253 nm; ^{1}H NMR (60 MHz, DMSO-d_6) δ 3.93 (s, 3H, $ArOCH_3$, 5.87 (s, 1H, C5-H), 6.87–7.30 (m, 3H, ArH and -CH=CH-CO), 7.43–8.17 (m, 3H, ArH and -CH=CH-CO), 11.17 (bs, 2H, 2 × NH). Anal. ($C_{14}H_{12}N_2O_4$), C,H,N.

1-[(2-Acetoxyethoxy)methyl]-6-iodouracil (23). A mixture of 6-iodouracil (**11**, 1.5g, 6.3mmol), 1,1,1,3,3,3-hexamethyldisilazane (HMDS, 6.31 g, 8.25 mL, 39.1 mmol) and chlorotrimethyl-silane (1 mL) in dry benzene was refluxed with stirring under dry nitrogen atmosphere for 4–6 h when a clear solution was obtained. The solvents were removed and the residue dried by distilling dry benzene from it twice and then under vacuum to yield 6-iodo-2,4-bis-*O*-trimethylsilyloxypyrimidine (**21**) as a pale-yellow oil. To an ice-cold solution of **21** in 1,2-dichloroethane (15 mL) (2-acetoxyethoxy)methyl acetate[52] (**22**, 1.23 g, 6.98 mmol) was added followed by the addition of tin(IV) chloride (1 mL). The mixture was stirred overnight at room temperature. The reaction mixture was poured into a cold satd soln of $NaHCO_3$ (50 mL) and CH_2Cl_2 (150 mL). The resulting emulsion was filtered over a bed of celite and the filtrate was extracted with CH_2Cl_2 (2 × 100 mL). The combined CH_2Cl_2 layers were washed with H_2O (2 × 100 mL) and dried. After removal of solvent, a gum was obtained which was purified by column chromatography over silica gel (60–120 mesh) with $CHCl_3$ as eluent to obtain 1-[(2-acetoxyethoxy)methyl-6-iodouracil (**23**, 1.08 g, 3.05 mmol, 48%) as a gum which solidified on keeping under vacuum for a long time; crystallized from $CHCl_3$–Et_2O, off-white solid, mp 112 °C; IR (KBr) ν_{max} 3020, 1730, 1710, 1670, 1565 cm^{-1} ; UV (EtOH) λ_{max} 270 nm; ^{1}H NMR (60 MHz, DMSO-d_6) δ

2.07 (s, 3H, $OCOCH_3$), 3.67–3.93 (A_2B_2m, 2H, OCH_2CH_2O), 4.07–4.37 (A_2B_2m, 2H, OCH_2CH_2O), 5.50 (s, 2H, NCH_2O), 6.47 (s, 1H, C5-H), 10.15 (bs, 1H, N3-H); Anal. ($C_9H_{11}N_2O_5$) C,H,N.

1-[(2-acetoxyethoxy)methyl]-[*E*]-6-(2-acylvinyl)uracils (24–27). General procedure. A mixture of 1-[(2-acetoxyethoxy)methyl]-[*E*]-6-iodouracil (**23**, 300 mg, 0.85 mmol), Et_3N (390 mg, 3.85 mmol), $(Ph_3P)_2PdCl_2$ (35 mg, 0.05 mmol), and CuI (20 mg, 0.11 mmol) was stirred at room temperature for 10 min. 1-Arylprop-2-yn-1-ol (**12–15**, 1.21 mmol) was then added to the mixture which was stirred at 55 °C for 6 h. After removal of the solvent, the brown residue was worked up with H_2O (50 mL) and $CHCl_3$ (3 × 100 mL) and dried (anhydrous Na_2SO_4). After removal of the solvent, the residue was chromatographed on a column of silica gel (60–120 mesh), [eluent acetone in $CHCl_3$ (1:9)] to obtain 1-[(2-acetoxyethoxy)methyl]-[*E*]-6-(2-acylvinyl)uracils (**24–27**).

1-[(2-Acetoxyethoxy)methyl]-[*E*]-6-(2-benzoylvinyl)-uracil (24). 92%, Mp 158 °C (acetone); IR (KBr) ν_{max} 3170, 3060, 1740, 1730, 1710, 1670, 1620 cm^{-1} ; UV (EtOH) λ_{max} 246 nm; ^1H NMR (60 MHz, $CDCl_3$) δ 2.05 (s, 3H, $OCOCH_3$), 3.79–3.99 (A_2B_2m, 2H, OCH_2CH_2O), 4.14–4.35 (A_2B_2m, 2H, OCH_2CH_2O), 5.37 (s, 2H, NCH_2O), 6.07 (s, 1H, C5-H), 7.43-7.71 (m, 5H, $ArH_{m,p}$ and -CH=CHCO), 7.95–8.11 (m, 2H, ArH_o), 8.99 (bs, 1H, N3-H); Anal. ($C_{18}H_{18}N_2O_6$) C,H,N.

1-[(2-Acetoxyethoxy)methyl]-[*E*]-6-(2-*p*-toluoylvinyl)-uracil (25). 71%, Mp 178–179 °C (acetone); IR (KBr) ν_{max} 3010, 1720, 1710, 1665, 1620 cm^{-1} ; UV (EtOH) λ_{max} 251 nm; ^1H NMR (60 MHz, $CDCl_3$) δ 2.07 (s, 3H, $OCOCH_3$), 2.47 (s, 3H, $ArCH_3$), 3.77–4.00 (A_2B_2m, 2H, OCH_2CH_2O), 4.13–4.37 (A_2B_2m, 2H, OCH_2CH_2O), 5.37 (s, 2H, NCH_2O), 6.08 (app. d, 1H, C5-H), 7.33 (d, 2H, J = 8 Hz, ArH_m), 7.67 (s, 2H, -CH=CHCO), 7.93 (d, 2H, J = 8 Hz, ArH_o), 8.99 (bs,1H, N3-H); ^1H NMR (300 MHz, DMSO-d_6) δ 1.95 (s, 3H, $OCOCH_3$), 2.40 (s, 3H, $ArCH_3$), 3.68–3.74 (A_2B_2m, 2H, OCH_2CH_2O), 4.04–4.17 (A_2B_2m, 2H, OCH_2CH_2O), 5.30 (s, 2H, NCH_2O), 6.38 (s, 1H, C5-H), 7.38 (d, 2H, J = 7.5 Hz, ArH_m), 7.54 (d, 1H, J = 15.3 Hz, -*CH*=CHCO), 7.97 (d, 1H, J = 15.9 Hz, -CH=*CH*CO), 8.06 (d, 2H, J = 7.5Hz, ArH_o) 11.28 (app. t, 1H, N3-H); Anal. ($C_{19}H_{20}N_2O_6$) C,H,N.

1-[(2-Acetoxyethoxy)methyl]-[*E*]-6-(2-*p*-anisoylvinyl)-uracil (26). 91%, Mp 180–181 °C (acetone–methanol); IR(KBr) ν_{max} 3170, 3040, 1730, 1710, 1670, 1620, 1600 cm^{-1} ; UV (EtOH) λ_{max} 322, 242 nm; ^1H NMR (100 MHz, $CDCl_3$) δ 2.06 (s, 3H, $OCOCH_3$), 3.76–4.00 (A_2B_2m, 2H, OCH_2CH_2O), 3.92 (s, 3H, $ArOCH_3$) 4.14–4.34 (A_2B_2m, 2H, OCH_2CH_2O), 5.36 (s, 2H, NCH_2O), 6.05 (app. d, 1H, C5-H), 7.02 (d, 2H, J = 8 Hz, ArH_m), 7.64 (app. d, 2H, -CH=CHCO), 8.02 (d, 2H, J = 8 Hz, ArH_o), 8.80 (bs, 1H, N3-H); Anal. ($C_{19}H_{20}N_2O_7$) C,H,N.

1-[(2-Acetoxyethoxy)methyl]-[*E*]-6-(2-*o*-anisoylvinyl)-uracil (27). 70%, Mp 162 °C (acetone); IR (KBr) ν_{max} 3290, 1740, 1735, 1700, 1670, 1620, 1600 cm^{-1}; UV (EtOH) λ_{max} 320, 244 nm; ^1H NMR (100 MHz, $CDCl_3$) δ 2.08 (s, 3H, $OCOCH_3$), 3.80–4.04 (A_2B_2m, 2H, OCH_2CH_2O), 3.96 (s, 3H, $ArOCH_3$), 4.16–4.36 (A_2B_2m, 2H, OCH_2CH_2O), 5.38 (s, 2H, NCH_2O), 6.04 (app. d, 1H, C5-H), 7.08 (app. t, 2H, J = 8 Hz, ArH), 7.56 (s, 2H, -CH=CHCO), 7.76 (dd, 1H, J = 8 Hz, 2Hz, ArH), 8.96 (bs, 1H, N3-H); Anal. ($C_{19}H_{20}N_2O_7$) C,H,N.

[*E*]-6-(2-Acylvinyl)-1-[(2-hydroxyethoxy)methyl]uracils(7–10). General procedure. 1[(2-Acetoxyethoxy)methyl]-[*E*]-6-(2-acylvinyl)uracil (**24–27**, 0.47 mmol) was added to a solution of sodium methoxide (0.52 mmol) in methanol (12 mL). The mixture was stirred at room temperature and then neutralized with Dowex-50-X6 (H^+) resin. The solvent was then removed to yield [*E*]-6-(2-acylvinyl)-1-[(2-hydroxyethoxy)methyl]uracils (**7–10**).

[*E*]-6-(2-Benzoylvinyl)-1-[(2-hydroxyethoxy)methyl]-uracil (7). 81%, Mp 155 °C, (methanol); IR (KBr) ν_{max} 3470, 3070, 1720, 1700, 1675, 1625 cm^{-1}; UV (EtOH) λ_{max} 247 nm; ^1H NMR (100 MHz, DMSO-d_6) δ 3.56 (s, 4H, OCH_2CH_2O), 4.68 (app. t, H, OH), 5.32 (s, 2H, NCH_2O), 6.38 (s, 1H, C5-H), 7.48–7.84 (m, 4H, $ArH_{m,p}$,-CH=CH-CO), 8.00 (d, 1H, J = 16 Hz, -CH=CHCO), 8.20 (d, 2H, J = 8 Hz, ArH_o), 11.56 (bs, 1H, N3-H); Anal. ($C_{16}H_{16}N_2O_5$) C,H,N.

1-[(2-Hydroxyethoxy)methyl]-[*E*]-6-(2-*p*-toluoylvinyl)-uracil (8). 95%, Mp 174–175 °C, (methanol); IR (KBr) ν_{max} 3480, 3220, 1710, 1690, 1675, 1620 cm^{-1}; UV (EtOH) λ_{max} 300, 242 nm; ^1H NMR (100 MHz, DMSO-d_6) δ 2.40 (s, 3H, $ArCH_3$), 3.52 (s, 4H, OCH_2CH_2O), 4.66 (app. t, H, OH), 5.28 (s, 2H, NCH_2O), 6.36 (s, 1H, C5-H), 7.40 (d, 2H, J = 8 Hz, ArH_m) 7.60 (d, 1H, J = 16 Hz, -CH=CH-CO), 8.00 (d, 1H, J = 16 Hz, -CH=CHCO), 8.12 (d, 2H, J = 8 Hz, ArH_o), 11.54 (bs, 1H, N3-H); Anal. ($C_{17}H_{18}N_2O_5$) C,H,N.

[*E*]-6-(2-*p*-Anisoylvinyl)-1-[(2-hydroxyethoxy)methyl]-uracil (9). 96%, Mp 180 °C, (methanol); IR (KBr) ν_{max} 3400, 3220, 1690, 1660, 1620, 1590 cm^{-1} ; UV (EtOH) λ ^1H NMR (100 MHz, DMSO-d_6) δ 3.52 (s, 4H, OCH_2CH_2O), 3.86 (s, 3H, $ArOCH_3$), 4.64 (app. t, H, OH), 5.28 (s, 2H, NCH_2O), 6.32 (s, 1H, C5-H), 7.08 (d, 2H, J = 8 Hz, ArH_m) 7.56 (d, 1H, J = 16 Hz, -CH=CH-CO), 8.00 (d, 1H, J = 16 Hz, -CH=CHCO), 8.18 (d, 2H, J = 8 Hz, ArH_o), 11.48 (bs,1H, N3-H); Anal. ($C_{17}H_{18}N_2O_6$) C,H,N.

[*E*]-6-(2-*o*-Anisoylvinyl)-1-[(2-hydroxyethoxy)methyl]-uracil (10). 90%, Mp 178–180 °C, (methanol); IR (KBr) ν_{max} 3500, 3010, 1705, 1680, 1615, 1600 cm^{-1}; UV (EtOH) λ_{max} 320, 244 nm; ^1H NMR (100 MHz, DMSO-d_6) δ 3.34 (s, 4H, OCH_2CH_2O), 3.90 (s, 3H, $ArOCH_3$), 4.66 (app. t, H, OH), 5.26 (s, 2H, NCH_2O), 6.08 (s, 1H, C5-H), 6.91–7.82 (m, 6H, $ArH_{m,p}$ and

-CH=CH-CO), 11.50 (bs, 1H, N3-H); Anal. ($C_{17}H_{18}N_2O_6$) C,H,N.

Biology

Inhibition of L1210, FM3A, Molt and CEM cell proliferation. All assays were performed in flat-bottomed 96-well microtiter plates as previously described.[53] Briefly, the cells were suspended in growth medium and added to the microplate wells at a density of 5×10^4 L1210 or FM3A cells/well (200 μL), or 7.5×10^4 Molt and CEM cells/well in the presence of varying concentrations of the test compounds. The cells were then allowed to proliferate for 48 h (L1210, FM3A) or 72 h (Molt, CEM) at 37 °C in a humidified CO_2-controlled atmosphere. At the end of the incubation period, the cells were counted in a Coulter counter. The IC_{50} was defined as the compound concentration that reduced the number of viable cells by 50%.

Antiviral assays. The antiviral assays were based on an inhibition of virus-induced cytopathicity in either HeLa (vesicular stomatitis virus, Coxsackie virus B4, and respiratory syncytial virus), Vero (parainfluenza-3 virus, reovirus-1, Sindbis virus, Coxsackie B4, and Punta Toro virus), E_6SM (human embryonic skin-muscle) [herpes simplex virus type 1 (HSV-1) (strain KOS), HSV-2 (strain B), vaccinia virus, vesicular stomatitis virus, and the thymidine kinase-deficient HSV-1 strains B2006 and VMW 1837] or HEL (human embryonic lung) [varicella zoster virus (strains OKA, YS, 07/1 and YS/R) and cytomegalovirus (strains AD-169 and Davis)] cell cultures, following previously established procedures.[54,55] Briefly, confluent cell cultures in microtiter trays were inoculated with 100 $CCID_{50}$ of virus, 1 $CCID_{50}$ being the virus dose required to infect 50% of the cell cultures. After a 1 h virus adsorption period, residual virus was removed, and the cell cultures were incubated in the presence of varying concentrations (400, 200, 100, ..., μg/mL) of the test compounds. Viral cytopathogenicity was recorded as soon as it reached completion in the control virus-infected cell cultures.

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