Synthesis and Biological Activities of Novel 5-(2-Acylethynyl)uracils[†,1]

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5-(2-Acylethynyl)-2,4-dimethoxypyrimidines (3–6) were synthesized in excellent yields from 2,4-dimethoxy-5-[2-(trimethylsilyl)ethynyl]pyrimidine (2) by treatment with acid chlorides in the presence of anhydrous aluminum chloride. Compounds 3–6 were deblocked with chlorotrimethylsilane and sodium iodide in acetonitrile to the corresponding 5-[(2-acyl-1-iodo)vinyl]uracils (7–10), which on treatment with potassium hydroxide in dioxane yielded the corresponding 5-(2-acylethynyl)uracils (11–14). The 5-(2-acylethynyl)uracils were found to be active against Ehrlich ascites carcinoma (EAC) cells in vivo, the most active compounds being 5-(2-benzoylethynyl)uracil (11) and 5-(2-*p*-toluoylethynyl)uracil (12). The T/C values of 281 and 300 were obtained for compounds 11 and 12, respectively, in the case of mice bearing EAC cells. The 5-(2-acylethynyl)uracils have also shown in vitro activity against CCRF-CEM and L1210/0 tumor cell lines. The lead compound 5-(2-*p*-toluoylethynyl)uracil effectively inhibited thymidylate synthetase.

Various 5-substituted derivatives of uracil have acquired importance as anticancer and antiviral agents. Notable among them are 5-fluorouracil (5-FU)[2] and the corresponding 2′-deoxyribonucleoside (FUdR), which have been used in cancer chemotherapy for decades. 5-(Trifluoromethyl)-2′-deoxyuridine (F₃TdR)[3] has been used in the treatment of ocular herpes keratitis. Recently (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU)[4] has been found to be one of the most potent drugs effective against herpes simplex virus type I (HSV-I). Among 5-substituted uracils, 3′-azido-3′-deoxythymidine (AZT) has been found to be a potent inhibitor of the human immunodeficiency virus (HIV) and is being used among AIDS patients.[5] This has prompted the development of other 3′-azido analogues of pyrimidine deoxyribonucleosides as potent inhibitors of human immunodeficiency virus.[6]

Inherent in the choice of 5-substituted derivatives of uracil as anticancer agents is the possibility that they could act as inhibitors of thymidylate synthetase (TS), an essential enzyme required for the growth of cells. Indeed, 5-fluorouracil in the form of 5-fluoro-2′-deoxyuridylate has been shown to form a tight ternary complex with thymidylate synthetase and the cofactor methylene tetrahydrofolic acid.[7,8] On the basis of the structure of the ternary complex involved in the thymidylate synthetase reaction, several potent inhibitors of TS have been developed.[9–11] Pronounced cytotoxicity and significant antiviral activity have also been reported for 5-ethynyl-2′-deoxyuridine.[12–14] The corresponding 5′-phosphate, e.g., 5-ethynyl-2′-deoxyuridylate, has been shown to be a strong inhibitor of TS.[15,16] Several 5-alkynyl-2′-deoxyuridines have also been reported.[17] The compounds have antiviral activity.[18]

We have a long-term interest in the development of various inhibitors of thymidylate synthetase and dihydroorotate dehydrogenase, essential enzymes required for the growth of cells. With that objective, we have synthesized various 5- and 6-substituted derivatives of uracil[19,20] and dihydrouracil.[21] Recently, we became interested in uracil derivatives of structure 1 with an ethynyl side chain at C₅ position which is further conjugated with an acyl group.

We felt that such molecules (after being converted to the corresponding 2′-deoxyribonucleotides) could act as
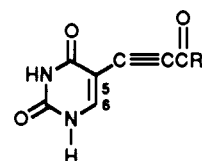
1: R = aromatic group

potent inhibitors of thymidylate synthetase (TS) for the following reasons: (i) the attack by the thiol group of

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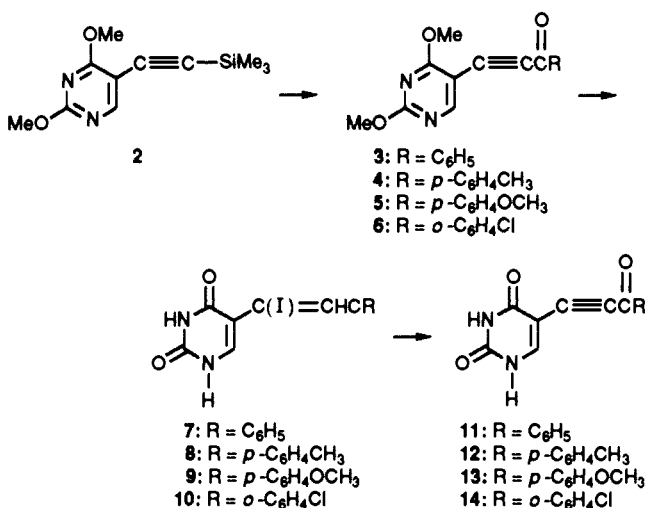(19) Bhatt, R. S.; Kundu, N. G.; Chwang, T. L.; Heidelberger, C. *J. Heterocycl. Chem.* 1981, *18*, 771.

Scheme I

2

3: R = C_6H_5
4: R = p-$C_6H_4CH_3$
5: R = p-$C_6H_4OCH_3$
6: R = o-C_6H_4Cl

7: R = C_6H_5
8: R = p-$C_6H_4CH_3$
9: R = p-$C_6H_4OCH_3$
10: R = o-C_6H_4Cl

11: R = C_6H_5
12: R = p-$C_6H_4CH_3$
13: R = p-$C_6H_4OCH_3$
14: R = o-C_6H_4Cl

cysteine of thymidylate synthetase[22] at the C_6 position of the 2'-deoxyribonucleotide derived from 1 will be facilitated due to the presence of the activating group at C_5; (ii) the attack by the thiol group of cysteine or some other nucleophilic moiety in TS could also occur at the acyl conjugated acetylenic function at C_5; (iii) the aromatic group of the acyl side chain will increase the lipophilicity of the molecule and help with membrane permeability; (iv) the complex between the 2'-deoxyribonucleotide of 1 and TS mimics the TS–dUMP–methylene tetrahydrofolic acid complex. In this paper we report a general method for the synthesis of compounds of structure 1 and some preliminary biological results on these compounds.

Chemistry

Because of their biochemical and biological significance, various methods have been developed for the synthesis of 5-substituted derivatives of uracil.[23-31] Most of these methods are targeted toward the synthesis of a small group of uracil derivatives. Only a few general methods aimed toward the synthesis of a large number of 5-substituted uracils are available. Recently palladium-catalyzed reactions have been of greatest significance and have been utilized successfully to introduce alkyl, alkenyl, and alkynyl substituents at C_5 position of uracil nucleosides and heterocyclic bases.[32-36] C_5-lithiated species of protected uracil

nucleosides have been utilized for the synthesis of 5-substitued uridines and 2'-deoxyuridines.[37] Photochemical methods for the synthesis of C_5- and C_6-substituted uracils have also been described.[38,39]

Silylated acetylenes have been utilized for the synthesis of conjugated acetylenic ketones.[40] We have reported[20] a convenient method for the synthesis of a C-silylated pyrimidine, 2,4-dimethoxy-5-[2-(trimethylsilyl)ethynyl]-pyrimidine (2). When a mixture of compound 2 and an aromatic acid chloride in dichloromethane was treated with anhydrous aluminum chloride the corresponding acetylenic ketones (3–6) were obtained in excellent yield (Scheme I).

The acetylenic ketones, e.g., 5-(2-acetylethynyl)-2,4-dimethoxypyrimidines (3–6), were well-characterized crystalline compounds. In the infrared spectra, they exhibited a very strong band at 2200–2190 cm^{-1}, indicative of the conjugated acetylenic group. The ultraviolet spectra of these compounds had absorption at about 322 nm. In the proton NMR spectra, the acetylenic ketones exhibited signals at about δ 4.04 and δ 4.10 (due to the methoxy groups on the pyrimidine ring), signals between δ 8.54–8.60 (due to the C_6-H on the pyrimidine ring), and the appropriate signals due to the protons on the aromatic rings. It was observed that the C_6-H of the acetylenic ketones showed a much downfield absorption compared to the C_6-H of 5-[2-(trimethylsilyl)ethynyl]uracil, which showed an absorption at δ 7.55.[20]

Although various methods are available for the deblocking of alkoxypyrimidines to uracils, the presence of a conjugated acetylenic group at C-5 on the pyrimidine ring necessitated the use of a milder reagent. Iodotrimethylsilane has been successfully used for the cleavage of the aromatic ethers;[41] however, its application for the removal of the ether groups in methoxy pyrimidines has been limited.[42,20] When the acetylenic ketones were treated with iodotrimethylsilane, deblocking took place with concurrent addition of hydrogen iodide to the triple bond. Similar results were obtained by using chlorotrimethylsilane and sodium iodide in acetonitrile.[43] The products were, however, cleaner and easier to crystallize. The deblocked compounds were identified as 5-(2-acyl-1-iodovinyl)uracils (7–10) from spectroscopic results. Thus, in the infrared spectra, a complete lack of absorption between 2200 and 2000 cm^{-1} indicated the absence of the triple bond. The most positive evidence was obtained from the proton NMR spectra, where an extra peak was seen due to the vinylic hydrogen in the downfield region (usually around δ 8.0). The C_6 hydrogen on the uracil ring in compounds 7–10 had chemical shifts in the range δ 8.45–8.22. This assignment is in conformity with the chemical shifts of C_6 hydrogens of 5-(2-acylethynyl)uracils (δ 8.44–8.1) and that of 5-(2-p-

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Table I. Effect of 5-(2-Acylethynyl)uracils on Ehrlich Ascites Tumor in Mice[a]

no.	dose,[b] mg/kg	average[c] ascites cell: T/C	% inhibn of ascites cell: $(1 - T/C) \times 100$	average[d] ascites fluid: T/C	% inhibn of ascitic fluid: $(1 - T/C) \times 100$
11	50	completely dry		fluid could not be drawn	
11	20	35/150	76	0.8/2	60
12	50	completely dry		fluid could not be drawn	
12	20	completely dry		fluid could not be drawn	
13	20	100/123	19.1	2/2.5	20
14	20	59/165	64.25	2/3.2	37.5
5-FU	50	completely dry		fluid could not be drawn	

[a] Two groups of mice were inoculated ip with 1×10^6 Ehrlich ascites carcinoma cells according to standard protocol. [b] Administered to EAC-bearing Swiss mice once daily on days 1–7 after inoculation of the animals with tumor cells on day 0; groups of five animals per dose level were used with one control group for every six groups. [c] Drug evaluation was done by counting the number of cells present in treated group (T) and in control group (C) of mice. [d] The drugs were evaluated by calculating the fluid weight in treated (T) group and fluid weight in control group (C).

toluoylvinyl)uracil (δ 8.20).[44] The mass spectral results corroborated the structure of 5-(2-benzoyl-1-iodovinyl)-uracil (7), where a weak molecular ion at $m/z = 368$ was observed. From NMR, compounds 7–10 were seen as mixture of E and Z isomers.

The 5-(2-acyl-1-iodovinyl)uracils (7–10) were smoothly dehydrohalogenated with potassium hydroxide in dioxane to the corresponding 5-(2-acylethynyl)uracils (11–14), respectively. They were high-melting solids which had strong absorption at 2200 cm⁻¹ in the infrared and 324–335-nm absorption in the ultraviolet. Their proton NMR spectra were in conformity with their structures (see the Experimental Section).

Biological Results

The 5-(2-acylethynyl)uracils (AEUs) (11–14) were tested for biological activity against Ehrlich ascites carcinoma (EAC) cells in Swiss Albino mice in vivo. Three parameters were followed to observe the effect of these compounds on the EAC cells, e.g., the cell counts of EAC cells in the treated mice vs the control groups, the change in fluid weight in the treated vs the control groups, and the increase in survival of the treated mice.

In Table I are recorded the effects of these compounds as measured by cell counts and change in fluid weight. It is to be noted that the two parameters yielded fairly comparable results. Among the 5-(2-acylethynyl)uracils (11–14), 5-(2-benzoylethynyl)uracil (11) and 5-(2-p-toluoylethynyl)uracil (12) were found to be most effective in the inhibition of the growth of the EAC cells and were comparable to 5-fluoro uracil in their potency. 5-[2-(6-Chlorobenzoyl)ethynyl]uracil (14) was also found to be fairly active whereas 5-[2-(p-methoxybenzoyl)ethynyl]-uracil (13) was found to be the least active in the group.

The effect of 5-(2-acylethynyl)uracils on the survival of mice bearing EAC cells (Table II) was comparable to the inhibition of the EAC cells in vivo (Table I). Compounds 11 and 12 were found to be the most effective in prolonging the life span of mice bearing EAC cells. Indeed, p-toluoyl compound 12 was found to be the most effective (T/C = 300) when given at a single dose of 140 mg/kg and even superior to 5-fluorouracil at this dose level. However, at multiple dose (20 mg/kg given for 7 days), 5-fluorouracil was found to be better.

The acetylenic compounds (AEUs) have also been tested against L1210/0 mouse leukemia and CCRF-CEM human lymphoblastoid cells in vitro, and the results are shown

Table II. Effect of 5-(2-Acylethynyl)uracils on the Survival of Mice Bearing Ascites Tumor[a]

no.	dose,[b] mg/kg	no. of days treated	average controls	survival treated	extremes control	extremes treated	percent[c] of controls
11	140	1	13	36.5	11–15	34–39	281
11	210	1	13	21.5	11–15	21–22	165
11	20	7	13	22	11–15	21–23	169
11	30	7	13	17.5	11–15	17–18	134
12	70	1	11.5	30	10–13	29–31	260
12	140	1	11.5	34.5	10–13	34–35	300
12	10	7	11.5	13	10–13	12–14	113
12	20	7	11.5	20.5	10–13	18–23	178
13	140	1	11	14	10–12	13–15	127
13	210	1	11	14	10–12	12–16	127
13	20	7	11	18	10–12	17–19	164
13	30	7	11	12	10–12	11–13	109
14	140	1	13	16.5	12–14	15–18	127
14	210	1	13	26.5	12–14	26–27	204
14	20	7	13	16.5	12–14	16–17	127
14	30	7	13	18.5	12–14	17–20	142
5-FU	140	1	12	11	11–13	8–14	92
5-FU	20	7	12	41	11–13	37–45	342

[a] Two groups of mice were inoculated ip with 1×10^6 Ehrlich ascites cells according to standard procedure. [b] Starting from 24 h after tumor transplantation, the treated group received single doses or seven doses of ip injections of the synthesized compounds. The control group mice were injected with the vehicle only. Groups of five animals per dose level were used with one control group for every five groups. [c] Testing was evaluated by calculating median survival times (MST) of the treated and control groups of mice; percent of control >125 is considered active.

Table III. Effect of 5-(2-Acylethynyl)uracils on L1210/0 and CCRF-CEM Cells in Culture[a]

	IC_{50}, μM	
compound	CCRF-CEM	L1210/0
11	2.0	0.65
12	2.25	1.9
13	2.3	2.0
14	2.2	2.0
5-FU	2.0	0.3

[a] Incubation of the cells with the compounds was done at 37 °C for 48 h with different concentrations of the compounds. The compounds were added in Me_2SO (final Me_2SO concentration, 0.5%). For details see ref 47. L1210/0 cells were obtained from Dan Griswold of the Southern Research Institute in Birmingham, AL.

in Table III. As can be seen, all the AEUs (11–14) were highly active against both cell lines in vitro. They had almost equal activities, which were comparable to those of 5-fluorouracil.

We have also explored the mechanism of action of these compounds particularly as inhibitors of thymidylate synthetase (TS) enzyme, an essential enzyme required for the conversion of deoxyuridylic acid to thymidylic acid and, hence, essential for the multiplication of cells. The activity of TS was determined by measuring the tritiated water released from the conversion of [5-³H]-dUrd to dTMP in intact L1210/0 cells according to the procedure of Kalman and Yalowich.[45] Figure 1 shows the rate of TS inhibition by 5-(2-p-toluoylethynyl)uracil (12) (the most active compound in the group). The insert is a first-order replot of the data. As can be seen, there was a lag period (about 30 min) before TS inhibition became log-linear. This lag was probably due to relatively slow activation of 12 to the nucleotide level after transport. The inhibition of TS increased with the time of incubation with the compound. At the end of 4 h, almost 90% of the activity of TS was found to be inhibited.

(44) Unpublished results from our laboratory.

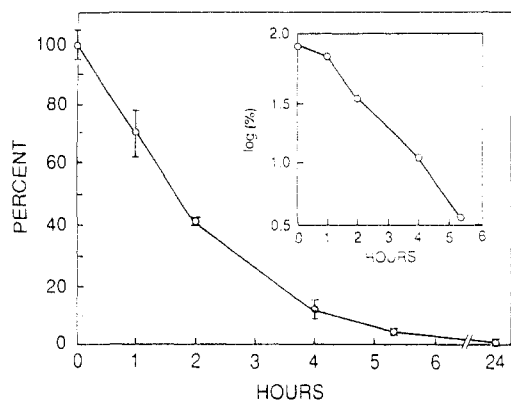(45) Kalman, T. I.; Yalowich, J. *Proc. Am. Assoc. Cancer. Res.* **1978**, *19*, 153.

Figure 1. The percent activity of thymidylate synthetase in L1210/0 cells for 5-^{3}HdUMP $\rightarrow$ dTMP + [^{3}H]H$_2$O conversion is shown against the time (in hours), the cells being incubated with 5-(2-p-toluoylethynyl)uracil (12). The insert is a first-order replot of the data. L1210/0 cells [in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% dialyzed fatal calf serum, 0.5 mL, 5 × 10^5 cells/mL] were incubated with 5-(2-p-toluoylethynyl)uracil (12) (final concentration of 12 = 16.67 μM with 1.3% DMSO) for varying time periods at 37 °C and 5% CO$_2$. At the end of the incubation period, [5-^{3}H]dUrd (Moravek Biochemicals, City of Industry, CA, 50 μL, 110000 cpm) was added and the mixture was incubated for a further period of 60 min. A 3% charcoal suspension (1 mL) in 0.2 N HCl was then added and the mixture was centrifuged at 4000g for 25 min at 4 °C. The supernatant liquid (1 mL) was taken and the radioactivity was counted (Beckmann LS 9000) using RIA Solve II (Research Products Int., Mount Prospect, IL). Corrections were made for the radioactivity released after tritiated dUrd addition at zero time. The corrected radioactivity was measured as the percent of radioactivity released under identical conditions without the drug.

A comparison of cytotoxicity (IC) and TS inhibition (ID) for 5-(2-p-toluoylethynyl)uracil (12) is shown in Figure 2. An excellent concordance was seen between the two curves. The slightly more powerful effect on cytotoxicity was then due to the slow rate of TS inactivation, since the ID curve represented the effect at 3 h, whereas the IC values were obtained at 48 h. This agreement between the IC values and ID values for compound 12, indicated that, for this compound, thymidylate synthetase inhibition was probably the primary factor responsible for the cytotoxicity of this compound.

It is evident from thymidylate synthetase inhibition studies that the 5-(2-acylethynyl)uracils, inspite of their bulky C$_5$-substituents, must be converted intracellularly to the corresponding nucleosides and subsequently to the nucleotides, and they were effective inhibitors of thymidylate synthetase like many other uracil nucleotides with bulky C$_5$-substituents.[11,14] The in vitro cell-culture studies together with the in vivo activities of these compounds strongly point out that these 5-substituted uracils themselves should be useful as antitumor agents. However, the syntheses of the 2′-deoxyribonucleosides, 3′-azido-2′,3′-dideoxyribonucleosides, and 2′-fluoro-2′-deoxyarabino-nucleosides and acylic nucleosides of these novel uracils are highly warranted to explore the wide applicability of these compounds as anticancer, antiviral, and anti-AIDS agents. These studies are in progress in our laboratory.

Experimental Section

Melting points were determined on a Reichert (285980) (Austria) melting point bath and are uncorrected. The ultraviolet spectra (UV) were recorded on a Hitachi 200-20 spectrometer in spectrophotometric-grade ethanol (Baker). The infrared (IR) spectra were taken on a Perkin-Elmer 298 instrument on KBr plates. The proton nuclear magnetic resonance spectra (^{1}H NMR) (reported in δ) were recorded on a Varian XL-200 spectrometer
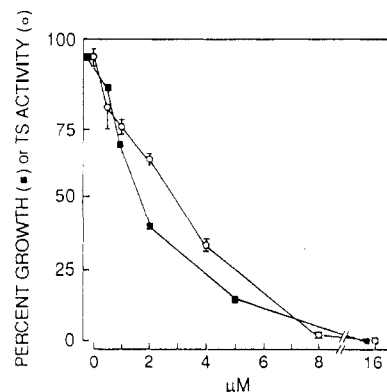

Figure 2. The percent growth (■) or TS activity (○) of L1210/0 cells in the presence of different concentrations of 5-(2-p-toluoylethynyl)uracil (12). The percent growth was determined according to the procedure under Table III after 48 h. TS activity was measured according to the legend under Figure 1 after incubation of the cells with compound 12 for 3 h. IC$_{50}$ = 1.9 μM vs ID$_{50}$ = 2.8 μM.

and a 100-MHz FX-100 spectrometer in solvents as indicated with tetramethylsilane as internal reference. Silica gel TLC was performed on 60 F-254 precoated sheets (E. Merck) and column chromatography was done on silica gel (60–120 mesh). Elemental analyses were performed on Perkin-Elmer Elemental Analyzer 240C and were within ±0.4% of the calculated values.

5-(2-Benzoylethynyl)-2,4-dimethoxypyrimidine (3). To a well-stirred, ice-cold solution of 2,4-dimethoxy-5-[2-(trimethylsilyl)ethynyl]pyrimidine (2) (0.1 g, 0.42 mmol) and benzoyl chloride (0.07 g, 0.53 mmol) in CH$_2$Cl$_2$ (12 mL) was added powdered anhydrous AlCl$_3$ (0.23 g, 1.72 mmol) in portions in 10 min. After the addition was complete, the mixture was stirred under a N$_2$ atmosphere in an ice bath for 5 h and then was gradually warmed to room temperature. The mixture was poured on ice (30 mL) and a hydrochloric acid (1 mL, 12 N) mixture, and the organic layer was separated. The aqueous layer was extracted with CHCl$_3$ (2 × 25 mL). The combined organic extracts were washed with H$_2$O (15 mL), NaHCO$_3$ (saturated, 2 × 15 mL), and then H$_2$O (15 mL) and dried (anhydrous Na$_2$SO$_4$). After removal of the solvent, a crystalline solid [90 mg, 82%, single spot in TLC, R$_f$ = 0.42 (CHCl$_3$–EtOAc = 20:1)] was obtained. It was crystallized from methanol as a yellow, crystalline solid: mp 122–125 °C; IR (KBr) ν_{max} 2200, 1630, 1590 cm^{-1}; UV λ_{max} 322 nm (ϵ 22 902); ^{1}H NMR (200 MHz, CDCl$_3$) δ 4.08 (s, 3 H, OMe), 4.16 (s, 3 H, OMe), 7.58 (t, 2 H, J = 7.0 Hz, Ar-H$_m$), 7.67 (t, 1 H, Ar-H$_p$, J = 7.0 Hz), 8.28 (d, 2 H, Ar-H$_o$, J = 7.0 Hz), and 8.60 (s, 1 H, C$_6$-H). Anal. (C$_{15}$H$_{12}$N$_2$O$_3$) C, H, N.

2,4-Dimethoxy-5-(2-p-toluoylethynyl)pyrimidine (4). It was synthesized from 2,4-dimethoxy-5-[2-(trimethylsilyl)-ethynyl]pyrimidine (2) (0.1 g), p-toluoyl chloride (0.07 g), and anhydrous AlCl$_3$ (0.22 g) in CH$_2$Cl$_2$ (12 mL) according to the procedure for 3. 2,4-Dimethoxy-5-(2-p-toluoylethynyl)pyrimidine (4) was obtained in 84% yield as a light yellow, crystalline solid: crystallized from methanol; mp 140–141 °C; R$_f$ = 0.38 (CHCl$_3$–EtOAc = 20:1); IR (KBr) ν_{max} 2200, 1635, 1595 cm^{-1}; UV λ_{max} 322 nm (ϵ 24675); ^{1}H NMR (100 MHz, CDCl$_3$) δ 2.44 (s, 3 H, Ar-CH$_3$), 4.04 (s, 3 H, OMe), 4.12 (s, 3 H, OMe), 7.32 (d, 2 H, J = 8.0 Hz, Ar-H$_m$), 8.13 (d, 2 H, J = 8.0 Hz, Ar-H$_o$), 8.56 (s, 1 H, C$_6$-H). Anal. (C$_{16}$H$_{14}$N$_2$O$_3$) C, H, N.

2,4-Dimethoxy-5-[2-(p-methoxybenzoyl)ethynyl]pyrimidine (5). This was obtained as a white, crystalline solid (crystallized from methanol) in 73% yield: mp 124–126 °C; IR (KBr) ν_{max} 2200, 1628, 1600 cm^{-1}; UV λ_{max} 330 nm (ϵ 24079); ^{1}H NMR (100 MHz, CDCl$_3$) δ 3.88 (s, 3 H, Ar-OMe), 4.04 (s, 3 H, OMe), 4.10 (s, 3 H, OMe), 6.98 (d, 2 H, J = 8.0 Hz, Ar-H$_m$), 8.20 (d, 2 H, J = 8.0 Hz, Ar-H$_o$), 8.54 (s, 1 H, C$_6$-H). Anal. (C$_{16}$H$_{14}$N$_2$O$_4$) C, H, N.

5-[2-(o-Chlorobenzoyl)ethynyl]-2,4-dimethoxypyrimidine (6) was obtained as a white, crystalline solid (methanol): mp 118–120 °C; IR (KBr) ν_{max} 2200, 1635, 1590 cm^{-1}; UV λ_{max} 320 nm (ϵ 21871); ^{1}H NMR (200 MHz, CDCl$_3$) δ 4.06 (s, 3 H, OMe), 4.10 (s, 3 H, OMe), 7.22–7.34 (m, 3 H, Ar-H$_{m,p}$), 8.17 (d, 1 H, J

= 8.0 Hz, Ar-H$_o$), 8.57 (s, 1 H, C$_6$-H). Anal. (C$_{15}$H$_{11}$N$_2$O$_3$Cl) C, H, N.

5-(2-Benzoylethynyl)uracil (11). A mixture of 5-(2-benzoylethynyl)-2,4-dimethoxypyrimidine (3) (0.25 g, 0.93 mmol), chlorotrimethylsilane (0.37 mL, 2.9 mmol), and sodium iodide (0.43 g, 2.87 mmol) in dry acetonitrile (12 mL) was stirred at room temperature for 24 h under a nitrogen atmosphere. The solvent was removed under reduced pressure and the residue was treated with a few drops of sodium metabisulfite solution, then water, filtered, and dried to yield 5-(2-benzoyl-1-iodovinyl)uracil (7) as a yellow solid (280 mg, 89%): crystallized from methanol; mp 220–222 °C; IR (KBr) ν_{max} 1715, 1665, 1612, 1600 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 7.59–7.78 (m, 3 H, Ar-H$_{m,p}$), 7.98 (d, 2 H, J = 8.0 Hz, Ar-H$_o$), 8.07 and 8.10 (2 s, 1 H, vinylic-H), 8.45 (s, 1 H, C$_6$-H); MS m/z 368 (M, 1.8), 240 (M – HI, 11.7), 105 (C$_7$H$_5$O, 100), 77 (C$_6$H$_5$, 41). To a suspension of 5-(2-benzoyl-1-iodovinyl)uracil (7) (100 mg, 0.26 mmol) in dioxane (3 mL) was added aqueous KOH solution (2 M, 1.15 mL, 2.14 mmol). The suspended material went into solution. It was stirred at room temperature for 7 h and then heated at 90 °C for 20 min. The mixture was cooled and neutralized with HCl (1 M) when a solid separated. It was filtered, dried (30 mg, 46%), and crystallized from hot methanol into a white, powdered solid, 5-(2-benzoylethynyl)uracil (11): mp >290 °C; IR (KBr) ν_{max} 3460, 3340, 2200, 1710, 1690, 1620, 1592 cm^{-1}; UV λ_{max} 328 nm (ϵ 18600); ^{1}H NMR (100 MHz, DMSO-d_6) δ 7.64 (m, 3 H, Ar-H$_{m,p}$), 8.24 (d, 2 H, J = 8.0 Hz, Ar-H$_o$), 8.40 (s, 1 H, C$_6$-H), 11.64 (b s, 2 H, NH). Anal. (C$_{13}$H$_8$N$_2$O$_3$) C, H, N.

5-(2-p-Toluoylethynyl)uracil (12). 2,4-Dimethoxy-5-(2-p-toluoylethynyl)pyrimidine (4) was deblocked with chlorotrimethylsilane and sodium iodide in acetonitrile to yield 5-(1-iodo-2-p-toluoylvinyl)uracil (8): 59%; mp 252–254 °C; IR (KBr) ν_{max} 1735, 1655, 1605 cm^{-1}; ^{1}H NMR δ 2.42 (s, 3 H, Ar-Me), 7.42 (d, 2 H, J = 8.0 Hz, Ar-H$_m$), 7.94 (m, 3 H, Ar-H$_o$ and vinylic-H), 8.32 (s, 1 H, C$_6$-H).

The above (iodovinyl)uracil (8) on treatment with KOH in dioxane as under 11 yielded 5-(2-p-tolylethynyl)uracil (12) in 61% yield as a white, powdered solid: mp >300 °C; IR (KBr) ν_{max} 3450, 2200, 1720, 1665, 1610, 1600 cm^{-1}; UV λ_{max} 326 nm (ϵ 18977); ^{1}H NMR (100 MHz, DMSO-d_6) δ 2.40 (s, 3 H, Ar-CH$_3$), 7.4 (d, 2 H, J = 8.0 Hz, Ar-H$_m$), 8.12 (d, 2 H, J = 8.0 Hz, Ar-H$_o$), 8.32 (s, 1 H, C$_6$-H), 11.6 (b s, 2 H, NH). Anal. (C$_{14}$H$_{10}$N$_2$O$_3$) C, H, N.

5-[2-(p-Methoxybenzoyl)ethynyl]uracil (13). 2,4-Dimethoxy-5-[2-(p-methoxybenzoyl)ethynyl]pyrimidine (5) was converted to 5-[1-iodo-2-(p-methoxybenzoyl)vinyl]uracil (9) with chlorotrimethylsilane and sodium iodide (82% yield). Compound 9 had the following spectroscopic characteristics: IR (KBr) ν_{max} 1710, 1670, 1600 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.86 (s, 3 H, OMe), 7.14 (d, 2 H, J = 8.0 Hz, Ar-H$_m$), 7.96 (d, 2 H, J = 8.0 Hz, Ar-H$_o$), 8.02 and 8.06 (2 s, 1 H, vinylic-H), 8.34 (s, 1 H, C$_6$-H). Compound 9 was converted to 5-[2-(p-methoxybenzoyl)ethynyl]uracil (13) on treatment with KOH in dioxane in 69% yield: crystallized from hot methanol; mp >290 °C; IR (KBr) ν_{max} 3450, 2205, 1710, 1685, 1645, 1610 cm^{-1}; UV λ_{max} 335 nm (ϵ 17700); ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.90 (s, 3 H, OMe), 7.18 (d, 2 H, J = 8.0 Hz, Ar-H$_m$), 8.24 (d, 2 H, J = 8.0 Hz, Ar-H$_o$), 8.42 (s, 1 H, C$_6$-H), 11.66 (b s, 2 H, NH). Anal. (C$_{14}$H$_{10}$N$_2$O$_4$) C, H, N.

5-[2-(o-Chlorobenzoyl)ethynyl]uracil (14). 5-[2-(o-Chlorobenzoyl)-1-iodovinyl]uracil (10) was obtained from 5-[2-(o-chlorobenzoyl)ethynyl]-2,4-dimethoxypyrimidine (6) in the usual way (60% yield): mp 222–225 °C; IR (KBr) ν_{max} 1715, 1665, 1612, 1590 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 7.42–7.70 (m, 4 H, Ar-H$_{m,p}$, vinylic-H), 8.13 (d, 1 H, J = 7.0 Hz, Ar-H$_o$), 8.22 (s, 1 H, C$_6$-H), 11.62 (b s, 1 H, NH), 12.02 (b s, 1 H, NH).

5-[2-(o-Chlorobenzoyl)ethynyl]uracil (14) was obtained from 10 on treatment with KOH in dioxane in 59% yield: crystallized from hot methanol; mp 248 °C; IR (KBr) ν_{max} 3460, 2200, 1725,

1648, 1610, 1590 cm^{-1}; UV λ_{max} 329 nm (ϵ 17362); ^{1}H NMR (100 MHz, DMSO-d_6) δ 7.64 (m, 3 H, Ar-H$_{m,p}$), 8.28 (dd, 1 H, J = 8.0 Hz, J' = 2.0 Hz, Ar-H$_o$), 8.36 (s, 1 H, C$_6$-H), 11.64 (s, 1 H, NH), 11.88 (b s, 1 H, NH). Anal. (C$_{13}$H$_7$N$_2$O$_3$Cl) C, H, N.

Biological Studies. In Vivo Animal Studies. Swiss Albino mice bearing Ehrlich ascites carchinoma cells (EAC) were obtained from the Chittaranjan National Cancer Research Center, Calcutta, India (originally obtained from the Karolinska Institute, Sweden) and maintained in the same strain by serial transplantation. The EAC cells were drawn under aseptic condition from an adult Swiss mouse bearing a 10–20 day old tumor. The tumor cells were diluted and counted under a microscope. Two groups of mice (18–20 g), five in each group, were inoculated intraperitoneally with 0.2 mL of diluted solution containing 1 × 10^6 cells on day 0. The compounds were given by the intraperitoneal route starting on day 1 and continued only upto the 7th day. The test solutions of different doses of compounds were prepared by homogenization in normal saline containing 10% propylene glycol. The control group of mice were injected vehicle only.

The doses for compounds 11 and 12 were optimized and the best T/C values are being reported. The doses reported for compounds 13 and 14 were selected on the basis of guidelines obtained with compounds 11 and 12. The 5-FU dose was selected to conform with the doses selected for compounds 11 to 14 and in general agreed with the values reported in literature.[46] In case of median survival time, the body weight of the animals were recorded on days 0, 5, and 10. The ascites tumors were measured by three parameters—cell count, fluid weight, and median survival time.

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