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Synthesis and biological activities of [*E*]-5-(2-acylvinyl)uracils

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Summary — The synthesis of a number of 5-substituted uracils, *eg* [*E*]-5-(2-acylvinyl)uracils **8a–8g** is described. These compounds were found to have cytotoxic activities against CCRF-CEM human lymphoblastoid cells, HT-29 colon carcinoma cells and L1210/0 mouse leukemia cells. These compounds were also found to inhibit thymidylate synthase.

[*E*]-5-(2-acylvinyl)uracils / anticancer agents / thymidylate synthase inhibitors

Introduction

The importance of 5-substituted derivatives of uracil as anticancer and antiviral (including anti-AIDS) agents is well-established. For example, 5-fluorouracil (5-FU) and the corresponding 2'-deoxyribonucleoside (FUdR) [1] have been of importance in cancer chemotherapy for decades, whereas 5-(trifluoromethyl)- 2'-deoxyuridine (F_3TdR) [2] is an important agent for the treatment of ocular herpes keratitis. [*E*]-5-(2-Bromovinyl)-2'-deoxyuridine (BVDU) [3] has been found to be a potent agent against herpes simplex virus type I (HSV-I). Recently, 3'-azido-3'-deoxythymidine (AZT) [4, 5] has been found to be an effective inhibitor of the human immunodeficiency virus (HIV) and is being used in AIDS patients. A number of other 5-substituted derivatives of uracil [6–15] have also been developed which have been found to be inhibitors of thymidylate synthase (TS) [16–18], an essential enzyme required for cellular multiplication processes.

In view of our interest in the development of inhibitors of TS which could act as anticancer agents, we recently synthesized 5-(acylethynyl) uracils (5-AEUs), a group of novel 5-substituted uracils of structure I [19–20]. These compounds were found to have antitumour properties and act as potent inhibitors of the thymidylate synthase enzyme [21].

I II

In order to find the structure–activity relation among 5-substituted uracils as inhibitors of thymidylate synthase it became desirable to synthesize the vinyl analogues of 5-AEUs, *eg* [*E*]-5-(2-acylvinyl)uracils of structure II. Because of the presence of an active substituent at the C-5 position of the uracil ring these compounds could act, after being con-verted to the corresponding 2'-deoxyribonucleotides, as inhibitors of the TS enzyme for the following reasons: 1) the attack on the thiol group of the cysteine moiety of the TS enzyme at the C-6 carbon atom of the 2'-deoxyribonucleotides derived from II will be facilitated due to the inductive and mesomeric effects of the C-5 substituent; 2) the attack by the thiol group could also take place at the vinylic group at C-5 position; 3) the attack by the thiol group at C-6 of the uracil ring and another nucleophilic group on the enzyme at the vinylic moiety at C-5 position will lead to a very tight enzyme–inhibitor complex; 4) furthermore, hydrophobic interactions between the R group on the C-5 substituent and hydrophobic groups on the

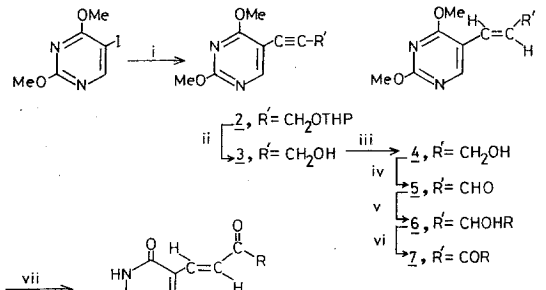
TS enzyme [18] could also strengthen the complex. Such considerations thus led us to the design of compounds of structure II and this paper reports a facile and efficient method for the synthesis of [*E*]-5-(2-acylvinyl)uracils and their biological properties.

Chemistry

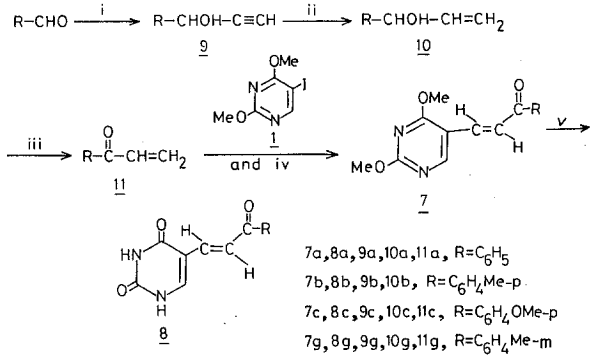
Although the synthesis of 5-vinyluracil and the corresponding nucleosides has been reported [22–25], to our knowledge there is only one report of the synthesis of 5-(2-acylvinyl)uracils [26]. However, their biological properties have not been extensively explored. We have recently reported the synthesis of [*E*]-5-(2-acylvinyl)uracils according to scheme 1, as shown below [27–29].

Starting from 5-iodo-2,4-dimethoxypyrimidine **1**, [*E*]-5-(2-acylvinyl) uracils **8a–8f** were obtained through a 7-step procedure in an overall yield of 12–19% (**8a**, 15%; **8b**, 15%; **8c**, 17%; **8d**, 14%; **8e**, 12% and **8f**, 19%). Due to the multistep nature of scheme 1 and poor overall yields, we were prompted to develop an alternative method for the synthesis of [*E*]-5-(2-acylvinyl)uracils. Palladium-catalysed reactions have been successfully utilised for C–C bond formation in aromatic and heterocyclic systems [30–37]. We have utilised this reaction for the synthesis of [*E*]-5-(2-acylvinyl)uracils according to scheme 2.

The acetylenic alcohols **9** were synthesised from the condensation of acetylene with the appropriate aldehydes in the presence of sodium in liquid ammonia [38] and were obtained in 49–82% yields. These on reduction with lithium aluminium hydride in ether [39] or by hydrogenation (Lindlar catalyst) afforded the corresponding allyl alcohols **10** in excellent yields. The oxidation of the allyl alcohols 10 under mild conditions [40] gave the vinylic ketones (**11**; **11a**, R = C_6H_5; **11b**, R = C_6H_4Me-*p*; **11c**, R = C_6H_4OME-*p* and **11g**, R = C_6H_4Me-*m*). 5-Iodo-2,4-dimethoxypyrimidine **[1]** [41] on treatment with the vinylic ketones **11** in the presence of bis(triphenylphosphine) palladium(II)chloride and cuprous iodide in triethylamine afforded [*E*]-5-(2-acylvinyl)-2,4-dimethoxypyrimidines **7** in modest yields. These were demethylated with chlorotrimethylsilane and sodium iodide in acetonitrile [28, 29] to yield [*E*]-5-(2-acylvinyl)uracils **8**. The vinylic hydrogens in compounds **8** had the *E*-configuration as was evident from their NMR spectra where they were coupled with a J = 16 Hz. The overall yields of the [*E*]-5-(2-acylvinyl) uracils starting from 5-iodo-2,4-dimethoxypyrimidine **1** were in the following range: 27–35%; **8a**, 29%: **8b**, 39%; **8c**, 27%; and **8g**, 35%. Thus it appears that the palladium catalysed reaction for the synthesis of [*E*]-5-(2-acylvinyl)uracils (scheme 2) is much superior to the Grignard procedure (scheme 1) by its simplicity (fewer steps), less time and material consumption, and better overall yields.



Scheme 1. i) Cu^{I}-C≡C-CH_2-OTHP in Py; ii) *p*-TSA, MeOH; iii) LAH in THF; iv) MnO_2 in CH_2Cl_2; v) RMgX in THF; vi) MnO_2 in CH_2Cl_2; vii) Me_3SiCl, NaI in CH_3CN.



Scheme 2. i) CH≡CH, Na in liquid NH_3; ii) LAH, rt or Lindlar's catalyst, H_2; iii) CrO_3, H_2SO_4 in acetone, 0°C; iv) $[Ph_3P]_2PdCl_2$, CuI, Et_3N, reflux, 24 h; v) Me_3SiCl, NaI, CH_3CN.

Biological results

The [*E*]-5-(2-acylvinyl)uracils **8a–8f** were tested against tumour cell lines *in vitro, eg* against CCRF–CEM human lymphoblastoid cells, HT-29 colon carcinoma cells [42] and against L1210/0 mouse leukemia cells. The results are shown in table I.

It can be seen from table I that [*E*]-5-(2-acylvinyl)uracils showed some activity against all 3 cell

Table I. Cytotoxicity of [*E*]-5-(2-acylvinyl)uracils against tumour cells in culture.

Compound	*IC_{50} (μM)*		
	CCRF/CEM	*HT-29*	*L1210/0*
8a	12	23	7.4
8b	26	12	7.4
8c	26	–	–
8d	31	54	–
8e	56	66	–
8f	40	–	–
5-FU	2.0	–	0.3

lines *in vitro*. However, the corresponding acetylenic compounds of structure I were ≈ 3 times more active against L1210/0 cell line and 10–20 times more active against CCRF–CEM cells [21] compared to the vinylic compounds. This could be due to the different stereo-electronic properties of the AEUs compared to the AVUs.

Amongst the 5-AVUs themselves, the nature of the acyl group played some role in determining the biological activities of these compounds. A comparison of the IC_{50}'s against CCRF–CEM cell line showed compound **8a**, where the aromatic ring is unsubstituted, to be the most active. The substitution of hydrogen with an electron-donating group in the aromatic ring (compounds **8b–8d**) reduced the activity by 2–fold. Also the replacement of the aromatic group with an aliphatic group in the acyl moiety (compounds **8e** and **8f**) reduced the activity to a greater extent, indicating the importance of the aromatic group in the acyl moiety.

A comparison of the IC_{50}'s of compounds **8a** and **8b** against L1210/0 mouse leukemia cell line with those of 5-vinyluracil (IC_{50} = 1 x 10^{-4} M), 5-dibromo-vinyl uracil (IC_{50} = 1 x 10^{-4} M) [43] shows the importance of acyl conjugation in potentiating the reactivities of the 5-AVUs.

Thymidylate synthase inhibition studies in CCRF-CEM cells

We have also tested the thymidylate synthase inhibitory activities of [*E*]-5-(2-acylvinyl)uracils in intact CCRF–CEM and L1210/0 cell lines according to the procedure of Kalman and Yalowich [44].

In figure 1, the thymidylate synthase inhibition in CCRF–CEM cells by [*E*]-5-[2-(3',4'-dimethoxybenzoyl)vinyl]uracil **8d** is shown. A plot of TS activity (as measured by tritiated water released) against time of incubation of the cells with the drug at 2 different concentrations of the drug is shown. It should be

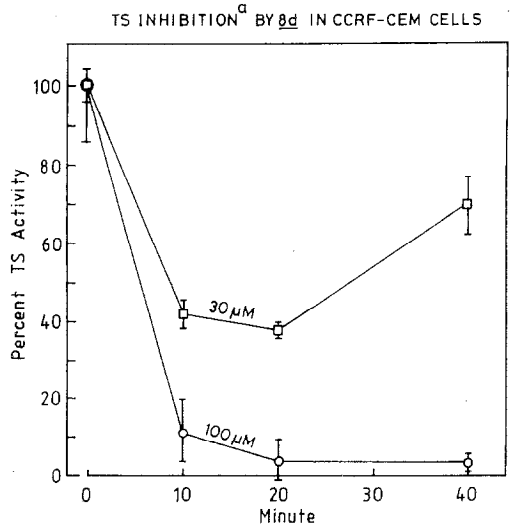


Fig 1. [a]TS inhibition by [^{3}II]II_2O-release assay, [5-^{3}H]dUrd added for 10 min x 37° at the times indicated.

observed that TS inhibition is clearly rapid with this drug. Beyond 20 min incubation of the cells with the drug at 100 μM concentration, the thymidylate synthase enzyme is practically totally inhibited. At 30 μM concentration (IC_{50} of compound **8d**), however, > 50% inhibition of the enzyme was observed beyond 20 min of exposure to the drug. The decrease in inhibition at 40 min for the 30 μmol concentration may just be scatter, but perhaps some mechanism of resistance could also take place.

In figure 2, the percent of thymidylate synthase activity is plotted against the concentration of **8d** used to treat the CCRF–CEM cells. A 30 ≈ min exposure of the cells took place before tritiated dUrd was added. It should be observed that under these conditions, 50% inhibition of TS took place at 30 μM concentration (IC_{50}) of **8d**, whereas at 100 μM concentration > 80% inhibition of TS was observed. It should also be noted that TS inhibition by **8d** was of first order in the drug (log 'pseudo first-order' *vs* lincar drug concentration) at 30 min, but this saturated at ≥100 μM, meaning that we had reached a rate-limiting step of either transport, activation or of binding of the activated nucleoside to TS.

Thymidylate synthase inhibition studies in L1210/0 cells

The thymidylate synthase inhibitory activities of [*E*]-5-(2-acylvinyl)uracils were also tested in L1210/0 mouse leukemia cells. In figure 3A, the percentage activity was plotted against concentration of com-

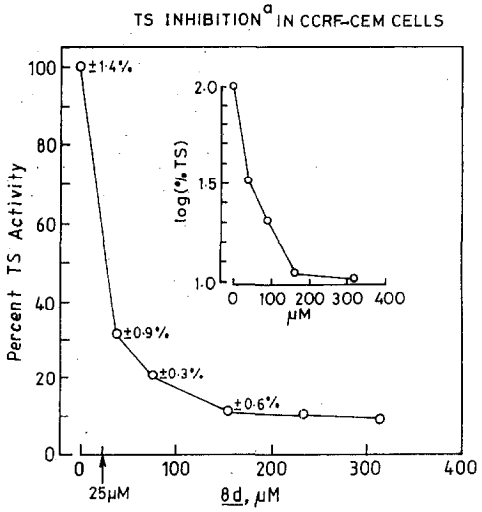


Fig 2. [a]30-Min drug exposure prior to adding [5-^{3}H]dUrd for $3H_2O$-release assay (60 min x 37°).

pound **8a** which was used for incubation of the L1210/0 cells for 2 h. A reference study was also carried out for 5-fluorouracil and plotted in figure 3B. It is to be observed that whereas 50% inhibition of TS activity for compound **8a** is at 140 μM, the corresponding value for 5-FU is ≈ 2.5 μM.

A comparison of the inhibition of TS activity in L1210/0 cells by the other 5-AVUs is shown in table II.

From table II, it is to be noted that electron donating substitution in the aromatic ring of the C-5 side chain decreased the TS inhibitory activity of [*E*]-5-(2-acylvinyl)uracils to a small extent.

A comparison of TS inhibitory activities of [*E*]-5-(2-acylvinyl)uracils with those of 5-(2-acylethynyl)uracils [21] shows the former compounds to be less potent than the latter compounds, which agrees with their comparative cytotoxic properties.

It is to be noted from TS inhibition studies that the [*E*]-5-(2-acylvinyl)uracils (**8a–8f**) like the 5-(2-acylethynyl)uracils, in spite of their bulky C_5-substituents, must be converted intracellularly to the corresponding nucleosides and subsequently to the nucleotides and

Table II. Inhibition of TS activity in L1210/0 cells of [*E*]-5-(2-acylvinyl)uracils at IC_{90}.

Compound	*% Inhibition*
8a	59.0
8b	59.1
8c	53.5
8d	48.2

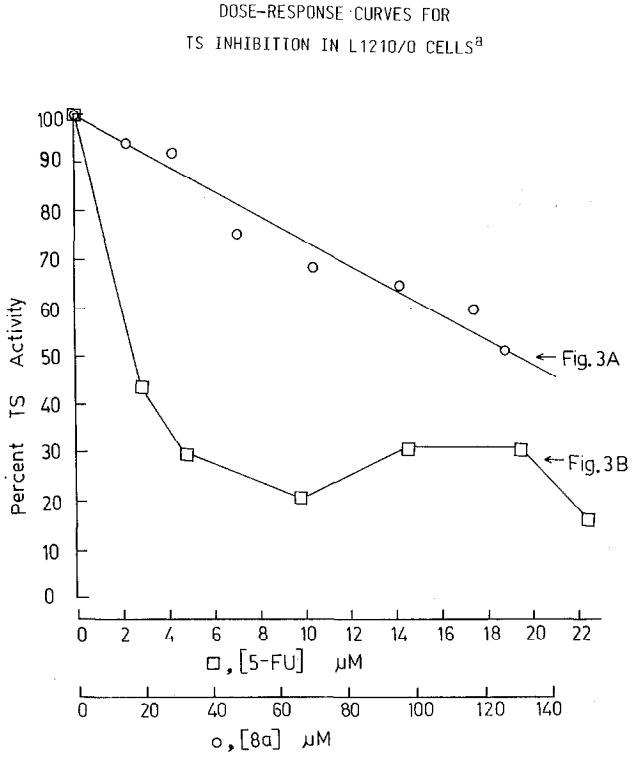


Fig 3A, B. [a]Drugs at IC_{90}; drug x 120 min; ([^{3}H]dUrd x 20 min).

that they are effective inhibitors of TS like many other uracil nucleotides with bulky C-5 substituents [9, 10]. This corroborates our working hypothesis but does not, however, rule out the possibility of other modes of action for the AVUs.

Experimental protocols

Melting points were determined on a Reichert (28590) (Austria) melting point bath and in open capillaries in a sulphuric acid 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, as KBr plates for solids and as thin films (neat) for liquid samples. The proton nuclear magnetic resonance spectra (^{1}H-NMR) (reported in δ) were recorded on a Varian EM-360L 60 MHz spectrometer in solvents as indicated with tetramethyl–silane as internal reference. Silica-gel TLC was performed on 60F-254 precoated sheets (E Merck, Darmstadt) and column chromatography was carried out on neutral alumina or silica gel. Analyses indicated by elemental symbols were within ± 0.4% of the theoretical values and were performed on Perkin–Elmer Elemental Analyzer 240C.

The 5-(2-acylvinyl)uracils **8a, 8b, 8c** and **8g** were synthesised by the palladium-catalysed reaction procedure

(scheme 2) and reported below, whereas 5-(2-acylvinyl)uracils **8d, 8e** and **8f** were synthesised according to the Grignard procedure (scheme 1) which has been previously reported [20, 27–29].

*Acetylenic carbinols **9a, 9b, 9c** and **9g***
These were synthesised according to the literature procedure [38]. Their IR spectra showed the characteristic absorption bands at 3300 cm^{-1} (C≡C-H), 2120 cm^{-1} (w, C≡C). The ^{1}H-NMR spectra (60 MHz, CCl_4, TMS) had the following values:

*1-Phenylprop-2-yn-1-ol **9a.*** δ 2.43 (d, 1H, *J* = 2 Hz, *H*-C≡C-), 3.63 (bs, lH,H-C-O*H*), 5.20 (bs, 1H, *H*-C-OH), 7.20 (m, 5H, Ar-H).

*1-p-Tolylprop-2-yn-1-ol **9b.*** δ 2.23 (s, 3H, Ar-Me), 2.39 (d, 1H, *J* = 2 Hz, *H*-C≡C-), 3.85 (s, 1H, HC-O*H*), 5.17 (d, 1H, 2 Hz, *H*-C-OH), 7.00 (d, 2H, *J* = 8 Hz, Ar-H), 7.27 (d, 2H, *J* = 8 Hz, Ar-H).

*1-p-Anisylprop-2-yn-1-ol **9c.*** δ 2.50 (d, 1H, *J* = 2 Hz, *H*-C≡C-), 3.60 (s, 3H , Ar-OMe), 3.85 (s , 1H, CH-O*H*), 5.16 (d, 1H, *J* = 2 Hz, *H*-C-OH), 6.67 (d, 2H, *J* = 8 Hz, Ar-H), 7.26 (d, 2H, *J* = 8 Hz, Ar-H).

*1-m-Tolylprop-2-yn-1-ol **9g.*** δ 2.26 (s, 3H, Ar-Me), 2.43 (d, 1H, *J* = 2 Hz, *H*-C≡C-), 3.60 (bs, 1H, CHO*H*), 5.17 (d, 1H, *J* = 2 Hz, *H*COH), 7.06 (m, 4H, Ar-H).

*Substituted allyl alcohols **10a, 10b** and **10g***
10a, **10b** and **10g** were obtained by the lithium aluminium hydride reduction of the corresponding acetylenic carbinols. A typical reaction procedure is as follows:

*Synthesis of 1-phenylprop-2-en-1-ol **10a.*** To a cooled (0°C) solution of **9a** (**1g**, 7.57 mmol) in dry ether, lithium aluminium hydride (350 mg, 9.22 mmol) was added portionwise. After the addition was complete, the mixture was further stirred at room temperature for 18 h and then decomposed with saturated Na_2SO_4 solution. It was extracted with ether (3 x 50 ml) and the ether layer was washed with water (3 x 50 ml) and dried (anhydrous Na_2SO_4). After the removal of solvent and sublimation (oven temp 72°C at 1 mm Hg), a viscous liquid (910 mg, 89%) was obtained. ^{1}H-NMR (60 MHz, CCl_4) δ 4.07 (bs, lH, CHO*H*), 4.73–5.33 (m, 3H, C*H*$_2$=CH-C*H*OH), 5.56–6.16 (m, lH, CH_2 = C*H*-CHOH), 6.96–6.33 (m, 5H, Ar-H).

*1-p-Tolylprop-2-en-1-ol **10b.*** Yield 91%, liquid, sublimated at 85°C at 1 mm Hg. ^{1}H-NMR (60 MHz, CCl_4) δ 2.26 (s, 3H, Ar-Me), 3.92 (bs, 1H, CHO*H*), 4.73–5.33 (m, 3H, C*H*$_2$ = CH–C*H*OH), 5.56-6.20 (m, 1H, CH_2 = C*H*-CHOH), 6.82–7.26 (m, 4H, Ar-H).

*1-m-Tolylprop-2-en-1-ol **10g.*** Yield 90%, liquid, sublimated at 80°C under 1 mm Hg. ^{1}H-NMR (60 MHz, CCl_4) δ 2.26 (s, 3H, Ar-Me), 4.36 (bs, 1H, CHO*H*), 4.82–5.40 (m, 3*H*, C*H*$_2$ = CH-C*H*OH), 5.63–6.23 (m, 1H, CH_2 = C*H*-CHOH), 6.86–7.23 (m, 4H, Ar-H).

*1-p-Anisylprop-2-en-1-ol **10c.*** (1-*p*-Anisylprop-2-yn-1-ol **9c,** 500 mg, 3.09 mmol) was hydrogenated in alcohol (20 ml) in the presence of Lindlar catalyst (5% palladium on calcium carbonate, poisoned with lead) (30 mg) by stirring under hydrogen atmosphere at ordinary pressure and temperature for 36 h. After the usual work-up, a liquid was obtained which was purified by sublimation at 118° under 1.5 mm Hg (400 mg, 79%). ^{1}H-NMR (60 MHz, CCl_4) δ 3.73 (s, 3H, Ar-OMe), 4.86–5.36 (m, 3H, C*H*$_2$ = CH–C*H*OH), 5.56–6.20 (m, 1H, CH_2 = C*H*-CHOH), 6.74 (d, 2H, *J* = 8 Hz, Ar-H), 7.10 (d, 2H, *J* = 8 Hz, Ar-H).

*3-Aryl-3-oxo-prop-1-enes **11a, 11b, 11c, 11g.** A typical procedure*
*3-oxo-3-phenylprop-1-ene **11a.*** The allyl alcohol (**10a**, 1 g, 7.46 mmol) was dissolved in acetone, cooled to 0°C and then Jones' reagent (CrO_3 in sulphuric acid-water) was added to it until the colour of the Jones' reagent persisted. It was further stirred for 30 min, then the mixture was poured into water and extracted vigorously with ether (3 x 50 ml). The organic phase was washed with water (3 x 50 ml), $NaHCO_3$ solution (10%), then with water (3 x 50 ml) again and dried (anhydrous sodium sulphate). After removal of solvent, a residue was obtained which was purified by rapid chromatography on a column of alumina (neutral), the eluting solvent being 5% ethyl acetate in petroleum ether (60–80°C fraction) (yield 790 mg, 80%), bp: 80°C/2 mm Hg. IR ν_{max} 1660 cm^{-1} (conjugated C = O). ^{1}H-NMR (60 MHz, CCl_4) δ 4.73 (dd, lH, *J* = 10 Hz, 2 Hz, vinylic-H, cis) 6.26 (dd, 1H, *J* = 16 Hz, 2 Hz, vinylic, *trans*), 6.79–8.03 (m, 6H, Ar-H and one vinylic-H).

*3-Oxo-3-p-tolylprop-1-ene **11b.*** An oil (yield 86%), bp: 45°C/0.15 mm Hg. IR ν_{max} 1665 cm^{-1}; ^{1}H-NMR (60 MHz, CCl_4) δ 2.40 (s, 3H, Ar-Me), 5.76 (dd, lH, *J* = 10 Hz, 2 Hz, vinylic-H, *cis*), 6.30 (dd, 1H, *J* = 16 Hz, 2 Hz, vinylic-H, *trans*), 6.89–7.89 (m, 5H, Ar-H and one vinylic-H).

*3-p-Anisyl-3-oxo-prop-1-ene **11c.*** An oil (yield 79%), bp: 70°C/0.15 mm Hg. IR ν_{max} 1660 cm^{-1}; ^{1}H-NMR (60 MHz, CCl_4) δ 3.82 (s, 3H, Ar-OMe), 5.79 (dd, 1H, *J* = 10 Hz, 2 Hz, vinylic-H, *cis*), 6.33 (dd, lH, *J* = 16 Hz, 2 Hz, vinylic-H, *trans*), 6.76–8.03 (m, 5H, Ar-H and one vinylic-H).

*3-Oxo-3-m-tolylprop-1-ene **11g.*** An oil (yield 83%), bp: 40°C/0.15 mm Hg. IR ν_{max}1660 cm^{-1}; ^{1}H-NMR (60 MHz, CCl_4) δ 2.33 (s, 3H, Ar-Me), 5.73 (dd, 1H, *J* = 10 Hz, 2 Hz, vinylic-H, *cis*), 6.26 (dd, 1H, *J* = 16 Hz, 2Hz, vinylic-H, *trans*), 6.83-7.76 (m, 5H, Ar-H and one vinylic-H).

*Synthesis of [E]-5-(2-acylvinyl)-2,4-dimethoxypyrimidines **7a, 7b, 7c** and **7g**. A general procedure*
*[E]-5-(2-Benzoylvinyl)-2,4-dimethoxypyrimidine **7a.*** A mixture of 5-iodo-2,4-dimethoxypyrimidine (**1**, 250 mg, 0.93 mmol), bis(triphenylphosphine) palladium(II)chloride (10 mg, 0.014 mmol) and cuprous iodide (10 mg, 0.052 mmol) in dry triethylamine (15 ml) was stirred under nitrogen atmosphere for 15 min. 3-Oxo-3-phenylprop-1-ene (**11a,** 250 mg, 1.9 mmol) was then added to this. The mixture was then further stirred at room temperature for 15 min and refluxed for 24 h. Triethylamine was then removed under reduced pressure. The residue was treated with water (50 ml) and then extracted with chloroform (3 x 50 ml). The combined chloroform extracts were washed with water (3 x 50 ml), dried (anhydrous Na_2SO_4) and solvent was removed to yield a gum. This was purified by chromatography on a column of silica gel (60–120 mesh). The desired compound was eluted with chloroform (75 mg, 29%), mp: 134–136°C (lit [28, 29] mp. 134°C), identical to an authentic sample from IR, UV and ^{1}H-NMR comparison.

Compounds **7b** and **7c** were obtained by the above procedure in 39% and 27% yields respectively. They were identical to authentic samples [28, 29] from IR, UV and ^{1}H-NMR comparisons.

[E]-2,4-Dimethoxy-5-(2-m-toluoylvinyl)pyrimidine **7g.** This was obtained in 35% yield by following the general procedure as described under **7a**; mp: 136°C.IR ν_{max} 1660(s), 1600 cm^{-1}; UV (ethanol) λ_{max} 322 nm (ε 26, 475); ^{1}H-NMR, δ 2.43 (s, 3H, Ar-Me), 4.03 and 4.13 (2s, 6H, OMe), 7.30–7.40 (m, 2H, Ar-H), 7.66 (s, 2H, vinylic-H), 7.79 (m, 2H, Ar-H), 8.56 (s, 1H, C_6-H). Anal $C_{16}H_{16}N_2O_3$ (C, H, N).

Synthesis of [E]-5-(2-acylvinyl)uracils ***8a, 8b, 8c, 8g.*** *A typical procedure*

[E]-5-(2-m-Toluoyl vinyl)uracil ***8g.*** A mixture of [*E*]-2,4-dimethoxy-5-(2-*m*-toluoylvinyl)pyrimidine (**7g,** 100 mg, 0.35 mmol), anhydrous sodium iodide (130 mg, 0.87 mmol) and chlorotrimethylsilane (0.1 ml, 0. 87 mmol) in dry acetonitrile (4 ml) was stirred under nitrogen atmosphere for 24 h. The solvent was then removed under reduced pressure. The residue was treated with cold water (5 ml), filtered, washed with sodium metabisulfite solution (10%), again with water, filtered and dried. This was then crystallized from methanol/water (4:1) into a cream-coloured solid (63 mg, 70%), mp: > 300°C. IR ν_{max} 3160, 1710, 1680, 1590 cm^{-1}; UV (ethanol) λ_{max} 328, 270 nm; ^{1}H-NMR (DMSO–d_6, 60 MHz), δ 2.40 (s, 3H, Ar-Me), 7.26–8.20 (m, 7H, Ar-H, vinylic-H and C_6-H).

Cytotoxicity studies of [E]-5-(2-acylvinyl)uracils ***8a–8f*** *against CCRF-CEM and L1210/0 cells*

Human lymphoblastoid CCRF-CEM cells were seeded at (4.0–5.0) x 10^4 cells/ml in duplicate for each drug concentration in borosilicate test tubes containing Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% dialysed fetal calf serum, 16 mM HEPES and 8 mM MOPS buffer, pH 7.2. The test compounds dissolved in Me_2SO were added to the cell cultures, with rapid mixing with the use of a 1:200 dilution to obtain desired drug concentrations (final Me_2SO concentration 0.5%). Each compound was tested at 7 or more different concentrations. Incubation of cells with the compounds was performed at 37°C for 48 h. L1210/0 cells were obtained from D Griswold of the Southern Research Institute in Birmingham, Alabama.

Cytotoxicity studies of [E]-5-(2-acylvinyl)uracils against HT-29 colorectal carcinoma cells

HT-29 colorectal carcinoma cells were cultured and tested according to the procedure of Link *et al* [42]. The HT-29 cells grew attached and were trypsinised for cell counting at 48 h of continuous drug exposure.

Typical experimental procedure for thymidylate synthase inhibition studies

L1210/0 cells in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% dialysed fetal calf serum, 0.5 ml, 5 x 10^5 cells/ml were incubated with the drug (in different concentrations in 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, 110 000 cpm) was added and the mixture was further incubated for a period of 60 min. A 3% charcoal suspension (1 ml) in O.2 N HC1 was then added and the mixture was centrifuged at 4 000 *g* 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, USA). 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 (see fig 1). The measurements were made in triplicate with 5-FU in parallel as a positive control. The error bars thus indicate the experimental SDs.

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