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UNUSUAL CYTOTOXICITIES OF 5-(ACYLETHYNYL)-1-(2-HYDROXYETHOXY)METHYLURACILS[1]

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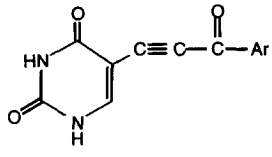
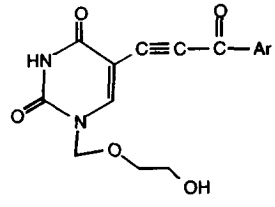
Abstract 5-(Acylethynyl)-1-(2-hydroxyethoxy)methyl uracils (**2**)-(**6**) were synthesised in excellent yields from 1-(2-acetoxyethoxy)methyl-5-iodouracil (**7**) utilising palladium-copper catalysed reactions. Compounds (**2**)-(**5**) were shown to be highly active against CCRF-CEM (IC_{50} 0.6-1.6 μM) and L1210/0 cells (IC_{50} 0.7-2.2 μM) in culture, and thus exhibit greater activity than their parent bases.

Chemistry

5-Substituted uracils and their nucleosides are important compounds in cancer chemotherapy.[2] Thus, we became interested in novel 5-substituted uracils as possible anticancer and antiviral agents.[1,3,4] We recently developed a number of novel 5-substituted uracils (**1a-d**) which displayed promising cytotoxic activity against CCRF-CEM human lymphoblastoid cells (IC_{50} 2.0-2.3 μM) and L1210 mouse leukemia cells (IC_{50} 0.65-2.0 μM) in culture. These compounds were also shown to be inhibitors of thymidylate synthase enzyme.[3] In an attempt to improve the biological activities of this series (**1a-d**), we recently synthesised the corresponding 2'-deoxyribonucleoside analogues.[5] However, to our surprise we found these compounds to be less active against CCRF-CEM cells in culture.[6] This was in contrast to many other 2'-deoxyribonucleosides (FUdR *vs.* FU) .[2]





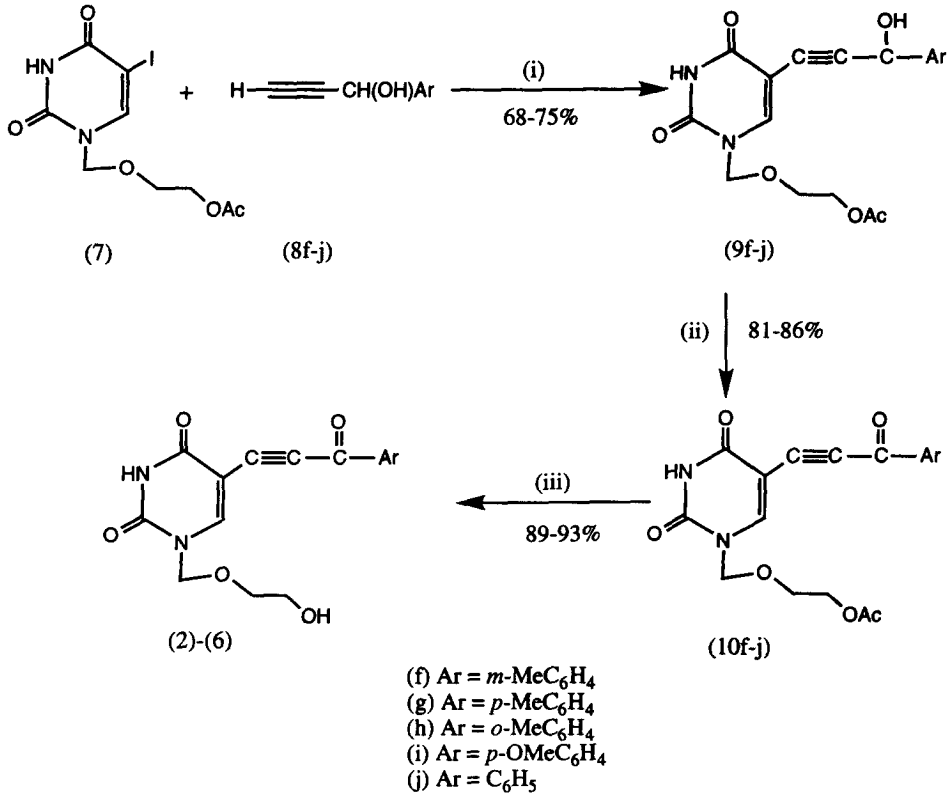
(1a) Ar = C_6H_5
(1b) Ar = *p*-$OMeC_6H_4$
(1c) Ar = *p*-MeC_6H_4
(1d) Ar = *o*-ClC_6H_4

(2) Ar = *m*-MeC_6H_4
(3) Ar = *p*-MeC_6H_4
(4) Ar = *o*-MeC_6H_4
(5) Ar = *p*-$OMeC_6H_4$
(6) Ar = C_6H_5

The attachment of a 2-hydroxyethoxymethyl group to purine and pyrimidine bases has given rise to compounds of notable biological significance, *e.g.* acyclovir and HEPT.[7-13] Thus, we synthesised the series of acyclonucleosides (**2**)-(**6**) possessing a hydroxyethoxymethyl group on the N1-atom. Compounds (**2**)-(**6**) could also be considered as substituted acetylenic ketones and could act as Michael acceptors. The biological activity of several acetylenic ketones has been reported.[3,5,14].

In this letter we describe the synthesis of compounds (**2**)-(**6**) as shown in the Scheme below and their unusual cytotoxicities as given in the Table.

Scheme



(f) Ar = *m*-MeC_6H_4
(g) Ar = *p*-MeC_6H_4
(h) Ar = *o*-MeC_6H_4
(i) Ar = *p*-$OMeC_6H_4$
(j) Ar = C_6H_5

(i) $(Ph_3P)_2PdCl_2$, CuI, DMF, Et_3N, 55°C, 6h; (ii) PCC, CH_2Cl_2, r.t., 3h;
(iii) NaOMe, MeOH, r.t., 4h.

The facile synthesis of 1-(2-acetoxyethoxy)methyl-5-iodouracil (**7**)[15,16] was carried out according to the glycosylation procedure of Vorbrüggen.[17] Compound (**7**) was heated in DMF at 55°C with the appropriately substituted 1-arylprop-2-yn-1-ols[18] (**8f-j**) in the presence of bis(triphenylphosphine)palladium(II) chloride (6 mol%) and cuprous iodide (9-11 mol%), with triethylamine (2.5-2.8 equivalents) as the base. Under these conditions, the 1-(2-acetoxyethoxy)methyl-5-(3-aryl-3-hydroxyprop-1-ynyl)uracils (**9f-j**) were obtained in good yields (68-75%). However, due to their instability, these compounds could not be fully characterised, but were oxidised directly with pyridinium chlorochromate in dichloromethane at room temperature to yield the 1-(2-acetoxyethoxy)methyl-5-(acylethynyl)uracils (**10f-j**) in excellent yields (81-86%).[19] Following deacylation with sodium methoxide

in methanol at room temperature, the 5-(acylethynyl)-1-(2-hydroxyethoxy)methyluracils series (**2**)-(**6**) were obtained in excellent yields (89-93%). The compounds (**2**)-(**6**) were fully characterised by IR, UV and ^{1}H NMR spectroscopy.[20]

Biological Results

The compounds (**2**)-(**5**) were tested[21] for their biological activities against CCRF-CEM, L1210/0 and HT-29 colon carcinoma cell lines *in vitro*. The results of the tests are shown in the Table. Unfortunately compound (**6**) could not be tested because of a solubility problem.

Table

Cytotoxicitity results of compounds (2)-(5) against tumor cells in culture[a]

		IC_{50} (μM)		
Entry	**Compound**	**CCRF-CEM**	**L1210/0**	**HT-29**
1	2	0.6	0.7	14.0
2	3	0.7	0.9	8.0
3	4	0.9	2.2	25.0
4	5	1.6	1.5	20.0
5[b]	1b	2.3	2.0	-----
6[b]	1c	2.25	1.9	-----
7[b]	5-FU	2.0	0.3	-----

[a] Incubation of the cells with the compounds was carried out at 37° C for 48 h with varying concentrations of the compounds. The compounds were added in solutions of DMSO (final DMSO concentration, 0.5%)[21] The L1210/0 cells were obtained from Dan Griswold of the Southern Research Institute in Birmingham, AL. [b] Data from ref. 3.

All the acyclonucleosides were found to be highly active against CCRF-CEM and L1210/0 cells in culture, displaying increased potency compared to the corresponding free bases. They were also more potent than 5-fluorouracil in the CCRF-CEM trial. For example, 1-(2-hydroxyethoxy)methyl-5-(*p*-toluoylethynyl)uracil (**3**) was found to be three times more effective against CCRF-CEM cells and twice as effective against L1210/0 cells than its corresponding free base 5-(*p*-toluoylethynl)uracil (**1c**) (Entry 2 *vs.* 6). Similarly, 1-(2-hydroxyethoxy)methyl-5-(*p*-methoxybenzoylethynyl)uracil (**5**) was more potent than its corresponding base 5-(*p*-methoxybenzoylethynyl)uracil (**1b**). These results were in contrast to those of a similar nature, 2-hydroxyethoxymethyl substitution of the N1 position of 5-fluorouracil [22,23] and that of other 5-substituted uracils[24,25] lead to reduced biological activities. Thus, 1-(2-hydroxyethoxy)methyl-5-fluoro uracil had an IC_{50} value of 1.7×10^{-5} M and greater when tested against L1210/0 cells in culture; this

was in comparison to an IC_{50} value of 1.0-2.0 μM for 5-FU.[3,22] However, the acyclonucleosides (**2**)-(**5**) were only moderately active against the HT-29 cell line.

The nature of the aryl substituents appeared to play a significant role in determining the biological activity of the compounds (**2**)-(**5**). Thus, the *m*-methyl compound (**2**) exhibited the greatest biological activity, whereas the *o*-methyl compound (**4**) was not as active in the tests (see Entries 1 and 3). A *p*-methoxy substituent as in compound (**5**) appeared to reduce the activity even further. These results seem to indicate that steric hinderance and the electron donating capacity of the substituents on the aryl function are a factor in controlling cytotoxicity.

Conclusion

The substitution of the N1-position in 5-(acylethynyl)uracils with a 2-hydroxyethoxymethyl group conferred increased cytotoxicity to the resulting compounds against CCRF-CEM and L1210/0 cells in culture. This was an unprecedented observation, and was in contrast to the results obtained so far with other 5-substituted uracils. The mode of action of these interesting compounds and their activities against other cell lines including normal mammalian cell lines, are presently under active study.

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19. **Experimental synthesis of 1-(2-acetoxyethoxy)methyl-5-(acylethynyl)uracils (10f-j). A typical procedure. 1-(2-Acetoxyethoxy)methyl-5-(*p*-methoxybenzoylethynyl)uracil (10i)** : to a mixture of 1-(2-acetoxyethoxy)methyl-5-iodouracil (**7**, 500 mg, 1.41 mmol), bis(triphenylphosphine)palladium (II) chloride (60 mg, 0.085 mmol), copper(I) iodide (30 mg, 0.158 mmol) (well stirred at room temperature for 15 min.) under a nitrogen atmosphere, 1-(*p*-methoxyphenyl)prop-2-yn-1-ol (320 mg, 1.97 mmol) was added, followed by triethylamine (400 mg, 3.95 mmol). The reaction mixture was stirred and heated at 55° C for 6 h until TLC indicated that the reaction was complete. After removal of solvent under reduced pressure, the residue was treated with H_2O (20 ml) and extracted with $CHCl_3$ (3 x 50 ml). The $CHCl_3$ extracts were combined, washed with 10 % aqueous solution of sodium EDTA (3 x 50 ml), H_2O (3 x 50 ml) and dried (anhydrous sodium sulfate). After removal of solvent, a residue was obtained as a gum which was purified by chromatography on a column of silica gel (60-120 mesh) with chloroform-acetone (2 : 1) as eluent to yield 1-(2-acetoxyethoxy)methyl-5-(3-hydroxy-3-*p*-methoxyphenyl prop-1-ynyl)uracil **9i** as a light brown foam which turned into a gum.

Compound **9i** (250 mg, 0.64 mmol) was oxidized using pyridinium chlorochromate (PCC, 560 mg, 2.60 mmol) in dichloromethane (20 ml) by stirring at room temperature for 3 h. The mixture was filtered through a bed of Celite® and the filtrate was washed with H_2O and dried. The residue was purified by chromatography on silica gel using 15 % acetone in chloroform as eluent to yield 1-(2-acetoxyethoxy)-methyl-5-(*p*-methoxybenzoylethynyl)uracil **10i** (200 mg, 81.2 %; crystallized from benzene, m.p. 174° C; IR (KBr) : υ_{max} 2200 (C≡C), 1725, 1675, 1620, 1590 cm^{-1}; UV (EtOH) : λ_{max} 335.8 nm; ^1H NMR (DMSO-d_6, 100 MHz) : δ 2.00 (s, 3H, CH_3COO), 3.68-3.86 (m, A_2B_2, 2H, $AcOCH_2CH_2$), 3.90 (s, 3H, $ArOCH_3$), 4.04-4.28 (m, A_2B_2, 2H, $AcOCH_2CH_2$), 5.20 (s, 2H, OCH_2-uracil), 7.16 (d, 2H, J = 8 Hz, ArH_m), 8.20 (d, 2H, J = 8 Hz, ArH_0), 8.74 (s, 1H, C-6 H), 12.00 (bs, 1H, N-3 H); anal. calcd. for $C_{19}H_{18}N_2O_7$: C, 59.06; H, 4.70; N, 7.25 found : C, 59.22; H, 4.69; N, 7.01.

20. **Experimental synthesis of 5-(acylethynyl)-1-(2-hydroxyethoxy)methyluracils (2-6). A typical procedure. 1-(2-Hydroxyethoxy)methyl-5-(*p*-methoxybenzoyl ethynyl)-uracil (5).** 1-(2-Acetoxyethoxy)methyl-5-(*p*-methoxybenzoylethynyl)uracil (**10i**, 120 mg, 0.31 mmol) was stirred with sodium methoxide solution [8.60 ml, containing sodium (8.60 mg, 0.37 mmol)] under nitrogen atmosphere for 4 h and then neutralized by addition of Dowex 50-X8 (H^+) resin. The mixture was filtered and the resin was washed with methanol (3 x 10 ml). The filtrates were combined and the solvent was removed under reduced pressure, afforded a white solid (100 mg, 93 %); recrystallized from ethanol, m.p. 186° C; IR (KBr) : υ_{max} 2200 (C≡C), 1720, 1680, 1630, 1610, 1590 cm^{-1}; UV (EtOH) : λ_{max} 332.0 nm, 233.0 nm; ^1H NMR (DMSO-d_6, 100 MHz) : δ 3.56 (s, 4H, OCH_2 CH_2O), 3.88 (s, 3H, $ArOCH_3$), 4.68 (bs, 1H, OH), 5.20 (s, 2H, OCH_2-uracil), 7.16 (d, 2H, J = 8 Hz, ArH_m), 8.20 (d, 2H, J = 8 Hz, ArH_0), 8.72 (s, 1H, C-6 H), 11.96 (bs, 1H, N-3 H); anal. calcd. for $C_{17}H_{16}N_2O_6$: C, 59.30; H, 4.68; N, 8.14 found : C, 59.68; H, 4.51; N, 8.15.

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