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.
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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]
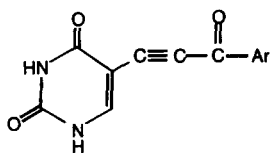
(1a) Ar = C$_6$H$_5$
(1b) Ar = *p*-OMeC$_6$H$_4$
(1c) Ar = *p*-MeC$_6$H$_4$
(1d) Ar = *o*-ClC$_6$H$_4$
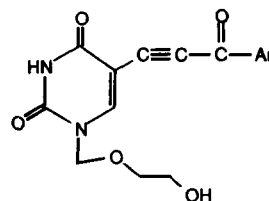
(2) Ar = *m*-MeC$_6$H$_4$
(3) Ar = *p*-MeC$_6$H$_4$
(4) Ar = *o*-MeC$_6$H$_4$
(5) Ar = *p*-OMeC$_6$H$_4$
(6) Ar = C$_6$H$_5$

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$_6$H$_4$
(g) Ar = p-MeC$_6$H$_4$
(h) Ar = o-MeC$_6$H$_4$
(i) Ar = p-OMeC$_6$H$_4$
(j) Ar = C$_6$H$_5$
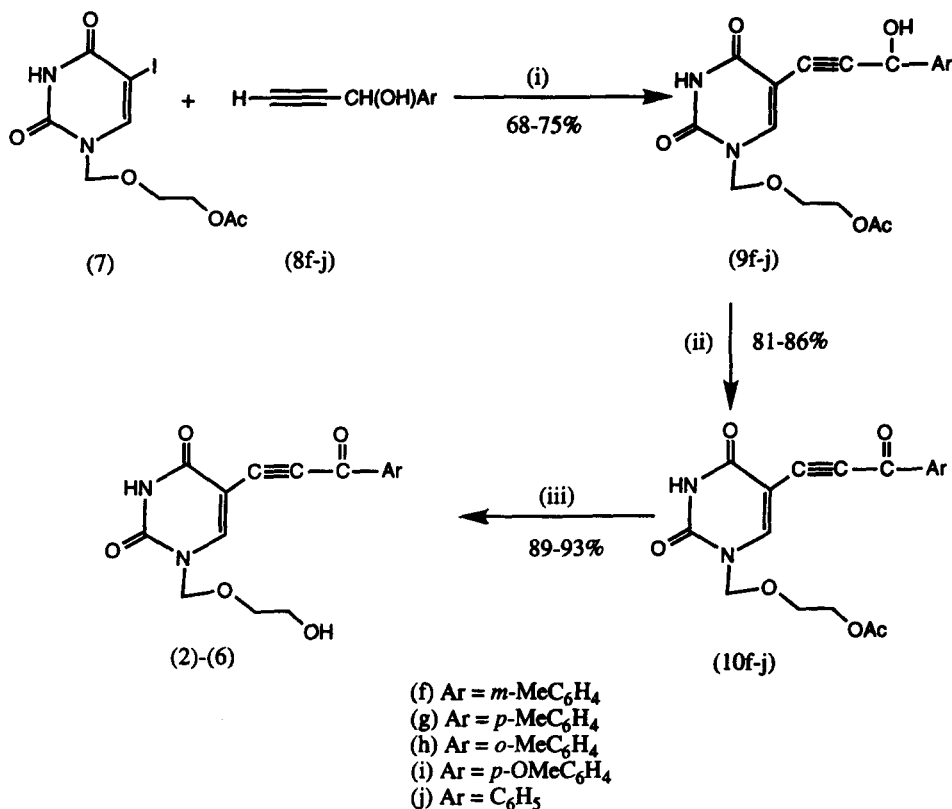
(i) (Ph$_3$P)$_2$PdCl$_2$, CuI, DMF, Et$_3$N, 55°C, 6h; (ii) PCC, CH$_2$Cl$_2$, r.t., 3h; (iii) NaOMe, MeOH, r.t., 4h.

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]

Entry	Compound	IC$_{50}$ (μM)		
		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.

Acknowledgements

References and Notes

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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; ^{1}H NMR (DMSO-d$_6$, 100 MHz) : δ
    3.56 (s, 4H, OCH$_2$ CH$_2$O), 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$_2$O$_6$: C, 59.30; H, 4.68; N, 8.14 found : C, 59.68; H, 4.51; N,
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