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QUARTERLY

Acyclic analogues of 5-fluoro-dUMP and 5-fluoro-2'-deoxyuridine: Synthesis and inhibition of thymidylate synthase and tumour cell growth*°

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1-[(2-Hydroxyethoxy)methyl]-5-fluorouracil (HEMFU) and 1-[(1,3-dihydroxy-2-propoxy)methyl]-5-fluorouracil (DHPFU) were prepared by alkylation of the di-O-TMS derivative of 5-fluorouracil and phosphorylated with the use of the wheat shoot phosphotransferase system to their monophosphates, HEMFUMP and DHPFUMP. 1-(2-Phosphonylmethoxyethyl)-5-fluorouracil (PMEFU) was obtained by condensation of diethyl-2-chloroethoxymethanephosphonate with 5-fluorouracil and cleavage of the alkylphosphoester with trimethylbromosilane.

Inhibition of highly purified thymidylate synthase from mouse tumour Ehrlich carcinoma and leukemia L1210 cells by each of the nucleotide analogues, DHPFUMP, PMEFU and HEMFUMP, and of L5178Y mouse leukemia cell growth by the nucleoside (HEMFU) analogue, were studied.

DHPFUMP proved to be the strongest inhibitor, non-competitive *vs* dUMP, with K_i^{app} 2.8 μM for time-independent interaction with the enzyme and N^5,N^{10}-methylenetetrahydrofolate (CH_2H_4PteGlu). In the presence of CH_2H_4PteGlu, DHPFUMP exhibited time-dependent inactivation of the enzyme, the inactivation rate plots being biphasic and pointing to K_i values in the μM range (10^3-fold higher than for 5-fluoro-

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Abbreviations: b.i.d., bis in die; CEMP, 2-chloroethoxymethylene diethylphosphonate; CH_2H_4PteGlu, N^5,N^{10}-methylenetetrahydrofolate; DHPFU, 1-[(1,3-dihydroxy-2-propoxy)methyl]-5-fluorouracil; di-O-TMS, 2,4-di-*O*-trimethylsilyl; FUra, 5-fluorouracil; HEMFU, 1-[(2-hydroxyethoxy)methyl]-5-fluorouracil; HMDS, hexamethyldisilazane; PMEMeU, 1-(2-phosphonylmethoxyethyl)-5-methoxyuracil; PMEU, 1-(2-phosphonylmethoxyethyl)uracil; q.d. quod die; TS, thymidylate synthase.

dUMP). HEMFUMP and PMEFU were much weaker inhibitors of the enzyme, with K_i^{app} values of 0.26 mM (non-competitive *vs* dUMP) and 30 mM (non-competitive *vs* dUMP), respectively. HEMFU, despite the weak interaction of its nucleotide analogue with the enzyme, proved to be a strong cell (L5178Y) growth inhibitor, with IC_{50} in the range 10^{-5} M.

Adenine, guanine and cytosine acyclic nucleosides, nucleotides, and their phosphonates, potent antiviral agents, have been intensively investigated [1] but little is known about the biological properties of uracil and especially 5-fluorouracil analogues [2–4]. The latter compounds could be inhibitors of thymidylate synthase (TS), a key enzyme in antitumour therapy, and potential antitumour agents.

One of these, 1-[(2-hydroxyethoxy)methyl]-5-fluorouracil (HEMFU, Fig. 1), was reported to be cytotoxic (ID_{50} 1.7×10^{-5} M, as compared with 1×10^{-6} M for 5-fluorouracil), against L1210 mouse leukemia cells in culture, and effective in *in vivo* P388 mouse leukemia treatment: at 400 mg/kg (b.i.d. × 4) or 240 mg/kg (q.d. 1–9), increasing survival up to 75% with no evidence of host toxicity [4]. However another publication [3] reported lack of activity against Detroit 98 and mouse L cells in culture up to 10^{-4} M. To clarify these discrepancies we decided to synthesize HEMFU and some other new acyclo derivatives of 5-fluorouracil (FUra) and investigate their biological activities.

MATERIALS AND METHODS

Chemistry

General methods. Melting points (uncorrected) were measured on a Boetius micro-

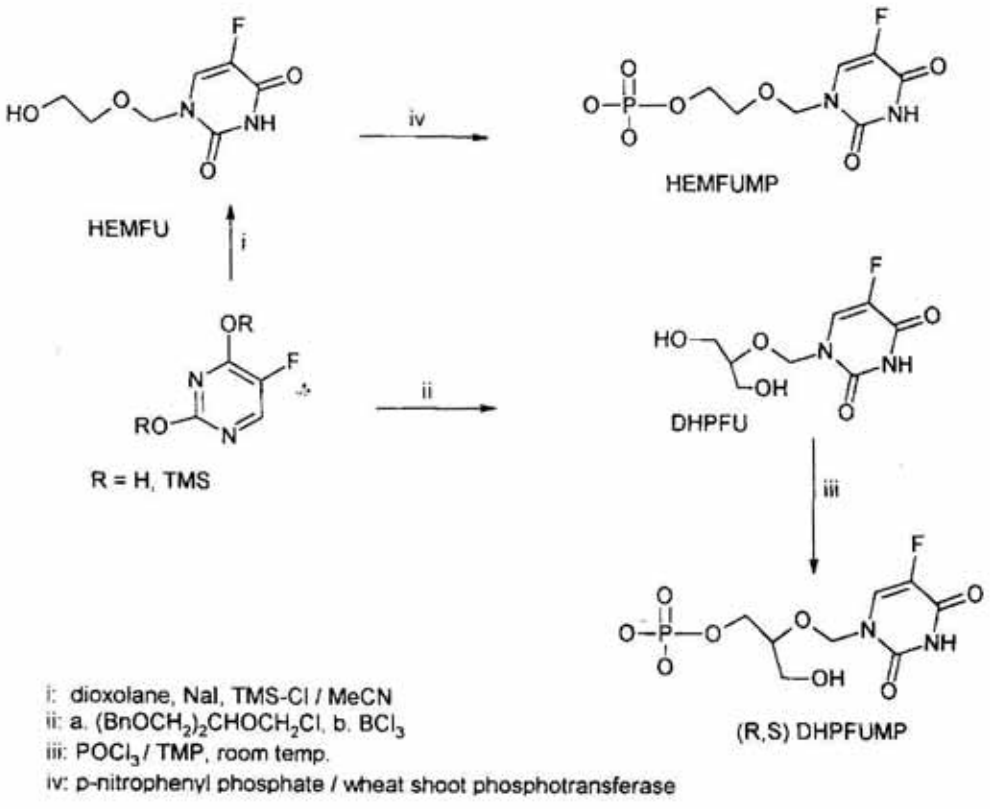


Figure 1. Synthesis of 1-[(2-hydroxyethoxy)methyl]-5-fluorouracil (HEMFU) and 1-[(1,3-dihydroxy-2-propoxy)methyl]-5-fluorouracil (DHPFU) and their monophosphates HEMFUMP and DHPFUMP.

scopic hot stage. UV spectra were recorded on a Cary 3 instrument. Analytical TLC and PLC made use of Merck silicagel F_{254} plates (DC 0.25 mm, No. 5717; silicagel PSC plates, 2 mm, No. 1.05717), and Merck cellulose F plates (0.2 mm, No. 5574). Paper chromatography was performed on Whatman 3MM paper. Chromatograms were developed in the following solvent systems (by vol.): (A) n-BuOH/ AcOH/Me_2CO-conc./NH_4OH/H_2O, 35:25:10:3:27; (B) $CHCl_3$/MeOH, 95:5; (C) n-PrOH/ toluene, 1:1; (D) EtOAc/hexane, 6:4; (E) MeOH/CH_2Cl_2, 1:1; (F) i-PrOH-conc./ NH_4OH/H_2O, 7:1:2. All evaporations were under vacuum at 35°C. Relation of extinction coefficient to phosphorus (ε/P) was measured by the micromethod of Chen *et al.* [5].

1-[(2-Hydroxyethoxy)methyl]-5-fluorouracil (HEMFU)

An application of the one-pot procedure of Ubasawa *et al.* [6] was used. 1.44 g (10 mmol) of 5-fluorouracil (FUra) was suspended in 15 ml of CH_3CN and stirred under reflux with 5.4 ml (22 mmol) of *N,O*-bis(trimethylsilyl)acetamide to obtain a clear solution. To this solution was added 0.7 ml (10 mmol) of 1,3-dioxolane, 1.7 g (10 mmol) KI and 1.38 ml (10 mmol) of chlorotrimethylsilane. The mixture was stirred at room temp. overnight and monitored on silica gel plates developed in solvent A. The reaction was quenched by addition of 20 ml of MeOH, followed by neutralization with about 4 g of $NaHCO_3$. Solid materials were removed by filtration and the filtrate concentrated under reduced pressure to an oil. The residual oil was dissolved in 5 ml of MeOH, adsorbed on a small volume of silica gel (230–400 mesh) and applied on a silica gel column (3 cm × 35 cm). The products were eluted with solvent B. Fractions containing the main compound were concentrated to a clear oil that crystallized. The crude product was recrystallized from solvent C to yield 1.57 g (77%) of colourless HEMFU, m.p. 151–153°C (lit. m.p. 154–156°C [6]), R_F (Whatman 3MM, solvent F) 0.54. UV: λ_{max} (pH 1) 266 nm (ε 8040), λ_{max} (pH 13) 265 nm (ε 5980). Lit. [7] UV: λ_{max} (pH 1) 266 nm (ε 8200), λ_{max} (pH 13) 265 nm (ε 6100).

1-[(2-Hydroxyethoxy)methyl]-5-fluorouracil monophosphate (HEMFUMP)

HEMFUMP was prepared by enzymatic phosphorylation of HEMFU with a crude extract of wheat shoot phosphotransferase, using *p*-nitrophenylphosphate as the phosphate donor (Fig. 1) as described by Giziewicz & Shugar [8]. The compound was slowly converted to the parent nucleoside DHPFU with *Crotalus adamanteus* 5'-nucleotidase.

1-[(1,3-Dihydroxy-2-propoxy)methyl]-5-fluorouracil (DHPFU)

This was prepared by the following modification of a previously reported procedure [9]. A mixture of 0.7 g (3.5 mmol) of well-dried FUra, 12.5 ml of hexamethyldisilazane (HMDS), and 100 mg of ammonium sulphate was heated under reflux, with protection from moisture, for 2 h. Excess HMDS was evaporated and the oily residue subjected to fractional distillation under vacuum. The main fraction (0.825 g; 3 mmol) was dissolved in benzene (7.5 ml) and mercuric cyanide (1.5 g; 2 mmol) and chloromethyl ether (1.44 g; 4.5 mmol) was added. The mixture was heated under reflux under nitrogen for 3 h, evaporated to a gum, dichloromethane (22.5 ml) added and the solution extracted with 30% KI and saturated aqueous NaCl. The organic layer was dried over anhydrous sodium sulphate and evaporated to dryness. The residue was chromatographed on PLC plates with solvent D, the main band eluted with ethyl acetate and evaporated to dryness to give 0.83 g (67%) of the dibenzyloxy derivative of DHPFU. R_F (silicagel, solvent D) 0.30; UV: λ_{max} (EtOH) 264 nm (lit. [9] λ_{max} (EtOH) 265 nm; R_F (silicagel, solvent D) 0.33). This material was subjected to debenzylation with the use of BCl_3 (0.116 g; 1 mmol) in dry

dichloromethane (8 ml). The mixture was stirred at −78°C for 4 h, and the reaction quenched with 75 ml of solvent E. The mixture was filtered, evaporated to dryness and crystallized from ethanol to give 0.379 g (81%) of DHPFU, m.p. 128–130°C; UV: λ_{max} (pH 1) 264 nm (ε 7450), λ_{max} (pH 13) 265 nm (ε 5580) (lit. [9] m.p. 130–131°C; UV: λ_{max} (pH 1) 265 nm (ε 7600), λ_{max} (pH 13) 266 nm (ε 5700)).

(*R,S*)-1-[(1,3-Dihydroxy-2-propoxy)methyl]-5-fluorouracil 5′-monophosphate (R,S-DHPFUMP)

DHPFU was subjected to chemical phosphorylation with the use of $POCl_3$ in trimethyl phosphate as described by Scheit & Faerber [10] and chromatographed on Whatman 3MM paper with solvent F to give (R,S)-DHPFUMP as the disodium salt in 35% yield. The compound was slowly converted to the parent nucleoside DHPFU with *Crotalus adamanteus* 5′–nucleotidase.

1-(2-Phosphonylmethoxyethyl)-5-fluorouracil (PMEFU)

Method A. FUra (0.524 g; 4 mmol) was dissolved in 14 ml of dry dimethylsulfoxide followed by addition of 2-chloroethoxymethylene diethylphosphonate (CEMP) (0.26 g; 1.13 mmol) and anhydrous K_2CO_3 (0.55 g; 4.4 mmol). The resulting suspension was stirred at room temp. for 12 h, filtered and dimethylsulfoxide removed under diminished pressure. The residue was mixed with 50 ml H_2O and extracted three times with 30 ml portions of chloroform. Extracts were dried over anhydrous sodium sulphate, evaporated to dryness and chromatographed on PLC plates with solvent B to give the diethyl ester of PMEFU (ester I, 0.076 g; 0.3 mmol), in 30% yield relative to CEMP. R_F (Whatman 3MM paper, solvent F) 0.72; UV: λ_{max} (MeOH) 270 nm, λ_{max} (MeOH -aq. 1 N NaOH, 100:1) 268.5 nm; A_{max} (MeOH)/A_{max} (MeOH-aq. 1 N NaOH, 100:1) 1.32. This material was dissolved in 0.4 ml CH_3CN followed by addition of trimethylsilylbromide (153 μL, 1 mmol) and stirred 16 h at room temp. in the dark. The resulting mixture was evaporated to dryness. The residue was co-distilled with CH_3CN (2 ml) and MeOH (3 × 3 ml), dissolved in 1 M triethylammonium hydrogen carbonate, stirred 1 h, evaporated to dryness at 50°C, and co-distilled with MeOH. The methanolic solution was applied on one sheet of Whatman 3MM paper, and developed in solvent F. The main band (R_F 0.12) was eluted with water and evaporated to dryness to give PMEFU as the triethylammonium salt. UV: λ_{max} (pH 1) 267 nm (ε 6350), λ_{max} (pH 13) 266 nm (ε 5080); ε/P 0.99.

Method B. A mixture of FUra (0.37 g; 2.84 mmol), HMDS (7 ml) and ammonium sulphate (0.18 g) was heated 8 h under reflux and evaporated to dryness. The residue was dissolved in 3 ml of anhydrous CH_3CN, and CEMP (0.75 g; 3.24 mmol) was added. The mixture, in a sealed heavy-wall tube, was heated for 48 h at 110°C, cooled to room temp., and diluted with 5 ml of 1,2-dichloroethane, and extracted with 7 ml of aqueous saturated sodium bicarbonate. The organic layer was separated, dried with anhydrous sodium sulphate, concentrated, applied on PLC silicagel plates and developed with solvent B. The main band was eluted with solvent G and evaporated to dryness to give 0.61 g (0.2 mmol) of the diethyl ester of PMEFU. This material was deblocked and purified as described in Method A to give a product identical with that prepared by Method A.

1-(2-Phosphonylmethoxyethyl)-5-methoxyuracil (PMEMeU)

Application of method A to uracil and CEMP gave, unexpectedly, PMEMeU in 11% yield. UV: λ_{max} (pH 2) 276 nm (ε 9800), λ_{max} (pH 13) 275 nm (ε 7300); pK_a 9.3 (lit. for 5-HOMeUrd; UV: λ_{max} (pH 2) 277 nm, λ_{max} (pH 13) 273 nm; pK_a 9.33 [11]), ε/P 1.01. This unexpected result was probably due to the fact that bromine-contaminated trimethylsilylbromide

was used in the deblocking procedure, followed by several coevaporations of the reaction mixture (containing traces of K_2CO_3) with methanol.

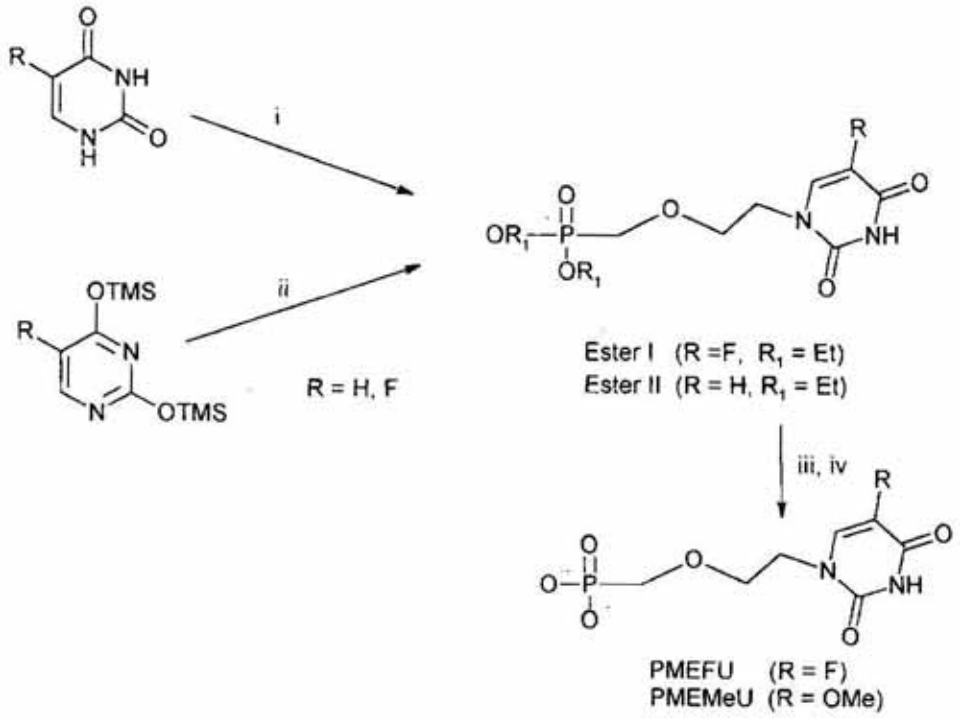
Biochemistry

Enzyme studies. 5'-Nucleotidase (EC 3.1.3.5) was from Sigma. Highly purified preparations of thymidylate synthases from mouse leukemia L1210 and Ehrlich ascites carcinoma cells were employed [12]. The [5-^{3}H]dUMP tritium release assay was used in inhibition studies. Identification of the type of inhibition involved and quantitative analysis of thymidylate synthase inhibition by DHPFUMP, leading to time-dependent biphasic inactivation of the enzyme, were performed as previously described [13].

In vitro cell growth inhibition. Mycoplasma-free (with the Gibco MycoTect test) L5178Y mouse leukemia cells were grown as a suspension in Fisher's medium, supplemented with 8% newborn calf serum, in 5% CO_2 atmosphere at 37°C (doubling time: 8 h). The influence of the analogue on exponentially growing cell viability, and [^{14}C]leucine and [^{3}H]thymidine incorporation was followed as previously described [13].

RESULTS AND DISCUSSION

Syntheses. 1-[(2-Hydroxyethoxy)methyl]-5-fluorouracil (HEMFU) and 1-[(1,3-dihydroxy-2-propoxy)methyl]-5-fluorouracil (DHPFU) were prepared by modification of previously described procedures involving alkylation of



i: $(EtO)_2P\text{-}CH_2\text{-}O\text{-}CH_2\text{-}CH_2Cl$ / K_2CO_3 Anh. / DMSO, rt., 12h
ii: $(EtO)_2P\text{-}CH_2\text{-}O\text{-}CH_2\text{-}CH_2Cl$ / DCE, 110°C, 48h
iii: Me_3SiBr / MeCN / Br_2, rt., 16h
iv: MeOH / K_2CO_3, 50°C, 2h

Figure 2. Synthesis of 1-(2-phosphonylmethoxyethyl)-5-fluorouracil (PMEFU) and 1-(2-phosphonylmethoxyethyl)-5-methoxyuracil (PMEMeU).

the di-O-TMS derivative of 5-fluorouracil [6, 9] (Fig. 1).

HEMFUMP was prepared by enzymatic phosphorylation of HEMFU with the wheat shoot phosphotransferase system [8], using *p*-nitrophenylphosphate as phosphate donor (Fig. 2). DHPFU was monophosphorylated with the use of $POCl_3$-TMP (Scheit & Faerber, 1978 [10]) to give a mixture of *R* and *S* enantiomers of 1-[(1,3-dihydroxy-2-propoxy)methyl]-5-fluorouracil monophosphate[(*R,S*)-DHPFUMP]. Both nucleotides, on treatment with *Crotalus adamanteus* 5′-nucleotidase, were slowly converted to the parent nucleosides HEMFU and DHPFU.

1-(2-Phosphonylmethoxyethyl)-5-fluorouracil (PMEFU) was prepared by two procedures: (a) condensation of 2-chloroethoxymethylenephosphonate (CEMP) with 5 fluorouracil in DMSO, in the presence of K_2CO_3, and (b) by condensation of di-TMS-5-fluorouracil with CEMP and cleavage of the resulting alkylphosphoester I with the use of trimethylbromosilane (Fig. 2). 1-(2-Phosphonylmethoxyethyl)uracil (PMEU) was synthesized as above from uracil and CEMP and then converted to 1-(2-phosphonylmethoxyethyl)-5-methoxyuracil (PMEMeU) by bromination and methoxylation reactions.

Biochemistry. Inhibition of highly purified thymidylate synthase (TS) from mouse tumour Ehrlich carcinoma or leukemia L1210 cells by each of the nucleotide analogues, DHPFUMP, PMEFU, PMEMeU and HEMFUMP, and of L5178Y mouse leukemia cell growth by the nucleoside (HEMFU) analogue, were studied.

DHPFUMP proved to be the strongest inhibitor, non-competitive *vs* dUMP, with K_i^{app} 2.8 μM for time-independent interaction with the enzyme and N^5,N^{10}-methylenetetrahydrofolate ($CH_2H_4PteGlu$). In the presence of $CH_2H_4PteGlu$, DHPFUMP exhibited time-dependent inactivation of the enzyme, the inactivation rate plots being biphasic (Fig. 3), pointing to differing reactions of the inhibitor with the two binding sites on the enzyme molecule, as for FdUMP. Inhibition constants and inactivation rate constants (presented as the mean of three experiments ± SEM), calculated with the use of apparent inactivation rate constants during the initial (0.0–1.5 min) and later (4–10 min) periods of preincubation with various inhibitor concentrations, were $K_{i'}$ 2.3 ± 0.6 μM and $k_{2'}$ 0.35 ± 0.15 min^{-1}, and $K_{i''}$ 2.5 ± 0.9 μM and $k_{2''}$ 0.14 ± 0.04 min^{-1}, respectively (corresponding values for FdUMP are $K_{i'}$ 5.5 nM and $k_{2'}$ 0.18 min^{-1}, and $K_{i''}$ 71 nM and $k_{2''}$ 0.17 min^{-1}). HEMFUMP, PMEFU and PMEMeU were much weaker inhibitors of the enzyme, non-competitive *vs* dUMP, with K_i^{app} values of 0.3 mM, 0.4 M and 0.3 M, respectively (Table 1).

HEMFU, despite weak interaction of its nucleotide analogue with the enzyme, proved to be a moderately strong L5178Y cell growth inhibitor, with IC_{50} in the range 10^{-5} M (Table 2), hence 100-fold weaker than 5-fluoro-dUrd [13]. This result is similar to that reported for L1210 leukemia cells in culture [4]. Similar cell growth inhibitor activities of other acyclic 5-fluorodeoxyuridine derivatives, 1-[3-(2-hydroxyethoxy)-1-cyclopentoxy]propyl-5-fluorouracil (against human larynx tumour

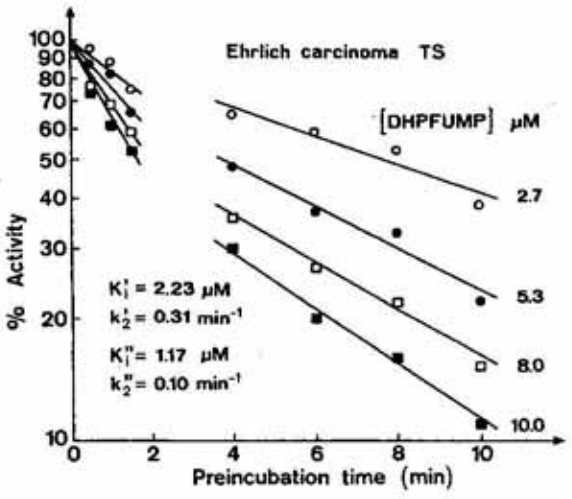


Figure 3. Slow-binding inhibition of Ehrlich carcinoma thymidylate synthase by DHPFUMP.

Table 1. Apparent K_i values for inhibition of mammalian tumour thymidylate synthase by acyclic analogues of 5-FdUMP and 5-methoxy-dUMP

Compound	Enzyme source	K_i^{app} (μM)	Inhibition type
DHPFUMP	Ehrlich carcinoma	2.8	Competitive
HEMFUMP	L1210 leukemia	270	Non-competitive
PMEMeU	L1210 leukemia	33000	Non-competitive
PMEFU	L1210 leukemia	40000	Non-competitive

Table 2. Inhibition of murine leukemia L5178Y cell growth by HEMFU

Compound	IC_{50}[a] (μM)		
	Growth assay	[^{14}C]Leu incorporation	[^{3}H]Thd incorporation
HEMFU	9.4[b]	18.7[b]	42.6[b]

[a]IC_{50} is the concentration for 50% reduction in cell number, [^{14}C]Leu or [^{3}H]Thd incorporation. [b]Results are means of two experiments which did not differ by more than 12%.

Hep-2) and 1-[3-(3-chloro-2-hydroxypropoxy)-1-methoxy]propoxy-5-fluorouracil (against colon carcinoma HT-29) have been reported [14, 15].

REFERENCES

1. Naesens, L., Snoeck, R., Andrei, G., Balzarini, J., Neyts, J. & De Clercq, E. (1996) HPMPC (cidofovir), PMEA (adefovir) and related acyclic nucleoside phosphonate analogues: A review of their pharmacology and clinical potential in the treatment of viral infections. *Antiviral Chem. Chemother.* **8**, 1–23.
2. Holy, A., Rosenberg, I. & Dvorakova, H. (1989) Synthesis of (3-hydroxy-2-phosphonylmethoxypropyl) derivatives of heterocyclic bases. *Collect. Czech. Chem. Commun.* **54**, 2470–2501.
3. Kelley, J.L., Kelsey, J.E., Hall, W.R., Krochmal, M.P. & Schaeffer, H.J. (1981) 1-[(2-Hydroxyethoxy)methyl] pyrimidines as candidate antivirals. *J. Med. Chem.* **24**, 753–756.
4. Rosowsky, A., Kim, S.-H. & Wick, M. (1981) Synthesis and antitumor activity of an acyclonucleoside derivative of 5-fluorouracil. *J. Med. Chem.* **24**, 1177–1181.
5. Chen, P.S., Toribara, T.Y. & Warner, H. (1956) Microdetermination of phosphorus. *Anal. Chem.* **28**, 1756–1758.
6. Ubasawa, M., Takashima, H. & Sekiya, K. (1995) A convenient one-pot synthesis of acyclonucleosides. *Chem. Pharm. Bull.* **43**, 142–143.
7. Robins, M.J. & Hatfield, P.W. (1982) Nucleic acid related compounds. 37. Convenient and high-yield syntheses of *N*-[(2-hydroxyethoxy)methyl] heterocycles as "acyclic nucleoside" analogues, *Can. J. Chem.* **60**, 547–553.
8. Giziewicz, J. & Shugar, D. (1978) Enzymatic phosphorylation of nucleosides to the 5'-phosphates; in *Nucleic Acid Chemistry* (Townsed, L.B. & Tipson, R.S., eds.) pp. 955–961, John Wiley, New York.
9. Ogilvie, K.K., Hamilton, R.G., Gillen, M.F., Radatus, B.K., Smith, K.O. & Golloway, K.S. (1984) Uracil analogues of the acyclonucleoside 9-[[(2-hydroxy-1-hydroxymethyl)ethoxy]-methyl]guanine (BIOLF-62). *Can. J. Chem.* **62**, 16–21.
10. Scheit, K.H. & Faerber, P. (1978) The synthesis of pyrimidinethione nucleoside 5'-mono-, -di-, -tri-phosphates; in *Nucleic Acid Chemistry* (Townsed, L.B. & Tipson, R.S., eds.) pp. 793–799, John Wiley, New York.

11. Hayes, S.J. & Lis, A.W. (1971) Studies on 5-hydroxyuridine (isobarbituridine): IV. Correlation of the spectral properties of 5-hydroxyuridine and its N- and O- methyl derivatives. *Physiol. Chem. & Physics* **3**, 205–225.

12. Rode, W., Zielinski, Z., Dzik, J.M., Kulikowski, T., Bretner, M., Kierdaszuk, B., Ciesla, J. & Shugar, D. (1990) Mechanism of inhibition of mammalian tumor and other thymidylate synthases by N^4-hydroxy-dCMP, N^4-hydroxy-5-fluoro-dCMP, and related analogues. *Biochemistry* **29**, 10835–10842.

13. Dzik, J.M., Bretner, M., Kulikowski, T., Golos, B., Jarmula, A., Poznanski, J., Rode, W. & Shugar, D. (1996) Synthesis and interactions with thymidylate synthase of 2,4 dithio analogues of dUMP and 5-fluoro-dUMP. *Biochim. Biophys Acta* **1293**, 1–8.

14. Campos, J., Pineda, M.J., Gómez, J.A., Entrena, A., Trujillo, M.A., Gallo, M.A. & Espinosa, A. (1996) 5-Fluorouracil derivatives. 1. Acyclonucleosides through a tin (IV) chloride-mediated regiospecific ring opening of alkoxy-1,4-diheteroepanes. *Tetrahedron* **52**, 8907–8924.

15. Gómez, J.A., Campos, J., Marchal, J.A., Trujillo, M.A., Melguizo, C., Prados, J., Gallo, M.A., Arnega, A. & Espinosa, A. (1997) Chemical modifications on the acyclic moiety of 3-(2-hydroxyethoxy)1-alkoxypropyl nucleobases. 2. Differentiation and growth inhibition in Rhabdomyo sarcoma cells after exposure to a novel 5-fluorouracil acyclonucleoside. *Tetrahedron* **53**, 7319–7334.