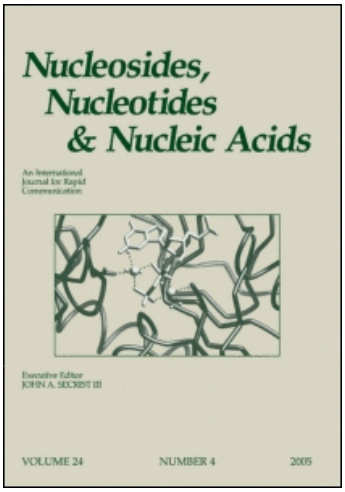

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Synthesis And Biological Activity Op Hydpdxymetflyl Analogs op 5-Benzylacyclouridine and 5-Benzyloxybenzylacycloaridine

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SYNTHESIS AND BIOLOGICAL ACTIVITY OF HYDROXYMETHYL ANALOGS OF 5-BENZYLACYCLOURIDINE AND 5-BENZYLOXYBENZYLACYCLOURIDINE

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Abstract: Hydroxymethyl analogs of 5-benzylacyclouridine (BAU) and 5-benzyloxybenzylacyclouridine (BBAU) were synthesized by the condensation of appropriately blocked 2-(chloromethyl)glycerols with substituted 2,4-dimethoxypyrimidines. The HM derivatives were found to be potent inhibitors of the enzyme uridine phosphorylase and to potentiate significantly the growth-inhibiting action of FdUrd in cell culture.

Abbreviations:

Acyclo-:	2-Hydroxyethoxymethyl-
Acyclovir:	9-(2'-hydroxyethoxymethyl)guanine
BAU:	5-Benzylacyclouridine
BBAU:	5-(m-Benzyloxybenzyl)acyclouridine
FdUrd:	5-Fluoro-2'-deoxyuridine (FUDR)
FUra:	5-Fluorouracil (5-FU)
HM-BAU:	Hydroxymethylbenzylacyclouridine or 5-Benzyl-1-[(1,3-dihydroxy-2-propoxy)methyl]-uracil
HM-BBAU:	Hydroxymethylbenzyloxybenzylacyclouridine or 5-(m-Benzyloxybenzyl)-1-[1,3-dihydroxy-2-propoxy) methyl]uracil

BAU and BBAU, first synthesized in our laboratory, are potent inhibitors of uridine phosphorylase[1], an important enzyme of the pyrimidine salvage pathway in nucleic acid synthesis. The N-1 acyclo side chain of these two compounds is identical to the N-9 substituent of Acyclovir (9-(2'-hydroxyethoxymethyl)guanine, the antiviral agent first synthesized by Schaeffer[2]), and amounts to a part of the

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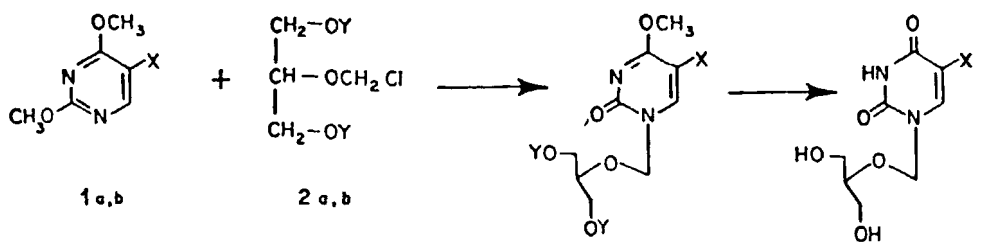
ribofuranose portion of natural nucleosides. The substitution of a benzyl, or m-benzyloxybenzyl group into the 5-position of uracil has been shown by Baker and Kelley[3] to give the most active of uracil derivatives tested against the FdUrd phosphorylase of Walker 256 Rat Tumor. Although BAU and BBAU are not cytotoxic by themselves, they are excellent potentiators of the antitumor activity of FdUrd[4], presumably because they inhibit uridine phosphorylase, thus preventing phosphorolytic degradation to the less active 5-FUra[1], and hence have clinical potential.

Recently, four independent groups of investigators[5-8] have reported that a hydroxymethyl analog of Acyclovir, (referred to as DMPG[5], 2'NDG[6] or BIOLF-62[7]), resembling Acyclovir but more water-soluble and closer to a natural furanoside in structure, was more active than its prototype Acyclovir in both in vitro and in vivo testing against Herpes Simplex Virus types 1 and 2. We therefore undertook the synthesis of BAU and BBAU derivatives with a second hydroxymethyl group in the side chain to determine whether their biological properties might similarly be enhanced.

Chemical syntheses

HM-BAU and HM-BBAU were synthesized according to Scheme 1. Appropriately blocked 2-O-chloromethylated glycerols were condensed with 5-benzyl- or 5-benzyloxybenzyluracil derivatives according to



a. X= Y= $C_6H_5CH_2$

b. X=m-$C_6H_5CH_2OC_6H_4CH_2$
Y= C_6H_5CO

SCHEME 1

the methods used by Schaeffer[2] or Robins[9], and the products deblocked by catalytic hydrogenation or alkaline hydrolysis. It was necessary to change the blocking group from benzyl (higher yielding) to benzoyl for the BAU analog, to avoid conversion of BBAU to BAU analogs during deblocking. The possibility of rearrangement of 1,3-glyceryl diesters to 1,2-diesters under the acidic conditions of chloromethylation should be noted[10]. We have concluded on the basis of NMR spectra that such a rearrangement did not take place, since there is no 2-proton signal at δ3.5 corresponding to $NCOCH_2$ in the spectrum of **3b**. Instead, all of the glyceryl proton signals have been shifted to δ4.47, the location of CH_2-OBzoyl in the spectra of BAU and BBAU benzoates[1]. Diethers do not rearrange under these conditions[10]. We have therefore assigned the structures as shown in Scheme 1, in conformity with the literature[5-8].

Melting points were determined on a Gallenkamp melting point apparatus. Ultraviolet absorption spectra were measured on a Perkin-Elmer Model 402 recording spectrophotometer, and ^1H NMR spectra were run on a Varian A60-A or a Bruker WM-250 machine in $CDCl_3$ or DMSO-d_6, using trimethylsilane as an internal standard. Analyses were performed by the Baron Consulting Co. of Orange, CT, and by the Galbraith Laboratories of Knoxville, TN.

5-Benzyl-1-[(1,3-bis(benzyloxy)-2-propoxy)methyl]uracil (3a).

1,3-Di-O-benzylglycerol was prepared by treating epichlorohydrin with benzyl alcohol and NaOH[5]. To obtain the chlorinated side chain, hydrogen chloride gas was dried by passing through concentrated H_2SO_4 and bubbled into a stirred mixture of paraformaldehyde (0.8 g, 26 mmol) and 1,3-di-O-benzylglycerol (1.5 g, 5.6 mmol) in 20 ml of dry methylene chloride at 0° for 3 hrs until all the paraformaldehyde had dissolved. The resulting solution was stored at 0° for 16 hrs and then dried over $CaCl_2$. After filtering, solvent and excess HCl were removed in vacuo. The oily residue was dissolved in 20 ml of methylene chloride and added to a mixture of **1a** (1 g, 4.3 mmol) finely ground potassium carbonate (1.5 g), and 30 ml of dry methylene chloride. The reaction mixture was stirred overnight under nitrogen at room temperature. After filtering, the solvent was removed under

diminished pressure and the residual oil passed through a silica gel column and eluted with methylene chloride-methanol (3:1). Evaporation of solvent then yielded 0.61 g of pure **3a** (43%) as a colorless oil. UV(EtOH): λ_{max} 282 nm (5500). NMR($CDCl_3$): 3.47-3.60 (m, 6H, CH_2OBzl and CH_2 at C_5 overlap), 3.95 (s, 3H, OCH_3), 3.99-4.10 (m, 1H, tert. H) 4.49 (s, 4H, OBzl), 5.35 (s, 2H, CH_2 at N_1), 7.09-7.37 (m, 16H, ArH and C_6-H overlap).

Anal. Calcd for $C_{30}H_{32}N_2O_5$: C, 71.98; N, 6.44; N, 5.60. Found: C,71.80, H,6.50; N, 5.51.

5-(m-Benzyloxybenzyl)-1-[(1,3-bis(benzoyloxy)-2-propoxy)methyl]uracil (3b).

1,3-Di-O-benzoylglycerol was prepared by treating commercially available 1,3-dichloroacetone with sodium benzoate, followed by catalytic reduction of the ketone to alcohol. Dry hydrogen chloride gas was bubbled into a stirred mixture of paraformaldehyde (0.8 g, 26 mmol) and 1,3-di-O-benzoylglycerol (1.5 g) in 20 ml of dry methylene chloride at 0° for 3 hrs. After separating water and further drying over $CaCl_2$, the solvent and excess HCl were removed under vacuum. The residue was dissolved in 20 ml of dry methylene chloride, added to a mixture of **1b** (1 g, 3.0 mM), potassium carbonate (1.5 g), and 30 ml of dry methylene chloride and stirred overnight at room temperature. After filtering, the solvent was removed under reduced pressure and the residual oil passed through a silica gel column. Elution was with methylene chloride-methanol (30:1) to give 0.7 g of **3b** as pure oily product giving a single spot on TLC (34%). UV(EtOH): λ_{max} 275 nm (8000) and 282 nm (7900). NMR($CDCl_3$): δ 3.38 (s, 2H, CH_2 at C_5), 3.93 (s, 3H, OCH_3), 4.40-4.65 (m, 5H, $CH_2OBzoyl$ and tert. H), 5.00 (s, 2H, OBzl), 5.37 (s, 2H, CH_2 at N_1) 6.60-8.07 (m, 20 H, ArH and C_6-H).

Anal. Calc'd. for $C_{37}H_{34}N_2O_8$: C,70.02; H, 5.40; N, 4.42. Found: C,70.36; H, 5.80; N, 4.36.

5-Benzyl-1-[(1,3-dihydroxy-2-propoxy)methyl]uracil (4a).

2 g (4.6 mmol) of **3a** was dissolved in 100 ml of glacial acetic acid and 0.2 ml of concentrated HCl. 5% Palladium on charcoal (1 g) was then added and the reaction mixture hydrogenated under 50 lbs

pressure. After the theoretical amount of hydrogen had been absorbed, the solution was filtered and evaporated to dryness. The residue was placed on a silica gel column and eluted with methylene chloride-methanol (12:1) to give 1.1 g of 5a (87%). It was isolated as an oil and dried under vacuum over P_2O_5 for several days. UV(pH 1): λ_{max} 266 nm (9400); (pH 11): λ_{max} 266 nm (6400). NMR (DMSO-d_6): δ3.47(s, 4H, CH_2CH_2OH), 3.52 (s, 3H, CH_2 at C_5 and tert. H), 4.66 (t, 2H, OH) 5.20 (s, 2H, CH_2 at N_1), 7.24 (s, 5H, ArH), 7.65(s, 1H, C_6-H), 11.33 (s, 1H, NH).

Anal. Calc'd for $C_{15}H_{18}N_2O_5 \cdot H_2O$: C,55.55; H,6.22; N,8.64. Found: C,55.27, H,5.82; N,8.66.

5-(m-Benzyloxybenzyl)-1-[(1,3-dihydroxy-2-propoxy)methyl]uracil (4b).

To a solution of 1.0 g of **3b** in 35 ml of EtOH there was added 33 ml of 2N NaOH and the reaction mixture stirred overnight at room temperature. The solution was neutralized with HCl or alternatively with Dowex 50 (H^+), filtered, and the resin washed thoroughly with aqueous alcohol. After evaporation of the combined filtrates, the residue was passed through a silica gel column and the product eluted with methylene chloride-methanol (15:1) to yield 0.45 g of **4b**. It was recrystallized with some difficulty from methylene chloride, M.p. 113° C. UV(pH 1): λ_{max} 266 nm (10,600); (pH 11): λ_{max} 267 nm (7700). NMR ($CHCl_3$): δ 3.53-3.69 (m, 7H, CH_2 at C_5, CH_2OH and tert. H. overlap), 5.02 (s, 2H, CH_2 of OBzl), 5.20 (s, 2H, CH_2 at N_1), 6.80-7.44 (m, 10H, ArH and C_6-H overlap).

Anal.Calc'd for $C_{22}H_{24}N_2O_6 \cdot 0.75\ H_2O$: C, 62.04; H, 6.03; N, 6.58. Found C, 62.26; H, 5.95; N, 6.84.

Biological Evaluation

Testing of compounds HM-BAU **(4a)** and HM-BBAU **(4b)** as inhibitors of uridine phosphorylase was performed using 105,000 xg supernatant of mouse liver and human liver homogenates. Enzyme assays were carried out as previously described[1]. Apparent K_i values are related to K_i by the following equation:

$$\text{apparent } K_i = \frac{K_{is}(1 + [S]/K_m)}{1 + ([S]/K_m)(K_{is}/K_{ii})}$$

where K_{is} and K_{ii} are the inhibition constants that would have been estimated from the replots of slope and intercept, respectively, of a Lineweaver-Burk plot vs [I]. If a compound is a competitive inhibitor with respect to the substrate uridine. $K_{ii} = \infty$ and $K_{is} = K_i$. Therefore:

$$\text{apparent } K_i = K_i(1 + [S]/K_m)$$

Thus, the true K_i values would be lower than the apparent K_i values by a factor of $(1 + S/K_m)$. It must be noted, however, that these compounds were not characterized with regard to the type of inhibition (i.e. competitive, non-competitive or uncompetitive), nor were they tested as substrates for uridine phosphorylase.

Apparent K_i values of 1.60 and 0.32 μM were estimated for HM-BAU and HM-BBAU, respectively for enzyme from mouse liver. When tested against uridine phosphorylase from human liver, the two compounds HM-BAU and HM-BBAU were confirmed as excellent inhibitors of this enzyme; Apparent K_i values being 2.5 and 0.65 μM, respectively.

Determination of the activities of HM-BAU **(4a)** and HM-BBAU **(4b)** as potentiators of FdUrd anti-neoplastic activity was carried out using a human pancreatic carcinoma (DAN) in culture. Details of the procedure are described in reference 11.

TABLE 1

% Growth Inhibition of DAN cells in culture

	Potentiator		
FdUrd μM	None	HM-BAU 50 μM	HM-BBAU 50 μM
0.1	20.2 ± 1.1[a]	63.4 ± 1.1[a]	73.6 ± 0.3[a]
0.3	33.8 ± 1.3	67.8 ± 1.7	83.2 ± 1.3
1.0	66 ± 1.4	> 100	> 100

a. Significantly different at $P < 0.001$ from control value and $P < 0.01$ from one another.

Table 1 summarizes the effect of HM-BAU and HM-BBAU upon the inhibitory effect of FdUrd on the growth of pancreatic DAN cells in culture. FdUrd alone at 0.1 and 0.3 μM inhibited the cell growth by 20% and 34% respectively. However, when 50 μM HM-BAU was added 5 minutes prior to the addition of FdUrd, the growth inhibition by 0.1 and 0.3 μM FdUrd increased significantly ($P < 0.001$) to 63 and 68% respectively. HM-BBAU (50 μM) was even more effective, increasing growth inhibition to 74 and 83%. At 1.0 μM FdUrd there was a 66% inhibition by FdUrd alone, which was enhanced to 100% or greater cell-kill in addition to inhibition of growth) by pre-treatment with either HM-BAU or HM-BBAU.

As with the parent compounds (BAU and BBAU[4,11]) the hydroxymethyl analogs did not affect the growth of pancreatic DAN when given alone.

The activity of FdUrd appears to be limited by the action of phosphorylases in the cell which degrade it to the much less active 5-fluorouracil. In cells with little thymidine phosphorylase activity or which have lost thymidine activity and hence are dependent on uridine phosphorylase, blocking of uridine phosphorylase by an inhibitor should extend the existence and effectiveness of FdUrd[1]. The present results demonstrate that the in vitro antineoplastic activity of FdUrd can be significantly potentiated by the uridine phosphorylase inhibitors HM-BAU and HM-BBAU in a similar manner to the parent compounds BAU and BBAU. HM-BAU and HM-BBAU alone at 50 μM concentration do not affect the growth of DAN in culture; instead the cytotoxic effects of FdUrd (at 0.1 μM) were enhanced from 20% to 63 and 74% respectively; and of FdUrd (0.3 μM) from 34% to 60 and 83%.

If the enhancement of FdUrd chemotherapy can be utilized clinically, it should offer a substantial improvement in the effectiveness of FdUrd chemotherapy against certain solid human tumors at concentrations less toxic to the host.

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