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Synthesis and Biological Evaluation of Neutral Derivatives of 5-Fluoro-2′-deoxyuridine 5′-Phosphate

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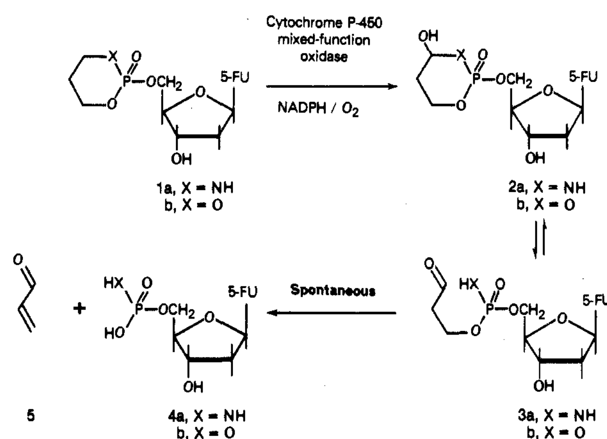
5-Fluoro-5′-(2-oxo-1,3,2-oxazaphosphorinan-2-yl)-2′-deoxyuridine (**1a**) and 5-fluoro-5′-(2-oxo-1,3,2-dioxaphosphorinan-2-yl)-2′-deoxyuridine (**1b**) were prepared by reaction of 5-fluoro-2′-deoxyuridine (**7a**) and phosphoryl chloride with 3-amino-1-propanol and 1,3-propanediol, respectively. The thymidine analogues, **1c** and **1d**, were prepared similarly from thymidine. Compound **1b** was synthesized in better yield from **13a** and trimethylene phosphate with triphenylphosphine/diethyl azodicarboxylate as a condensing agent. Compounds **1a–d** were resistant to degradation by 5′-nucleotidase, alkaline phosphatase, venom phosphodiesterase, and crude snake venom. None of these compounds were significantly biotransformed when incubated with mouse hepatic microsomal preparations in the presence of an NADPH-generating system. When administered intraperitoneally (ip) for 5 consecutive days, **1a** was nearly as effective as 5-fluorouracil at prolonging the life spans of BDF_1 mice implanted intraperitoneally with leukemia P-388. However, much larger dosages of **1a** were required for optimal activity. Compound **1b** administered similarly was only marginally effective. Neither **1a** nor **1b** was active against a P-388 mutant resistant to 5-fluorouracil.

5-Fluorouracil (5-FU, NSC-19893) is used extensively, often in combination with other drugs, in the palliative management of a number of human neoplasms, including carcinomas of the colon, breast, and ovary.[1-3] In common with most other anticancer drugs, resistance to 5-FU constitutes a serious and vexing clincal problem. Thus, although 5-FU is the most effective single agent for the treatment of disseminated colon cancer, only 20% of patients with this disease achieve an objective response.[4,5] Despite continued drug treatment, most, if not all, of these patients eventually relapse. Notwithstanding its widespread clinical use, the precise molecular mechanism of antitumor action of 5-FU has not been determined. However, it is generally accepted that the cytotoxicity of the drug is due to one or both of the following biochemical mechanisms: (1) biotransformation to 5-fluoro-2′-deoxyuridylic acid (5-FdUMP), which, in the presence of N^5,N^{10}-methylenetetrahydrofolic acid binds covalently to thymidylate synthetase and inhibits de novo thymidylate synthesis and, consequently, DNA synthesis;[6-8] (2) biotransformation to 5-fluorouridine 5′-triphosphate (5-FUTP), which can be incorporated into mRNA, tRNA, or rRNA, with possible alteration of RNA structure and function.[9-15] Although the biochemical pharmacological determinants of clinical antitumor response to 5-FU has not been established, several impressive correlates have been reported in experimental tumor systems.[16-27] A frequent finding[18-27] is that therapeutic response correlates with the persistence of 5-FdUMP in tumor cells. Conversely, resistance appears to be associated with the rapid clearance of this metabolite from tumors. This resistance mechanism cannot be overcome merely by administering 5-FdUMP. First, this metabolite is anionic at physiological pH and, therefore, is unlikely to penetrate into cells.[28,29] Second, it is susceptible to rapid dephosphorylation by phosphohydrolases in plasma and in other tissues. To circumvent this problem, we have attempted to devise a strategy to generate 5-FdUMP directly within cells that obviates the requirement for intracellular anabolism from 5-FU or the corresponding ribo- or deoxyribonucleoside. Our approach is based upon the mechanism of action of cyclophosphamide.

Cyclophosphamide is not independently cytotoxic.[30,31] It is biotransformed in vivo predominantly by hepatic cytochrome P-450 dependent mixed-function oxidase[32] to

Scheme I



4-hydroxycyclophosphamide. This metabolite exists in equilibrium with aldophosphamide, its ring-open tautom-

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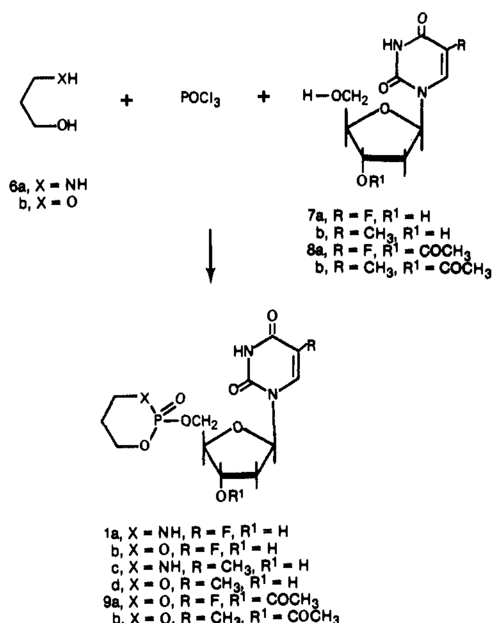
Scheme II



er.[33,34] Aldophosphamide is chemically unstable and spontaneously dissociates via an E2 elimination mechanism to give phosphorodiamidic mustard and acrolein. Substantial evidence has accumulated that phosphorodiamidic mustard is the metabolite responsible for the antitumor activity of cyclophosphamide.[35-38] However, 4-hydroxycyclophosphamide and aldophosphamide probably contribute significantly to the antitumor selectivity of the drug. Although the precise roles of these metabolites have not been established, several plausible hypotheses have been advanced.[38-43] One important role may be the transportion of the hydrophilic phosphordiamidic mustard across cell membranes.[38,41] If this postulate is correct, then the mechanism potentially can be exploited to transport other ionic organophosphates, such as 5-FdUMP, into cells (Scheme I).

Thus, biooxidation of the cyclic 5′-phosphoramidate **1a** in a manner similar to cyclophosphamide would give the hydroxy analogue **2a**, which, conceivably, could penetrate cell membranes by passive diffusion or facilitated transport and then ring open to the acyclic analogue **3a**. Elimination of acrolein from **3a** would yield the phosphoramidate 4a, which could be converted to 5-FdUMP either by chemical[44,45] or enzymic[45-47] hydrolysis. A similar oxidation–elimination sequence beginning with the cyclic 5′-phosphotriester **1b** would yield 5-FdUMP directly.

A preliminary account of this work has appeared.[48]

Chemistry. Our approach to the synthesis of **1a** and **1b** (Scheme II) is based upon Yoshikawa's method for the preparation of 5′-nucleotides from unsubstituted nucleosides.[49]

Thus, 5-FUdR (**7a**) was reacted with partially hydrolyzed phosphoryl chloride in triethyl phosphate, and the product was treated in situ with a solution of 3-amino-1-propanol (**6a**) in chloroform in the presence of triethylamine. The crude reaction product was desalted by acetone precipitation and by ion exchange on DEAE-cellulose (bicarbonate form), and the neutral residue was chromatographed on silica to give unchanged 5-FUdR and a product that appeared homogeneous by TLC. However, analysis of the product by HPLC in the reverse-phase mode (μ-Bondapak C_{18}) showed that it consisted of a mixture of four components in the ratio 5:5:1:1. These components were separated by reverse-phase semipreparative chromatography. The UV spectrum of each component was almost identical with that of 5-FUdR. The mass spectra of the trimethylsilyl derivatives[50] showed similar fragmentation patterns, each with a molecular ion at m/e 581 corresponding to the trisubstituted parent compounds. The ^{1}H NMR spectra at 360 MHz indicated that these compounds were diastereomeric pairs of positional isomers derived by substitution of the cyclic phosphoramidate moiety at the 5′- and 3′-hydroxyl groups. The diastereomers arise from the presence of a chiral phosphorus atom in these compounds. The spectrum of 5-FUdR in Me_2SO-d_6 shows the 3′-OH and the 5′-OH resonances as a one-proton doublet (J_{HOCH} = 4 Hz) and a

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one-proton triplet (J_{HOCH} = 4 Hz) at δ 5.20 and 5.09, respectively. In the spectra of the two major isomers, which were closely similar, the 3'-OH doublet is present at δ 5.42 (J_{HOCH} = 4 Hz). However, the 5'-OH triplet is absent, indicating substitution at this position. This interpretation is supported by the downfield shift of the 5'-H resonances from δ 3.53–3.67 in 5-FUdR to 3.97–4.09 in the product. The chemical shift of the 3'-H resonances, by comparison, is virtually unchanged (δ 4.22–4.27 in 5-FUdR vs. 4.17–4.33 in **1a**). Bonding of the nitrogen atom to phosphorus is indicated by the presence of a multiplet at δ 5.21 attributable to the NHP(O) proton. The methylene resonances of the cyclic phosphoramide moiety appear as complex two-proton multiplets at δ 4.17–4.33 ($OCH_2CH_2CH_2NH$), 1.51–1.67 (OCH_2CH_2CHNH), and 2.96–3.16 ($OCH_2CH_2CH_2NH$). The ^1H NMR spectra of the minor pair of diastereomers are generally similar to those of **1a**, except that the 5'-OH resonance occurs as a one-proton triplet at δ 5.37, while the 3'-OH resonance is absent. In addition, the chemical shift of the 3'-H signal occurs approximately 0.5-ppm downfield from that of the corresponding protons of 5-FUdR. The position of the 5'-H resonance, however, is unchanged.

Similar spectral analysis of the product derived from **7a** and 1,3-propanediol (**6b**) showed that it consisted of a 5:1 mixture of the cyclic 5'- and the 3'-phosphate positional isomers. Stereoisomers are not possible for these compounds because the phosphorus atom is not chiral.

These data provide further support for the observation of Dunlap et al.[51] that the Yoshikawa reaction for the preparation of 5'-nucleotides from unsubstituted nucleosides is not regiospecific but gives substantial amounts of the 3'-isomers.

The thymidine analogues **1c** and **1d** were prepared similarly. Although HPLC analyses of these compounds indicated that they contained a mixture of 5'- and 3'-isomers, detailed spectral analysis of each component was not attempted.

Proof that the cyclic phosphate group in **1b** and **1d** was present in the 5'-position of the molecule was obtained from chemical interconversion studies. Thus, the reaction of **1b** and **1d** with acetic anhydride in pyridine gave **9a** and **9b**, respectively; these products were spectrally and chromatographically identical with the products obtained from the reaction of 3'-*O*-acetyl-5-fluoro-2-deoxyuridine (**8a**) and 3'-*O*-acetylthymidine (**8b**) with phosphoryl chloride and 1,3-propanediol (Scheme II). This result precludes the possibility that the phosphoryl group in **1b** and **1d** is bonded to the 3'-oxygen atom and, therefore, eliminates alternative structures such as the isomeric cyclic 3',5'-nucleotides.

Although the modified Yoshikawa synthesis provided a direct route to the desired cyclic 5'-nucleotides, the yields from these reactions were disappointingly low. However, considering the greater steric requirements of the cyclization reactions, this result is consistent with the report of Dunlap et al.[51] that the Yoshikawa procedure gives only a 28% yield of 5-FdUMP from 5-FUdR. Since the amount of **1a** formed by this method was insufficient for antitumor screening, an alternative synthesis of this compound was attempted from 5-FUdR and trimethylene phosphate (**10**) by using triphenylphosphine/diethyl azodicarboxylate[52] as the condensing agent (Scheme III). Chromatography of the crude product on silica gel gave **1b** in 64% yield free

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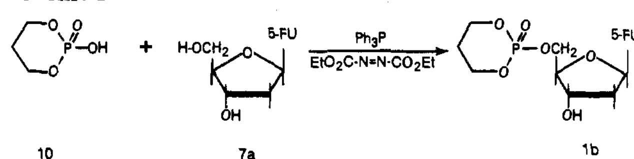
Scheme III



of the 3'-isomer. Because of the comparatively good yield and high isomeric purity of the product and the ease of workup of the reaction, this is clearly the best method for the preparation of **1b**. A similar synthesis of **1a** was not attempted because of the unavailability of the azo analogue of trimethylene phosphate. Because of the pronounced acid lability of phosphoramidates, it seems likely that this compound would undergo facile self-addition to yield a polymeric product.

All of the cyclic 5'-phosphates were stable in neutral aqueous buffers for up to 24 h at room temperature. Although the corresponding phosphoramidates were also stable in neutral buffers, they were extremely susceptible to acid-induced decomposition. Therefore, care was taken to ensure neutral or slightly basic (pH 7–8) conditions during all chemical and biological manipulations.

For the enzyme studies, the cyclic 5'-nucleotides were purified to homogeneity by reverse-phase semipreparative chromatography. However, since the separation of **1a** into its component isomers in gram quantities was not possible, this compound was screened as a mixture of isomers.

Enzyme Studies. Since it is important to the success of our strategy that the cyclic 5'-nucleotides are resistant to degradation by nucleotide-catabolizing enzymes, the stability of **1a–d** was examined in the presence of 5'-nucleotidase (EC 3.1.3.5), alkaline phosphatase (EC 3.1.3.1), phosphodiesterase 1 (EC 3.1.4.1), and crude snake venom. None of the compounds showed measurable degradation when incubated with these enzymes for 2 h at 37 °C.

The susceptibility of these same compounds to oxidative biotransformation by cytochrome P-450 dependent mixed-function oxidases was examined by incubating them with hepatic microsomal preparations from phenobarbital-induced mice. All four compounds showed a 5 to 10% decrease in concentration over the 2-h incubation period. HPLC analysis of the supernatants from ethanol-quenched incubations showed a different chromatographic profile from NADPH-deficient or heat-deactivated controls, but no major new peaks were detected that were unambiguously derived from the 5'-cyclic compounds. Cyclophosphamide used as control was extensively metabolized under these conditions.

Antitumor Screening. Compounds 1a and **1b** were screened against murine leukemia P-388 both sensitve and resistant to 5-FU (Table I). Administered intraperitoneally (ip) daily for 5 consecutive days at maximally tolerated dosages, 1a was only slightly less effective than 5-FU at prolonging the life spans of mice bearing the 5-FU-sensitive tumor. However, much higher dosages were required for optimal activity. Compound **1b**, administered similarly, was only marginally active at the highest dose tested. Neither compound was effective against a P-388 mutant resistant to 5-FU.

Discussion

Our objectives in this study were to synthesize neutral analogues of 5-FdUMP which incorporate the structural features that contribute to the selectivity of cyclophosphamide. We hoped these analogues might penetrate into cells and overcome innate or acquired resistance to 5-FU due to the deletion of anabolic enzymes. The success of this strategy is critically dependent on the oxidative

Table I. Effect of Neutral 5-FdUMP Analogues on the Survival of BDF_1 Mice[a] Implanted Intraperitoneally with P-388 Leukemia[b] Both Sensitive and Resistant to 5-FU

		P-388/0			P-388/5-FU		
compd	dose, mg/kg, (qd, 1-5)	MST,[c] days	% ILS[d]	% wt[e] change	MST,[c] days	% ILS[d]	% wt[e] change
saline		9		9.4	10		2.1
5-FU	35	8	−11	−21.8	9	−10	−14.2
	20	19	111	−16.5	11	10	−3.9
	15				11	10	−6.2
1a	500	17	89	−17.9			
	300	14	55	−15.7	9	−10	−5.0
	180	13	44	−13.9	10	0	−3.7
	108	12	33	−6.6	10	0	−3.4
	65				9	−10	1.5
1b	500	12	33	−12.4	10	0	0.6
	300	11	22	−8.8	10	0	1.2
	180	10	11	−0.0	10	0	4.1
	108	10	11	1.7	10	0	6.3

[a] Six mice per group; average weight 23 g. [b] 1×10^6 cells inoculated intraperitoneally on day 0. [c] Median survival time (MST) of 20 mice used as control was 9 days. [d] Percentage increase in life span of treated animals (T) compared with saline-treated controls (C) was determined by the formula: $(T/C - 1) \times 100$. [e] The average percentage weight change on day 6 was taken as a measure of drug toxicity.

bioactivation of the analogues by cytochrome P-450 dependent mixed-function oxidases. Although the cyclic phosphoramidate **1a** was almost as active as 5-FU against leukemia P-388, much higher dosages were required for optimal activity. This result is consistent with the minimal extent of biotransformation of **1a** when incubated with mouse hepatic microsomes in the presence of an NADPH-generating system. The marginal activity of the cyclic phosphate **1b**, moreover, parallels the weak activity of the dioxo analogue of cyclophosphamide.[53] However, it cannot be assumed that the antitumor activity of **1a** at high dosages is due to oxidative bioactivation. Metabolites anticipated from this mode of biotransformation were not conclusively detected in microsomal incubates. Although **1a**, in common with the other cyclic 5′-nucleotides, was not degraded in vitro by several different phosphohydrolases, conceivably it is catabolized in vivo to 5-FU or 5-FUdR by some other enzymes. This possibility is consistent with the inactivity of **1a** against the 5-FU-resistant tumor. However, since the mechanism of drug resistance in this tumor has not been established, this interpretation is entirely speculative. Apart from the failure of the cells to anabolize 5-FU to 5-FdUMP, other resistance mechanisms are possible, such as increased thymidine salvage, altered thymidylate synthetase, or decreased drug incorporation into RNA.

Subsequent to our initial report on the synthesis and biological evaluation of the cyclic nucleotides described herein, Hunston et al.[54] reported the synthesis of a similar series of compounds. Compound **1a** was marginally active against sarcoma 180 at a dose of 100 mg/kg but inactive against leukemia L1210 at 50 mg/kg (schedules not specified). Enzymes studies were not reported.

In summary, it appears that the poor substrate affinity of the 5′-cyclic 5-FdUMP analogues for cytochrome P-450 mixed-function oxidase inherently limits their potential as latent precursors of the parent nucleotide. Currently, we are investigating approaches to overcome this problem.

Experimental Section

Proton nuclear magnetic resonance spectra (^{1}H NMR) were recorded at ambient temperature on a Varian Associates T-60A spectrometer or on a Bruker HX-360 spectrometer in the Fourier transform mode, in $CDCl_3$ or Me_2SO-d_6, with tetramethylsilane as an internal standard. Mass spectra were obtained on a Finnegan Model 3300 quadrupole spectrometer in the electron-impact or chemical-ionization mode with a direct-inlet probe. Elemental analyses were performed by Galbraith Laboratories Inc., Knoxville, TN, and the results are within ±0.4% of the theoretical values. All solvents were distilled immediately before use and were stored over freshly activated (300 °C/4 h) molecular sieves (type 4Å). Nucleosides were dried over P_2O_5 under vacuum at 100 °C for 24 h before use. All reactions were conducted in dry glassware and were protected from atmospheric moisture. Evaporations were carried out on a rotaty evaporator under high vacuum at a bath temperature of <40 °C. The homogeneity of products was determined by ascending TLC on silica-coated glass plates (silica gel 60 F 254, Merck) with ethyl acetate–2-propanol–water (4:2:1, top layer) as the eluting solvent. Compounds were visualized under UV light (254 nm output) or with iodine vapor. Preparative separations were performed on glass plates (20 × 20 cm) coated with a 2-mm layer of silica gel F254 (Merck) or on columns of silica gel 70–230 mesh (Woelm). Analytical HPLC was carried out in the reverse phase on μ-Bondapak C-18 columns (Waters Associates). All phoshohydrolases were obtained from Sigma Chemical Co., St. Louis, MO.

5-Fluoro-5′-(2-oxo-1,3,2-oxazaphosphorinan-2-yl)-2′-deoxyuridine (1a). A solution of 5-FUdR (2.5 g, 10.16 mmol) in $(EtO)_3PO$ (40 mL), contained in a 2-L round-bottomed flask, was cooled to 0 °C in an ice–salt bath; $POCl_3$ (0.95 mL, 10.16 mmol) was added with stirring, followed by H_2O (92 μL, 5.08 mmol). After 3 h at 0 °C, more $POCl_3$ (0.95 mL, 10.16 mmol) and H_2O (92 μL, 5.08 mmol) were added. The reaction mixture was stirred for 2 h at 0 °C and then stored for 18 h in a cold room at 2 °C. The flask was then immersed in an ice–salt bath and dry toluene (ca. 250 mL) was added with stirring until the reaction mixture developed a slight turbidity. A solution of 3-amino-1-propanol (1.56 mL, 20.32 mmol) and Et_3N (9.84 mL, 71.14 mmol) in $CHCl_3$ (50 mL) was then added dropwise with stirring over 3 h. A white precipitate separated. After the mixture was stirred at room temperature for 24 h, toluene (700 mL) was added, and the flask was placed in the cold room for 20 h. The clear supernatant was carefully decanted, and the copious white residue was washed by decantation with toluene (2 × 300 mL), the solid being allowed to settle for 1 h each time. Dry acetone (200 mL) was added, and the mixture was stirred vigorously for 20 min. The insoluble salts (which consisted mainly of $Et_3N{\cdot}HCl$) were filtered off and washed with acetone 50 mL). The filtrate and washings were combined and evaporated. This process was repeated three to four more times with 50-mL aliquots of acetone until no more insoluble residue remained. During this time, care was taken to ensure that the acetone solutions remained neutral, a drop or two of Et_3N being added if necessary. The clear viscous residue, which consisted mainly of a mixture of **1a** and unreacted

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5-FUdR, was taken up in 0.01 M triethylammonium bicarbonate (TEAB) buffer, pH 7.5 (20 mL), and applied to a column (5 × 60 cm) of DEAE-cellulose (Whatman DE 32) in the bicarbonate form. Elution was carried out with a linear gradient of TEAB, pH 7.5 (1.5 L of 0.01 M buffer in the mixing chamber, 1.5 L of 0.2 M buffer in the reservoir) at a flow rate of 1.5 mL/min. Fractions of 15 mL were collected. The composition of the eluate was monitored by UV absorption at 254 nm and by HPLC (see below). The 0.14–0.23 M fraction containing **1a** and 5-FUdR was lyophilized, and the residue was coevaporated with EtOH (4 × 50 mL) to remove residual TEAB. The remaining oil (1.42 g) was dissolved in MeOH (50 mL), silica (3.0 g) was added, and the solution was evaporated to dryness. After further evaporations from MeOH (2 × 30 mL) and EtOAc (2 × 30 mL), a free-flowing powder was obtained, which was transferred to a column (2.5 × 60 cm) of silica (105 g) previously made up in ethyl acetate. Elution was carried out with ethyl acetate–2-propanol–water (4:2:1, v/v, top phase). Fractions of 10 mL were collected. The eluent was monitored by UV absorption at 254 nm and by HPLC (0.05 M, pH 7.4, Tris buffer–methanol, 85:15, 2.0 mL/min). 5-FUdR was eluted first, followed by **1a**. Fractions containing **1a** were combined, evaporated, and dried under vacuum over P_2O_5 for 24 h. A clear viscous oil remained. The yield was 693 mg. HPLC analysis of this product showed that it contained four components in the ratio 5:5:1:1. A sample (20 mg) of the mixture was resolved into its individual components by chromatography on a Magnum 9 ODS 2 column (Whatman) with water–methanol (85:15) as the eluent at a flow rate of 1.5 mL/min. The two major components (fractions 1 and 2), which were identified as diastereomers of **1a**, eluted first, followed by the two minor components (fractions 3 and 4), which were identified as diastereomers of 5-fluoro-3'-(2-oxo-1,3,2-oxazaphosphorinan-2-yl)-2'-deoxyuridine. The fractions corresponding to individual isomers were evaporated and dried over P_2O_5 in vacuo. Compound **1a** was obtained as a glass. The yield (for both diastereomers) was 16%. Spectral characteristics (first diastereomer): ^{1}H NMR (Me_2SO-d_6) δ 7.95 (d, 1 H, C_6 H, J = 7 Hz), 6.16 (tt, 1 H, $C_{1'}$ H, J = 7 Hz), 5.42 (d, 1 H, $C_{3'}$ OH, J = 4 Hz), 5.21 (m, 1 H, NHP(O), J = 7 Hz), 4.17–4.33 (m, 3 H, $C_{3'}$ H, $OCH_2CH_2CH_2NH$), 3.97–4.09 (m, 2 H, $C_{5'}$ H), 3.92–3.97 (m, 1 H, $C_{4'}$ H), 2.96–3.16 (m, 2 H, $NHCH_2CH_2CH_2O$), 2.11–2.21 (m, 2 H, $C_{2'}$ H), 1.51–1.67 (m, 2 H, $OCH_2CH_2CH_2NH$); MS, m/e 581 (M^+) [tris(trimethylsilyl) derivative]; UV (EtOH) λ_{max} 269 nm (ϵ 7700). The second diastereomer gave virtually identical spectral characteristics. Anal. (diastereomer mixture) ($C_{12}H_{17}FN_3O_7P$) C, H, N.

5-Fluoro-3'-(2-oxo-1,3,2-oxazaphosphorinan-2-yl)-2'-deoxyuridine. The yield (both diastereoisomers) was 3%. Spectral properties (first diastereomer): ^{1}H NMR (Me_2SO-d_6) δ 8.13 (d, 1 H, C_6 H, J = 7 Hz), 6.17 (t, 1 H, $C_{1'}$ H, J = 7 Hz), 5.35 (t, 1 H, $C_{5'}$ OH, J = 4 Hz), 5.19–5.26 (m, 1 H, NHP(O), J = 7 Hz), 4.76–4.82 (m, 1 H, $C_{4'}$ H), 4.18–4.44 (m, 2 H, $OCH_2CH_2CH_2NH$), 4.08–4.13 (m, 1 H, $C_{4'}$ H), 3.63–3.68 (m, 2 H, $C_{5'}$ H), 3.01–3.08 (m, 2 H, $NHCH_2CH_2CH_2O$), 2.22–2.32 (m, 2 H, $C_{2'}$ H), 1.56–1.85 (m, 2 H, $OCH_2CH_2CH_2NH$); MS, m/e 581 (M^+) [tris(trimethylsilyl) derivative]; UV (EtOH) λ_{max} 268 nm (ϵ 8000). The second diastereomer gave virtually identical spectral characteristics.

5-Fluoro-5'-(2-oxo-1,3,2-dioxaphosphorinan-2-yl)-2'-deoxyuridine (1b). Method 1. The compound was prepared from 5-FUdR (2.5 g, 10.16 mmol) exactly as described for **1a** except that 1,3-propanediol (1.50 mL, 20.32 mmol) was used instead of 3-amino-1-propanol. The compound was obtained as a glass. The yield was 282 mg (7.6%): ^{1}H NMR (Me_2SO-d_6) δ 7.95 (d, 1 H, C_6 H, J = 7 Hz), 6.16 (tt, 1 H, $C_{1'}$ H, J = 7 Hz), 5.47 (d, 1 H, $C_{3'}$ OH, 4 Hz), 4.24–4.44 (m, 5 H, $C_{3'}$ H, $OCH_2CH_2CH_2O$), 4.08–4.23 (m, 2 H, $C_{5'}$ H), 3.92–3.97 (m, 1 H, $C_{4'}$ H), 2.03–2.26 (m, 2 H, $C_{2'}$ H), 1.77–2.14 (m, 2 H, $OCH_2CH_2CH_2O$); MS, m/e 510 (M^+) [bis(trimethylsilyl) derivative]; UV λ_{max} (EtOH) 268 nm (ϵ 8300). Anal. ($C_{12}H_{16}FN_2O_8P$) C, H, N.

Method 2. A solution of diethyl azodicarboxylate (3.84 mL, 24.4 mmol) in dimethylacetamide (15 mL) was added, dropwise, with stirring over 1 h at room temperature to a solution of 5-FUdR (4.0 g, 16.3 mmol), trimethylene phosphate[55] (3.37 g, 24.4 mmol), and triphenylphosphine (6.39 g, 24.4 mmol) in dimethylacetamide (15 mL). After 24 h at room temperature, the solvent was evaporated in vacuo, and the yellow oily residue was partitioned between H_2O (100 mL) and $CHCl_3$ (100 mL). The aqueous layer was washed with $CHCl_3$ (2 × 50 mL) and then lyophilized. The clear viscous residue was preadsorbed on silica (17 g) by successive evaporation from MeOH (100 mL) and EtOAc (3 × 50 mL). The free-flowing powder obtained was transferred to a column (5 × 60 cm) of silica (375 g) that had previously been made up in EtOAc. The products were eluted with EtOAc–*i*-PrOH–H_2O (4:2:1, top layer). Fifteen-milliter fractions were collected. 5-FUdR eluted first, followed by **1b**. Fractions 95–118, which contained pure **1b**, were combined and evaporated. The yield was 2.87 g. Fractions 79–94, which were contaminated with 5-FUdR, were rechromatographed on silica (150 g) to give a further 0.94 g of **1b**. The product was dried over P_2O_5 in vacuo. The total yield was 3.79 g (64%). The purity of this preparation by HPLC analysis was 100%. The product was spectrally and chromatographically identical with that obtained by method 1.

5'-(2-Oxo-1,3,2-oxazaphosphorinan-2-yl)thymidine (1c). This compound was obtained from thymidine (2.46 g, 10.16 mmol) and 3-amino-1-propanol (1.56 mL, 20.32 mmol) by the same procedure used for the synthesis of **1a**. The crude product, as evidenced by HPLC and MS analysis, consisted of a mixture of isomers in the ratio 5:5:1:1. However, no attempt was made to resolve the mixture on a preparative scale. The yield was 710 mg of a visous oil (19%).

5'-(2-Oxo-1,3,2-dioxaphosphorinan-2-yl)thymidine (1d). This compound was prepared from thymidine (0.5 g, 2.07 mmol), phosphoryl chloride (0.39 mL, 4.18 mmol), and 1,3-propanediol (0.3 mL, 4.13 mmol) by the general procedure described for **1a**. It was obtained as a viscous oil. The yield was 302 mg (40%): ^{1}H NMR (Me_2SO-d_6) δ 7.48 (d, 1 H, C_6 H, J = 2.5 Hz), 6.19 (t, 1 H, $C_{1'}$ H, J = 7 Hz), 5.41 (d, 1 H, $C_{3'}$ OH), 4.28–4.44 (m, 5 H, $C_{3'}$ H, $OCH_2CH_2CH_2O$), 4.07–4.23 (m, 2 H, $C_{5'}$ H), 3.92–3.97 (m, 1 H, $C_{4'}$ H), 2.08–2.22 (m, 2 H, $C_{2'}$ H), 1.74–2.08 (m, 2 H, $OCH_2CH_2CH_2O$), 1.79 (d, 3 H, CH_3, J = 2.5 Hz); MS, m/e 506 [bis(trimethylsilyl) derivative]; UV (EtOH) λ_{max} 267 nm (ϵ 8500). Anal. ($C_{13}H_{19}N_2O_8P$) C, H, N.

3'-*O*-Acetyl-5-fluoro-5'-(2-oxo-1,3,2-dioxaphosphorinan-2-yl)-2'-deoxyuridine (9a). Method 1. This compound was prepared from 3'-*O*-acetyl-5-fluoro-2'-deoxyuridine[56] (250 mg, 0.87 mmol), phosphoryl chloride (162 μL, 1.74 mmol), H_2O (15.7 μL), 1,3-propanediol (0.126 mL, 1.74 mmol), and Et_3N (0.85 mL, 6.09 mmol) in triethyl phosphate (3.5 mL) and $CHCl_3$ (4.5 mL) by the general method described for **1a**. However, since the product was not readily precipitated from the reaction mixture by the addition of toluene, a modified workup procedure was used. The crude reaction mixture was evaporated under reduced pressure to remove chloroform, and the remaining solution was extracted exhaustively with *n*-hexane (8 × 25 mL) to remove $(EtO)_3PO$. The viscous residue was taken up in acetone (15 mL) and, after removal of insoluble salts by filtration, the solution was concentrated and applied to four preparative thick-layer plates. After developing in ethyl acetate–methanol (8:2), the forerunner product band was extracted with EtOH–$CHCl_3$ (1:1). The yield was 35 mg of a viscous oil (11%): ^{1}H NMR ($CDCl_3$) δ 7.82 (d, 1 H, C_6 H, J = 7 Hz), 6.28 (t, 1 H, $C_{1'}$ H, J = 7 Hz), 5.20–5.50 (m, 1 H, $C_{3'}$ H), 4.13–4.83 (m, 7 H, $C_{4'}$ H, $C_{5'}$ H, $OCH_2CH_2CH_2O$), 1.87–2.73 (m, 2 H, $OCH_2CH_2CH_2O$), 2.10 (s, 3 H, $COCH_3$); MS, m/e (MH^+ 409); UV (EtOH) λ_{max} 268 nm (ϵ 8800). Anal. ($C_{14}H_{18}FN_2O_9P$) C, H, N.

Method 2. A solution of **1b** (250 mg, 0.68 mmol) in dry pyridine (10 mL) was cooled to 4 °C in an ice bath, and acetic anhydride (0.64 mL, 6.83 mmol) was added with stirring. After 24 h at room temperature, the pyridine was evaporated, and the remaining oil was dried in vacuo. The residue was preadsorbed on silica (0.5 g) and transferred to a column (65 × 1 cm) of silica (23 g) previously made up in EtOAc. The product was eluted with EtOAc–*i*-PrOH–H_2O (4:2:1, top layer). The yield was 185 mg of a viscous oil (66%). The spectral and chromatographic properties

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of the compound were identical with those of the product prepared by Method 1.

3′-O-Acetyl-5′-(2-oxo-1,3,2-dioxaphosphorinan-2-yl)thymidine (9b). Method 1. The compound was prepared from 3-O-acetylthymidine (500 mg) as described for **9a**, Method 1, in 10% yield: ^{1}H NMR (Me_2SO-d_6) δ 7.56 (s, 1 H, C_6 H), 6.17–6.24 (t, 1 H, $C_{1'}$ H), 5.24–5.29 (m, 1 H, $C_{3'}$ H), 4.31–4.36 (m, 5 H, $C_{4'}$ H, $OCH_2CH_2CH_2O$), 4.14–4.28 (m, 2 H, $C_{5'}$ H), 2.25–2.44 (m, 2 H, $C_{2'}$ H), 2.08 (s, 3 H,$COCH_3$), 1.81 (s, 3 H, CH_3), 1.76–2.17 (m, 2 H, $OCH_2CH_2CH_2O$); MS, *m/e* 405 (MH^+); UV (EtOH) λ_{max} 268 (ε 9400). Anal. ($C_{15}H_{21}N_2O_9P$) C, H, N.

Method 2. The compound was prepared from **1d** (200 mg, 0.55 mmol) and acetic anhydride (520 μL) in pyridine (10 mL). The product was purified by preparative chromatography on two thick layers of silica with $CHCl_3$–CH_3OH (95:5) as eluent. The plates were twice developed. The yield was 155 mg (69%). The product was identical with that obtained by method 1.

Enzyme Experiments. 1. Phosphohydrolases. The substrate (0.7 μmol) in water (20 μL) was incubated at 37 °C for 2 h with (a) 5′-nucleotidase (*Crotalus adamanteus*) (20 μL, 2.25 units/mL) in 0.1 M Tris-HCl buffer–0.01 mM $MgCl_2$, pH 9.0 (100 μL); (b) alkaline phosphatase (*Escherichia coli*) (20 μL, 2.18 units/mL) in 0.1 M glycine–sodium hydroxide buffer, pH 10.4 (100 μL); (c) phosphodiesterase I (*Crotalus adamanteus*) (20 μL, 1.69 units/mL) in 0.1 M Tris-HCl buffer–0.01 mM $MgCl_2$, pH 9.0 (100 μL); (d) snake venom (*Crotalus adamanteus*) (20 μL, 1.25 mg/mL) in 0.1 M Tris-HCl buffer–0.1 mM $MgCl_2$, pH 9.0 (100 μL). After incubations were complete, 0.9 mL of EtOH was added, and the solutions were centrifuged at 2000 rpm for 5 min. Aliquots of the supernatants were analyzed by HPLC in the reverse-phase mode (μ-Bondapak C-18) with 0.05 M Tris-HCl buffer, pH 7.0, and methanol (75:25) as eluent.

2. Cytochrome P-450 Dependent Mixed-Function Oxidases. BDF_1 mice were injected intraperitoneally with sodium phenobarbital (75 mg/kg) for 4 consecutive days. The animals were killed 24 h after the last injection, and their livers were excised. A 33% liver homogenate in 0.05 M Tris-HCl/0.15 M KCl/0.01 M $MgCl_2$ buffer, pH 7.4, was centrifuged at 10000*g* for 20 min at 4 °C. The supernatant fraction was aspirated and centrifuged at 105000*g* for 60 min at 4 °C. The microsomal pellet was washed by resuspension in the original volume of buffer containing 0.01 M EDTA and then resedimented at 105000*g* for 30 min. The final pellet was reconstituted in the Tris/KCl/$MgCl_2$ buffer such that each milliliter of suspension contained microsomes from 0.33 g wet weight of liver. Each incubation mixture contained cyclic 5′-nucleotide (1 mM), NADP (0.4 mM), glucose 6-phosphate (5.0 mM), glucose-6-phosphate dehydrogenase (0.6 units/mL), and 0.25 mL of microsomal suspension in a total volume of 1.25 mL. After 1 h at 37 °C, the incubates were transferred to Amicon Centriflo CF 25 membrane cones (Amicon Corp. Lexington, MA) and centrifuged at 2000 rpm (<1000*g*) in a swinging-bucket centrifuge for 75 min at 4 °C. The filtrates were analyzed by HPLC on a μ-Bondapak C-18 column with 0.05 M Tris buffer, pH 7.4, and methanol (85:15) as eluent at a flow rate of 2 mL/min or on a Partisil 10-SAX column (25 cm × 4.6 mm i.d.) (Whatman) with 0.05 M NaOAc buffer, pH 5.0, at a flow rate of 2 mL/min.

Antitumor Screening. Mice weighing 18–20 g were obtained from Jackson Laboratories, Madison, WI. Murine leukemia P-388, both sensitive and resistant to 5-FU (P-388/0 and P-388/5-FU, respectively), was obtained from Dr. Arthur E. Bogden, Mason Research Institute, Worcester, MA. The P-388/0 and the P-388/5-FU tumors were maintained by weekly intraperitoneal passage in female DBA/2 and male BDF_1 mice, respectively. For antitumor screening, 1×10^6 cells were inoculated intraperitoneally into male BDF_1 mice. The test compounds, dissolved in 0.9% salaine, were administered intraperitoneally daily for 5 consecutive days beginning 24 h after tumor transplantation. Animals were observed for 60 days or until the time of death. Antitumor activity was determined by comparing the median survival time of treated animals (*T*) with that of saline-treated controls (*C*) and was expressed as a percentage increase in life span (% ILS), where % ILS = $(T/C - 1) \times 100$.

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Registry No. β-**1a**, 85954-65-0; α-**1a**, 85954-66-1; β-**1a** (3′-derivative), 85894-72-0; α-**1a** (3′-derivative), 85894-73-1; **1b**, 78000-60-9; β-**1c**, 85954-67-2; α-**1c**, 85954-68-3; β-**1c** (3′-derivative), 85894-74-2; α-**1c** (3′-derivative), 85894-75-3; **1d**, 67803-64-9; **6a**, 156-87-6; **6b**, 504-63-2; **7a**, 50-91-9; **7b**, 50-89-5; **8a**, 2059-38-3; **8b**, 21090-30-2; **9a**, 85894-76-4; **9b**, 85894-77-5; **10**, 13507-10-3; 5′-nucleotidase, 9027-73-0; alkaline phosphatase, 9001-78-9; phosphodiesterase, 9025-82-5.

N-(4-Substituted-thiazolyl)oxamic Acid Derivatives, a New Series of Potent, Orally Active Antiallergy Agents

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A series of *N*-(4-substituted-thiazolyl)oxamic acid derivatives were synthesized and tested for antiallergy activity in the rat PCA model. These compounds were conveniently prepared by treatment of the appropriate acetophenone with thiourea and iodine or by reaction of the chloroacetylbenzene with thiourea to give the corresponding aminothiazoles; subsequent condensation with ethyloxalyl chloride gave the thiazolyloxamates. Many of the analogues showed a 50% inhibition at <2 mg/kg po or <0.4 mg/kg iv and were significantly more potent than disodium cromoglycate, which in the rat PCA model is orally inactive and gives a 50% inhibition at 1.2 mg/kg iv. Hydrolysis of the oxamates generally resulted in enhanced activities, while substitution of the phenyl ring with a variety of substituents (e.g., 4-F, 4-OEt, and 4-$NHCOCH_3$) did not significantly enhance the activity of the unsubstituted phenyl derivative. One of the ethanolamine salts, *N*-[4-(1,4-benzodioxan-6-yl)-2-thiazolyl]oxamic acid ethanolamine salt (**61**, PRH-836-EA), has been selected for further pharmacological evaluation.

The clinical utility of disodium cromoglycate (DSCG) as a prophylactic antiallergy agent is based on its ability to inhibit the release of mediators initiated by antigen–antibody interactions. DSCG, however, suffers from the fact that it is not orally absorbed but must be administered as a finely powdered aerosol.[1] As a result, the focus of attention for more than a decade has been on the development of more potent, orally active DSCG-like compounds.[2,3]

In 1975, it was reported[4] that a number of oxanilates and *N*-heteroaryloxamates were active in the rat passive cutaneous anaphylaxis (PCA) assay. More recently, *N,N′*-

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