Journal of Medicinal Chemistry

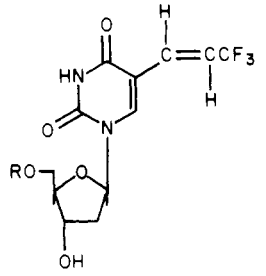
Volume 22, Number 4 April 1979

Communications to the Editor

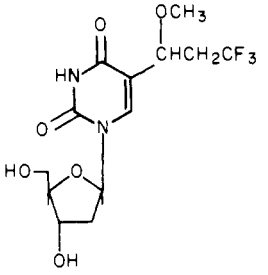
trans-5-(3,3,3-Trifluoro-1-propenyl)-2′-deoxyuridylate: A Mechanism-Based Inhibitor of Thymidylate Synthetase

Sir:

Thymidylate (dTMP) synthetase (EC 2.1.1.45) catalyzes the reductive methylation of 2′-deoxyuridylate (dUMP) to 2′-deoxythymidylate (dTMP) with the concomitant conversion of 5,10-methylenetetrahydrofolic acid to 7,8-dihydrofolic acid. Because this enzyme represents the sole de novo pathway for dTMP synthesis, it is not surprising that it has received much attention as a target for inhibitors with potential chemotherapeutic utility. Recent reviews have been published on the catalytic mechanism[1] and important inhibitors[2] of this enzyme. The most potent inhibitors of this enzyme thus far discovered are 5-substituted 2′-deoxyuridylates which act as *mechanism-based* inhibitors. An early event in the reaction catalyzed by dTMP synthetase involves attack of a nucleophilic group of the enzyme at the 6 position of dUMP to form a variety of 5,6-dihydropyrimidine intermediates which remain covalently bound to the enzyme during the catalytic sequence. The known mechanism-based inhibitors of dTMP synthetase undergo similar nucleophilic attack at the 6 position; as a consequence, the analogue either remains attached to the enzyme or a latent moiety at the 5 position of the inhibitor is activated. The mechanism-based inhibitors of dTMP synthetase thus far known include 5-fluoro-, 5-trifluoromethyl-, and 5-nitro-2′-deoxyuridylates.[1-4] Here, we describe a preliminary report of a new mechanism-based inhibitor of this enzyme related to CF_3dUMP: *trans*-5-(3,3,3-trifluoro-1-propenyl)-2′-deoxyuridylate, 1. In addition to its inhibition of dTMP



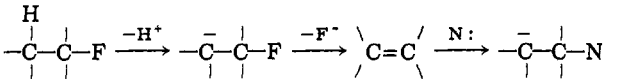
1, R = PO_3H_2
2, R = H



3

synthetase, this compound illustrates how latent reactive C–F bonds may be activated through vinylogous conjugation so they may *reach out* to form covalent bonds adjacent to or removed from the active site of the target enzyme. This principle should be applicable to a growing number of mechanism-based inhibitors which utilize latent-reactive C–F bonds.[5-7]

Scheme I



trans-5-(3,3,3-Trifluoro-1-propenyl)-2′-deoxyuridine (**2**) was prepared by the general method of Bergstrom and Ruth.[8] A mixture of 5-chloromercuri-2′-deoxyuridine (2.64 mmol) and Li_2PdCl_4 (2.9 mmol) was stirred in methanol under an atmosphere of 3,3,3-trifluoropropene for 3.5 h at room temperature. The mixture was filtered, treated with H_2S to precipitate PdS and HgS, and refiltered. Column chromatographies on silica gel eluting with methanol–chloroform and then on Bio-Gel P-2 eluting with H_2O resulted in two products. Recrystallization from H_2O yielded *trans*-5-(3,3,3-trifluoro-1-propenyl)-2′-deoxyuridine (**2**, 17%) and 5-(1-methoxy-3,3,3-trifluoropropyl)-2′-deoxyuridine (**3**, 36%). Compound **2** decomposes above 140 °C and shows λ_{max} (H_2O) 285 nm (ϵ 6500), 243 (7010). Anal. ($C_{12}H_{13}F_3N_2O_5$) C, H, N. Compound **3** shows λ_{max} (H_2O) 264 nm. Anal. ($C_{13}H_{17}F_3N_2O_6$) C, H, N. Both **2** and **3** showed single spots on TLC and had ^1H NMR and IR spectra consistent with their assigned structures. The nucleosides **2** and **3** showed single UV-absorbing peaks on LC using a Lichosorb C_{18} column (4.6 × 250 mm) with 40% MeOH–water (v/v) as eluant. The 5′-nucleotide 1 was prepared in ca. 30% yield using carrot phosphotransferase.[9] It was purified by DEAE-cellulose chromatography[10] followed by paper chromatography on Whatman no. 1 filter paper using isobutyric acid–0.5 N NH_4OH (10:6). Thymidylate synthetase was obtained from a methotrexate-resistant strain of *L. casei* and purified as previously described.[10] Initial velocities were determined as previously described.[11]

Although carbon–fluorine bonds are generally quite strong, when a transient or stable negative charge exists on the carbon adjacent to a C–F bond, β-elimination of fluoride ion may occur as depicted in Scheme I.[12] The resultant olefinic carbon originally bearing the fluorine atom may undergo attack by a nucleophile, and if additional fluorine atoms are attached to this carbon successive elimination–addition reactions may result in cleavage of all C–F bonds. In addition, C–F bonds which are labilized by this mechanism may be extended by carbon–carbon double bonds and retain their reactivity.[12]

CF_3dUMP, a potent inhibitor of dTMP synthetase, has been reported to irreversibly modify the enzyme from Ehrlich Ascites cells.[13] The corresponding pyrimidine, 5-trifluoromethyluracil, was found to acylate amines to give uracil-5-carboxamides, and it was suggested that a similar reaction might occur between CF_3dUMP and dTMP synthetase.[14] Model studies of the hydrolysis of 1-substituted 5-trifluoromethyluracils have demonstrated that

0022-2623/79/1822-0339$01.00/0

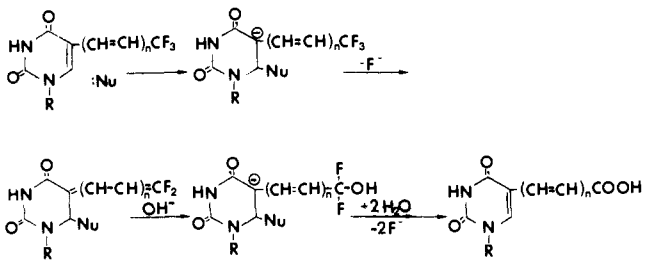


Figure 1. Mechanism of hydrolysis of CF_3dUrd (n = 0) and **2** (n = 1), where R is 1-β-deoxyribofuranosyl and :Nu is either OH^- or the anion of the 5′-hydroxyl of the sugar.

β-carbanion activation of the C–F bonds occurs upon addition of a nucleophile at the 6 position of the heterocycle to generate an incipient carbanion at C-5 and activate the CF_3 moiety.[15] We have found that the CF_3 group of **2** is only slightly less susceptible toward base-catalyzed hydrolysis than is CF_3dUrd (k_{obsd} = 1.13 h^{-1} vs. k_{obsd} = 1.37 h^{-1} in 1 N NaOH at 50 °C). That the CF_3 group of **2** was converted to the corresponding carboxylic acid was verified by comparison of its UV spectrum with that of an authentic sample obtained by hydrolysis of methyl *trans*-3-[5-(2′-deoxyuridyl)]propenoate.[16] However, in **3** the CF_3 group is completely inert toward base-catalyzed hydrolysis, as demonstrated by LC analysis. The general mechanism of hydrolysis of CF_3dUrd and **2** is depicted in Figure 1.

Compound **1** was a competitive inhibitor of dTMP synthetase (K_i = 8.6 μM) when assayed in the presence of 5,10-methylenetetrahydrofolate under standard conditions.[11] When variable excess amounts of **1** were incubated with dTMP synthetase at 25 °C in the *absence* of 5,10-methylenetetrahydrofolate there was a first-order decrease in enzyme activity which is protected by the substrate dUMP. A replot of these data ($1/k_{obsd}$ vs. $1/[I]$) is linear and provides the dissociation constant, $K_d = 2.5 \times 10^{-5}$ M, and the unimolecular rate constant for inactivation, k = 0.133 min^{-1}. The fact that K_i is lower than the K_d is a result of the synergistic effect of 5,10-methylenetetrahydrofolate on nucleotide binding.[11] Preliminary data demonstrates that inactivation also occurs in the presence of cofactor. That the CF_3 moiety of **1** had been transformed is indicated by UV spectral evidence. Compound **1** has λ_{max} at 285 and 244 nm with λ_{min} at 262. A difference spectra of **1** and dTMP synthetase vs. dTMP synthetase shows a single maximum at 272 nm; denaturation of the enzyme with 3% $NaDodSO_4$ does not effect a change of the UV spectrum of the product. A similar result is obtained when 5,10-methylenetetrahydrofolate is included in the reaction mixture. Although the nature of the product has not yet been established, this result clearly demonstrates that covalent modification of the 5 substituent of **1** occurs upon interaction with the enzyme.

While the results described here are too preliminary to allow definitive mechanistic conclusions, they clearly demonstrate an irreversible or pseudoirreversible inhibition of dTMP synthetase initiated by nucleophilic attack at the 6 position of **2**; current evidence suggests that the mechanism of inhibition by this analogue may be similar to that of CF_3dUMP. In addition, the fact that the reactivity of C–F bonds can be retained by vinylogous conjugation adds great diversity to such inhibitors, as the latent reactive group can be extended until it is suitably juxtaposed to a nucleophile of the target enzyme to react with it. Utilization of latently reactive C–F bonds as mechanism-based inhibitors has achieved much recent success,[5-7] and vinylogous activation of the C–F bond should increase the utility of such analogues.

Acknowledgment. This work was supported by USPHS Grants CA 14394 (D.V.S.) and CA 2-493 (D.E.B.) from the National Cancer Institute. D.V.S. is the recipient of a NIH Career Development Award.

References and Notes

(1) A. L. Pogolotti, Jr., and D. V. Santi in "Bioorganic Chemistry", Vol. 1, E. E. van Tamelen, Ed., Academic Press, New York, N.Y., pp 277–311.
(2) P. V. Danenberg, *Biochim. Biophys. Acta*, **473**, 73 (1977).
(3) D. V. Santi, A. L. Pogolotti, Jr., T. L. James, Y. Wataya, K. M. Ivanetich, and S. S. M. Lam, *ASC Symp. Ser.* **no. 28**, 57–76.
(4) A. Matsuda, Y. Wataya, and D. V. Santi, *Biochem. Biophys. Res. Commun.*, **84**, 654 (1978).
(5) R. B. Silverman and R. H. Abeles, *Biochemistry*, **15**, 4718 (1976).
(6) R. B. Silverman and R. H. Abeles, *Biochemistry*, **16**, 5515 (1977).
(7) E. Wong and C. Walsh, *Biochemistry*, **17**, 1313 (1978).
(8) D. E. Bergstrom and J. L. Ruth, *J. Am. Chem. Soc.*, **98**, 1587 (1976).
(9) C. L. Harvey, E. M. Clericuzio, and A. I. Nussbaum, *Anal. Biochem.*, **36**, 413 (1970).
(10) Y. Wataya and D. V. Santi, *Methods Enzymol.*, **46**, 307 (1977).
(11) Y. Wataya, D. V. Santi, and C. Hansch, *J. Med. Chem.*, **20**, 1469 (1977).
(12) T. T. Sakai and D. V. Santi, *J. Med. Chem.*, **16**, 1079 (1973).
(13) P. Reyes and C. Heidelberger, *Mol. Pharmacol.*, **1**, 14 (1965).
(14) C. Heidelberger, D. G. Parsons, and D. C. Remy, *J. Med. Chem.*, **7**, 1 (1964).
(15) D. V. Santi and T. T. Sakai, *Biochemistry*, **10**, 3598 (1971).
(16) Synthesized by the method described in ref 8; D. E. Bergstrom and J. L. Ruth, unpublished results.

Yusuke Wataya, Akira Matsuda, Daniel V. Santi*
Department of Biochemistry and Biophysics and Department of Pharmaceutical Chemistry University of California, San Francisco, California 94143

Donald E. Bergstrom, Jerry L. Ruth
Department of Chemistry University of California, Davis, California 95616

Received November 10, 1978