addition, the model allows one to go beyond the correlation between activity and *hydrophilicity* and to suggest that some of the reasons for this correlation is that the more hydrophilic substituents include many that are effective proton donors in hydrogen bonds to the DNA. The examination of substituents such as 4-SO_3^- would further validate this suggestion, since such a compound would be very hydrophilic but not a proton donor. The two outliers in the current Hansch analysis are R = $NHCH_2CO_2H$, which is likely to be anionic at physiological pH and, thus, less effective than suggested by its hydrophilicity, and R = $NHCONH_2$, for which our model cannot suggest a reason for inactivity. It is possible that the presence of the CO prevents as effective hydrogen bonding as found in the OH and NH_2 substituents.

An obvious problem in correlating biological activity with models based on molecular mechanics is the separation of binding effects from physicochemical properties such as partition coefficient that can cause parallel changes in activity. It should be possible to include binding energies in the multiple linear regression analysis. Unfortunately, the mitomycins are not a good family of compounds for this purpose because only one type of substituent tried thus far, the N^7-phenyl derivatives, gave any useful regression analysis. At this time, one can at least design structural modifications that fit the model well while making certain that they enhance hydrophilicity and do not increase the difficulty of quinone reduction.

Application of the mitomycin binding model to nonmitomycins such as **16** is the least well-founded aspect of our study, yet it might hold the greatest promise for future drug design. This approach should be tested further by the synthesis and evaluation of simpler molecules that can interact with the same binding sites as mitomycins.

Acknowledgment. This investigation was supported by the NIH, National Research Service Award CA-07822 from the National Cancer Institute (to W.R.) and by National Cancer Institute Grant CA-25644 (to P.K.). We also gratefully acknowledge use of the facilities of the UCSF computer Graphics Laboratory (R. Langridge, director, and T. Ferrin, facility manager), supported by NIH RR-1081, and helpful discussions with Professor I. D. Kuntz.

Registry No. 1, 50-07-7; 1·2GC2ATGC2, 102046-81-1; 5·2GC10, 102046-79-7; 10·2GC2ATGC2, 102046-83-3; 10·2GC10, 102130-36-9; 11·2GC10, 102046-85-5; 12·2GC10, 102046-87-7; 13-2GC10, 102072-56-0; 16·2GC10, 102046-89-9; adenine, 73-24-5.

Supplementary Material Available: Quantum mechanically derived charges for mitomycin fragments, conformational analysis of distorted segments in the sugar–phosphate backbone of DNA, and stereo pairs for the monocovalent adducts of mitomycin C analogues to $d(GC10)_2$ (7 pages). Ordering information is given on any current masthead page.

Folate Analogues. 25. Synthesis and Biological Evaluation of N^{10}-Propargylfolic Acid and Its Reduced Derivatives[1]

Maryam Ghazala,[†] M. G. Nair,*[†] Tahereh R. Toghiyani,[†] R. L. Kisliuk,[‡] Y. Gaumont,[‡] and T. I. Kalman[§]

Department of Biochemistry, University of South Alabama, Mobile, Alabama 36688, Department of Biochemistry and Pharmacology, Tufts University School of Medicine, Boston, Massachusetts 02111, and Department of Medicinal Chemistry, State University of New York, Buffalo, New York 14260. *Received September 3, 1985*

N^{10}-Propargylfolic acid (**2**), which is the closest pteridine analogue of the thymidylate synthase inhibitor N^{10}-propargyl-5,8-dideazafolic acid (PDDF), was synthesized starting from diethyl [*p*-(*N*-propargylamino)benzoyl]-L-glutamate (**5**) and *N*-(3-bromo-2-oxopropyl)phthalimide (**8**). The 7,8-dihydro derivative of propargylfolic acid served as a synthetic substrate of *Lactobacillus casei* dihydrofolate reductase. Propargylfolic acid and its reduced derivatives were weak inhibitors of *L. casei* thymidylate synthase compared to PDDF. All derivatives of propargylfolate were active against the growth of *Streptococcus faecium*, but with the exception of 7,8-dihydropropargylfolic acid, all were inactive against *L. casei*. Although less potent than PDDF, marked inhibition of thymidylate synthase by **2** was observed in permeabilized L1210 cells.

Specific inhibitors of thymidylate synthase[2] (EC 2.1.1.45) are useful chemotherapeutic agents for the treatment of various forms of human cancers.[2-4] Most inhibitors of this enzyme are analogues of the nucleotide substrate deoxyuridine monophosphate (dUMP), and relatively few are coenzyme analogues structurally related to folic acid (**1**). Recently, Jones and co-workers reported[5] that the quinazoline derivative 5,8-dideaza-N^{10}-propargyl folic acid (PDDF) is an excellent inhibitor of L1210 thymidylate synthase and that it exhibited remarkable activity against the L1210 tumor in vivo. We have subsequently determined the antifolate activity of this compound using both methotrexate- (MTX-) sensitive and resistant strains of *Lactobacillus casei* (ATCC 7469) and *Streptococcus faecium* (ATCC 8043).[6] The propargyl derivative was as active as MTX against *S. faecium* and showed good activity against the MTX-resistant strain of this organism. It was a very powerful inhibitor of *L. casei* and *S. faecium* thymidylate synthases. The poly-γ-glutamyl derivatives of PDDF were remarkably more active than the parent compound in inhibiting thymidylate synthase derived from several species,[7] including man.[8] Since the introduction

[†] University of South Alabama.
[‡] Tufts University School of Medicine.
[§] State University of New York.

(1) Previous paper: Nair, M. G. *J. Org. Chem.* **1985**, *50*, 1879.
(2) Friedkin, M. *Adv. Enzymol.* **1973**, *38*, 235.
(3) Santi, D. V. *J. Med. Chem.* **1980**, *23*, 103.
(4) Heidelberger, C.; Danenberg, P. V.; Moran, R. G. *Adv. Enzymol.* **1983**, *54*, 57.
(5) Jones, T. R.; Calvert, A. H.; Jackman, A. L.; Brown, S. J.; Jones, M.; Harrap, K. R. *Eur. J. Cancer* **1981**, *17*, 11.
(6) Nair, M. G.; Salter, D. C.; Kisliuk, R. L.; Gaumont, Y.; North, G.; Sirotnak, F. M. *J. Med. Chem.* **1983**, *26*, 605.
(7) Kisliuk, R. L.; Gaumont, Y.; Kumar, P.; Coutts, M.; Nair, M. G.; Nanavati, N. T.; Kalman, T. I. In *Proceedings of the Second Workshop on Folyl and Antifolyl Polyglutamates*; Goldman, I. D., Ed.; Praezer Scientific: New York, 1985; pp 319–328.
(8) Cheng, Y. C.; Dutschman, G. E.; Starnes, M. C.; Fisher, M. H.; Nanavati, N. T.; Nair, M. G. *Cancer Res.* **1985**, *45*, 598.

0022-2623/86/1829-1263$01.50/0

of a propargyl substituent at the 5,8-dideazafolate framework had such a profound influence on the thymidylate synthase inhibition, it was of interest to examine the biological effects of substituting the hydrogen at the 10-position of folic acid with a propargyl substituent.

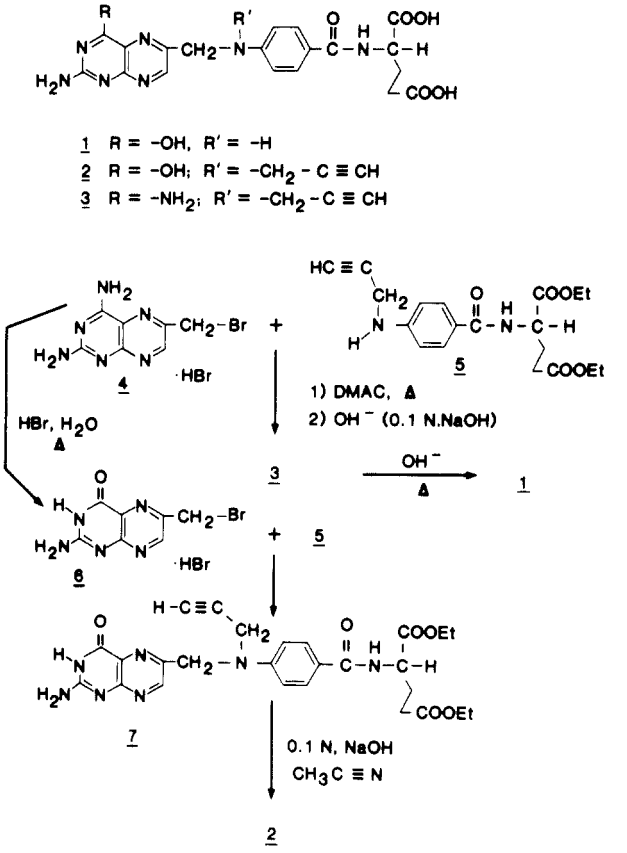
A second rationale for the synthesis of N^{10}-propargylfolic acid (2) was the following: A large number of MTX-resistant tumors are known to possess elevated levels of the enzyme dihydrofolate reductase (DHFR) without the concurrent elevation of other folate-dependent enzymes.[9,10] Therefore, if 7,8-dihydro-N^{10}-propargylfolate can serve as a synthetic substrate of DHFR, and the resulting tetrahydro derivative with the natural configuration at C-6 can either inhibit thymidylate synthase effectively or interfere with tetrahydrofolate utilization, it might be capable of exhibiting selective toxicity against those MTX-resistant tumors having high levels of DHFR.[9,11] As part of a continuing program[12] aimed at developing synthetic substrates of DHFR that are potential inhibitors of thymidylate synthase, this paper details the chemical synthesis and biological evaluation of N^{10}-propargylfolic acid and its reduced derivatives.

Chemistry

Our initial synthetic strategy was the preparation of N^{10}-propargylaminopterin (3)[13] and its subsequent deamination at the 4-position to the target compound. Reaction of 6-(bromomethyl)-2,4-diaminopteridine hydrobromide (4)[14] with diethyl *p*-[(*N*-propargylamino)benzoyl]-L-glutamate (5)[5,6] in dimethylacetamide (DMAC) at elevated temperatures gave the expected alkylation product, which on mild hydrolysis gave N^{10}-propargylaminopterin (3). Attempted deaminations of 3 to the title compound, propargylfolic acid (2), under a variety of conditions were unsuccessful. The experimental conditions that were required to induce deamination at the 4-position of 3 also resulted in depropargylation at the 10-position to yield folic acid (1) as the exclusive product. After a series of experiments, it was concluded that selective deamination of 3 without depropargylation is difficult, and therefore, this strategy for the synthesis of 2 was abandoned. Although the observed depropargylation appeared to be an interesting transformation with possible synthetic applications, alternate strategies were called for the synthesis of 10-propargylfolic acid (2).

Treatment of 6-(bromomethyl)-2,4-diaminopteridine hydrobromide[14] with aqueous hydrogen bromide at elevated temperature was reported to give the corresponding deamination product[14] 2-amino-6-(bromomethyl)-4-hydroxypteridine hydrobromide (6). Compound 6, prepared according to the procedure of Piper and Montgomery,[14] was not soluble in a suitable solvent to carry out the alkylation reaction with 5 at a desired temperature. Although a small amount of 2 was indeed isolated (vide infra)

Scheme I



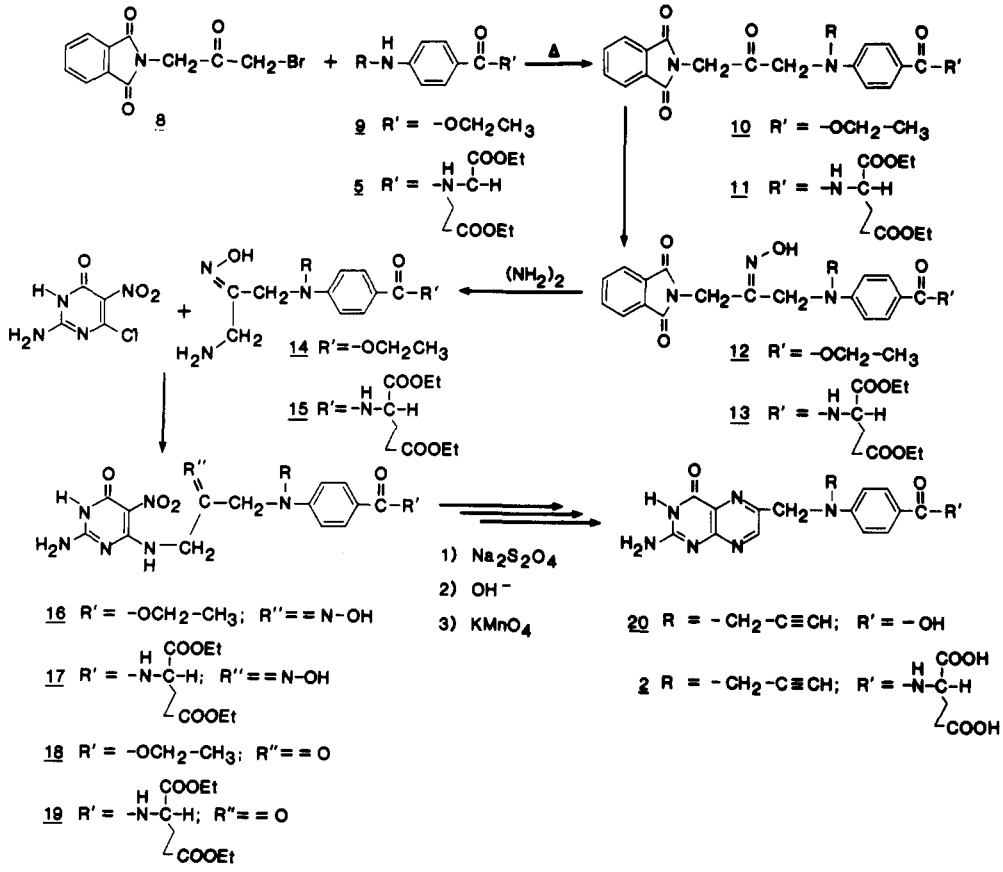
by hydrolysis of the alkylation product 7 obtained from such a reaction carried out in DMAC or Me_2SO at high temperatures, this method was found unsuitable for the preparation of a large amount of 2, which was required for obtaining the preliminary biological data (Scheme I). Due to the unacceptability of these two apparently simple procedures for the preparation of the required amount, we decided to embark upon a more elaborate synthetic strategy for the preparation of the title compound 2.

The use of substituted pthalimide intermediates for the construction of the desired side chains of several classical analogues of folic acid has been previously reported from this laboratory.[1,15] These side chains were masked α-amino ketones possessing either a substituted ethyl ester of *p*-aminobenzoic acid or a substituted diethyl ester of (*p*-aminobenzoyl)-L-glutamic acid, which are obtainable from their respective phthalimide intermediates by hydrazinolysis. The use of the former intermediate in the classical Boon–Leigh procedure of pteridine synthesis will lead to the formation of a pteroic acid analogue, while the latter will give rise to the respective folate derivative. Since both N^{10}-propargylpteroic acid and N^{10}-propargylfolic acid were biologically relevant, due to the potential antibacterial activity of the former and the antitumor activity of the latter, we decided to synthesize and evaluate both of these compounds by modifications of the Boon–Leigh procedure.

The preparation of *N*-(3-bromo-2-oxopropyl)phthalimide has been previously reported.[1,16] Fusion of this bromo ketone 8 with ethyl *p*-(*N*-propargylamino)benzoate (9) or diethyl [*p*-(*N*-propargylamino)benzoyl]-L-glutamate (5) gave the corresponding phthalimide intermediates 10 and 11, respectively, in acceptable yields after column chro-

(9) Friedkin, M.; Crawford, E. J.; Plante, L. *Ann. N.Y. Acad. Sci.* **1971**, *186*, 209.
(10) Misra, D. K.; Humphreys, S. R.; Friedkin, M.; Goldin, A.; Crawford, E. J. *Nature (London)* **1961**, *189*, 39.
(11) Nair, M. G.; Saunders, C.; Chen, S. Y.; Kisliuk, R. L.; Gaumont, Y. *J. Med. Chem.* **1980**, *23*, 59.
(12) Nair, M. G.; Salter, O. C.; Kisliuk, R. L.; Gaumont, Y.; North, G. *J. Med. Chem.* **1983**, *26*, 1164.
(13) Piper, J. R.; McCaleb, G. S.; Montgomery, J. A.; Kisliuk, R. L.; Gaumont, Y.; Sirotnak, F. M. *J. Med. Chem.* **1982**, *25*, 877.
(14) Piper, J. R.; Montgomery, J. A. *J. Org. Chem.* **1977**, *42*, 208. Also see: Montgomery, J. A.; Rose, J. D.; Temple, C., Jr.; Piper, J. R. In *Chemistry and Biology of Pteridines*; Pfleiderer, W., Ed.; Walter De Gruyter: Berlin, New York, 1975; p 485.
(15) Nair, M. G.; Chen, S. Y.; Kisliuk, R. L.; Gaumont, Y.; Strumpf, D. *J. Med. Chem.* **1979**, *22*, 850.
(16) Nair, M. G.; Campbell, P. T.; Baugh, C. M. *J. Org. Chem.* **1975**, *40*, 1745.

Scheme II



matography over silica gel. These intermediates were converted to their respective oximes **12** and **13** by reaction with hydroxylamine hydrochloride in a pyridine–methanol mixture.[1,15,16] The oximes, which were obtained as a mixture of the syn and anti isomers, after characterization, were subjected to hydrazinolysis. The hydrazinolysis products **14** and **15** were allowed to react with an equimolar amount of 2-amino-6-chloro-4-hydroxy-5-nitropyrimidine[17] in refluxing ethanol containing 1 equiv of 4-methylmorpholine. The pyrimidine intermediates **16** and **17** thus obtained respectively from **14** and **15** were deprotected at the carbonyl function by hydrolysis of the oxime at 60 °C with a 1:1 mixture of trifluoroacetic acid and 1.0 N HCl for 20 min.

The deprotected pyrimidine intermediates **18** and **19** were reduced with sodium dithionite in aqueous DMF, and the corresponding amino compounds were subsequently cyclized and simultaneously hydrolyzed to the respective dihydropteridines. These compounds, without isolation, were oxidized with a solution of 5% $KMnO_4$ in methanolic sodium hydroxide to the final products, N^{10}-propargylpteroic acid (**20**) and N^{10}-propargylfolic acid (**2**), respectively. Both compounds were purified by ion-exchange chromatography over DEAE cellulose by elution with a linear NaCl gradient from 0 to 0.5 M at pH 7.00. The NMR spectra of **20** and **2** in TFA were very similar to those of pteroic acid and folic acid and had additional signals due to the propargyl group appearing at the expected chemical shifts. These spectral observations were in complete harmony with the structures as written for **2** and **20**.

Biological Evaluation and Discussion

The primary reason for the synthesis of N^{10}-propargylfolate was its close analogy with the quinazoline analogue PDDF, which, with the exception of its poly-γ-glutamyl derivatives,[7,8] was shown to be the most potent inhibitor of L1210 thymidylate synthase of the coenzyme analogue class developed thus far.[5] Although it has been known for some time that the 6-substituted quinazoline analogues are better inhibitors of thymidylate synthase than their pteridine counterparts,[18-20] it was of interest to evaluate the magnitude of this difference in inhibition caused by the replacement of the fused benzene ring in PDDF with a fused pyrazine ring. Since these rings are both isosteric compatable and planar in geometry, any significant differences in their thymidylate synthase inhibitory potency must be due to the replacement of the methenyl groups of PDDF at the 5- and 8-positions with nitrogen atoms, which in turn will be accompanied by dipolar and hydrophobic perturbations. Comparative biological data obtained with PDDF and 10-propargylfolate were expected to be useful for the futute design of inhibitors of thymidylate synthase of the coenzyme analogue class. Due to these reasons the preliminary biological evaluations of **2** were undertaken using the following biological test systems.

(a) Effects on Microbial Growth. N^{10}-Propargylfolate derivatives were effective inhibitors of the growth of *S. faecium* (Table I). Reduction of 10-propargylfolate to its dihydro and tetrahydro forms enhanced inhibitory potency for *S. faecium* 5-fold to a level comparable with that of methotrexate. Dihydro-10-propargylfolate was the only compound of the series active against methotrexate-re-

(17) Stuart, A.; Wood, H. C. S. *J. Chem. Soc.* **1963**, 4186.

(18) Jones, T. R. *Eur. J. Cancer* **1980**, *16*, 707.

(19) Carlin, S. C.; Rosenberg, R. N.; Vandeventer, L.; Friedkin, M. *Mol. Pharmacol.* **1974**, *10*, 194.

(20) Calvert, A. H.; Jones, T. R.; Dady, P. J.; Grzelakowska-Sztabert, B.; Paine, R. M.; Taylor, G. A.; Harrap, K. R. *Eur. J. Cancer* **1980**, *16*, 713.

Table I. Summary of Microbiological and Enzyme Tests on N^{10}-Propargylfolate Derivatives

compd	IC_{50}, ng/mL				IC_{50}, M (enzymes derived from *L. casei*)	
	Streptococcus faecium		*Lactobacillus casei*		dihydrofolate reductase	thymidylate synthase
	ATCC 8043	MTX-resist.	ATCC 7469	MTX-resist.		
PDDF	0.12	18	0.29	2000	2.2×10^{-5}	1.3×10^{-8}
N^{10}-propargylpteroic acid	0.76	>2000	>2000	>2000	$>1 \times 10^{-4}$	$>2.9 \times 10^{-4}$
N^{10}-propargylfolic acid	0.56	>2000	>2000	>2000	$>1 \times 10^{-4}$	3.9×10^{-6}
7,8-dihydro-N^{10}-propargylfolic acid[a]	0.13	24	36	>2000	$>5 \times 10^{-5}$	5.0×10^{-5}
5,6,7,8-tetrahydro-N^{10}-propargylfolic acid[b]	0.08	>2000	>2000	>2000	$>1.4 \times 10^{-4}$	3.2×10^{-5}
MTX	0.12	2900	0.02	>500000	8.0×10^{-9}	6.0×10^{-5}

[a] 7,8-Dihydro-N^{10}-propargylfolate is a substrate for *L. casei* dihydrofolate reductase, giving 40% the rate obtained with 7,8-dihydrofolate when tested under the same conditions. [b] 5,6,7,8-Tetrahydro-N^{10}-propargylfolate was prepared enzymatically using *L. casei* dihydrofolate reductase and therefore contains the natural configuration at carbon 6.

sistant *S. faecium* as well as against *L. casei*. None of the compounds showed activity against methotrexate-resistant *L. casei*. The observed potent inhibitory activity of N^{10}-propargylpteroate against *S. faecium* is consistent with the fact that this organism can convert pteroate to folate.[21]

(b) Effects on Enzyme Activity. Inhibition of *L. casei* dihydrofolate reductase by N^{10}-propargylfolate derivatives was negligible. Dihydro-N^{10}-propargylfolate was found to be a substrate for this enzyme, giving 40% of the rate obtained with dihydrofolate. N^{10}-Propargylfolate inhibited *L. casei* thymidylate synthase 50% at 3.9×10^{-6} M (Table I). Dihydro-N^{10}-propargylfolate and tetrahydro-N^{10}-propargylfolate were respectively 5- and 3-fold less inhibitory for this enzyme than was N^{10}-propargylfolate. Tetrahydro-N^{10}-propargylfolate was inactive as a substrate for *L. casei* thymidylate synthase.

Since N^{10}-propargylfolate derivatives were found to be poor inhibitors of dihydrofolate reductase and thymidylate synthase, alternative inhibitory mechanisms must be considered to explain the observed growth inhibition. Possibilities include (1) metabolism of N^{10}-propargylfolate derivatives to inhibitory compounds by modification of the propargyl group or by the addition of γ-glutamate residues, 2) inhibition of serine hydroxymethyltransferase, the third enzyme of the thymidylate cycle, (3) inhibition of folate uptake, and (4) inhibition of GAR transformylase or AICAR transformylase.

(c) Inhibitory Studies Utilizing Intact and Permeabilized L1210 Cells. The enzyme inhibitory activity of **2** in intact L1210 cells was determined as described previously.[22] In contrast to the strong inhibition of thymidylate synthase activity by PDDF ($IC_{50} = 8.2 \times 10^{-6}$ M), **2** had no significant effect in this system even at 1 mM concentration; 3 mM of **2** caused only 25% inhibition.

The direct effect of **2** on L1210 thymidylate synthase was determined in comparison with N^{10}-methylfolate, unsubstituted folate, and PDDF after permeabilization of the cells with dextran sulfate.[23] The results are summarized in Table II. Marked inhibition of thymidylate synthase by **2** was observed ($IC_{50} = 7.5$ μM), although this analogue was less potent than its 5,8-dideaza derivative (PDDF; $IC_{50} = 0.022$ μM). The importance of the N-10 substituent is indicated by the much lower inhibitory activity of N^{10}-methylfolate ($IC_{50} = 66$ μM) and folate ($IC_{50} = 1.8$ mM). A similar trend was observed previously with the corresponding quinazoline series,[24] N^{10}-methyldideazafolate being 200-fold less active than PDDF and 5,8-dideazafolate even less active ($IC_{50} = 10$ μM), comparable to **2** in the present study.

Table II. Inhibition of Thymidylates Synthase Activity in Intact and Permeabilized L1210 Cells

compd	IC_{50}, M	
	intact	permeabilized[a]
PDDF	8.2×10^{-6}	2.2×10^{-8}
N^{10}-propargylfolate (**2**)	$>3 \times 10^{-3}$	7.5×10^{-6}
N^{10}-methylfolate	$>10^{-3}$	6.6×10^{-5}
folate	$>10^{-3}$	1.8×10^{-3}

[a] L1210 cells[22] were permeabilized[23] by incubation at 4 °C for 20 (or 40) min in the presence of 400 μg/mL of Na Dextran sulfate (M_r 500 000; Pharmacia) followed by washing and resuspension. Enzyme activity was determined at pH 7.4 by measuring the tritium released into water[22] from I μM 5-[^{3}H]-dUMP by 5×10^6 cells during 30 min at 37 °C. The assay mixture contained 0.4 mM *d,l*-L-methylenetetrahydrofolate.

It is apparent from the biological data that propargylfolic acid is considerably weaker in biological activity compared to PDDF in all the test systems examined, except *S. faecium*. The 7,8-dihydro derivative of **2** had comparable activity to PDDF and MTX against the growth of this organism, and the enzymatically reduced tetrahydro derivative was even more active than either PDDF or MTX against the MTX-sensitive strain of *S. faecium*. A lower degree of thymidylate synthase inhibition by **2** was not unexpected, but the magnitude of this difference between PDDF and **2** is striking. As anticipated, 7,8-dihydropropargylfolate served as a synthetic substrate of DHFR, but the tetrahydro derivative thus obtained was not an effective inhibitor of thymidylate synthase. From these data, it appears that the presence of the electronegative N atoms at the 5- and 8-positions of classical folate analogues is detrimental to thymidylate synthase inhibition irrespective of the substituents present at the 10-position.

Experimental Section

Melting points were determined on a Fisher Model 355 digital melting point analyzer. NMR spectra were run in $CDCl_3$ or CF_3COOH on a 90-MHz Perkin-Elmer spectrometer with Me_4Si as an internal standard unless otherwise mentioned. Field strength of the various proton resonance is expressed in parts per million, and peak multiplicity is depicted as follows: s, singlet; d, doublet; t, triplet; q, quartet; c, unresolved multiplet, the center of which is given. Ultraviolet spectra were recorded on a Bausch and Lomb spectronic 2000 spectrometer interfaced with a Commodore superpet computer. Elemental analysis were done by Galbraith Laboratories, Inc., Knoxville, TN. Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements or functions were within ±0.4% of the theoretical values.

Ethyl *p*-(*N*-Propargylamino)benzoate (9). A mixture of 13.2 g (80 mmol) of ethyl *p*-aminobenzoate, 250 mL of reagent alcohol, and 11.2 g (80.5 mmol) of K_2CO_3 contained in a 500-mL

(21) Stokstad, E. L. R. *The Vitamins*; Sebrell, W. H., Jr., Harris, R. S.; Eds.; Academic: New York, 1954; Vol. III p 143.
(22) Yalowich, J. C.; Kalman, T. I. *Biochem. Pharmacol.* **1985**, *34*, 2319.
(23) Kucera, R.; Paulus, H. *Arch. Biochem. Biophys.* **1982**, *214*, 102.
(24) Kalman, T. I.; Hsiao, M. C. *Proc. Am. Assoc. Cancer. Res.* **1984**, *25*, 311.

round-bottomed flask was heated in an oil bath for 18 h at 70 °C under vigorous stirring. The reaction mixture was cooled, filtered, and concentrated under reduced pressure to give a dark gum that was dissolved in 100 mL of EtOAc. The EtOAc layer was washed with water, dried over anhydrous Na_2SO_4, and evaporated. Repeated column chromatography of the crude mixture over silica gel CC 7 using 25% petroleum ether in methylene chloride as the eluting solvent gave white crystals: yield 10.38 g (63.9%); mp 76–78 °C; NMR ($CDCl_3$) δ 8.0, 6.72 (d, 4 H, Ar), 4.42 (q, 2 H, ethoxy), 4.06 (d, 2 H, methylene), 2.33 (s, 1 H, acetylene), 1.46 (t, 3 H, methyl). Anal. ($C_{12}H_{13}NO_2$) C, H, N.

Ethyl *p*-[*N*-Propargyl-*N*-(3-phthalimido-2-oxopropyl)-amino]benzoate (10). A finely ground mixture of 6.9 g (24.4 mmol) of *N*-(3-bromo-2-oxopropyl)phthalimide (8) and 10 g (49.2 mmol) of 9 was melted under stirring in a 100-mL round-bottomed flask placed in a silicone oil bath at 80 °C. After 3 h a bright yellow solid was formed that was dissolved in 100 mL of CH_2Cl_2, washed with water, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The more polar desired product was isolated as pale yellow crystals by column chromatography over silica gel 60 (70–230 mesh) using 1% MeOH in CH_2Cl_2 as the eluting solvent: yield 4.44 g (44.6%); mp 164–167 °C; NMR ($CDCl_3$) δ 8.02, (d, 2 H, ar), 7.83 (c, 4 H, phthalimide), 6.80 (d, 2 H, Ar), 4.62 (s, 4 H, $NCH_2(C{=}O)CH_2$), 4.29 (c, 4 H, ethoxy and propargyl methylene), 2.4 (s, 1 H acetylene), 1.38 (t, 3 H, methyl). Anal. ($C_{23}H_{20}N_2O_5$) C, H, N.

Diethyl [*p*-[*N*-Propargyl-*N*-(3-phthalimido-2-oxopropyl)amino]benzoyl]-L-glutamate (11). A round-bottomed flask containing a mixture of 1.13 g of bromo ketone 8 (4 mmol), 1.44 g of the propargylamine 5 (4 mmol), 0.16 g (4 mmol) of MgO, and 5 mL of dimethylacetamide (DMAC) was placed in an oil bath maintained at 75 °C and stirred for 48 h. The reaction mixture was poured over 100 g of crushed ice, triturated, and filtered. The precipitate thus obtained was dissolved in 100 mL of CH_2Cl_2, dried over anhydrous Na_2SO_4, and concentrated by rotary evaporation to 25 mL. This crude reaction product was applied on a column made of silica gel (100 g) and eluted with CH_2Cl_2. The product was more polar than either of the starting materials, as judged by TLC (1% MeOH–$CHCl_3$) on silica gel plates. The fractions containing this more polar material were pooled and evaporated, and the residue was recrystallized from MeOH: mp 141–142 °C; yield 1.15 g (50%); NMR ($CDCl_3$) δ 8.8 (c, 6 H, phthalimido, Ar), 6.75 (d, 2 H Ar), 4.6 (s, 2 H propargylmethylene), 4.3 (s, 2 H, methylene), 4.15 (s, 2 H, methylene), 4.8 (1 H, α proton of glutamate), 4.2 (c, 4 H, ethoxy), 2.5–2.0 (c, 4 H, glutamate), 1.3 (2 superimposed t, 6 H, ethoxy). Anal. ($C_{30}H_{31}N_3O_8$) C, H, N.

Ethyl *p*-[*N*-Propargyl-*N*-(3-phthalimido-2-oxopropyl)-amino]benzoate Oxime (12). A 6.87-g (17.0 mmol) sample of 10 and 1.74 g (25.00 mmol) of hydroxylamine hydrochloride were dissolved in 100 mL of 1:1 MeOH–pyridine, and the mixture was heated under reflux with stirring under nitrogen for 2 h. Evaporation of the reaction mixture resulted in a gum that was partitioned between EtOAc and water. The oxime was obtained from the organic layer as a pale yellow solid upon evaporation, which was crystallized from MeOH as a mixture of two isomers: yield 5.74 g (80.5%); mp 148–149 °C; NMR ($CDCl_3$) δ 7.68 (c, 6 H, 2 Ar and 4 phthalimido), 6.7 (d, 2 H, Ar), 4.58 (S, 4 H, methylenes of phthalimide moiety), 4.25 (q, 2 H, ethoxy), 4.03 (s, 2 H, propargylmethylene), 2.55 (s, 1 H, acetylene), 1.34 (t, 3 H, methyl). Anal. ($C_{23}H_{21}N_3O_5$) C, H, N.

Diethyl [*p*-[*N*-Propargyl-*N*-(3-phthalimido-2-oxopropyl)amino]benzoyl]-L-glutamate Oxime (13). To a solution of 50 mL of pyridine and 50 mL of MeOH were added 2.24 g (4 mmol) of compound 11 and 0.313 g (4.5 mmol) of hydroxylamine hydrochloride, and the resultant mixture heated under reflux for 1.5 h. The reaction mixture was evaporated under reduced pressure, and the gummy residue was dissolved in 100 mL of EtOAc. The EtOAc layer was washed three times with 50-mL portions of water, dried over Na_2SO_4, and evaporated to obtain the expected syn and anti mixture of the oxime (TLC, NMR) in quantitative yield. Examination of this product by TLC (silica gel plates, 3% MeOH–$CHCl_3$) clearly revealed the formation of two more polar products in 3:2 ratio corresponding to the two isomers. An NMR spectrum of this compound in $CDCl_3$ exhibited signals corresponding to the three methylene groups as three doublets with each component of the doublets in a 3:2 ratio of integrated intensity due to the presence of the isomers. Attempts to recrystallize this oxime were unsuccessful, and it was used for the next step without further characterization.

Ethyl *p*-[*N*-Propargyl-*N*-(3-aminoacetonyl)amino]-benzoate Oxime (14). In a 500-mL three-neck flask a homogeneous solution of 5.64 g (13.46 mmol) of 12 in 300 mL of MeOH was made by refluxing under nitrogen and cooled to 50 °C. To this solution in a nitrogen atmosphere was added 720 mg (22.46 mmol) of 95% hydrazine in three portions at an interval of 24 h each. After 72 h, TLC showed the complete conversion of the starting material to the products. The reaction mixture was acidified with 23 mL of 1 N HCl and concentrated at 30 °C under reduced pressure to a small volume, triturated with 100 mL of dilute HCl, and filtered. The filtrate was adjusted to pH 8.9 by NH_4OH whereupon a gum was obtained that was exhaustively extracted with 200 mL of EtOAc. After the EtOAc layer was washed with water, it was dried over anhydrous Na_2SO_4, filtered, and evaporated to obtain the hydrazinolysis product as a gum. The NMR spectrum of this compound did not show any resonances due to the phthalimide moiety. Without further characterization, it was immediately used for the next step.

Diethyl [*p*-[*N*-Propargyl-*N*-(3-aminoacetonyl)amino]-benzoyl]-L-glutamate Oxime (15). A solution of 2.3 g (4 mmol) of 13 in 400 mL of MeOH was stirred with 144 mg (4.5 mmol) of anhydrous hydrazine under nitrogen, until all the starting material was consumed (~72 h) as judged by TLC. At this point, 4 mL of 1 N HCl was added and the solution evaporated to dryness. The gummy residue thus obtained was triturated with 100 mL of ice-cold 0.05 N HCl and filtered. This clear filtrate was adjusted to pH 9 by the addition of 35% NH_4OH, whereupon 15 separated as a thick oil, which was extracted in 200 mL of EtOAc. After it was washed three times with 50-mL portions of distilled water, the EtOAc layer was dried over Na_2SO_4 and evaporated to obtain 1.4 g of the desired hydrazinolysis product in ~75% yield. The NMR spectrum of 15 was completely devoid of the resonances due to the phthalimide protons, indicating complete hydrazinolysis. Significant resonances appeared at δ 7.8 (d, 2 H, Ar), 7.1 and 6.9 (dd, 2 H, Ar), 4.9 (t, 1 H, α-proton of glutamate), 4.4–4.1 (c, 10 H, 3 methylenes, 2 ethoxy), 2.5–2.2 (5 H, glutamate, acetylene), and 1.35 (c, 6 H, ethoxy). The TLC of this compound showed two spots in a ratio of 3:2 on silica gel plates (5% MeOH–$CHCl_3$).

Ethyl *p*-[*N*-Propargyl-*N*-[3-[(2-amino-4-hydroxy-5-nitropyrimidin-6-yl)amino]acetonyl]amino]benzoate Oxime (16). A solution of 3.0 g (15.7 mmol) of 2-amino-6-chloro-4-hydroxy-5-nitropyrimidine in 300 mL of methanol was added to 14 obtained from the previous reaction that was contained in a 500-mL round-bottomed flask. The mixture was refluxed under nitrogen for 45 min, 1.7 mL of *N*-methylmorpholine was added, and the refluxing was continued for an additional 3 h. The reaction mixture was evaporated to dryness and triturated with ice, and the resulting brown solid was filtered and used for further reaction. An analytical sample was obtained by recrystallization from MeOH: mp 300 °C dec; UV (0.1 N NaOH) λ_{max} 310 nm; NMR (TFA) δ 7.94, 7.38 (d, 4 H, Ar), 4.54 (s, 4 H, methylenes), 4.20 (c, 4 H, ethoxy and propargylmethylene), 2.5 (s, 1 H, acetylene), 1.14 (t, 3 H, methyl). Anal. ($C_{19}H_{21}N_7O_6$) C, N, H: calcd, 4.77; found, 4.25.

Diethyl [*p*-[*N*-Propargyl-*N*-[3-[(2-amino-4-hydroxy-5-nitropyrimidin-6-yl)amino]acetonyl]amino]benzoyl]-L-glutamate Oxime (17). A solution of 600 mg (~3 mmol) of 2-amino-6-chloro-4-hydroxy-5-nitropyrimidine in 200 mL of MeOH was mixed with a solution of 1.34 g (3 mmol) of the hydrazinolysis product 15 in 100 mL of MeOH. After 4-methylmorpholine (3 mmol) was added to the reaction mixture, it was heated under reflux for 4 h. The mixture was filtered hot to remove traces of insoluble material and the filtrate evaporated to dryness, whereupon a yellow semisolid of 17 was obtained. On trituration with 200 g of ice and overnight refrigeration a yellow solid was formed, which was filtered and recrystallized from MeOH: mp 175 °C; yield 1.35 g (68%). Anal. ($C_{26}H_{32}N_8O_9 \cdot 0.75H_2O$) C, H, N.

Ethyl *p*-[*N*-Propargyl-*N*-[3-[(2-amino-4-hydroxy-5-nitropyrimidin-6-yl)amino]acetonyl]amino]benzoate (18). In a 500-mL round-bottomed flask, 3.5 g (7.9 mmol) of oxime 16

was dissolved in 100 mL of TFA. The flask was transferred to a water bath maintained at 65–70 °C, and an equal volume of 1 N HCl was added over 30 min. After the addition was complete, the mixture was concentrated to approximately one-third of its original volume under reduced pressure at 65–70 °C, cooled, and triturated with ice. The brown solid thus obtained was filtered, washed several times with water, and dried. A portion was recrystallized from methanol: mp 217 °C; yield 3.54 g; UV (0.1 N NaOH) λ_{max} 336.6 nm. Anal. ($C_{19}H_{20}N_6O_6$·HCl) C, H, N.

Diethyl [*p*-[*N*-Propargyl-*N*-[3-[(2-amino-4-hydroxy-5-nitropyrimidin-6-yl)amino]acetonyl]amino]benzoyl]-L-glutamate (19). To a solution of 1.2 g (2 mmol) of **17** in 50 mL of TFA was added 50 mL of 1.0 N HCl portionwise at 60–65 °C during a period of 20 min. The clear solution thus obtained was evaporated almost to dryness under reduced pressure and the residue triturated with 150 g of ice and filtered. The precipitate of **19** thus obtained was washed with distilled water and dried: mp 131–132 °C; yield 100%. Anal. ($C_{26}H_{31}N_7O_9$·0.5HCl) C, H, N.

Conversion of 18 to N^{10}-Propargylpteroic Acid (20). (a) Dithionite Reduction. In an Erlenmeyer flask, 3 g (6.46 mmol) of compound **18** was dissolved in 70 mL of DMF at 65–70 °C. While the temperature was maintained, 25 g of sodium dithionite was added portionwise to the solution with stirring during 20 min. During the same time 70 mL of distilled water was also added. After the addition was complete, the solution was poured over 500 g of ice and the resulting precipitate was filtered after 2 h. This product exhibited a UV maximum at 298.6 nm in 0.1 N NaOH that indicated the reduction of the nitro to the amino group; yield 1.67 g (65%).

(b) Cyclization, Hydrolysis, and Oxidation. The dithionite reduction product obtained from the previous reaction was dissolved in 600 mL of 0.1 N NaOH under stirring in a nitrogen atmosphere. The solution was made homogeneous by portionwise addition of 1.25 mL of acetonitrile and the stirring continued at room temperature for 18 h. The reaction mixture was adjusted to pH 7 with 1 N HCl and concentrated under vacuum to half the original volume. The oxidation was carried out by adding 100 mL of methanol to the above dihydropteridine solution followed by portionwise addition of 17.5 mL of 5% $KMnO_4$ in water. After 2 h, when the UV spectrum (0.1 N NaOH) indicated complete oxidation as judged by the appearance of a well-defined λ_{max} between 360 and 365 nm, the solution was filtered, and the filtrate was ajusted to pH 3 by glacial HOAc where upon a bright yellow precipitate was formed, which after refrigerating for 1 h was filtered, washed with water, and dried over P_2O_5 under vacuum to give 1.12 g of crude **20**. The product was purified by ion-exchange chromatography over DEAE cellulose in chloride form using a linear NaCl gradient from 0 to 0.5 M: yield 760 mg; UV (0.1 N NaOH) λ_{max} 365.2, 279.2, 255.4 nm; NMR (TFA) δ 8.45 (s, C_7H) 7.85, 7.40 (d, 4 H, Ar), 5.09 (s, 2 H, C_9 methylene), 4.33 (s, 2 H propargylmethylene), 2.4 (s, 1 H, acetylene). Anal. ($C_{17}H_{14}N_6O_3$·H_2O) C, H, N.

Preparation of N^{10}-Propargylfolic Acid (2). A 1.75-mmol portion of pteroic acid (**20**) was dissolved in 50 mL of dry Me_2SO by heating. After the solution was cooled to room temperature, 25 mL of tetrahydrofuran was added and the solution chilled at 0 °C. A 2.19-mmol sample of freshly distilled 4-methylmorpholine and 1.75 mmol of isobutyl chloroformate were added to the chilled solution, kept at 0 °C for an additional 15 min, and allowed to warm to room temperature during 20 min. The solution of this mixed anhydride was added to a solution of 2 mmol of diethyl glutamate hydrochloride and 2 mmol of *N*-methylmorpholine in 15 mL of Me_2SO and the reaction mixture stirred for 14 h. The solvents were removed under reduced pressure, and the residue was triturated with 50 g of ice, basified with 50 mL of saturated $NaHCO_3$, and filtered. The precipitate of the diethyl ester thus obtained was hydrolyzed with 250 mL of freshly prepared 0.1 N NaOH at 25 °C for 18 h. The reaction mixture was diluted to 600 mL with distilled water, the pH adjusted to 8–7.5 by 1 N HCl, and the product purified by ion exchange chromatography over a DEAE cellulose column: yield 166.9 mg; UV (0.1 N NaOH) λ_{max} (ϵ) 366.1 (9450), 291 (26 070), 255 nm (27 170); NMR (TFA) δ 8.40 (s, 1 H, C_7H), 7.41 (c, 4 H, Ar), 5.01 (s, 2 H, C_9 methylene), 4.46 (s, α-H of glutamate moiety), 4.2 (s, 2 H, propargylmethylene), 2.31–1.96 (s, 5 H, 1 H acetylene and 4 H glutamate). Anal. ($C_{22}H_{21}N_7O_6$·H_2O) C, H, N, O.

Conversion of 19 to N^{10}-Propargylfolic Acid (2). These reactions were carried out in an analogous manner, as described for the conversion of **18** to **20**. In a 500-mL Erlenmeyer flask was suspended 1.17 g (2 mmol) of the deprotected oxime in 30 mL of DMF and heated under stirring until all the compound had dissolved. The solution was placed in a water bath maintained at 60 °C and 10 g of sodium hydrosulfite added. To this vigorously stirring slurry was added 60 mL of distilled water portionwise during a period of 20 min and the mixture diluted to 500 mL with ice-cold water. After refrigeration overnight, the precipitated reduction product was collected by filtration, suspended in 200 mL of 0.1 N NaOH, and stirred for 8 h at 25 °C to facilitate cyclization and hydrolysis to 7,8-dihydropropargylfolic acid. After addition of 30 mL of MeOH, the solution was oxidized with 8 mL of 5% $KMnO_4$ for 30 min and filtered. Examination of the UV spectrum of the filtrate in 0.1 N NaOH indicated complete oxidation to a 6-substituted pteridine, which was characterized by the appearance of a well-defined adsorption maximum between 360 and 365 nm. The filtrate (adjusted to pH 7.5 with 1 N HCl) was evaporated to ~50 mL, chilled, and acidified to pH 3.5 with 1 N HCl. A bright yellow precipitate of **2** was formed, which was filtered, washed with distilled water, and dried; yield 350 mg.

Final purification of this crude product was achieved by ion-exchange chromatography over a DEAE cellulose column by elution with a linear NaCl gradient ranging from 0 to 0.5 M at pH 7.0 in 0.005 M phosphate buffer; yield 256 mg. This sample was identical in all respects with the samples obtained either by the elaboration of **20** or by the hydrolysis of the alkylation product **7**.

Conversion of 6 to N^{10}-Propargylfolic Acid (2). The required 6-(bromomethyl)pterin hydrobromide (**6**) was prepared from **4** by the procedure of Piper and Montgomery.[14] A stirring suspension of 336 mg (1 mmol) of **6** and 360 mg (1 mmol) of **5** was heated in an oil bath to 90–110 °C for 18 h and then poured over 50 g of crushed ice and triturated. The water-insoluble solid was separated by filtration, suspended in a mixture of 50 mL of 0.1 N NaOH and 10 mL of MeCN, and stirred overnight. The turbid solution was filtered, and the filtrate adjusted to pH 7.5 with 1 N HCl and chromatographed over a DEAE cellulose column as described previously for the purification of **2**. The NMR and UV spectra of the pure compound isolated after chromatography (15 mg) were indistinguishable from the authenic sample of **2** prepared by the Boon–Leigh procedure.

Preparation of Reduced Derivatives of N^{10}-Propargylfolate. 7,8-Dihydro-N^{10}-propargylfolate was prepared by dithionite reduction of N^{10}-propargylfolate.[25] 5,6,7,8-Tetrahydro-N^{10}-propargylfolate having the natural configuration at carbon 6 was prepared[26] by enzymatic reduction of 7,8-dihydro-N^{10}-propargylfolate using *L. casei* dihydrofolate reductase.[27] Both reduced compounds were purified by DEAE cellulose chromatography.[27] The dihydro compound had a UV absorbance peak at 285 nm with a shoulder at 304 nm. The absorbance ratios 285 nm/304 nm and 285 nm/345 nm were 1.2 and 4.0, respectively. The tetrahydro compound had a single peak at 300 nm with an absorbance ratio 300 nm/340 nm of 14. The UV spectra were taken in a solution containing 0.005 M Tris, 0.2 M NaCl, and 0.2 M-2-mercaptoethanol at pH 7.4.

Acknowledgment. This investigation was supported by PHS Grant CA 27101 awarded by the National Cancer Institute, DHHS. Other support for this work was provided by PHS Grants CA 10914 (R.L.K.), CA 32687 (M.G.N.), and CA 35212 (T.I.K.) from NCI, DHHS, and Grant CH 192 (T.I.K.) from the American Cancer Society.

Registry No. **2**, 101760-45-6; **5**, 76858-72-5; **6**, 59212-10-1; **7**, 101760-46-7; **8**, 6284-26-0; **9**, 101248-36-6; **10**, 101760-47-8; **11**,

(25) Friedkin, M.; Crawford, E. J.; Misra, D. *Fed. Proc.* **1962**, *21*, 176.
(26) Fontecilla-Camps, J. C.; Bugg, C. E.; Temple, C., Jr.; Rose, J. D.; Montgomery, J. A.; Kisliuk, R. L. *J. Am. Chem. Soc.* **1979**, *101*, 6114.
(27) Kisliuk, R. L.; Gaumont, Y.; Baugh, C. M. *J. Biol. Chem.* **1974**, *249*, 4100.

101773-16-4; (Z)-12, 101760-48-9; (E)-12, 101760-57-0; (Z)-13, 101760-49-0; (E)-13, 101760-58-1; 14, 101760-50-3; (Z)-15, 101760-51-4; (E)-15, 101760-59-2; 16, 101760-52-5; 17, 101760-53-6; 18, 101760-54-7; 19, 101760-55-8; 20, 101760-56-9; ethyl p-aminobenzoate, 94-09-7; hydroxylamine hydrochloride, 5470-11-1; 2-amino-6-chloro-4-hydroxy-5-nitropyrimidine, 1007-99-4; ethyl p-[N-propargyl-N-[3-[(2,5-diamino-3,4-dihydro-4-oxopyrimidin-6-yl)amino]acetonyl]amino]benzoate, 101760-60-5; diethyl glutamate hydrochloride, 1118-89-4; 7,8-dihydro-10-propargylfolic acid, 101760-61-6; 5,6,7,8-tetrahydro-10-propargylfolic acid, 101760-62-7; dihydrofolate reductase, 9002-03-3; thymidylate synthase, 9031-61-2.

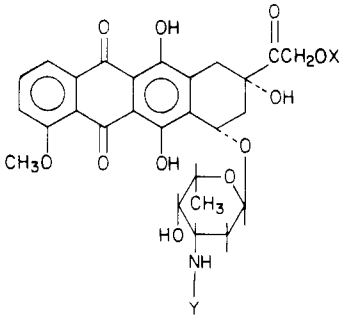
Adriamycin Analogues. Preparation and Biological Evaluation of Some Thio Ester Analogues of Adriamycin and N-(Trifluoroacetyl)adriamycin 14-Valerate[1]

Ramakrishnan Seshadri, John M. Idriss, and Mervyn Israel*

Department of Pharmacology, University of Tennessee—Memphis College of Medicine, Memphis, Tennessee 38163.
Received December 9, 1985

On the consideration that the highly active DNA-nonbinding adriamycin analogues N-(trifluoroacetyl)adriamycin 14-valerate and N-(trifluoroacetyl)adriamycin 14-O-hemiadipate undergo initial metabolic conversion to N-(trifluoroacetyl)adriamycin by the action of nonspecific serum and tissue esterases, a number of N-(trifluoroacetyl)-adriamycin 14-thio esters have been prepared and studied for in vitro growth inhibition, vs. human-derived CCRF-CEM leukemic lymphocytes, and in vivo antitumor activity, vs. murine P388 leukemia, relative to the rate of thio ester deacylation induced by esterases present in mouse serum. Products were obtained by reaction of N-(trifluoroacetyl)-14-bromodaunorubicin with thioacetic, thiopropionic, thiobutyric, thiovaleric, and thiobenzoic acids in ethanol, in the presence of potassium carbonate. Because little is known about similar thio ester derivatives of adriamycin itself, the corresponding adriamycin 14-thio esters were also prepared and evaluated for antitumor activity; with these products, determination of their extent of interaction with calf thymus DNA was also performed. For the adriamycin thio ester products, significant in vivo anti-P388 activity was seen with the thioacetate, thiovalerate, and thiobenzoate derivatives, although no compound matched the curative effects of N-(trifluoroacetyl)adriamycin 14-valerate in this system. With respect to the N-(trifluoroacetyl)adriamycin 14-thio ester products, although the corresponding oxo ester analogues are all significantly biologically active, none of the thio ester derivatives showed activity in vitro or in vivo.

For some time these laboratories have been engaged in the search for improved analogues of the antitumor antibiotic adriamycin (doxorubicin, 1). Since its clinial introduction in the early 1970s, 1 has become the most widely used chemotherapeutic agent in cancer medicine. While most text and reference sources continue to suggest that the antitumor and cytotoxic properties of this agent are due mechanistically to direct drug–DNA binding, we questioned the validity of this hypothesis and the reported structural requirement for a basic 3′-aminoglycoside function as a necessary condition for the expression of biological activity in the anthracycline system.[2] Accordingly, we set about investigating a broad range of semisynthetic structural variants of 1 with altered DNA-binding properties. This approach led to the design, synthesis, preclinical development, and clinical introduction of the novel DNA-nonbinding analogue N-(trifluoroacetyl)-adriamycin 14-valerate (AD 32, 2).[3,4] In animal model systems, 2 shows the double advantage, compared to 1, of being therapeutically superior and very much less toxic.[3,5-8] Extensive clinical trials with 2 have demonstrated significant activity against human disease, with little of the toxicities normally seen with 1.[9-11] No patient receiving 2 has experienced cardiac toxicity, regardless of the total accumulated dose received. Because of solubility problems with 2, N-(trifluoroacetyl)adriamycin 14-O-hemiadipate, a second-generation DNA-nonbinding analogue of 2 with improved water solubility, has been prepared[12,13] and is expected to enter clinical trials shortly. Various pharmacology studies with 2 and its newer hemiadipate analogue in vitro and in vivo have established that these agents have broad-range intrinsic biological activity, without the



1: X=Y=H (adriamycin)
2: X=CO(CH$_2$)$_3$CH$_3$; Y=COCF$_3$ (AD 32)

(1) Work started at Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA.
(2) DiMarco, A.; Arcamone, F. *Arzneim.-Forsch.* **1975**, *25*, 368 and earlier references cited therein.
(3) Israel, M.; Modest, E. J.; Frei, E., III *Cancer Res.* **1975**, *35*, 365.
(4) Israel, M.; Modest, E. J. U.S. Patent 4 055 566, July 12, 1977.
(5) Parker, L. M.; Hirst, M.; Israel, M. *Cancer Treatment Rep.* **1978**, *62*, 119.
(6) Vecchi, A.; Cairo, M.; Mantovani, M.; Sironi, M.; Spreafico, F.; *Cancer Treatment Rep.* **1978**, *62*, 111.
(7) Henderson, I. C.; Billingham, M.; Israel, M.; Krishan, A.; Frei, E., III *Proc. Am. Assoc. Cancer Res.* **1978**, *19*, 158.
(8) Dantchev, D.; Slioussartchouk, V.; Paintrand, M.; Hayat, M.; Bourut, C.; Mathe, G. *Cancer Treatment Rep.* **1979**, *63*, 875.
(9) Blum, R. H.; Garnick, M. B.; Israel, M.; Cannellos, G. P.; Henderson, I. C.; Frei, E., III *Cancer Treatment Rep.* **1979**, *63*, 919.
(10) Blum, R. H.; Garnick, M. B.; Israel, M.; Cannellos, G. P.; Henderson, I. C.; Frei, E., III *Recent Results Cancer Res.* **1981**, *76*, 7.
(11) Garnick, M. B.; Griffin, J. D.; Sack, M. J.; Blum, R. H.; Israel, M.; Frei, E., III *Anthracycline Antibiotics in Cancer Therapy*; Muggia, F. M., Young, C. W., Carter, S. K., Eds.; Martinus Nijhoff: The Hague, 1982; p 541.
(12) Israel, M.; Potti, P. G. U.S. Patent 4 299 822, November 10, 1981.
(13) Israel, M.; Potti, P. G.; Seshadri, R. *J. Med. Chem.* **1985**, *28*, 1226.

0022-2623/86/1829-1269$01.50/0