Quinazoline Antifolates Inhibiting Thymidylate Synthase: Benzoyl Ring Modifications

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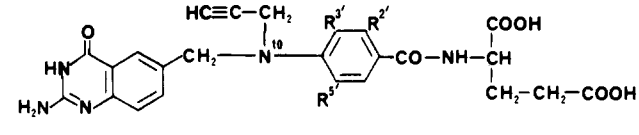
Four new analogues of the antifolate N^{10}-propargyl-5,8-dideazafolic acid were prepared that were substituted in the benzoyl ring. The 2′-chloro and 2′-methyl analogues were prepared from the appropriately substituted *p*-nitrobenzoic acids. The route to the 3′-chloro and 3′,5′-dichloro analogues was by chlorination of diethyl N^{10}-propargyl-5,8-dideazafolate and diethyl *N*-[4-(prop-2-ynylamino)benzoyl]-L-glutamate, respectively, using sulfuryl chloride. The compounds were tested for their inhibition of purified L1210 thymidylate synthase (TS), for their inhibition of purified L1210 dihydrofolate reductase (DHFR), and for their inhibition of the growth of L1210 cells in culture. The 2′-chloro substituent reduced the TS inhibition by twofold and the 2′-methyl substituent reduced it by 20-fold; the 3′-chloro and 3′,5′-dichloro derivatives were very poor inhibitors. The substituents only slightly affected the DHFR inhibition. None of the compounds improved upon N^{10}-propargyl-5,8-dideazafolic acid in inhibiting the growth of L1210 cells in culture.

There have been many examples of modifications of the benzoyl ring in the folic acid, aminopterin, and methotrexate series. 3′,5′-Dichloromethotrexate has been the only compound of interest to emerge. It showed improved inhibition of dihydrofolate reductase (DHFR, EC 1.5.1.4) compared to methotrexate[1] and, at higher doses, was more active in vivo against murine tumors.[2-4] However, it was more rapidly inactivated by hepatic aldehyde oxidase.[5] Clinical trials showed that it was no better than methotrexate.[6,7]

Recently we have shown that N^{10}-propargyl-5,8-dideazafolic acid (**1a**, Chart I)[8] is a potent inhibitor of thymidylate synthase (TS, EC 2.1.1.45)[9] and that its in vivo antitumor activity appears to stem from this inhibition alone.[10,11] A subsequent series of 5,8-dideazafolic acids exemplified a range of N^{10} substituents, but none improved upon propargyl in conferring TS inhibitory activity.[12] This paper describes further analogues of **1a** in which the benzoyl ring has been modified.

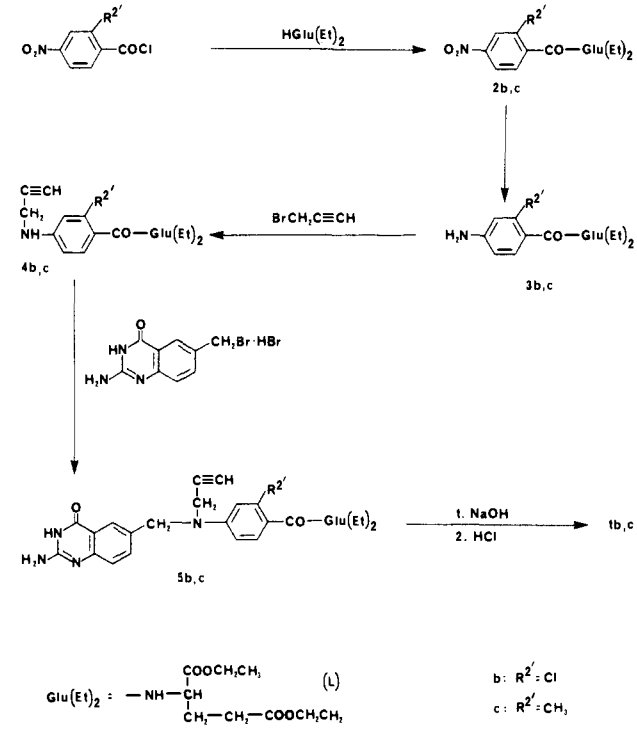
Previous studies of the effect of benzoyl ring modification in the binding of folates to TS have been few. 3′,5′-Dichloro substitution in the tetrahydrofolate and tetrahydroaminopterin series abolished substrate and inhibitory activity, respectively. However, this study utilized bacterial enzyme, and the weak inhibitors were 2,4-diamino compounds.[13] Also using bacterial enzyme, Plante and co-workers noted that either 3′-iodo or 3′,5′-dibromo substitution of tetrahydrofolic acid caused a loss of TS cofactor activity.[14] In contrast, 3′-iodo substitution of tetrahydrohomofolic acid, a recognized TS inhibitor in vitro,[15] caused only a marginal loss of inhibitory property.[14] Lastly, Nair and colleagues recently synthesized and tested 1′,2′,3′,4′,5′,6′-hexahydrohomofolic acid but found that this radical alteration destroyed enzyme binding.[16] We decided to examine the effects of simple substituents in the benzoyl ring of **1a**; the compounds **1b–e** (Chart I) with methyl and chlorine substituents were prepared and tested.

Chart I



	$R^{2'}$	$R^{3'}$	$R^{5'}$
1a	H	H	H
1b	Cl	H	H
1c	CH_3	H	H
1d	H	Cl	H
1e	H	Cl	Cl

Scheme I



Chemistry

The analogues **1b,c** (Chart I) bearing 2′-chlorine and 2′-methyl substituents were prepared (Scheme I) via the appropriately substituted (nitrobenzoyl)glutamate esters **2b,c**. Reduction to the amino derivative was achieved by catalytic hydrogenation in the case of **3c** and dithionite was used for **3b** in order to preserve the chlorine substituent. Alkylation with propargyl bromide gave the sec-

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Scheme II

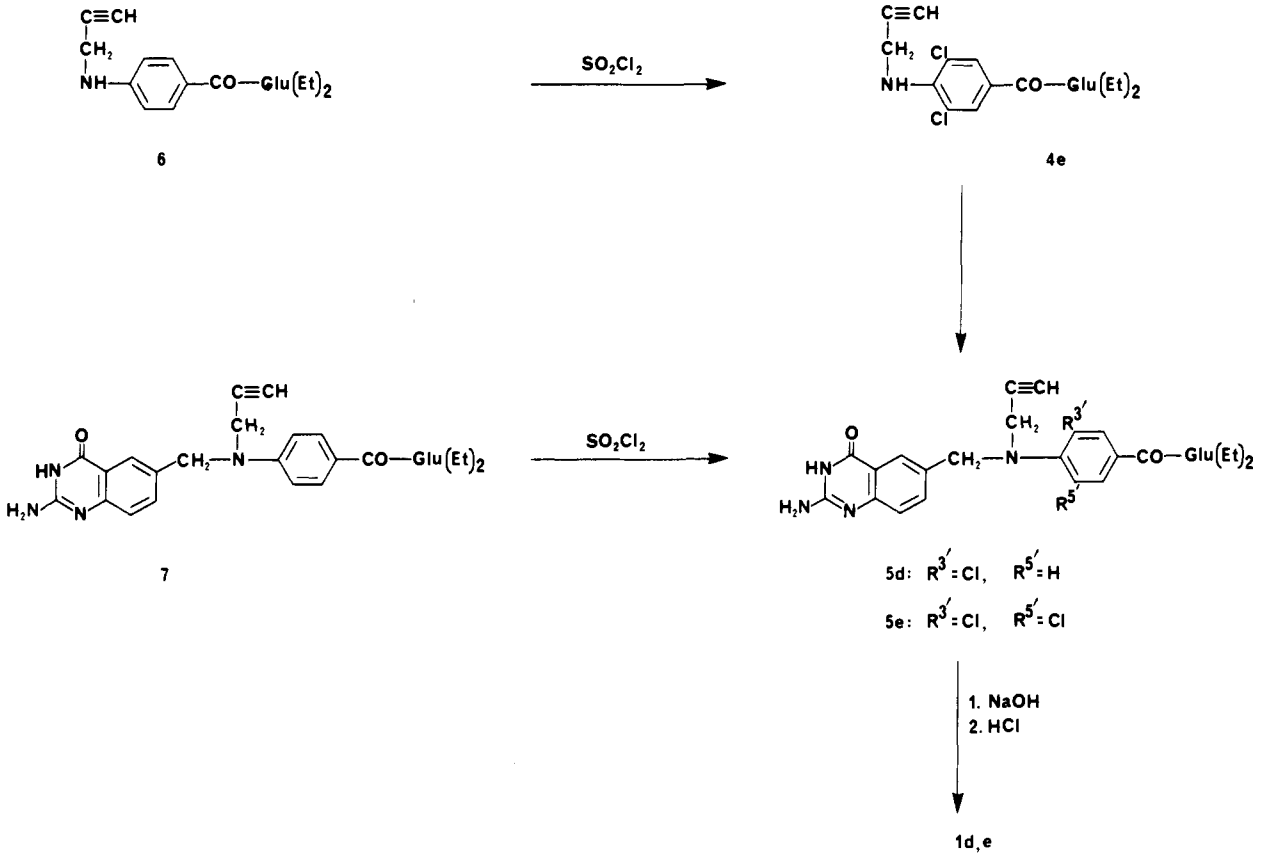


Table I. Preparation of Propargylamines 4

no.	eluant	yield, %	mp, °C	NMR	formula	anal.
4b	20% EtOAc–CH_2Cl_2	63	gum	*a*	$C_{19}H_{23}ClN_2O_5$	C, H, N, Cl
4c	30% EtOAc–CH_2Cl_2	37	85–87		$C_{20}H_{26}N_2O_5$	C, H, N
4e	15% EtOAc–CH_2Cl_2	91	gum	*b*	$C_{19}H_{22}Cl_2N_2O_5$	C, H, N; Cl[c]

[a] $CDCl_3$: 6.60 (m, 2 H, 3-H and 5-H), 7.68 (d, J = 8 Hz, 1 H, 6-H). [b] $CDCl_3$: 7.71 (s, 2 H, 2-H and 6-H). [c] Cl: calcd, 16.6; found, 15.9.

Table II. Preparation of Antifolate Diesters 5

no.	eluant	yield, %	mp, °C	NMR	formula	anal.
5b	10% CH_3OH–CH_2Cl_2	51	138–141	*a*	$C_{28}H_{30}ClN_5O_6$	C, H, N
5c	10% CH_3OH–CH_2Cl_2	39	162–165		$C_{29}H_{33}N_5O_6 \cdot 0.5H_2O$	C, H, N
5d	15% CH_3OH–CH_2Cl_2	42	gum	*b*	$C_{28}H_{30}ClN_5O_6 \cdot H_2O$	C, H, N
5e	7% CH_3OH–CH_2Cl_2	12	141–143		$C_{28}H_{29}Cl_2N_5O_6 \cdot H_2O$	C, H, N

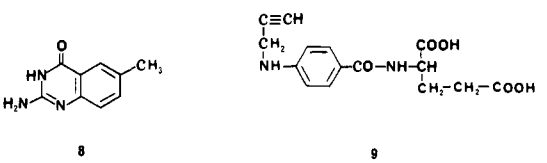
[a] Me_2SO-d_6: 6.82 (m, 2 H, 3′ + 5′ protons), 7.34 (d, J = 8 Hz, 1 H, 6′ proton). [b] Me_2SO-d_6: 7.35 (d, J = 8 Hz, 1 H, 5′ proton), 7.77 (dd, J = 8, 2 Hz, 1 H, 6′ proton), 7.92 (d, J = 2 Hz, 1 H, 2′ proton).

ondary amines **4b,c**. Further alkylation with 2-amino-6-(bromomethyl)-4-hydroxyquinazoline hydrobromide[17]

Table III. Preparation of Antifolate Diacids 1

	yield, %	mp, °C	formula	anal.
1b	79	224–227 dec	$C_{24}H_{22}ClN_5O_6 \cdot 1.5H_2O$	C, H, N, Cl
1c	38	226–228 dec	$C_{25}H_{25}N_5O_6 \cdot H_2O$	C, H, N
1d	83	185–190 dec	$C_{24}H_{22}ClN_5O_6 \cdot 2H_2O$	C, H, N, Cl
1e	91	200–210 dec	$C_{24}H_{21}Cl_2N_5O_6 \cdot H_2O$	C, H, N, Cl

Chart II



gave the antifolate diesters **5b,c**, which on saponification yielded the desired diacids **1b,c**.

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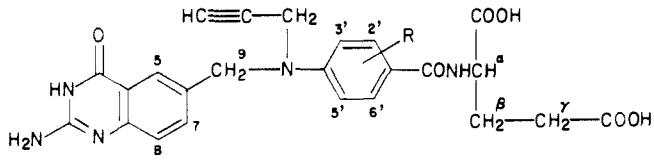
Table IV. ^{1}H NMR Spectral Data of Antifolate Diacids 1



compd		Glu CH_2^β	Glu CH_2^γ	propargyl H	propargyl CH_2	Glu CH^α	CH_2^9	NH_2	H^8	H^7	H^5	$H^{2'}$	$H^{3'}$	$H^{5'}$	$H^{6'}$	amidic NH
1b	δ	1.95	2.3	3.2		4.3	4.65	6.5	7.15	7.5	7.8	-		6.8	7.25	8.35
	app	m	m	br s		m	s	br	d[b]	dd[c]	d[d]	-		m	d[b]	d[b]
1c[e]	δ	2.0	2.3	3.1	4.2	4.3	4.6	6.1–6.6	7.15	7.5	7.8	-		6.7	7.25	8.1
	app	m	m	t[f]	br s	m	s	br	d[b]	dd[c]	d[d]			m	d[b]	d[b]
1d	δ	2.05	2.3	3.15	3.90	4.4		6.1–6.8	7.15	7.55	7.9	7.94	-	7.35	7.75	8.5
	app	m	m	t[i]	br s	m		br	d[g]	dd[h]	d[i]	d[i]		d[g]	dd[h]	d[b]
1e	δ	2.05	2.35	3.05	3.90	4.35	4.45	6.0–6.8	7.15	7.55	7.9		-	-	7.9	8.7
	app	m	m	t[i]	d[i]	m	s	br	d[g]	dd[h]	part of m				part of m	d[g]

[a] Spectra determined in Me_2SO-d_6; the signals from the lactam and carboxyl hydrogens were not recorded. [b] J = 7 Hz. [c] J = 7, 3 Hz. [d] J = 3 Hz. [e] 2′-Methyl substituent: 2.4 (s, 3 H). [f] J = 1.5 Hz. [g] J = 8 Hz. [h] J = 8, 2 Hz. [i] J = 2 Hz.

The chlorine substituents ortho to the bridge nitrogen atom in the analogues **1d** and **1e** were introduced into precursors with use of sulfuryl chloride (Scheme II). Thus treatment of the known[9] propargylamine **6** yielded the dichloro derivative **4e**, which after alkylation with the (bromomethyl)quinazoline gave the antifolate diester **5e**. Monochlorination of diethyl N^{10}-propargyl-5,8-dideazafolate **7**[9] afforded the diester **5d**. Saponification of these diester products yielded the required diacids **1d,e**. The position of chlorination in the compounds **4e** and **5d** were clearly shown by NMR. In the case of **5d** additional evidence came from NMR comparison with **5b**, which was prepared by an unequivocal route. The gelatinous diacids **1b–e** were all isolated by centrifugation; their melting points and microanalytical data are detailed in Table III and their NMR spectra in Table IV. The UV spectra are detailed in Table V, together with that of **1a** and (Chart II) 2-amino-4-hydroxy-6-methylquinazoline (**8**)[18] and *N*-[4-(prop-2-ynylamino)benzoyl]-L-glutamic acid (**9**) for comparison. All products and intermediates had microanalytical and NMR spectroscopic data to establish structure and purity.

The antifolate diacids **1b–e** were tested for their inhibition of purified L1210 thymidylate synthase, for their inhibition of purified L1210 dihydrofolate reductase, and for their inhibition of the growth of L1210 cells in culture. These results are expressed in Table VI.

Table V. Ultraviolet Spectral Data of Antifolates and Component Molecules[a]

compd	max, nm	ε	min, nm	ε
1a	301.5	26600	284	23700
	279	23900	251.5	9800
	229	50700		
1b	275.5	28800	248.5	13900
	227.5	51800		
1c	277	26600	249.5	14000
	229.5	49100		
1d	277	23200	252.5	14900
	229	52400		
1e	309	8700	292	8200
	276	15100	261	13900
	228.5	54600		
8	329	3800	286	800
	264–271.5	9200	249.5	7000
	228	41000		
9	281.5	19500	238	3500

[a] Spectra determined in 0.1 N NaOH (aqueous).

Results and Discussion

The values of inverse relative potency of the analogues for the inhibition of purified L1210 thymidylate synthase, expressed in Table VI, show a wide variation; all are greater than unity, indicating that no improvement of TS inhibition had been achieved. The introduction of a 2′-chloro substituent into **1a** giving compound **1b** essentially preserves the inhibition of TS. However, the 2′-methyl substituent (compound **1c**) reduces the inhibition by 21-fold. These inhibitions differ by an order of magnitude which is intriguing. The substituents are of similar size (van der Waals radii 1.8 and 2.0 Å, respectively[19]) so that a steric effect can be discounted. However, the dipoles of the substituents are in opposite directions, and this may account for the results observed.[20] In accord with this interpretation, 2′-azidoaminopterin (N^1–N^3 distance in the substituent ca. 2.73 Å[21]) in which the dipole is similar to that of chlorine was found to inhibit TS equally as aminopterin itself.[22] The 3′-chloro (**1d**) and 3′,5′-dichloro (**1e**) derivatives with substituent(s) ortho to nitrogen are very poor TS inhibitors. This result for compound **1e** accords with previous studies of 3′,5′-dichloro substitution in the

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Table VI. Inhibition of Enzymes and in Vitro Cytotoxicity of Folate Analogues[a]

compd	inhibition of L1210 thymidylate synthase: IC_{50}, nm	IC_{50} for CB3717 as control, nM	inverse rel potency[b]	IC_{50} for purified L1210 dihydrofolate reductase, μM	ID_{50} for the growth of L1210 cells in culture, μM
1a			1	7	7
1b	30	14	2.1	11	12
1c	400	19	21	25	24
1d	~5000	14.4	~347	3	72
1e	>5000	14.4	>347	3	37

[a] For methods, see ref 12. [b] Defined as IC_{50}(compound)/IC_{50}(1a).

tetrahydrofolic acid and tetrahydroaminopterin series.[13]

The UV spectra of compounds **1b–e** and **8** are drawn out (Figure 1, supplementary material) for it to be seen that the spectrum of the leastmost TS inhibitor **1e** more closely resembles the spectrum of the quinazoline **8** than that of **1a** (Figure 2, supplementary material). The 4-aminobenzoyl chromophore has thus been greatly attenuated. We presume that the two chlorines ortho to the bridge nitrogen induce steric inhibition of resonance (this has precedent in the 3′,5′-halogenated derivatives of methotrexate[23]) and we hypothesize that the associated change in conformation or conformational opportunity is inimical to TS binding. A similar but lesser effect can be seen in the spectrum of **1d**.

Substitution of the benzoyl ring giving compounds **1b–e** had little effect on DHFR inhibition (Table VI). Inhibition was slightly reduced in compounds **1b** and **1c** and slightly enhanced in compounds **1d** and **1e**. The enhancement for **1e** was similar to that observed with 3′,5′-dichloromethotrexate relative to methotrexate.[1]

The effects of the compounds **1a–1c** in inhibiting the growth of L1210 cells in tissue culture (Table VI) are roughly parallel their TS inhibitions. But this correlation does not extend to compounds **1d** and **1e** where the ID_{50} values are lower than expected. The lipophilic chlorine substituents in **1d** and **1e** may promote intracellular transport, and the higher drug levels thus achieved may compensate for the weakened TS binding.

Experimental Section

Melting points were determined on a Büchi apparatus and are uncorrected. NMR spectra were run in $CDCl_3$ or Me_2SO-d_6 on a 90-MHz spectrometer (Bruker HX90E). Field strengths are expressed in units of δ (ppm) and peak multiplicities are designated thus: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; br s, broad singlet; br, broad signal; m, multiplet. UV spectra were determined on a Pye Unicam SP8-150 spectrophotometer. Elemental analyses were determined by ICI Pharmaceuticals Division. Analysis indicated by a symbol for the element implies a result within ±0.4% of the theoretical value. Merck silica 60 (Art 7734) in gravity columns was used for chromatographic separations. DMA is *N*,*N*-dimethylacetamide.

Diethyl *N*-(2-Chloro-4-nitrobenzoyl)-L-glutamate (2b) and Diethyl *N*-(2-Methyl-4-nitrobenzoyl)-L-glutamate (2c). To a stirred suspension of diethyl L-glutamate (1 mol equiv) in toluene (0.5 mL/mmol) cooled to 0 °C was added pyridine (2.5 mol equiv) followed by the dropwise addition of a solution of 1.5 mol equiv of the appropriate acid chloride[24] dissolved in toluene (0.7 mL/mmol) over 20 min. Stirring was continued for 30 min at 0 °C and then for 1 h at 25 °C. Following dilution with toluene, the mixture was successively extracted with H_2O, 2 N HCl, 5% $NaHCO_3$, and H_2O. The organic phase was dried ($MgSO_4$) and the solvent removed in vacuo to give an oil. Compound **2b** was purified by recrystallization from 2% EtOAc–cyclohexane: yield 72%; mp 97–98 °C (lit.[26] mp 98–98.5 °C); NMR ($CDCl_3$, 90 MHz) δ 4.81 (m, 1 H, Glu CH$^\alpha$), 7.01 (d, J = 7 Hz, 1 H, NH), 7.80 (d, J = 8 Hz, 1 H, 6-H), 8.20 (m, 2 H, 3-H and 5-H). Anal. ($C_{16}H_{19}ClN_2O_7$) C, H, N. Compound **2c** was purified by column chromatography eluting with 50% EtOAc in petrol: yield 77%; mp 67–68 °C; NMR ($CDCl_3$, 90 MHz) δ 4.77 (m, 1 H, Glu CH$^\alpha$), 6.76 (d, J = 8 Hz, 1 H, NH), 7.56 (m, 1 H, 6-H), 8.06 (m, 2 H, 3-H and 5-H). Anal. ($C_{17}H_{22}N_2O_7$) C, H, N.

Diethyl *N*-(4-Amino-2-chlorobenzoyl)-L-glutamate (3b). To a stirred solution of **2b** (8.30 g, 21.5 mmol) in 55% EtOH–H_2O (450 mL) at room temperature was added $NaHCO_3$ (10.92 g, 130 mmol) followed by the portionwise addition of sodium dithionite monohydrate (16.51 g, 86 mmol) during 20 min. After the mixture was stirred for an additional hour, the EtOH was removed in vacuo and the residue diluted with brine. The product was extracted into EtOAc and purified by back-extraction into 2 N HCl, neutralization with solid $NaHCO_3$, and reextraction into ether. The dried ($MgSO_4$) ether extract was evaporated in vacuo to give an oil, which crystallized on standing and which was used without further purification (3.40 g, 44%): mp 73–75 °C; NMR ($CDCl_3$, 90 MHz) δ 4.10 (br s, 2 H, NH_2), 4.80 (m, 1 H, Glu CH$^\alpha$), 6.52 (m, 2 H, 3-H and 5-H), 7.10 (d, J = 8 Hz, 1 H, NH), 7.61 (d, J = 8 Hz, 1 H, 6-H). Anal. ($C_{16}H_{21}ClN_2O_5$) C, H, N.

Diethyl *N*-(4-Amino-2-methylbenzoyl)-L-glutamate (3c). Compound **2c** (4.30 g, 11.75 mmol) in EtOH (100 mL) was hydrogenated at atmospheric pressure with use of 10% Pd–C catalyst (0.50 g). Filtration and removal of the solvent in vacuo gave an oil, which crystallized on standing and which was used without further purification (3.20 g, 81%): mp 98–100 °C; NMR ($CDCl_3$, 90 MHz) δ 3.36 (br s, 2 H, NH_2), 4.77 (m, 1 H, Glu CH$^\alpha$), 6.43 (m, 3 H, NH, 3-H, and 5-H), 7.30 (m, 1 H, 6-H). Anal. ($C_{17}H_{24}N_2O_5$) C, H, N.

Diethyl *N*-[2-Chloro-4-(prop-2-ynylamino)benzoyl]-L-glutamate (4b) and Diethyl *N*-(2-Methyl-4-(prop-2-ynylamino)benzoyl)-L-glutamate (4c). A mixture of the appropriate primary amine **3b,c** (1 mol equiv), 2,6-lutidine (1.1 mol equiv), and propargyl bromide (1.1 mol equiv) in DMA (2 mL/mmol of amine) was stirred at room temperature (16 h for **4b**, 48 h for **4c**). The mixture was poured into H_2O and extracted with EtOAc, the organic phase washed with H_2O and dried ($MgSO_4$), and the solvent removed in vacuo. The resulting oil was purified by column chromatography (Table I).

Diethyl *N*-[3,5-Dichloro-4-(prop-2-ynylamino)benzoyl]-L-glutamate (4e). To a stirred solution of **6**[9] (7.30 g, 20.25 mmol) in $CHCl_3$ (150 mL) was added during 10 min a solution of SO_2Cl_2 (6.75 g, 50 mmol) in $CHCl_3$ (50 mL). After stirring overnight at room temperature, the $CHCl_3$ solution was washed with H_2O and dried ($MgSO_4$) and the solvent removed in vacuo. The resulting oil was purified by column chromatography (Table I).

Diethyl *N*-[2-Chloro-4-[*N*-[(2-amino-4-hydroxy-6-quinazolinyl)methyl]prop-2-ynylamino]benzoyl]-L-glutamate (5b), Diethyl *N*-[2-Methyl-4-[*N*-[(2-amino-4-hydroxy-6-quinazolinyl)methyl]prop-2-ynylamino]-benzoyl]-L-glutamate (5c) and Diethyl *N*-3,5-Dichloro-4-

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(24) Prepared by treating the appropriate commercially available acid chloride with an excess of refluxing thionyl chloride. Removal of the reagent in vacuo and distillation afforded 2-methyl-4-nitrobenzoyl chloride, bp 112–116 °C (0.7–0.8 mm) [lit.[25] bp 149–153 °C (14 mm)], and 2-chloro-4-nitrobenzoyl chloride, bp 112–116 °C (0.3–0.4 mm) [lit.[25] bp 158–160 °C (13 mm)].
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[*N*-[(2-amino-4-hydroxy-6-quinazolinyl)methyl]prop-2-ynylamino]benzoyl]-L-glutamate (5e). A mixture of 2-amino-6-(bromomethyl)-4-hydroxyquinazoline hydrobromide[17] (1 mol equiv), $CaCO_3$ (1 mol equiv), and the appropriate propargylamine **4b,c,e** (1 mol equiv) in DMA (5 mL/mmol) was stirred at room temperature for 48 h (**5e** for 6 days). The mixture was filtered, the solid washed with DMA, and the solvent removed in vacuo. The resulting gum was purified by column chromatography (Table II).

Diethyl *N*-[3-Chloro-4-[*N*-[(2-amino-4-hydroxy-6-quinazolinyl)methyl]prop-2-ynylamino]benzoyl]-L-glutamate (5d). To a stirred suspension of **7**[9] (1.00 g, 1.88 mmol) in $CHCl_3$ (100 mL) was added SO_2Cl_2 (0.64 g, 4.74 mmol), and the mixture was stirred for 40 min at room temperature. H_2O (50 mL) was added, the organic phase separated, washed twice with H_2O, dried ($MgSO_4$), and the solvent removed in vacuo. The resulting gum was purified by chromatography (Table II).

***N*-[2-Chloro-4-[*N*-[(2-amino-4-hydroxy-6-quinazolinyl)-methyl]prop-2-ynylamino]benzoyl]-L-glutamic Acid (1b), *N*-[2-Methyl-4-[*N*-[(2-amino-4-hydroxy-6-quinazolinyl)-methyl]prop-2-ynylamino]benzoyl]-L-glutamic Acid (1c), *N*-[3-Chloro-4-[*N*-[(2-amino-4-hydroxy-6-quinazolinyl)-methyl]prop-2-ynylamino]benzoyl]-L-glutamic Acid (1d), *N*-[3,5-Dichloro-4-[*N*-[(2-amino-4-hydroxy-6-quinazolinyl)methyl]prop-2-ynylamino]benzoyl]-L-glutamic Acid (1e).** The appropriate diester 4 (1 mol equiv) was suspended in 50% EtOH–H_2O (40 mL/mmol) and treated with 1.0 N NaOH [3 mol equiv (in the case of **1d** and **1e**, 6 mol equiv was used with an initial compound concentration of 1 mmol/60 mL)] and the mixture stirred at room temperature for 18 h. Addition of 3 mol equiv of 0.1 N HCl (6 mol equiv for **1d** and 1e) to pH 4.0 gave a gelatinous precipitate, which was purified by six cycles of centrifugation–decantation and resuspension in H_2O. The final aqueous suspension was freeze-dried to give an amorphous white solid. Details of the individual preparations are collected in Table III; spectroscopic data are in Tables IV and V.

***N*-[4-(Prop-2-ynylamino)benzoyl]-L-glutamic Acid (9).** NaOH (1 N; 38 mL, 38 mmol) was added to a stirred suspension of **6**[9] (3.44 g, 9.55 mmol) in 33% EtOH–H_2O (75 mL) and the mixture stirred for 18 h at room temperature. Some unreacted ester was removed by filtration and the basic solution extracted twice with ether, acidified to pH 4 with 2 N HCl, and extracted four times with EtOAc. The combined organic extracts were washed with brine and dried ($MgSO_4$), and the solvent was removed in vacuo to give a yellow oil, which solidified on standing (1.80 g, 58%): mp 90–95 °C dec; NMR satisfactory. Anal. ($C_{15}H_{16}N_2O_5 \cdot 0.25$ EtOAc) C, H, N. The EtOAc could not be removed by drying and it was confirmed by NMR spectroscopy.

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Registry No. **1a**, 76849-19-9; **1b**, 80014-98-8; **1c**, 80014-99-9; **1d**, 100020-40-4; 1e, 100020-41-5; **2b**, 80014-91-1; **2c**, 80015-10-7; **3b**, 100020-42-6; **3c**, 80014-85-3; **4b**, 80014-87-5; **4c**, 80014-86-4; **4e**, 100020-43-7; **5b**, 80014-79-5; **5c**, 80014-80-8; **5d**, 100020-44-8; **5e**, 100020-45-9; **6**, 76858-72-5; **7**, 76858-74-7; **9**, 100020-46-0; TS, 9031-61-2; DHFR, 9002-03-3; diethyl L-glutamate, 16450-41-2; 2-chloro-4-nitrobenzoyl chloride, 7073-36-1; 2-methyl-4-nitrobenzoyl chloride, 30459-70-2; propargyl bromide, 106-96-7; 2-amino-6-(bromomethyl)-4-hydroxyquinazoline hydrobromide, 77766-62-2.

Supplementary Material Available: Figure 1, UV spectra of the analogues **1b–e** and the quinazoline **8**. All solutions were 20 μM in 0.1 N NaOH. Figure 2, UV spectra of N^{10}-propargyl-5,8-dideazafolic acid (**1a**) and compounds **8** and **9**. All solutions were 20 μM in 0.1 N NaOH (2 pages). Ordering information is given on any current masthead page.

Potential Antitumor Agents. 46. Structure–Activity Relationships for Acridine Monosubstituted Derivatives of the Antitumor Agent *N*-[2-(Dimethylamino)ethyl]-9-aminoacridine-4-carboxamide

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A series of monosubstituted derivatives of the new antitumor agent *N*-[2-(dimethylamino)ethyl]-9-aminoacridine-4-carboxamide has been prepared, bearing methyl, methoxy, and chloro groups at available acridine positions. The physicochemical properties and antitumor activity of these compounds varied more with the position than with the nature of the substituent groups. The highest levels of both in vitro and in vivo antileukemic activity were shown by 5-substituted derivatives, while 7- and 8-substituted derivatives possessed the highest selectivity toward the HCT-8 human colon carcinoma line compared to the L1210 mouse leukemia line in vitro.

We recently reported the preparation and evaluation of the first examples of a new class of antitumor agent, the dibasic 9-aminoacridine-4-carboxamides.[1] These compounds fall into the general group of DNA-binding agents, binding tightly to double-stranded DNA by intercalation of the acridine chromophore between the base pairs.[1,2] The 9-aminoacridine-4-carboxamides (e.g., 1) show some selectivity for GC base pairs, suggesting that the cationic side chain makes additional binding contacts to accessible guanosine and/or cytosine residues.

Initial structure–activity relationships for this class of compound showed that a side chain that contained a cationic center positioned at a fixed distance (about 8 Å) from the acridine chromophore was essential. Significant attenuation of the pK_a of the side-chain nitrogen (e.g., to give **2** from **1**) or alteration of its position relative to the chromophore (e.g., compounds **3** and **4**) abolished cytotoxic activity, while compounds with the correct charge dispo-

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