

Quinazoline Antifolate Thymidylate Synthase Inhibitors: Benzoyl Ring Modifications in the C2-Methyl Series

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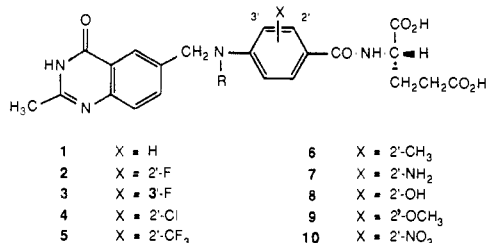
The synthesis of nine new 2-methyl-10-propargylquinazoline antifolates with substituents in the *p*-aminobenzoyl ring is described. In general the synthetic route involved the coupling of the appropriate ring-substituted diethyl *N*-(4-(prop-2-ynylamino)benzoyl)-L-glutamate with 6-(bromomethyl)-3,4-dihydro-2-methyl-4-oxoquinazoline (11) followed by deprotection using mild alkali. The compounds were tested as inhibitors of partially purified L1210 thymidylate synthase (TS). They were also examined for their inhibition of the growth L1210 cells in culture. Compared to the parent compound 1a the 2'-fluoro analogue 2a exhibited enhanced potency in both systems whereas the 3'-fluoro analogue 3a showed enhanced growth inhibitory properties against L1210 cells despite being a poorer inhibitor of the isolated enzyme. Chloro, hydroxy, methoxy, and nitro substituents in the 2'-position were also well tolerated by the enzyme but failed to give enhanced growth inhibition. The series was extended to cover analogues of the 2'-fluoro, 3'-fluoro, 2'-chloro, 2'-methyl, 2'-amino, 2'-methoxy, and 2'-nitro derivatives with modified alkyl substituents at N10.

Introduction

In the preceding article¹ we have demonstrated that the introduction of a fluorine atom into the 2'-position of a series of quinazoline antifolates enhances their potency as inhibitors of both thymidylate synthase (TS) and cell growth. This indicated that modification of the benzoyl ring could offer opportunities for further modulation of the biological activities of these compounds. In the work described below we have sought to optimize the stereoelectronic requirements of this region of the molecule by exploring, in the highly potent C2-methyl series,^{2,3} a wider range of ring substituents than hitherto studied.

Chemistry

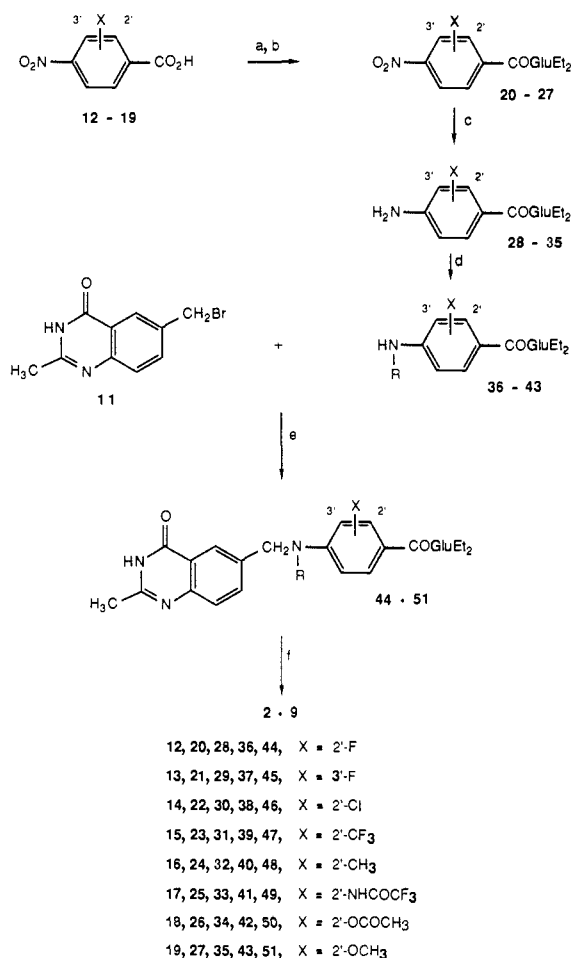
The majority of the compounds (2-9) were prepared by the route outlined in Scheme I and involved the condensation of the (bromomethyl)quinazolinone 11³ with the appropriate *N*-alkylated anilines 36-43 using either CaCO₃ (method A) or 2,6-lutidine (method P) to scavenge HBr.



See Table II for definition of R

The resulting antifolate diesters 44-51 were hydrolyzed by aqueous alkali (method B) to yield the antifolate diacids listed in Table II. The *N*-alkylated anilines 36a,¹ 38a,⁴ and 40a⁴ were known compounds. The others were prepared (Scheme I) from the appropriate nitrobenzoic acids 12-19. The derived acid chlorides were condensed with diethyl L-glutamate (methods K and O), and the resulting (nitrobenzoyl)-L-glutamate diesters 20-27 were reduced (H₂, 10% Pd-C; method C) to the anilines 28-35. The simple *N*-alkylated anilines 36-43 were derived from the parent anilines by reaction with the appropriate alkyl halide under a variety of conditions according to the reactivity of the halide (methods D, E, L, M; Table I). The precursor

Scheme I^{a,b}



^a See Table I for definition of R. ^b (a) (COCl)₂ or SOCl₂; (b) diethyl glutamate, Et₃N; (c) H₂, 10% palladium on carbon; (d) RX; (e) CaCO₃, DMA or 2,6-lutidine, DMF; (f) 1 N aqueous NaOH, EtOH.

aniline 36g (method F) for the 2'-fluoro-10-(hydroxyethyl)analogue 2g was prepared with the hydroxyl group

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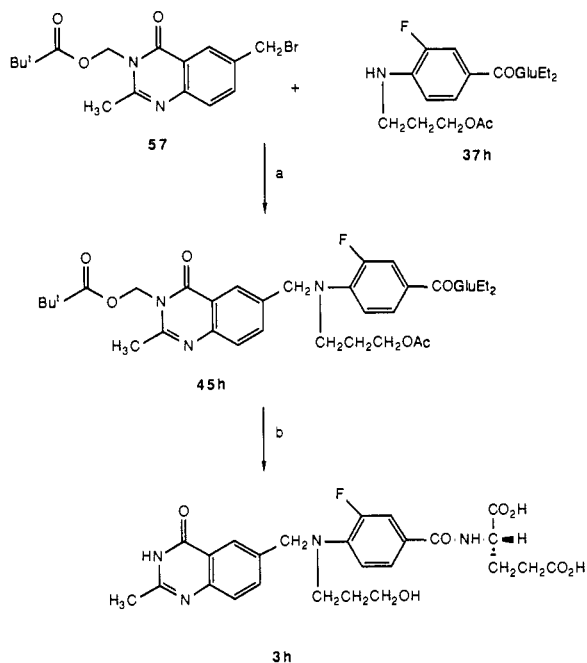
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Table I. Preparation of Substituted (Aminobenzoyl)glutamate Diesters 36-43, 53, and 55 and Derived Antifolate Diesters 44-51, 56, and 62

compd	starting amine	method (temp, °C)	% yield	derived antifolate diester	method	% yield	X	R
36a ^a	28	L(120) ^a	60	44a	A	37	2'-F	CH ₂ C≡CH
28		C(20)	99	44b	A	65	2'-F	H
36c	28	D(60)	31	44c	A	70	2'-F	CH ₃
36d	28	D(100)	52	44d	A	62	2'-F	CH ₂ CH ₃
36e	28	D(100)	51	44e	A	28	2'-F	CH ₂ CH=CH ₂
36f	28	E(120)	83	44f	A	38	2'-F	(CH ₂) ₂ F
36g	28	F(140)	47	44g	A	29	2'-F	(CH ₂) ₂ OCOC ₃ H ₇
36h	28	G(80)	31	44h	A	70	2'-F	(CH ₂) ₃ OCOCH ₃
53 ^b	52 ^b	H(130)	39	54 ^b	A	27	2'-F	(CH ₂) ₂ NPhth
55 ^b	52 ^b	F(85)	91	56 ^b	A	20	2'-F	CH ₂ CN
36k	28	F(110) ^c	41	44k	A	38	2'-F	CH ₂ CN
37a	29	L(120)	53	45a	A ^d	40	3'-F	CH ₂ C≡CH
29		C(20)	99	45b	A	40	3'-F	H
37c	29	D(100)	21	45c	A	81	3'-F	CH ₃
37d	29	D(100)	44	45d	A	48	3'-F	CH ₂ CH ₃
37e	29	D(100) ^e	36	45e ^f	N	50	3'-F	CH ₂ CH=CH ₂
37f	29	E(120)	78	45f	P	23	3'-F	(CH ₂) ₂ F
37g	29	D(100)	26	45g ^f	N	42	3'-F	(CH ₂) ₂ OCOCH ₃
37h	29	M(150)	27	45h ^f	N	56	3'-F	(CH ₂) ₃ OCOCH ₃
38a ^g	30 ^g	g	41	46a	P	23	2'-Cl	CH ₂ C≡CH
38d	30	M(85)	25	46d	P	38	2'-Cl	CH ₂ CH ₃
39a	31	L(100)	65	47a	P ^h	64	2'-CF ₃	CH ₂ C≡CH
40a ^g	32 ^g	g	66	48a	P	35	2'-CH ₃	CH ₂ C≡CH
40c	32 ^g	M(40)	22	48c	P	47	2'-CH ₃	CH ₃
40d	32 ^g	M(40)	68	48d	P	58	2'-CH ₃	CH ₂ CH ₃
41a	33	M(60)	62	49a	P	44	2'-NHCOCF ₃	CH ₂ C≡CH
41c	33	M(60)	44	49c	P	63	2'-NHCOCF ₃	CH ₃
41d	33	M(60)	54	49d	P	57	2'-NHCOCF ₃	CH ₂ CH ₃
42a	34	M(80)	61	50a	P	71	2'-OCOCH ₃	CH ₂ C≡CH
43a	35	M(80)	58	51a	P	35	2'-OCH ₃	CH ₂ C≡CH
43d	35	M(80)	48	51d	P	54	2'-OCH ₃	CH ₂ CH ₃
				62a	Q	73	2'-NO ₂	CH ₂ C≡CH
				62d	Q	41	2'-NO ₂	CH ₂ CH ₃

^aSee also ref 1. ^bDi-*tert*-butyl esters. ^cK₂CO₃ was used as the base. ^dProton Sponge was used as the base. ^e2,6-Lutidine was used as the base. ^fProtected as the 3-(pivaloyloxy)methyl derivative. ^gSee ref 4. ^hMgO was used as the base.

Scheme II^a

^a(a) 2,6-Lutidine, 105 °C (method N); (b) 1 N aqueous NaOH, EtOH (method B).

protected as the butyrate ester because the corresponding acetate ester was inseparable by chromatography from the

starting aniline **28**. For a similar reason, **36h** was prepared in two steps (method G) by alkylation of **28** with 1,3-dibromopropane followed by NaOAc displacement of the second bromide. For the synthesis of the 2'-fluoro-10-(aminoethyl) analogue **2i** the aniline di-*tert*-butyl ester **52^b** was alkylated with (2-bromoethyl)phthalimide to afford **53** (method H). This was coupled to **11** in the usual way and the resulting phthalimido di-*tert*-butyl ester **54** deprotected to **2i** in two stages by treatment with 3-(dimethylamino)propylamine followed by aqueous alkali at 60 °C (method I). The 2'-fluoro-10-cyanomethyl antifolate **2j** was also prepared via the di-*tert*-butyl ester **52**. In this case, *N*-cyanomethylation was achieved by reaction with bromoacetonitrile in the presence of 2,6-lutidine and NaI. The intermediate *N*-cyanomethylaniline **55** was coupled with **11** to give **56**, which was deprotected by treatment with CF₃CO₂H to yield **2j** (method J). When this sequence was repeated on the diethyl ester **28** and the *N*-cyano-methyl diester **44k** hydrolyzed with aqueous NaOH, complete hydration of the nitrile was observed, yielding the *N*-carbamoylmethyl antifolate **2k**. Some of the 3'-fluoro antifolates were prepared by the same methods as their 2'-fluoro counterparts, but in general the 3'-fluoro-*N*-alkylanilines **37a-h** were relatively poor nucleophiles, ne-

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Table II. Preparation and in Vitro Activities of Quinazoline Antifolate Diacids 2-10

compd	X	R	method	% yield	mp, °C	formula ^a	mass spectra <i>m/z</i> [M - H] ⁻	inhibn of TS: IC ₅₀ , μM	inhibn of L1210 cell growth in culture: IC ₅₀ , μM	L1210 cell growth in the presence of thymidine (% control)
1a ^b	H	CH ₂ C≡CH			165	C ₂₅ H ₂₄ N ₄ O ₆ ·2H ₂ O		0.040	0.090	100
1b ^b	H	H			197-201	C ₂₅ H ₂₂ N ₄ O ₆ ·H ₂ O		4.50	0.07	
1c ^b	H	CH ₃			254-257	C ₂₃ H ₂₄ N ₄ O ₆ ·0.75H ₂ O		0.30	0.11	100
1d ^b	H	CH ₂ CH ₃			221-225	C ₂₄ H ₂₆ N ₄ O ₆ ·0.5H ₂ O		0.17	0.36	88
1e ^b	H	CH ₂ CH=CH ₂			188	C ₂₅ H ₂₆ N ₄ O ₆ ·1.5H ₂ O		0.48	0.17	104
1f ^b	H	(CH ₂) ₂ F			207-210	C ₂₄ H ₂₅ FN ₄ O ₆ ·1.25H ₂ O		0.24	0.4	
1g ^b	H	(CH ₂) ₂ OH			>300	C ₂₄ H ₂₆ N ₄ O ₇ ·1.5H ₂ O		0.50	0.24	
1h ^b	H	(CH ₂) ₃ OH			300	C ₂₅ H ₂₈ N ₄ O ₇ ·H ₂ O		0.54	1.24	95
2a	2'-F	CH ₂ C≡CH	B	77	228-230	C ₂₅ H ₂₃ FN ₄ O ₆	493	0.020	0.027	97
2b	2'-F	H	B	90	190-194	C ₂₂ H ₂₁ FN ₄ O ₆ ·H ₂ O	455	3.78	0.064	94
2c	2'-F	CH ₃	B	83	224-226	C ₂₃ H ₂₃ FN ₄ O ₆ ·H ₂ O	469	0.12	0.019	98
2d	2'-F	CH ₂ CH ₃	B	94	214-217	C ₂₄ H ₂₅ FN ₄ O ₆ ·0.75H ₂ O	483	0.045	0.065	96
2e	2'-F	CH ₂ CH=CH ₂	B	84	209-211	C ₂₅ H ₂₅ FN ₄ O ₆	495	0.076	0.10	97
2f	2'-F	(CH ₂) ₂ F	B	63	220-225	C ₂₄ H ₂₄ F ₂ N ₄ O ₆ ·0.7H ₂ O	501	0.043	0.13	98
2g	2'-F	(CH ₂) ₂ OH	B	90	190-196	C ₂₄ H ₂₅ FN ₄ O ₇ ·H ₂ O	499	0.078	0.07	
2h	2'-F	(CH ₂) ₃ OH	B	77	132-136	C ₂₅ H ₂₇ FN ₄ O ₇ ·H ₂ O	513	0.59	0.030	95
2i	2'-F	(CH ₂) ₂ NH ₂	I	20	210-215 ^c	C ₂₅ H ₂₆ FN ₅ O ₆ ·1.25H ₂ O ^d	498	11.72	2.7	
2j	2'-F	CH ₂ CN	J	84	160-162 ^e	C ₂₄ H ₂₂ FN ₅ O ₆ ·0.5H ₂ O ^f	494	0.17	0.034	90
2k	2'-F	CH ₂ CONH ₂	B ^g	22	185 ^h	C ₂₄ H ₂₄ FN ₅ O ₇ ·H ₂ O	512	1.34	0.80	
3a	3'-F	CH ₂ C≡CH	B	79	156-160	C ₂₅ H ₂₃ FN ₄ O ₆ ·H ₂ O	493	1.43	0.052	102
3b	3'-F	H	B	92	220-222	C ₂₂ H ₂₁ FN ₄ O ₆ ·H ₂ O	455	9.34	0.12	86
3c	3'-F	CH ₃	B	94	210-211	C ₂₃ H ₂₃ FN ₄ O ₆ ·H ₂ O	469	1.14	0.14	92
3d	3'-F	CH ₂ CH ₃	B	86	167-168	C ₂₄ H ₂₅ FN ₄ O ₆ ·1.5H ₂ O	483	0.68	0.086	96
3e	3'-F	CH ₂ CH=CH ₂	B ⁱ	63	108-110	C ₂₅ H ₂₅ FN ₄ O ₆ ·2H ₂ O	495	5.16	0.14	97
3f	3'-F	(CH ₂) ₂ F	B	83	162-166	C ₂₄ H ₂₄ F ₂ N ₄ O ₆ ·1.5H ₂ O	501	1.75	0.069	
3g	3'-F	(CH ₂) ₂ OH	B ⁱ	28	120-122	C ₂₄ H ₂₅ FN ₄ O ₇ ·2H ₂ O	499	3.58	0.085	95
3h	3'-F	(CH ₂) ₃ OH	B ⁱ	70	127-132	C ₂₅ H ₂₇ FN ₄ O ₇ ·2H ₂ O	513	0.98	0.17	
4a	2'-Cl	CH ₂ C≡CH	B	66	162-164	C ₂₅ H ₂₃ ClN ₄ O ₆ ·0.5H ₂ O	509	0.074	0.25	
4d	2'-Cl	CH ₂ CH ₃	B	76	201-207	C ₂₄ H ₂₅ ClN ₄ O ₆ ·H ₂ O	499	0.12	0.87	
5a	2'-CF ₃	CH ₂ C≡CH	B	85	197-198	C ₂₆ H ₂₃ F ₃ N ₄ O ₆ ·1.25H ₂ O	543	0.48	1.2	
6a	2'-CH ₃	CH ₂ C≡CH	B	74	95 ^j	C ₂₆ H ₂₆ N ₄ O ₆ ·H ₂ O	489	0.50	0.36	97
6c	2'-CH ₃	CH ₃	B	62	219-221 ^k	C ₂₄ H ₂₆ N ₄ O ₆ ·2H ₂ O ^l	465	0.94	0.8	
6d	2'-CH ₃	CH ₂ CH ₃	B	67	212-215 ^k	C ₂₅ H ₂₈ N ₄ O ₆ ·0.5H ₂ O	479	0.32	1.2	94
7a	2'-NH ₂	CH ₂ C≡CH	B ^m	88	182-186	C ₂₅ H ₂₆ N ₅ O ₆ ·0.5H ₂ O	490	0.29	0.36	
7c	2'-NH ₂	CH ₃	B ^m	86	188-190 ^k	C ₂₃ H ₂₅ N ₅ O ₆ ·1.5H ₂ O	466	0.86	0.76	
7d	2'-NH ₂	CH ₂ CH ₃	B ^m	91	170-175 ^k	C ₂₄ H ₂₇ N ₅ O ₆ ·H ₂ O	480	0.31	1.3	
8a	2'-OH	CH ₂ C≡CH	B ^m	83	210 ^k	C ₂₅ H ₂₄ N ₄ O ₇ ·0.75H ₂ O	491	0.09	0.5	77
9a	2'-OCH ₃	CH ₂ C≡CH	B	50	175-180	C ₂₆ H ₂₆ N ₄ O ₇ ·2.5H ₂ O	505	0.067	3.0	95
9d	2'-OCH ₃	CH ₂ CH ₃	B	64	152-157	C ₂₅ H ₂₈ N ₄ O ₇ ·H ₂ O	495	0.19	18.0	
10a	2'-NO ₂	CH ₂ C≡CH	B	73	190-202	C ₂₅ H ₂₃ N ₅ O ₆ ·H ₂ O	520	0.13	0.40	
10d	2'-NO ₂	CH ₂ CH ₃	B	68	192-200 ^k	C ₂₄ H ₂₅ N ₅ O ₆ ·1.4H ₂ O ⁿ	510	0.145	1.5	

^a Anal. C, H, N except where stated otherwise. ^b See ref 3. ^c Softens >190 °C. ^d N: calcd, 13.4; found, 12.7. ^e Softens >145 °C. ^f N: calcd, 13.9; found, 13.3. ^g Prepared from the N10-cyanomethyl ester **44k**. ^h Softens >170 °C. ⁱ The starting quinazoline antifolate diethyl ester was protected as the N3-(pivaloyloxy)methyl derivative. ^j Sinters above this temperature but does not give a discrete melting point. ^k Decomposes at this temperature. ^l H: calcd, 5.9; found, 5.4. ^m Hydrolyses of **49a,c,d** and **50a** were performed at 50 °C for 2 h. ⁿ H: calcd, 5.2; found, 4.6.

series. A cyanomethyl group is a good isosteric replacement for propargyl since **2j** exhibits high potency against both parameters although the bulkier carbamoylmethyl group in **2k** causes diminished activity. The reduced potency of the aminoethyl (**2i**) analogue may result from repulsive electronic interactions with basic groups in this region of the enzyme. Steric factors alone will not explain this poor activity since the close hydroxyethyl isostere **2g** is a highly potent compound. In the range of N10-substituents studied 3'-fluoro substitution (**3a-h**) caused a drop in TS inhibitory potency although this effect is more pronounced in the propargyl analogue than in the others. The loss of enzyme inhibition seen with these analogues was not paralleled by a similar loss in L1210 growth inhibition. Indeed a notable improvement was found with aliphatic substituents other than methyl.

Of the 2'-substituents larger than fluorine that were incorporated some, chlorine (**4a,d**), hydroxide (**8a**), methoxide (**9a,d**), and the nitro group (**10a,d**) were well tolerated by TS while others, the amino (**7a,c,d**), methyl (**6a,c,d**), and trifluoromethyl (**5a**) groups, were less well accommodated. However all of these substituents caused diminished inhibition of L1210 cell growth.

Overall these results demonstrate that within this series of antifolates the simultaneous variation of the substituents

on N10, C2', and C3' has separate effects on the inhibitory potencies against TS and cell growth. In particular the combination of 2'-fluoro or 3'-fluoro substitution with appropriate N10-substituents has given the most potent cytotoxic TS inhibitors so far reported. The lack of direct correlation between potency against TS and the observed inhibition of cell growth suggests that other factors such as rate of uptake into the L1210 cells and degree of polyglutamation are also strongly influenced by modifications to these regions of the molecule.

Experimental Section

The general procedures used were described in the earlier paper¹⁰ in this series.

Diethyl N-[4-[N-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-N-prop-2-ynylamino]-2-fluorobenzoyl]-L-glutamate (44a**).** Method A. A mixture of **36a**¹ (1.10 g, 2.91 mmol), the bromomethyl compound **11**³ (736 mg, 2.91 mmol), and powdered CaCO₃ (583 mg, 5.83 mmol) in DMA (9 mL) was stirred for 24 h at 50 °C under argon. The cooled mixture was filtered and the filtrate was evaporated to dryness. The crude product was purified by chromatography using a gradient of 2-6%

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v/v EtOH in CH_2Cl_2 as eluent to give an amorphous solid: 589 mg (37%); mp 202–204 °C; NMR ($\text{Me}_2\text{SO}-d_6$) δ 1.2 (t, 6 H, 2 OCH_2CH_3), 2.0 (m, 2 H, $\text{CHCH}_2\text{CH}_2\text{CO}_2\text{Et}$), 2.35 (s, 3 H, CH_3), 2.4 (t, 2 H, $\text{CHCH}_2\text{CH}_2\text{CO}_2\text{Et}$), 3.25 (t, 1 H, $\text{C}\equiv\text{CH}$), 4.05, 4.1 (2 q, 4 H, 2 OCH_2CH_3), 4.35 (br s, 2 H, $\text{CH}_2\text{C}\equiv\text{C}$), 4.4 (m, 1 H, CH), 4.8 (br s, 2 H, $\text{ArCH}_2\text{N}<$), 6.65 (dd, 1 H, 3'-H), 6.7 (dd, 1 H, 5'-H), 7.5 (t, 1 H, 6'-H), 7.55 (d, 1 H, quinazoline 8-H), 7.7 (dd, 1 H, quinazoline 7-H), 7.95 (d, 1 H, quinazoline 5-H), 8.15 (dd, 1 H, CONH). Anal. ($\text{C}_{29}\text{H}_{31}\text{FN}_4\text{O}_6$) C, H, N, F.

The procedure was repeated with the appropriate amines to yield the antifolate diesters 44b–45d (Table I).

***N*-[4-[*N*-(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)-methyl]-*N*-prop-2-ynylamino]-2-fluorobenzoyl]-L-glutamic Acid (2a). Method B.** The diester 44a (360 mg, 0.65 mmol) was stirred for 5 h under argon in a mixture of 1 N aqueous NaOH (2.2 mL, 2.2 mmol), EtOH (12 mL), and H_2O (12 mL). The resulting solution was evaporated below 30 °C to ca. 10 mL, filtered into a centrifuge tube, and brought a pH 3.0 with 2 N aqueous HCl. The precipitate was isolated by centrifugation and freed from inorganic ions by repeated cycles of aqueous suspension–centrifugation–decantation until the supernatant was free of chloride ion (AgNO_3 test). The damp product was vacuum dried to give an amorphous white solid: 249 mg (77%); mp 228–230 °C; NMR ($\text{Me}_2\text{SO}-d_6$) δ 2.0 (m, 2 H, $\text{CHCH}_2\text{CH}_2\text{CO}_2\text{H}$), 2.35 (t, 2 H, $\text{CHCH}_2\text{CH}_2\text{CO}_2\text{H}$), 2.35 (s, 3 H, CH_3), 3.25 (t, 1 H, $\text{C}\equiv\text{CH}$), 4.35 (d, 2 H, $\text{CH}_2\text{C}\equiv\text{C}$), 4.35 (m, 1 H, CH), 4.8 (br s, 2 H, $\text{ArCH}_2\text{N}<$), 6.65 (dd, 1 H, 3'-H), 6.7 (dd, 1 H, 5'-H), 7.55 (t, 1 H, 6'-H), 7.55 (d, 1 H, quinazoline 8-H), 7.7 (dd, 1 H, quinazoline 7-H), 7.95 (d, 1 H, quinazoline 5-H), 7.95 (dd, 1 H, CONH); MS (FAB) m/z 493 [M – H][–]. Anal. ($\text{C}_{25}\text{H}_{23}\text{FN}_4\text{O}_6$) C, H, N.

The procedure was repeated with the appropriate diethyl esters 44b–51d and 62a,d to yield the antifolates 2b–10d (Table II). Some of the compounds gave poor melting points but all had correct elemental analyses (C, H, N) for the formulae listed in the table and NMR spectra consistent with the assigned structures.

Diethyl *N*-(4-Amino-2-fluorobenzoyl)-L-glutamate (28). Method C. A solution of the nitro compound 20 (20.0 g, 54 mmol) in EtOAc (400 mL) was stirred with 10% Pd–C (2.0 g) in an atmosphere of H_2 until complete reduction was indicated by TLC (ca. 4 h). The solution was filtered through Celite and evaporated to dryness to yield a white solid: 18.3 g (99%); mp 129–131 °C. Anal. ($\text{C}_{16}\text{H}_{21}\text{FN}_2\text{O}_5$) C, H, N, F.

The procedure was repeated with the appropriate nitro compounds 21, 23–27 to yield the amines 29, 31–35 (Table I).

Diethyl *N*-[2-Fluoro-4-(methylamino)benzoyl]-L-glutamate (36c). Method D. A mixture of 28 (1.0 g, 2.94 mmol) and MeI (0.55 mL, 8.82 mmol) in DMF (3 mL) was stirred for 1 h at 60 °C under argon. The cooled mixture was diluted with saturated aqueous NaHCO_3 and extracted with EtOAc. The dried EtOAc solution was evaporated and the residue was purified by chromatography using a gradient of 0–15% v/v EtOAc in CH_2Cl_2 as eluent. The product (321 mg, 31%) was isolated as a white solid: mp 97–98 °C; NMR (CDCl_3) δ 1.2, 1.3 (2 t, 6 H, 2 OCH_2CH_3), 2.0–2.55 (m, 4 H, $\text{CH}_2\text{CH}_2\text{CO}_2\text{Et}$), 2.9 (s, 3 H, NCH_3), 4.1, 4.2 (2 q, 4 H, 2 OCH_2CH_3), 4.85 (m, 1 H, CH), 6.2 (dd, 1 H, Ar 3-H), 6.4 (dd, 1 H, Ar 5-H), 7.1 (dd, 1 H, CONH), 7.9 (dd, 1 H, Ar 6-H). Anal. ($\text{C}_{17}\text{H}_{23}\text{FN}_2\text{O}_5$) C, H, N.

The procedure was repeated with the appropriate amines 28 and 29 using MeI, EtI, allyl bromide, and 2-bromoethyl acetate as the alkylating agents to yield 36d,e and 37c–e,g (Table I).

Diethyl *N*-[2-Fluoro-4-(2-fluoroethylamino)benzoyl]-L-glutamate (36f). Method E. A mixture of 28 (6.0 g, 17.6 mmol), 1-bromo-2-fluoroethane (2.0 mL, 26.4 mmol), and 2,6-lutidine (2.05 mL, 17.6 mmol) in DMA (30 mL) was heated for 24 h at 120 °C in a sealed tube. The cooled reaction mixture was partitioned between EtOAc (3 × 70 mL) and H_2O (150 mL). The EtOAc solution was dried and the solvent was evaporated. The crude product was purified by chromatography using a gradient of 0–20% v/v EtOAc in CH_2Cl_2 as eluent. The product (5.67 g, 83%) was isolated as a gum: NMR ($\text{Me}_2\text{SO}-d_6$) δ 1.15, 1.20 (2 t, 6 H, 2 OCH_2CH_3), 2.0 (m, 2 H, $\text{CHCH}_2\text{CH}_2\text{CO}_2\text{Et}$), 2.4 (t, 2 H, $\text{CHCH}_2\text{CH}_2\text{CO}_2\text{Et}$), 3.4 (dq, 2 H, CH_2N), 4.05, 4.1 (2 q, 4 H, 2 OCH_2CH_3), 4.4 (m, 1 H, CH), 4.55 (dt, 2 H, CH_2F), 6.4 (dd, 1 H, Ar 3-H), 6.5 (dd, 1 H, Ar 5-H), 6.7 (t, 1 H, >NH), 7.5 (dd, 1 H, Ar 6-H), 7.85 (dd, 1 H, CONH).

The procedure was repeated with the amine 29 to yield 37f (Table I).

Diethyl *N*-[4-[(2-Butyroxethylamino)-2-fluorobenzoyl]-L-glutamate (36g). Method F. A mixture of 28 (6.1 g, 17.9 mmol), 2-bromoethyl butyrate (18.9 g, 9.68 mmol), NaI (2.68 g, 17.9 mmol), and 2,6-lutidine (4.17 mL, 35.8 mmol) in DMF (24 mL) was stirred for 3 h at 140 °C under argon. The cooled reaction mixture was partitioned between EtOAc (2 × 200 mL) and H_2O (80 mL) containing 5 M H_2SO_4 (5 mL). The EtOAc solution was washed repeatedly with H_2O until the washings had pH >6. The organic solution was dried and evaporated to dryness. The crude product was purified by chromatography using a gradient of 0–10% v/v EtOAc in CH_2Cl_2 as eluent. The product (4.07 g, 47%) was isolated as an oil: NMR ($\text{Me}_2\text{SO}-d_6$) δ 0.9 (t, 3 H, CH_3), 1.15, 1.2 (2 t, 6 H, 2 OCH_2CH_3), 1.55 (m, 2 H, $\text{COCH}_2\text{CH}_2\text{CH}_3$), 2.0 (m, 2 H, $\text{CHCH}_2\text{CH}_2\text{CO}_2\text{Et}$), 2.3 (t, 2 H, CH_2CO_2), 2.35 (t, 2 H, $\text{CHCH}_2\text{CH}_2\text{CO}_2\text{Et}$), 3.4 (t, 2 H, CH_2N), 4.05, 4.1 (2 t, 4 H, 2 OCH_2CH_3), 4.15 (t, 2 H, $\text{OCH}_2\text{CH}_2\text{N}$), 4.45 (m, 1 H, CH), 6.4 (dd, 1 H, Ar 3-H), 6.5 (dd, 1 H, Ar 5-H), 7.5 (dd, 1 H, Ar 6-H), 7.85 (dd, 1 H, CONH).

Diethyl *N*-[4-[(3-Acetoxypropylamino)-2-fluorobenzoyl]-L-glutamate (36h). Method G. A mixture of 28 (2.0 g, 5.9 mmol), 1,3-dibromopropane (2.4 mL, 23.6 mmol), and 2,6-lutidine (0.76 mL, 6.5 mmol) in DMF (15 mL) was stirred for 5 h at 110 °C under argon. The reaction was worked up according to method F above to afford diethyl *N*-[4-[(3-bromopropylamino)-2-fluorobenzoyl]-L-glutamate (36i): 980 mg (36%); mp 60–61 °C. Anal. ($\text{C}_{19}\text{H}_{26}\text{BrFN}_2\text{O}_5$) C, H, N, Br, F.

A mixture of 36i (960 mg, 2.07 mmol) and powdered anhydrous NaOAc (1.7 g, 20.7 mmol) in DMF was stirred for 7.5 h at 80 °C under argon. The cooled reaction mixture was filtered and the filtrate evaporated to dryness. The crude product was purified by chromatography using 5% v/v EtOH in CH_2Cl_2 as eluent. The product 36h (770 mg, 85%) was isolated as a gum: NMR ($\text{Me}_2\text{SO}-d_6$) δ 1.15, 1.2 (2 t, 6 H, 2 OCH_2CH_3), 1.85 (m, 2 H, $\text{CH}_2\text{CH}_2\text{CH}_2$), 2.0 (s, 3 H, OCOCH_3), 2.05 (m, 2 H, $\text{CHCH}_2\text{CH}_2\text{CO}_2\text{Et}$), 2.40 (t, 2 H, $\text{CHCH}_2\text{CH}_2\text{CO}_2\text{Et}$), 3.15 (t, 2 H, CH_2N), 4.0–4.2 (m, 6 H, 3 OCH_2), 4.45 (m, 1 H, CH), 6.35 (dd, 1 H, Ar 3-H), 6.45 (dd, 1 H, Ar 5-H), 6.55 (t, 1 H, >NH), 7.45 (dd, 1 H, Ar 6-H), 7.85 (t, 1 H, CONH). Anal. ($\text{C}_{21}\text{H}_{29}\text{FN}_2\text{O}_7$) C, H, N.

Di-*tert*-butyl-*N*-[2-Fluoro-4-[(2-phthalimidoethylamino)benzoyl]-L-glutamate (53). Method H. A mixture of di-*tert*-butyl-*N*-(4-amino-2-fluorobenzoyl)-L-glutamate (52)⁵ (1.98 g, 5.0 mmol), 2-(bromoethyl)phthalimide (7.62 g, 30 mmol), NaI (4.5 g, 30 mmol), and 2,6-lutidine (3.5 mL, 30 mmol) in DMA (7.5 mL) was stirred for 5 h at 130 °C under argon. The reaction was worked up according to method F above to afford a gum: 1.10 g (39%); NMR (CDCl_3) δ 1.5 (s, 18 H, 2 *t*-Bu), 2.05 (m, 2 H, $\text{CHCH}_2\text{CH}_2\text{CO}_2$ -*t*-Bu), 2.3 (t, 2 H, $\text{CHCH}_2\text{CH}_2\text{CO}_2$ -*t*-Bu), 3.45 (br t, 2 H, CH_2NH), 4.0 (t, 2 H, CH_2NPhth), 4.4 (m, 1 H, CH), 6.25 (dd, 1 H, Ar 3-H), 6.4 (dd, 1 H, Ar 5-H), 7.2 (dd, 1 H, Ar 6-H), 7.65–7.9 (m, 5 H, phthalimide ArH's and CONH).

***N*-[4-[*N*-(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)-methyl]-*N*-(2-aminoethylamino)-2-fluorobenzoyl]-L-glutamic Acid (2i). Method I.** The amino compound 53 was alkylated with 11 according to method A above to give 54 in 27% yield.

A mixture of 54 (360 mg, 0.49 mmol), 3-(dimethylamino)propylamine (0.37 mL, 2.92 mmol) and *N,N*-diisopropylethylamine (0.17 mL, 0.97 mmol) in MeOH (6 mL) was stirred for 6 h under reflux and argon. The resulting solution was evaporated to dryness and the crude product was purified by chromatography using a gradient of 0–20% v/v MeOH in CH_2Cl_2 containing 1% Et_3N to provide the di-*tert*-butyl ester (164 mg, 55%) of 2i as a gum. Hydrolysis of this ester at 60 °C according to method B yielded an amorphous solid: 50 mg (37%; 20% overall from 54); mp 210–215 °C (softened >190 °C); NMR ($\text{Me}_2\text{SO}-d_6$) δ 2.0 (m, 2 H, $\text{CHCH}_2\text{CH}_2\text{CO}_2\text{H}$), 2.25 (t, 2 H, $\text{CHCH}_2\text{CH}_2\text{CO}_2\text{H}$), 2.3 (s, 3 H, CH_3), 3.05 (m, 2 H, CH_2NH_2), 3.7 (m, 2 H, $\text{CH}_2\text{N}<$), 4.3 (m, 1 H, CH), 4.8 (br s, 2 H, $\text{ArCH}_2\text{N}<$), 6.7 (dd, 1 H, 3'-H), 6.95 (dd, 1 H, 5'-H), 7.5–7.7 (m, 3 H, 6'-H, quinazoline 8-H and 7-H), 7.85 (br s, 1 H, quinazoline 5-H), 7.9 (dd, 1 H, CONH); MS (FAB) m/z 498 [M – H][–]. Anal. ($\text{C}_{24}\text{H}_{26}\text{FN}_5\text{O}_6 \cdot 1.25\text{H}_2\text{O}$) C, H, N.

***N*-[4-[*N*-(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)-methyl]-*N*-(cyanomethylamino)-2-fluorobenzoyl]-L-glut-**

amic Acid (2j). Method J. Alkylation of **52** with bromoacetonitrile at 95 °C using method F gave **55** (91%), which was further alkylated with **11** (method A) to afford **56** (20%) as a foam. Anal. (C₃₂H₃₈FN₅O₆·0.25H₂O) C, H, N.

A solution of **56** (615 mg, 1.01 mmol) in CF₃CO₂H (6.5 mL) was kept for 10 min and evaporated to dryness. The residue was dissolved in a solution of NaHCO₃ (840 mg, 10 mmol) in H₂O (10 mL). The solution was filtered into a centrifuge tube and brought to pH 3.0 with 2 N aqueous HCl. The precipitate was isolated by centrifugation and freed from inorganic ions by four cycles of aqueous suspension–centrifugation–decantation. The damp product was freeze-dried to give an off-white amorphous solid: 430 mg (84%); mp 160–162 °C (softened >145 °C); NMR (Me₂SO-*d*₆) δ 2.0 (m, 2 H, CHCH₂CH₂CO₂H), 2.3 (t, 2 H, CHCH₂CH₂CO₂H), 2.35 (s, 3 H, CH₃), 4.4 (m, 1 H, CH), 4.8 (br s, 2 H, CH₂CN), 4.85 (br s, 2 H, ArCH₂N<), 6.75 (dd, 1 H, 3'-H), 6.8 (dd, 1 H, 5'-H), 6.95–7.8 (m, 3 H, 6'-H, quinazoline 8-H and 7-H), 7.95 (d, 1 H, quinazoline 5-H), 8.0 (dd, 1 H, CONH); MS (FAB) *m/z* 494 [M - H]⁻. Anal. (C₂₄H₂₂FN₅O₆·0.5H₂O) C, H, N.

N-[4-[N-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyloxy)methyl]-N-(carbamoylmethyl)amino]-2-fluorobenzoyl]-L-glutamic Acid (2k). The diethyl ester **44k** prepared as for **56** above from **28** was hydrolyzed according to method B. The product **2k** (187 mg, 22%) had mp 185 °C (softened >170 °C); MS (FAB) *m/z* 512 [M - H]⁻. Anal. (C₂₄H₂₄FN₅O₇·H₂O) C, H, N.

Diethyl N-(3-Fluoro-4-nitrobenzoyl)-L-glutamate (2l). Method K. A stirred slurry of 3-fluoro-4-nitrobenzoic acid⁶ (18.5 g, 0.1 mol) in dry toluene (400 mL) was treated with SOCl₂ (11 mL, 0.15 mol) and the mixture was heated under reflux for 1.5 h. The solution was cooled, filtered through Celite, and evaporated to dryness to give the crude acid chloride as a brown oil which solidified on standing. A solution of this acid chloride in CH₂Cl₂ (400 mL) was added over 15 min to a stirred mixture of diethyl L-glutamate hydrochloride (24 g, 0.1 mol) and Et₃N (27.9 mL, 0.2 mol) in CH₂Cl₂ (400 mL) below 25 °C (with cooling). The resulting brown solution was stirred for a further 2 h and then washed with H₂O (2 × 500 mL), treated with charcoal, and dried. The solution was evaporated to dryness and the residue was recrystallized from toluene–cyclohexane to give yellow needles: 35.75 g (96%); mp 80–81 °C; NMR (CDCl₃) δ 1.25, 1.30 (2 t, 6 H, 2 OCH₂CH₃), 2.25 (m, 2 H, CHCH₂CH₂CO₂Et), 2.5 (t, 2 H, CHCH₂CH₂CO₂Et), 4.15, 4.25 (2 q, 4 H, 2 OCH₂CH₃), 4.7 (m, 1 H, CH), 7.7 (d, 1 H, CONH), 7.75 (dd, 1 H, 6-H), 7.8 (dd, 1 H, 2-H), 8.15 (dd, 1 H, 5-H). Anal. (C₁₆H₁₉FN₂O₇) C, H, N, F.

The procedure was repeated with 4-nitro-2-(trifluoromethyl)benzoic acid¹¹ to yield diethyl N-[4-nitro-2-(trifluoromethyl)benzoyl]-L-glutamate (**23**).

Diethyl N-[3-Fluoro-4-(prop-2-ynylamino)benzoyl]-L-glutamate (37a). Method L. A mixture of **29** (6.8 g, 20 mmol) (prepared from **21** according to method C), K₂CO₃ (2.76 g, 20 mmol), and propargyl bromide (4.46 mL of an 80% w/w solution in toluene, 40 mmol) in DMF (22 mL) was stirred for 3 h at 120 °C under argon. The cooled mixture was filtered and the filtrate was evaporated to dryness. The residue was partitioned between EtOAc (250 mL) and H₂O (250 mL). The organic phase was dried and the solvent was evaporated. The crude product was purified by gas chromatography using 40% v/v EtOAc in hexane as eluent. The product (4.02 g, 53%) was isolated as a yellow oil: NMR (CDCl₃) δ 1.25, 1.3 (2 t, 6 H, 2 OCH₂CH₃), 2.2 (m, 2 H, CHCH₂CH₂CO₂Et), 2.25 (t, 1 H, C≡CH), 2.45 (t, 2 H, CHCH₂CH₂CO₂Et), 4.0 (br s, 2 H, CH₂C≡C), 4.1, 4.45 (2 q, 4 H, 2 OCH₂CH₃), 4.55 (br s, 1 H, HN<), 4.75 (m, 1 H, CH), 6.8 (t, 1 H, 5-H), 6.9 (d, 1 H, CONH), 7.5 (dd, 1 H, 6-H), 7.55 (dd, 1 H, 2-H). Anal. (C₁₉H₂₃FN₂O₅) H, N, F; C: calcd, 59.6; found, 60.3.

Diethyl N-[4-[(3-Acetoxypropyl)amino]-3-fluorobenzoyl]-L-glutamate (37 h). Method M. A mixture of **29** (340 mg, 1.0 mmol), 3-bromopropyl acetate (1.09 g, 6 mmol), and 2,6-lutidine (0.13 mL, 1.1 mmol) in DMF (3 mL) was stirred for 9 h at 150 °C under argon. The cooled mixture was partitioned between EtOAc (50 mL) and 0.5 N aqueous H₂SO₄ (10 mL). The

organic solution was washed several times with brine until the washings had pH >6.0, dried, and concentrated to a gum. Purification was achieved by chromatography using a gradient of 0–10% v/v EtOAc in CH₂Cl₂ as eluent. The product (117 mg, 27%) was isolated as a gum: NMR (Me₂SO-*d*₆) δ 1.15 (2 t, 6 H, 2 OCH₂CH₃), 1.9 (m, 2 H, CH₂CH₂CH₂), 2.0 (s, 3 H, OCOCH₃), 2.05 (m, 2 H, CHCH₂CO₂Et), 2.4 (t, 2 H, CHCH₂CH₂CO₂Et), 3.2 (t, 3 H, NCH₂), 4.1 (m, 6 H, 3 OCH₂), 4.4 (m, 1 H, CH), 6.05 (br s, 1 H, amine NH), 6.7 (dd, 1 H, Ar 5-H), 7.55 (d, 1 H, Ar 6-H), 7.6 (d, 1 H, Ar 2-H), 8.3 (d, 1 H, CONH).

The procedure was repeated with the appropriate (4-amino-benzoyl)-L-glutamate esters and alkyl halides at the stated temperatures (Table I) to yield the amines **38d** and **40c–43d**.

Diethyl N-[4-[N-[(3,4-Dihydro-2-methyl-4-oxo-3-[(pivaloyloxy)methyl]-6-quinazolinyloxy)methyl]-N-(3-acetoxypropyl)amino]-3-fluorobenzoyl]-L-glutamate (45 h). Method N. A mixture of **37h** (110 mg, 0.25 mmol), the bromomethyl compound **57'** (183 mg, 0.5 mmol), and 2,6-lutidine (0.06 mL, 0.5 mmol) in DMF (2 mL) was stirred for 7.5 h at 105 °C under argon. The cooled mixture was evaporated to dryness and the residue was purified by chromatography using a gradient of 0–30% v/v EtOAc in CH₂Cl₂ as eluent. The product (102 mg, 56%) was isolated as a gum: NMR (Me₂SO-*d*₆) δ 1.15 (s, 9 H, *t*-Bu), 1.2 (2 t, 6 H, 2 OCH₂CH₃), 1.9 (m, 2 H, CH₂CH₂CH₂), 1.95 (s, 3 H, OCOCH₃), 2.05 (m, 2 H, CHCH₂CH₂CO₂Et), 2.4 (t, 2 H, CHCH₂CH₂CO₂Et), 2.6 (s, 3 H, CH₃), 3.35 (m, 2 H, CH₂N<), 4.05 (m, 6 H, 3 OCH₂), 4.4 (m, 1 H, CH), 4.6 (br s, 2 H, ArCH₂N<), 6.05 (s, 2 H, OCH₂N), 7.05 (dd, 1 H, 5'-H), 7.55 (d, 1 H, 6'-H), 7.6 (d, 1 H, 2'-H), 7.7 (m, 2 H, quinazoline 7-H and 8-H), 8.05 (d, 1 H, quinazoline 5-H), 8.5 (d, 1 H, CONH).

The procedure was repeated with the appropriate starting amines to yield the 3-[(pivaloyloxy)methyl]quinazoline diesters **45e** and **45g** (Table I).

Diethyl N-(2-Methoxy-4-nitrobenzoyl)-L-glutamate (27). Method O. Oxalyl chloride (2.21 mL, 25.3 mmol) was added dropwise over 30 min to a stirred suspension of 2-methoxy-4-nitrobenzoic acid¹² (5.0 g, 25.3 mmol) in CH₂Cl₂ (200 mL) containing DMF (1 drop). After 3 h the solvent was evaporated and the crude acid chloride was dissolved with stirring in a mixture of CH₂Cl₂ (200 mL) and Et₃N (17.5 mL) and cooled to 0 °C. A solution of diethyl L-glutamate hydrochloride (6.08 g, 25.3 mmol) in CH₂Cl₂ (50 mL) was added dropwise while the temperature was kept below 20 °C. Stirring was continued for 1 h and the mixture was washed with 2 N HCl (2 × 50 mL) and brine (50 mL). The organic phase was dried and evaporated to dryness. The product (8.98 g, 93%) had the following: NMR (CDCl₃) δ 1.2, 1.35 (2 t, 6 H, 2 OCH₂CH₃), 2.15 (m, 2 H, CHCH₂CH₂CO₂Et), 2.45 (t, 2 H, CHCH₂CH₂CO₂Et), 4.1, 4.25 (2 q, 4 H, 2 OCH₂CH₃), 4.35 (s, 3 H, OCH₃), 4.85 (m, 1 H, CH), 7.85 (d, 1 H, Ar 3-H), 7.9 (dd, 1 H, Ar 5-H), 8.35 (d, 1 H, Ar 6-H), 8.5 (d, 1 H, CONH).

The procedure was repeated with the substituted 4-nitrobenzoic acids **17**¹³ and **18**¹⁴ to yield the substituted (4-nitrobenzoyl)-glutamate esters **25** and **26**.

Diethyl N-[4-[N-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyloxy)methyl]-N-prop-2-ynylamino]-2-methoxybenzoyl]-L-glutamate (51a). Method P. A mixture of **11** (1.0 g, 3.95 mmol), **43a** (1.54 g, 3.95 mmol) (prepared from **27** according to methods C and M), and 2,6-lutidine (2.3 mL, 19.7 mmol) in DMF (10 mL) was stirred for 18 h at 80 °C under argon. The cooled mixture was partitioned between EtOAc (4 × 50 mL) and H₂O (100 mL). The combined organic phase was washed with brine, dried, and evaporated to dryness. The crude oil was purified by chromatography eluting with 5% v/v MeOH in EtOAc. The product (780 mg, 35%) was isolated as a gum: NMR (Me₂SO-*d*₆) δ 1.15, 1.2 (2 t, 6 H, 2 OCH₂CH₃), 2.0 (m, 2 H, CHCH₂CH₂CO₂Et), 2.3 (t, 2 H, CHCH₂CH₂CO₂Et), 2.35 (s, 3 H, CH₃), 3.25 (t, 1 H, C≡CH), 3.85 (s, 3 H, OCH₃), 4.1, 4.2 (2 q, 4 H, 2 OCH₂CH₃), 4.35 (d, 2 H, CH₂C≡C), 4.45 (m, 1 H, CH), 4.8 (br s, 2 H, ArCH₂N<), 6.45 (s, 1 H, 3'-H), 6.6 (d, 1 H, 5'-H), 7.55 (d, 1 H, quinazoline

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8-H), 7.7 (d and dd, 2 H, 6'-H and quinazoline 7-H), 8.0 (d, 1 H, quinazoline 5-H), 8.2 (d, 1 H, CONH).

The procedure was repeated with the appropriate amines to yield the quinazoline antifolate diesters **45f**, **46a-51d** (Table I).

Methyl 2-Nitro-4-(prop-2-ynylamino)benzoate (59). A mixture of methyl 4-amino-2-nitrobenzoate (**58**)⁸ (10.6 g, 54 mmol), 2,6-lutidine (8.14 mL, 70 mmol), and propargyl bromide (7.82 mL of an 80% w/w solution in toluene, 70 mmol) in DMA (50 mL) was stirred for 4 h at 80 °C under argon. A second portion of propargyl bromide (70 mmol) was added and stirring was continued for a further 4.5 h at 80 °C. The cooled reaction mixture was partitioned between EtOAc (300 mL) and H₂O (3 × 100 mL). The organic phase was dried and evaporated to dryness. The crude product was purified by chromatography using CH₂Cl₂ as eluent to yield a buff solid: 6.58 g (52%); mp 134-135 °C. Anal. (C₁₁H₁₀N₂O₄) C, H; N: calcd, 12.0; found, 11.5.

Methyl 4-[N-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-N-prop-2-ynylamino]-2-nitrobenzoate (60). A mixture of the amino ester **59** (2.0 g, 8.55 mmol), the bromomethyl compound **11** (2.6 g, 10.25 mmol), and 2,6-lutidine (2.0 mL, 17.1 mmol) in DMA (10 mL) was stirred for 4 h at 80 °C under argon. The cooled reaction mixture was partitioned between EtOAc (2 × 300 mL) and H₂O (150 mL). The organic phase was washed with H₂O, dried, and evaporated to dryness. Trituration of the residue with EtOAc gave an amorphous buff solid: 1.52 g (44%); NMR (Me₂SO-*d*₆) δ 2.35 (s, 3 H, CH₃), 3.3 (t, 1 H, C≡CH), 3.75 (s, 3 H, OCH₃), 4.45 (d, 2 H, CH₂C≡C), 4.9 (br s, 2 H, ArCH₂N<), 7.05 (dd, 1 H, 5'-H), 7.2 (d, 1 H, 3'-H), 7.55 (d, 1 H, quinazoline 8-H), 7.65 (dd, 1 H, quinazoline 7-H), 7.75 (d, 1 H, 6'-H), 7.95 (d, 1 H, quinazoline 5-H).

4-[N-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)-methyl]-N-prop-2-ynylamino]-2-nitrobenzoic Acid (61). The methyl ester **60** (1.50 g, 3.7 mmol) was stirred for 2 h under argon in a mixture of 1 N aqueous NaOH (18.5 mL, 18.5 mmol) and EtOH (18.5 mL). The resulting solution was evaporated below 30 °C to ca. 10 mL, filtered, and acidified to pH 1 with 2 N aqueous HCl. The precipitated buff solid was filtered off, washed with H₂O, and vacuum dried: 1.24 g (82%); mp 260-262 °C (dec). Anal. (C₂₀H₁₆N₄O₅·H₂O) C, H, N.

Diethyl N-[4-[N-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-N-prop-2-ynylamino]-2-nitrobenzoyl]-L-glutamate (62a). **Method Q**. A solution of the acid **61** monohydrate (392 mg, 0.96 mmol) and diethyl L-glutamate hydrochloride (720 mg, 3.0 mmol) in DMF (3.3 mL) was stirred at 0 °C during the dropwise addition over 15 min of diphenyl phosphorazidate (0.43 mL, 2.0 mmol) followed by Et₃N (0.98 mL, 7.0 mmol) again over 15 min. The reaction mixture was allowed to warm to room temperature overnight and then partitioned between CH₂Cl₂ (3 × 25 mL) and H₂O (25 mL). The organic phase was washed with H₂O, dried, and evaporated to dryness. The

crude product was purified by chromatography using a gradient of 0-6% v/v EtOH in CH₂Cl₂ as eluent to give a buff solid: 421 mg (73%); mp 158-163 °C; NMR (Me₂SO-*d*₆) δ 1.15, 1.2 (2 t, 6 H, 2 OCH₂CH₃), 2.0 (m, 2 H, CHCH₂CH₂CO₂Et), 2.35 (s, 3 H, CH₃), 2.4 (t, 2 H, CHCH₂CH₂CO₂Et), 3.25 (t, 1 H, C≡CH), 4.05, 4.1 (2 q, 4 H, 2 OCH₂CH₃), 4.35 (m, 1 H, CH), 4.45 (d, 2 H, CH₂C≡C), 4.85 (br s, 2 H, ArCH₂N<), 7.1 (dd, 1 H, 5'-H), 7.25 (d, 1 H, 3'-H), 7.45 (d, 1 H, 6'-H), 7.55 (d, 1 H, quinazoline 8-H), 7.7 (dd, 1 H, quinazoline 7-H), 7.95 (d, 1 H, quinazoline 5-H), 8.8 (br d, CONH). Anal. (C₂₉H₃₁N₅O₈·0.75H₂O) C, H; N: calcd, 11.85; found, 11.3.

Registry No. **2a**, 112887-67-9; **2b**, 112887-95-3; **2c**, 112887-65-7; **2d**, 112887-66-8; **2e**, 129175-19-5; **2f**, 112887-97-5; **2g**, 112887-96-4; **2h**, 129175-20-8; **2i**, 129175-21-9; **2j**, 129175-22-0; **2k**, 112887-98-6; **3a**, 112888-30-9; **3b**, 129175-23-1; **3c**, 129175-24-2; **3d**, 129175-25-3; **3e**, 129175-26-4; **3f**, 129175-27-5; **3g**, 129175-28-6; **3h**, 129175-29-7; **4a**, 112888-33-2; **4d**, 112888-32-1; **5a**, 112888-26-3; **6a**, 129175-30-0; **6c**, 129175-31-1; **6d**, 129175-32-2; **7a**, 129193-64-2; **7c**, 129175-33-3; **7d**, 129175-34-4; **8a**, 112888-31-0; **9a**, 112888-28-5; **9d**, 112888-27-4; **10a**, 129175-35-5; **10d**, 112888-34-3; **11**, 112888-43-4; **13**, 403-21-4; **15**, 320-37-6; **17**, 91533-09-4; **18**, 17336-10-6; **19**, 2597-56-0; **20**, 106585-55-1; **21**, 126632-34-6; **23**, 112888-88-7; **24**, 80015-10-7; **25**, 129175-84-4; **26**, 129175-85-5; **27**, 129175-86-6; **28**, 106585-56-2; **29**, 126632-35-7; **30**, 80014-92-2; **31**, 112888-89-8; **32**, 80014-85-3; **33**, 129175-80-0; **34**, 129175-81-1; **35**, 129175-82-2; **36a**, 106585-57-3; **36c**, 112888-51-4; **36d**, 129175-62-8; **36e**, 129175-63-9; **36f**, 129175-64-0; **36g**, 129175-65-1; **36h**, 129175-66-2; **36k**, 112888-53-6; **36l**, 129175-83-3; **37a**, 1128893-4; **37c**, 129175-69-5; **37d**, 129175-70-8; **37e**, 129175-71-9; **37f**, 129175-72-0; **37g**, 129175-73-1; **37h**, 129175-74-2; **38a**, 80014-87-5; **38d**, 112888-95-6; **39a**, 112888-86-5; **40a**, 80014-86-4; **40c**, 129175-75-3; **40d**, 129175-76-4; **41a**, 129175-77-5; **41c**, 129175-78-6; **41d**, 129175-79-7; **42a**, 112888-94-5; **43a**, 112888-91-2; **43d**, 112888-90-1; **44a**, 129175-36-6; **44b**, 129175-37-7; **44c**, 129175-38-8; **44d**, 129175-39-9; **44e**, 129175-40-2; **44f**, 129193-65-3; **44g**, 129175-41-3; **44h**, 129175-42-4; **44k**, 129193-66-4; **45a**, 129193-67-5; **45b**, 129175-43-5; **45c**, 129175-44-6; **45d**, 129175-45-7; **45e**, 129193-68-6; **45f**, 129193-69-7; **45g**, 129175-46-8; **45h**, 129175-47-9; **46a**, 129175-48-0; **46d**, 129175-49-1; **47a**, 112888-87-6; **48a**, 129175-50-4; **48c**, 129175-51-5; **48d**, 129175-52-6; **49a**, 129175-53-7; **49c**, 129175-54-8; **49d**, 129175-55-9; **50a**, 129175-56-0; **51a**, 129175-57-1; **51d**, 129175-58-2; **52**, 85803-27-6; **53**, 129175-67-3; **54**, 129175-60-6; **55**, 129175-68-4; **56**, 129175-61-7; **57**, 112888-39-8; **58**, 84228-45-5; **59**, 129175-87-7; **60**, 129175-88-8; **61**, 129175-89-9; **62a**, 129175-59-3; **62d**, 112888-98-9; TS, 9031-61-2; BrCH₂C≡CH, 106-96-7; BrCH₂CH=CH₂, 106-95-6; BrCH₂CH₂OAc, 927-68-4; BrCH₂CH₂F, 762-49-2; BrCH₂CH₂OC(O)CH₂CH₂CH₃, 6065-66-3; Br(CH₂)₃Br, 109-64-8; Br(CH₂)₃OAc, 592-33-6; H-Glu(OEt)-OEt-HCl, 1118-89-4; 2-(bromoethyl)-phthalimide, 574-98-1.