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

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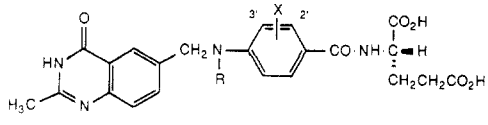
The synthesis of nine new 2-methyl-10-propargylquinazoline antifolates with substituents in the *p*-aminobenzoyl ring is described. In general the synthetic route involved the coupling of the appropriate ring-substituted diethyl *N*-[4-(prop-2-ynylamino)benzoyl]-L-glutamate with 6-(bromomethyl)-3,4-dihydro-2-methyl-4-oxoquinazoline (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 article[1] 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 **11**[3] with the appropriate N-alkylated anilines **36–43** using either $CaCO_3$ (method A) or 2,6-lutidine (method P) to scavenge HBr.

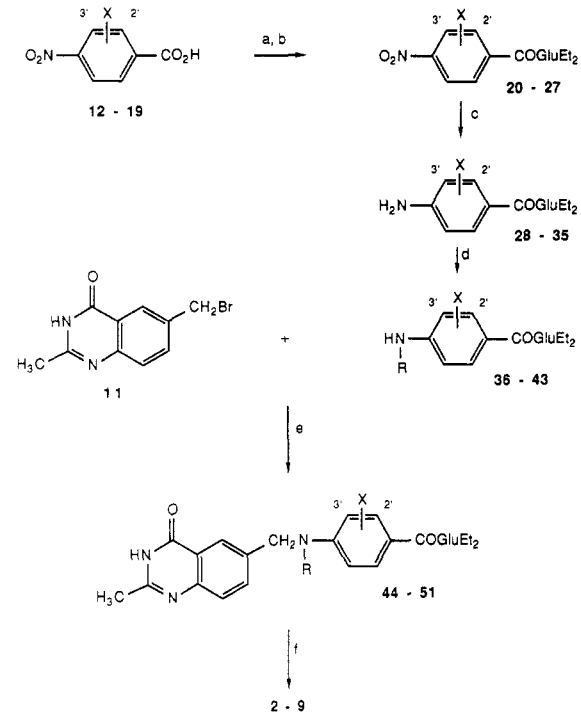


1	X = H	6	X = 2′-CH_3
2	X = 2′-F	7	X = 2′-NH_2
3	X = 3′-F	8	X = 2′-OH
4	X = 2′-Cl	9	X = 2′-OCH_3
5	X = 2′-CF_3	10	X = 2′-NO_2

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 **40a**[4] 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 (H_2, 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 aniline **36g** (method F) for the 2′-fluoro-10-(hydroxyethyl)analogue **2g** was prepared with the hydoxyl group

Scheme I[a,b]



12 - 19; 20 - 27; 28 - 35; 11; 36 - 43; 44 - 51; 2 - 9

12, 20, 28, 36, 44, X = 2′-F
13, 21, 29, 37, 45, X = 3′-F
14, 22, 30, 38, 46, X = 2′-Cl
15, 23, 31, 39, 47, X = 2′-CF_3
16, 24, 32, 40, 48, X = 2′-CH_3
17, 25, 33, 41, 49, X = 2′-$NHCOCF_3$
18, 26, 34, 42, 50, X = 2′-$OCOCH_3$
19, 27, 35, 43, 51, X = 2′-OCH_3

[a] See Table I for definition of R. [b] (a) $(COCl)_2$ or $SOCl_2$; (b) diethyl glutamate, Et_3N; (c) H_2, 10% palladium on carbon; (d) RX; (e) $CaCO_3$, DMA or 2,6-lutidine, DMF; (f) 1 N aqueous NaOH, EtOH.

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(1) Jackman, A. L.; Marsham, P. R.; Thornton, T. J.; Bishop, J. A. M.; O'Connor, B. M.; Hughes, L. R.; Calvert, A. H.; Jones, T. R. *J. Med. Chem.*, preceding paper in this issue.

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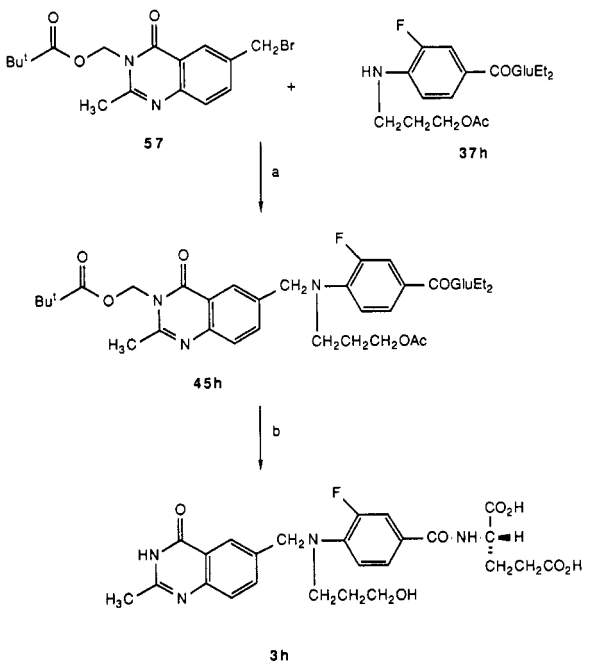
Table I. Preparation of Substituted (Aminobenzoyl)glutamate Diesters **36–43**, **53**, and **55** and Derived Antifolate Diesters **44–51**, **56**, and **62**

compd	starting amine	method (temp, °C)	% yield	derived antiofolate diester	method	% yield	X	R
36a[a]	**28**	L(120)[a]	60	**44a**	A	37	2′-F	$CH_2C{\equiv}CH$
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_2CH_3
36e	**28**	D(100)	51	**44e**	A	28	2′-F	$CH_2CH{=}CH_2$
36f	**28**	E(120)	83	**44f**	A	38	2′-F	$(CH_2)_2F$
36g	**28**	F(140)	47	**44g**	A	29	2′-F	$(CH_2)_2OCOC_3H_7$
36h	**28**	G(80)	31	**44h**	A	70	2′-F	$(CH_2)_3OCOCH_3$
53[b]	**52**[b]	H(130)	39	**54**[b]	A	27	2′-F	$(CH_2)_2NPhth$
55[b]	**52**[b]	F(85)	91	**56**[b]	A	20	2′-F	CH_2CN
36k	**28**	F(110)[c]	41	**44k**	A	38	2′-F	CH_2CN
37a	**29**	L(120)	53	**45a**	A[d]	40	3′-F	$CH_2C{\equiv}CH$
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_2CH_3
37e	**29**	D(100)[e]	36	**45e**[f]	N	50	3′-F	$CH_2CH{=}CH_2$
37f	**29**	E(120)	78	**45f**	P	23	3′-F	$(CH_2)_2F$
37g	**29**	D(100)	26	**45g**[f]	N	42	3′-F	$(CH_2)_2OCOCH_3$
37h	**29**	M(150)	27	**45h**[f]	N	56	3′-F	$(CH_2)_3OCOCH_3$
38a[g]	**30**[g]	*g*	41	**46a**	P	23	2′-Cl	$CH_2C{\equiv}CH$
38d	**30**	M(85)	25	**46d**	P	38	2′-Cl	CH_2CH_3
39a	**31**	L(100)	65	**47a**	P[h]	64	2′-CF_3	$CH_2C{\equiv}CH$
40a[g]	**32**[g]	*g*	66	**48a**	P	35	2′-CH_3	$CH_2C{\equiv}CH$
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_2CH_3
41a	**33**	M(60)	62	**49a**	P	44	2′-$NHCOCF_3$	$CH_2C{\equiv}CH$
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_2CH_3
42a	**34**	M(80)	61	**50a**	P	71	2′-$OCOCH_3$	$CH_2C{\equiv}CH$
43a	**35**	M(80)	58	**51a**	P	35	2′-OCH_3	$CH_2C{\equiv}CH$
43d	**35**	M(80)	48	**51d**	P	54	2′-OCH_3	CH_2CH_3
				62a	Q	73	2′-NO_2	$CH_2C{\equiv}CH$
				62d	Q	41	2-NO_2	CH_2CH_3

[a] See also ref 1. [b] Di-*tert*-butyl esters. [c] K_2CO_3 was used as the base. [d] Proton Sponge was used as the base. [e] 2,6-Lutidine was used as the base. [f] Protected as the 3-(pivaloyloxy)methyl derivative. [g] See ref 4. [h] MgO 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**[5] 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 CF_3CO_2H to yield **2j** (method J). When this sequence was repeated on the diethyl ester **28** and the *N*-cyanomethyl 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-

(2) Hughes, L. R.; Marsham, P. R.; Oldfield, J.; Jones, T. R.; O'Connor, B. M.; Bishop, J. A. M.; Calvert, A. H.; Jackman, A. L. *Proc. Am. Assoc. Cancer Res.* **1988**, *29*, 286.

(3) Hughes, L. R.; Jackman, A. L.; Oldfield, J.; Smith, R. C.; Burrows, K. D.; Marsham, P. R.; Bishop, J. A. M.; Jones, T. R.; O'Connor, B. M.; Calvert, A. H. *J. Med. Chem.*, first of three papers in this issue.

(4) Jones, T. R.; Smithers, M. J.; Taylor, M. A.; Jackman, A. L.; Calvert, A. H.; Harland, S. J.; Harrap, K. R. *J. Med. Chem.* **1986**, *29*, 468.

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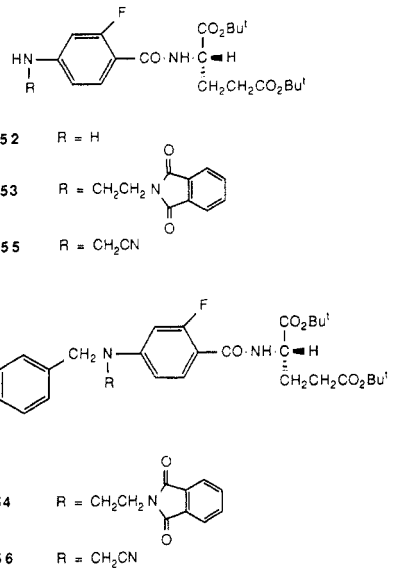
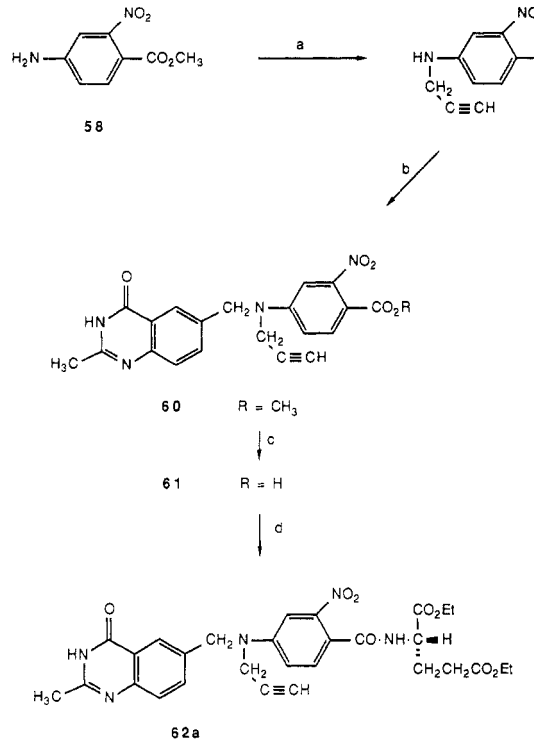


cessitating modified coupling procedures in some cases. In the coupling of **37a** to **11** the highest yield (40%) of **45a** was obtained when Proton Sponge[6] was used as base. If the coupling of **11** to anilines in the 3′-fluoro series was performed at elevated temperatures (>90 °C) in an attempt to accelerate the reaction, alkylation of the product on N3 by a second molecule of **11** became a serious problem. We circumvented this problem by protecting **11** as its N3-(pivaloyloxy)methyl derivative **57**.[7] This protected bromomethyl compound reacted cleanly with anilines at elevated at elevated temperatures (~105 °C) to give satisfactory yields of (pivaloyloxy)methyl-protected antifolate diesters in the 3′-fluoro series. A typical procedure of this type is outlined in Scheme II (method N).

In the synthesis of the 2′-amino (**7a,c,d**) and 2′-hydroxy (**8a**) analogues the functionalities were protected as the trifluoroacetyl and acetyl derivatives, respectively. These protecting groups were carried intact through the whole sequence and removed during the final hydrolysis with aqueous alkali. The synthesis of the 2′-nitro compound **10a** required a fundamentally different order of assembly of the synthons (Scheme III). Methyl 4-amino-2-nitrobenzoate (**58**)[8] was sequentially propargylated and alkylated with **11** to yield the tertiary amino ester **60** in 23% overall yield. This was saponified and the derived carboxylic acid **61** was coupled via the in situ generated azide with diethyl L-glutamate. The resulting antifolate diester **62a** was hydrolyzed according to method B to yield **10a** (Table II). The analogous sequence in which **58** was alkylated with iodoethane afforded **10d**.

Scheme III[a]



[a] (a) Propargyl bromide; (b) bromomethyl compound **11**, 2,6-lutidine, DMF; (c) 1 N aqueous NaOH, EtOH; (d) diethyl glutamate, diphenyl phosphorazidate, Et_3N, DMF.

Results and Discussion

The IC_{50} values for the inhibition of partially purified L1210 TS and for growth inhibition of L1210 cells were obtained as described in the preceding article[1] and are shown in Table II. The 2′-fluoro analogue **2a** showed a 2-fold enhancement over the parent **1a** in potency against TS and a 3-fold enhancement against L1210 cells. On the other hand, 3′-fluoro substitution (**3a**) was highly detrimental to TS inhibition. Nevertheless this 3′-fluoro derivative was still a more potent growth inhibitory agent than **1a**. The fact that thymidine alone prevents the growth-inhibitory properties of **1a**, **2a**, and **3a** suggests that TS is their sole locus of action of action. A possible explanation for this apparent discrepancy is that the 3-fluoro derivative is more readily transported into cells and/or metabolized intracellularly to polyglutamate derivatives that are not only retained within the cells but also have much greater TS inhibitory potency than the parent monoglutamates. In support of this hypothesis it has been demonstrated that these fluorinated compounds have retained or improved activity as substrates for folylpolyglutamate synthetase (FPGS).[9] We have reported[1] that there is a spin–spin coupling (J = 6.4 Hz) observed between the 2′-fluorine atom and the amide proton of **2a**, suggesting that there is a hydrogen bond between these two atoms that holds this region of the molecule in a favorable conformation for binding to TS. The sharp drop in affinity for TS caused by 3′-fluoro substitution is consistent with the resulting increase in electron density in the C3′ region giving rise to a direct unfavorable interaction with the enzyme or to effects on the conformation of the C9,N10 bridge region. Because of the enhanced inhibition of cell growth resulting from fluorine substitution we have replaced the N10-propargyl group of **2a** and **3a** with a series of alternative N10-substituents (**2b–k** and **3b–h**). In general, 2′-fluoro substitution resulted in improved TS and L1210 cell growth inhibition across the range of N10-substituents studied (for parent compound IC_{50} values,[3] see Table II). The enhancements were particularly marked (~5-fold) in N10-ethyl (**2d**), N10-fluoroethyl (**2f**), N10-hydroxyethyl (**2g**), and N10-alkyl (**2e**), whereas the N10-methyl (**2c**) analogue showed the greatest enhancement (6-fold) in growth inhibition. Three N10-substituents, namely cyanomethyl (**2j**), carbamoylmethyl (**2k**), and aminoethyl (**2i**) have been prepared only in the 2′-fluoro

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(7) Hughes, L. R. Eur. Pat. Appl. 239 362 A2, 1987.
(8) Hohn, J.; Hunger, K. German Patent 3 106 111, 1982.
(9) Jackman, A. L.; Marsham, P. R.; Hughes, L. R.; Thornton, T. J.; O'Connor, B. M.; Bishop, J. A. M.; Bisset, G.; Moran, R. G.; Calvert, A. H. *Proc. Am. Assoc. Cancer Res.* **1989**, *30*, 476.

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

compd	X	R	method	% yield	mp, °C	formula[a]	mass spectra *m/z* $[M - H]^-$	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	$CH_2C{\equiv}CH$			165	$C_{25}H_{24}N_4O_6{\cdot}2H_2O$		0.040	0.090	100
1b[b]	H	H			197–201	$C_{22}H_{22}N_4O_6{\cdot}H_2O$		4.50	0.07	
1c[b]	H	CH_3			254–257	$C_{23}H_{24}N_4O_6{\cdot}0.75H_2O$		0.30	0.11	100
1d[b]	H	CH_2CH_3			221–225	$C_{24}H_{26}N_4O_6{\cdot}0.5H_2O$		0.17	0.36	88
1e[b]	H	$CH_2CH{=}CH_2$			188	$C_{25}H_{26}N_4O_6{\cdot}1.5H_2O$		0.48	0.17	104
1f[b]	H	$(CH_2)_2F$			207–210	$C_{24}H_{25}FN_4O_6{\cdot}1.25H_2O$		0.24	0.4	
1g[b]	H	$(CH_2)_2OH$			>300	$C_{24}H_{26}N_4O_7{\cdot}1.5H_2O$		0.50	0.24	
1h[b]	H	$(CH_2)_3OH$			300	$C_{25}H_{28}N_4O_7{\cdot}H_2O$		0.54	1.24	95
2a	2′-F	$CH_2C{\equiv}CH$	B	77	228–230	$C_{25}H_{23}FN_4O_6$	493	0.020	0.027	97
2b	2′-F	H	B	90	190–194	$C_{22}H_{21}FN_4O_6{\cdot}H_2O$	455	3.78	0.064	94
2c	2′-F	CH_3	B	83	224–226	$C_{23}H_{23}FN_4O_6{\cdot}H_2O$	469	0.12	0.019	98
2d	2′-F	CH_2CH_3	B	94	214–217	$C_{24}H_{25}FN_4O_6{\cdot}0.75H_2O$	483	0.045	0.065	96
2e	2′-F	$CH_2CH{=}CH_2$	B	84	209–211	$C_{25}H_{25}FN_4O_6$	495	0.076	0.10	97
2f	2′-F	$(CH_2)_2F$	B	63	220–225	$C_{24}H_{24}F_2N_4O_6{\cdot}0.7H_2O$	501	0.043	0.13	98
2g	2′-F	$(CH_2)_2OH$	B	90	190–196	$C_{24}H_{25}FN_4O_7{\cdot}H_2O$	499	0.078	0.07	
2h	2′-F	$(CH_2)_3OH$	B	77	132–136	$C_{25}H_{27}FN_4O_7{\cdot}H_2O$	513	0.59	0.030	95
2i	2′-F	$(CH_2)_2NH_2$	I	20	210–215[c]	$C_{24}H_{26}FN_5O_6{\cdot}1.25H_2O$[d]	498	11.72	2.7	
2j	2′-F	CH_2CN	J	84	160–162[e]	$C_{24}H_{22}FN_5O_6{\cdot}0.5H_2O$[f]	494	0.17	0.034	90
2k	2′-F	CH_2CONH_2	B[g]	22	185[h]	$C_{24}H_{24}FN_5O_7{\cdot}H_2O$	512	1.34	0.80	
3a	3′-F	$CH_2C{\equiv}CH$	B	79	156–160	$C_{25}H_{23}FN_4O_6{\cdot}H_2O$	493	1.43	0.052	102
3b	3′-F	H	B	92	220–222	$C_{22}H_{21}FN_4O_6{\cdot}H_2O$	455	9.34	0.12	86
3c	3′-F	CH_3	B	94	210–211	$C_{23}H_{23}FN_4O_6{\cdot}H_2O$	469	1.14	0.14	92
3d	3′-F	CH_2CH_3	B	86	167–168	$C_{24}H_{25}FN_4O_6{\cdot}1.5H_2O$	483	0.68	0.086	96
3e	3′-F	$CH_2CH{=}CH_2$	B[i]	63	108–110	$C_{25}H_{25}FN_4O_6{\cdot}2H_2O$	495	5.16	0.14	97
3f	3′-F	$(CH_2)_2F$	B	83	162–166	$C_{24}H_{24}F_2N_4O_6{\cdot}1.5H_2O$	501	1.75	0.069	
3g	3′-F	$(CH_2)_2OH$	B[i]	28	120–122	$C_{24}H_{25}FN_4O_7{\cdot}2H_2O$	499	3.58	0.085	95
3h	3′-F	$(CH_2)_3OH$	B[i]	70	127–132	$C_{25}H_{27}FN_4O_7{\cdot}2H_2O$	513	0.98	0.17	
4a	2′-Cl	$CH_2C{\equiv}CH$	B	66	162–164	$C_{25}H_{23}ClN_4O_6{\cdot}0.5H_2O$	509	0.074	0.25	
4d	2′-Cl	CH_2CH_3	B	76	201–207	$C_{24}H_{25}ClN_4O_6{\cdot}H_2O$	499	0.12	0.87	
5a	2′-CF_3	$CH_2C{\equiv}CH$	B	85	197–198	$C_{26}H_{23}F_3N_4O_6{\cdot}1.25H_2O$	543	0.48	1.2	
6a	2′-CH_3	$CH_2C{\equiv}CH$	B	74	95[j]	$C_{26}H_{26}N_4O_6{\cdot}H_2O$	489	0.50	0.36	97
6c	2′-CH_3	CH_3	B	62	219–221[k]	$C_{24}H_{26}N_4O_6{\cdot}2H_2O$[l]	465	0.94	0.8	
6d	2′-CH_3	CH_2CH_3	B	67	212–215[k]	$C_{25}H_{28}N_4O_6{\cdot}0.5H_2O$	479	0.32	1.2	94
7a	2′-NH_2	$CH_2C{\equiv}CH$	B[m]	88	182–186	$C_{25}H_{25}N_5O_6{\cdot}0.5H_2O$	490	0.29	0.36	
7c	2′-NH_2	CH_3	B[m]	86	188–190[k]	$C_{23}H_{25}N_5O_6{\cdot}1.5H_2O$	466	0.86	0.76	
7d	2′-NH_2	CH_2CH_3	B[m]	91	170–175[k]	$C_{24}H_{27}N_5O_6{\cdot}H_2O$	480	0.31	1.3	
8a	2′-OH	$CH_2C{\equiv}CH$	B[m]	83	210[k]	$C_{25}H_{24}N_4O_7{\cdot}0.75H_2O$	491	0.09	0.5	77
9a	2′-OCH_3	$CH_2C{\equiv}CH$	B	50	175–180	$C_{26}H_{26}N_4O_7{\cdot}2.5H_2O$	505	0.067	3.0	95
9d	2′-OCH_3	CH_2CH_3	B	64	152–157	$C_{25}H_{28}N_4O_7{\cdot}H_2O$	495	0.19	18.0	
10a	2′-NO_2	$CH_2C{\equiv}CH$	B	73	190–202	$C_{25}H_{23}N_5O_8{\cdot}H_2O$	520	0.13	0.40	
10d	2′-NO_2	CH_2CH_3	B	68	192–200[k]	$C_{24}H_{25}N_5O_8{\cdot}1.4H_2O$[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 paper[10] 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 $CaCO_3$ (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%

(10) Marsham, P. R.; Chambers, P.; Hayter, A. J.; Hughes, L. R.; Jackman, A. L.; O'Connor, B. M.; Bishop, J. A. M.; Calvert, A. H. *J. Med. Chem.* **1989**, *32*, 569.

v/v EtOH in CH_2Cl_2 as eluent to give an amorphous solid: 589 mg (37%); mp 202–204 °C; NMR (Me_2SO-d_6) δ 1.2 (t, 6 H, 2 OCH_2CH_3), 2.0 (m, 2 H, $CHCH_2CH_2CO_2Et$), 2.35 (s, 3 H, CH_3), 2.4 (t, 2 H, $CHCH_2CH_2CO_2Et$), 3.25 (t, 1 H, C≡CH), 4.05, 4.1 (2 q, 4 H, 2 OCH_2CH_3), 4.35 (br s, 2 H, $CH_2C{\equiv}C$), 4.4 (m, 1 H, CH), 4.8 (br s, 2 H, $ArCH_2N<$), 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. ($C_{29}H_{31}FN_4O_6$) 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 (Me_2SO-d_6) δ 2.0 (m, 2 H, $CHCH_2CH_2CO_2H$), 2.35 (t, 2 H, $CHCH_2CH_2CO_2H$), 2.35 (s, 3 H, CH_3), 3.25 (t, 1 H, C≡CH), 4.35 (d, 2 H, $CH_2C{\equiv}C$), 4.35 (m, 1 H, CH), 4.8 (br s, 2 H, $ArCH_2N<$), 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. ($C_{25}H_{23}FN_4O_6$) 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. ($C_{16}H_{21}FN_2O_5$) 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, $CH_2CH_2CO_2Et$), 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. ($C_{17}H_{23}FN_2O_5$) 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-fluoroethyl)amino]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 (Me_2SO-d_6) δ 1.15, 1.20 (2 t, 6 H, 2 OCH_2CH_3), 2.0 (m, 2 H, $CHCH_2CH_2CO_2Et$), 2.4 (t, 2 H, $CHCH_2CH_2CO_2Et$), 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-Butyroxyethyl)amino]-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 (Me_2SO-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, $COCH_2CH_2CH_3$), 2.0 (m, 2 H, $CHCH_2CH_2CO_2Et$), 2.3 (t, 2 H, CH_2CO_2), 2.35 (t, 2 H, $CHCH_2CH_2CO_2Et$), 3.4 (t, 2 H, CH_2N), 4.05, 4.1 (2 t, 4 H, 2 OCH_2CH_3), 4.15 (t, 2 H, OCH_2CH_2N), 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-Acetoxypropyl)amino]-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-bromopropyl)amino]-2-fluorobenzoyl-L-glutamate (**36l**): 980 mg (36%); mp 60–61 °C. Anal. ($C_{19}H_{26}BrFN_2O_5$) C, H, N, Br, F.

A mixture of **36l** (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 (Me_2SO-d_6) δ 1.15, 1.2 (2 t, 6 H, 2 OCH_2CH_3), 1.85 (m, 2 H, $CH_2CH_2CH_2$), 2.0 (s, 3 H, $OCOCH_3$), 2.05 (m, 2 H, $CHCH_2CH_2CO_2Et$), 2.40 (t, 2 H, $CHCH_2CH_2CO_2Et$), 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. ($C_{21}H_{29}FN_2O_7$) C, H, N.

Di-tert-butyl N-[2-Fluoro-4-[(2-phthalimidoethyl)amino]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, $CHCH_2CH_2CO_2$-*t*-Bu), 2.3 (t, 2 H, $CHCH_2CH_2CO_2$-*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-aminoethyl)amino]-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 (Me_2SO-d_6) δ 2.0 (m, 2 H, $CHCH_2CH_2CO_2H$), 2.25 (t, 2 H, $CHCH_2CH_2CO_2H$), 2.3 (s, 3 H, CH_3), 3.05 (m, 2 H, CH_2NH_2), 3.7 (m, 2 H, $CH_2N<$), 4.3 (m, 1 H, CH), 4.8 (br s, 2 H, $ArCH_2N<$), 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. ($C_{24}H_{26}FN_5O_6 \cdot 1.25H_2O$) C, H, N.

N-[4-[N-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-N-(cyanomethyl)amino]-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. ($C_{32}H_{38}FN_5O_6 \cdot 0.25H_2O$) C, H, N.

A solution of **56** (615 mg, 1.01 mmol) in CF_3CO_2H (6.5 mL) was kept for 10 min and evaporated to dryness. The residue was dissolved in a solution of $NaHCO_3$ (840 mg, 10 mmol) in H_2O (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 (Me_2SO-d_6) δ 2.0 (m, 2 H, $CHCH_2CH_2CO_2H$), 2.3 (t, 2 H, $CHCH_2CH_2CO_2H$), 2.35 (s, 3 H, CH_3), 4.4 (m, 1 H, CH), 4.8 (br s, 2 H, CH_2CN), 4.85 (br s, 2 H, $ArCH_2N<$), 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. ($C_{24}H_{22}FN_5O_6 \cdot 0.5H_2O$) C, H, N.

***N*-[4-[*N*-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)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. ($C_{24}H_{24}FN_5O_7 \cdot H_2O$) C, H, N.

Diethyl *N*-(3-Fluoro-4-nitrobenzoyl)-L-glutamate (21). Method K. A stirred slurry of 3-fluoro-4-nitrobenzoic acid[5] (18.5 g, 0.1 mol) in dry toluene (400 mL) was treated with $SOCl_2$ (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 CH_2Cl_2 (400 mL) was added over 15 min to a stirred mixture of diethyl L-glutamate hydrochloride (24 g, 0.1 mol) and Et_3N (27.9 mL, 0.2 mol) in CH_2Cl_2 (400 mL) below 25 °C (with cooling). The resulting brown solution was stirred for a further 2 h and then washed with H_2O (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 ($CDCl_3$) δ 1.25, 1.30 (2 t, 6 H, 2 OCH_2CH_3), 2.25 (m, 2 H, $CHCH_2CH_2CO_2Et$), 2.5 (t, 2 H, $CHCH_2CH_2CO_2Et$), 4.15, 4.25 (2 q, 4 H, 2 OCH_2CH_3), 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. ($C_{16}H_{19}FN_2O_7$) C, H, N, F.

The procedure was repeated with 4-nitro-2-(trifluoromethyl)benzoic acid[11] 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), K_2CO_3 (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 H_2O (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 ($CDCl_3$) δ 1.25, 1.3 (2 t, 6 H, 2 OCH_2CH_3), 2.2 (m, 2 H, $CHCH_2CH_2CO_2Et$), 2.25 (t, 1 H, C≡CH), 2.45 (t, 2 H, $CHCH_2CH_2CO_2Et$), 4.0 (br s, 2 H, $CH_2C{\equiv}C$), 4.1, 4.45 (2 q, 4 H, 2 OCH_2CH_3), 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. ($C_{19}H_{23}FN_2O_5$) 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 H_2SO_4 (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 CH_2Cl_2 as eluent. The product (117 mg, 27%) was isolated as a gum: NMR (Me_2SO-d_6) δ 1.15 (2 t, 6 H, 2 OCH_2CH_3), 1.9 (m, 2 H, $CH_2CH_2CH_2$), 2.0 (s, 3 H, $OCOCH_3$), 2.05 (m, 2 H, $CHCH_2CO_2Et$), 2.4 (t, 2 H, $CHCH_2CH_2CO_2Et$), 3.2 (t, 3 H, NCH_2), 4.1 (m, 6 H, 3 OCH_2), 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-aminobenzoyl)-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-quinazolinyl]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**[7] (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 CH_2Cl_2 as eluent. The product (102 mg, 56%) was isolated as a gum: NMR (Me_2SO-d_6) δ 1.15 (s, 9 H, *t*-Bu), 1.2 (2 t, 6 H, 2 OCH_2CH_3), 1.9 (m, 2 H, $CH_2CH_2CH_2$), 1.95 (s, 3 H, $OCOCH_3$), 2.05 (m, 2 H, $CHCH_2CH_2CO_2Et$), 2.4 (t, 2 H, $CHCH_2CH_2CO_2Et$), 2.6 (s, 3 H, CH_3), 3.35 (m, 2 H, $CH_2N<$), 4.05 (m, 6 H, 3 OCH_2), 4.4 (m, 1 H, CH), 4.6 (br s, 2 H, $ArCH_2N<$), 6.05 (s, 2 H, OCH_2N), 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 acid[12] (5.0 g, 25.3 mmol) in CH_2Cl_2 (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 CH_2Cl_2 (200 mL) and Et_3N (17.5 mL) and cooled to 0 °C. A solution of diethyl L-glutamate hydrochloride (6.08 g, 25.3 mmol) in CH_2Cl_2 (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 ($CDCl_3$) δ 1.2, 1.35 (2 t, 6 H, 2 OCH_2CH_3), 2.15 (m, 2 H, $CHCH_2CH_2CO_2Et$), 2.45 (t, 2 H, $CHCH_2CH_2CO_2Et$), 4.1, 4.25 (2 q, 4 H, 2 OCH_2CH_3), 4.35 (s, 3 H, OCH_3), 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-quinazolinyl)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 H_2O (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 (Me_2SO-d_6) δ 1.15, 1.2 (2 t, 6 H, 2 OCH_2CH_3), 2.0 (m, 2 H, $CHCH_2CH_2CO_2Et$), 2.3 (t, 2 H, $CHCH_2CH_2CO_2Et$), 2.35 (s, 3 H, CH_3), 3.25 (t, 1 H, C≡CH), 3.85 (s, 3 H, OCH_3), 4.1, 4.2 (2 q, 4 H, 2 OCH_2CH_3), 4.35 (d, 2 H, $CH_2C{\equiv}C$), 4.45 (m, 1 H, CH), 4.8 (br s, 2 H), $ArCH_2N<$), 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 H_2O (3 × 100 mL). The organic phase was dried and evaporated to dryness. The crude product was purified by chromatography using CH_2Cl_2 as eluent to yield a buff solid: 6.58 g (52%); mp 134–135 °C. Anal. ($C_{11}H_{10}N_2O_4$) 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 H_2O (150 mL). The organic phase was washed with H_2O, dried, and evaporated to dryness. Trituration of the residue with EtOAc gave an amorphous buff solid: 1.52 g (44%); NMR (Me_2SO-d_6) δ 2.35 (s, 3 H, CH_3), 3.3 (t, 1 H, C≡CH), 3.75 (s, 3 H, OCH_3), 4.45 (d, 2 H, $CH_2C{\equiv}C$), 4.9 (br s, 2 H, $ArCH_2N<$), 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 H_2O, and vacuum dried: 1.24 g (82%); mp 260–262 °C (dec). Anal. ($C_{20}H_{16}N_4O_5 \cdot H_2O$) 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 Et_3N (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 CH_2Cl_2 (3 × 25 mL) and H_2O (25 mL). The organic phase was washed with H_2O, dried, and evaporated to dryness. The crude product was purified by chromatography using a gradient of 0–6% v/v EtOH in CH_2Cl_2 as eluent to give a buff solid: 421 mg (73%); mp 158–163 °C; NMR (Me_2SO-d_6) δ 1.15, 1.2 (2 t, 6 H, 2 OCH_2CH_3), 2.0 (m, 2 H, $CHCH_2CH_2CO_2Et$), 2.35 (s, 3 H, CH_3), 2.4 (t, 2 H, $CHCH_2CH_2CO_2Et$), 3.25 (t, 1 H, C≡CH), 4.05, 4.1 (2 q, 4 H, 2 OCH_2CH_3), 4.35 (m, 1 H, CH), 4.45 (d, 2 H, $CH_2C{\equiv}C$), 4.85 (br s, 2 H, $ArCH_2N<$), 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. ($C_{29}H_{31}N_5O_8 \cdot 0.75H_2O$) 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; $BrCH_2C{\equiv}CH$, 106-96-7; $BrCH_2CH{=}CH_2$, 106-95-6; $BrCH_2CH_2OAc$, 927-68-4; $BrCH_2CH_2F$, 762-49-2; $BrCH_2CH_2OCOCH_2CH_2CH_3$, 6065-66-3; $Br(CH_2)_3Br$, 109-64-8; $Br(CH_2)_3OAc$, 592-33-6; H-Glu(OEt)-OEt·HCl, 1118-89-4; 2-(bromoethyl)-phthalimide, 574-98-1.