Quinazoline Antifolate Thymidylate Synthase Inhibitors: Heterocyclic Benzoyl Ring Modifications

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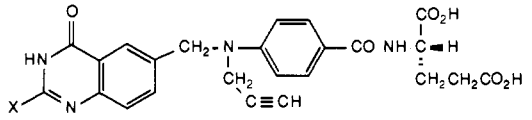
The synthesis is described of a series of C^2-methyl-N^{10}-alkylquinazoline-based antifolates in which the *p*-aminobenzoate ring is replaced by the heterocycles thiophene, thiazole, thiadiazole, pyridine, and pyrimidine. These were generally elaborated by the reaction of (bromomethyl)quinazoline **18** or its N^3-[(pivaloyloxy)methyl]-protected derivative **36** with suitable heterocyclic amines although each heterocyclic system required its own particular synthetic approach. The compounds were tested as inhibitors of partially purified L1210 thymidylate synthase (TS). They were also examined for their inhibition of the growth of L1210 cells in culture. The thiophene system **7** and its related thiazole **8** gave analogues that were considerably more potent than the parent benzene series **2** as inhibitors of L1210 cell growth although in general these heterocycles were somewhat poorer inhibitors of the isolated TS enzyme. The enhanced cytotoxicities of the thiophene and thiazole analogues result, at least in part, from their efficient transport into the cells via the reduced folate carrier mechanism and very good substrate activity for folylpolyglutamate synthetase. The replacement of the C^2-methyl group by C^2-(fluoromethyl) and C^2-(hydroxymethyl) substituents in the thiophene and thiazole series gave derivatives that were only slightly less potent inhibitors of the TS enzyme but which were considerably less cytotoxic.

Introduction

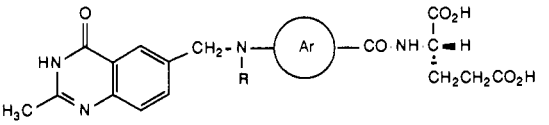
The principle of effective antitumor chemotherapy with a specific inhibitor of the enzyme thymidylate synthase (TS, EC 2.1.1.45) was established in clinical trials[1-3] of the quinazoline-based antifolate N^{10}-propargyl-5,8-dideazafolic acid (1).[4,5] The unacceptable hepatic and renal toxicities

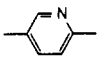
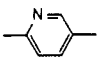
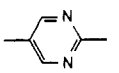


1	X = NH_2
2a	X = CH_3
3	X = H
4	X = CH_2F
5	X = CH_2OH
6	X = OCH_3

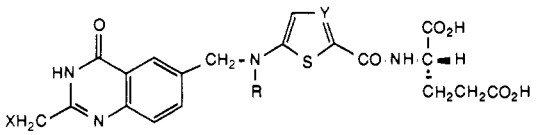
that caused this compound to be withdrawn from the clinic result from its poor aqueous solubility.[6,7] We have recently shown[8-10] that replacement of the C^2-amino group of 1 by a variety of small lipophilic substituents has given TS inhibitors that are considerably more cytotoxic and water soluble than 1. The C^2-methyl analogue **2a**[10] had the optimum activity in this series, although hydrogen (**3**),[8] fluoromethyl (**4**),[10] hydroxymethyl (**5**),[10] and methoxy (**6**)[9] C^2 substituents also gave highly potent compounds. Our hypothesis that a more soluble TS inhibitor would lack the hepatic and renal toxicity of 1 has been confirmed in studies with **2a** and **3-5** in mice.[11]

We have subsequently demonstrated[12] that fluorine substituents in the aminobenzoate moiety give further increases in cytotoxic potency. Moreover this level of potency is retained in a variety of alkyl substituents in the N^{10} position. These observations stimulated us to explore further modifications to this central region of the molecule. In this paper we describe the synthesis and biological activity of a series of quinazoline antifolates **7-17** which contain heterocyclic isosteres of the benzoyl ring.



7	Ar = 
8	Ar = 
9	Ar = 
10	Ar = 
11	Ar = 
12	Ar = 
13	Ar = 

See Table II for identities of R



14	X = F	Y = CH
15	X = F	Y = N
16	X = OH	Y = CH
17	X = OH	Y = N

Prior to this study the effect on TS inhibition of this type of modification had not been investigated although

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(1) Calvert, A. H.; Alison, D. L.; Harland, S. J.; Robinson, B. A.; Jackman, A. L.; Jones, T. R.; Newell, D. R.; Siddik, Z. H.; Wiltshaw, E.; McElwain, T. J.; Smith, I. E.; Harrap, K. R. *J. Clin. Oncol.* **1986**, *4*, 1245.

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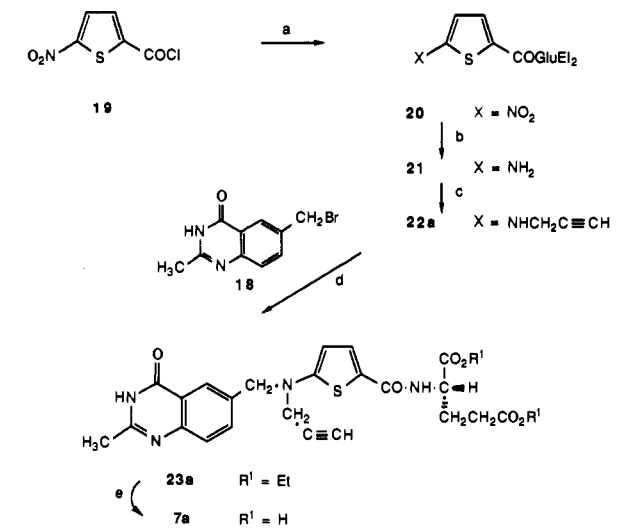
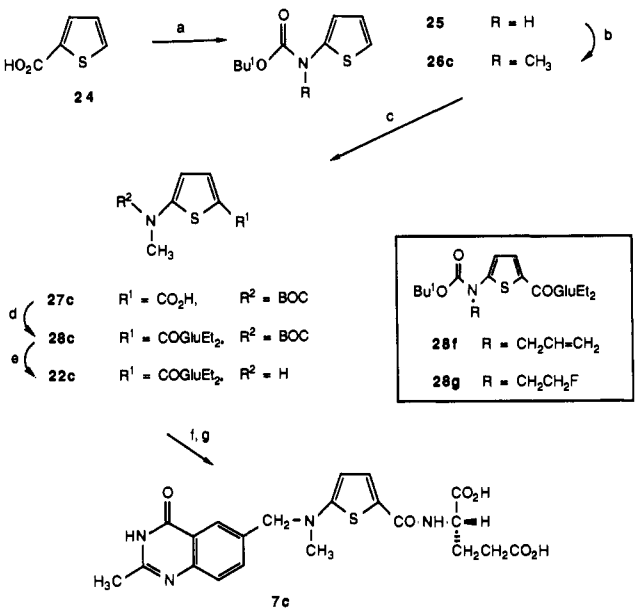
Scheme I[a]



[a] (a) Diethyl glutamate hydrochloride Et_3N, CH_2Cl_2 (method A); (b) iron powder, ferrous sulfate, aqueous MeOH, 70 °C (method B); (c) propargyl bromide, 2,6-lutidine, DMF, 50 °C (method C); (d) 2,6-lutidine, DMF, 60 °C (method D); (e) 1 N aqueous sodium hydroxide (method E).

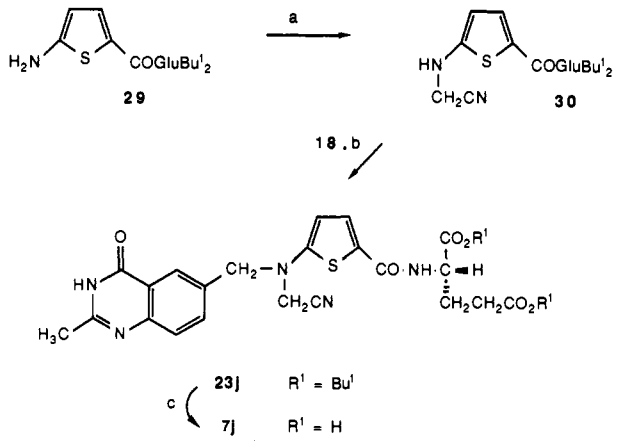
Scheme II[a]



[a] (a) Diphenyl phosphorazidate, Et_2N, *t*-BuOH, reflux (method F); (b) sodium hydride, iodomethane, DMF (method G); (c) (i) *n*-BuLi, (ii) CO_2 (method H); (d) (i) oxalyl chloride, DMF, (ii) diethyl glutamate, Et_3N, CH_2Cl_2 (method K); (e) trifluoroacetic acid (method I); (f) method D; (g) method E.

a number of heterocyclic benzoyl isosteres[13-16] of folic acid itself had been synthesized in the 1970s as potential inhibitors of dihydrofolate reductase (DHFR).

Scheme III[a]



[a] (a) Bromoacetonitrile (method C); (b) method D; (c) trifluoroacetic acid (method J).

Chemistry

The thiophene-linked compounds **7a–i** were prepared by the routes outlined in Schemes I and II (see also Tables I and II), which involved the condensation of (bromomethyl)quinazoline 18[10] with the appropriate N-alkylated (aminothenoyl)-L-glutamic esters **22a–i** using either 2,6-lutidine (method D) or $CaCO_3$ (method M) to scavenge HBr. The resulting antifolate diesters **23a–i** were hydrolyzed by aqueous alkali (method E) to yield the required antifolate diacids (Table II). Two approaches were developed for the preparation of the N-alkylated amines **22a–i**. The first approach (Scheme I) involved the reduction, by Fe–$FeSO_4$ (method B), of (5-nitrothenoyl)-L-glutamate ester **20** and alkylation of amine **21** using the appropriate alkyl halide (method C, see Table I). This

(2) Calvert, A. H.; Newell, D. R.; Jackman, A. L.; Gumbrell, L. A.; Sikora, E.; Grzelakowska-Sztabert, B.; Bishop, J. A. M.; Judson, I. R.; Harland, S. J.; Harrap, K. R. *NCI Monogr.* **1987**, *5*, 21.

(3) Bassendine, M. F.; Curtin, N. J.; Loose, H.; Harris, A. L.; James, D. F. *J. Hepatol.* **1987**, *4*, 349.

(4) Jones, T. R.; Calvert, A. H.; Jackman, A. L.; Brown, S. J.; Jones, M.; Harrap, K. R. *Eur. J. Cancer* **1981**, *17*, 11.

(5) Synonyms: ICI 155387; CB 3717; NSC 327182; *N*-[4-[*N*-[(2-amino-3,4-dihydro-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoyl]-L-glutamic acid.

(6) Newell, D. R.; Siddik, Z. H.; Calvert, A. H.; Jackman, A. L.; Alison, D. L.; McGhee, K. G.; Harrap, K. R. *Proc. Am. Assoc. Cancer Res.* **1982**, *23*, 181.

(7) Newell, D. R.; Alison, D. L.; Calvert, A. H.; Harrap, K. R.; Jarman, M.; Jones, T. R.; Manteuffel-Cymborowska, M.; O'Connor, P. *Cancer Treat. Rep.* **1986**, *70*, 971.

(8) Jones, T. R.; Thornton, T. J.; Flinn, A. J.; Jackman, A. L.; Newell, D. R.; Calvert, A. H. *J. Med. Chem.* **1989**, *32*, 847.

(9) 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.

(10) 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.* **1990**, *33*, 3060.

(11) Newell, D. R.; Maxwell, R. J.; Griffiths, J. R.; Bisset, G.; Hughes, L. R.; Calvert, A. H. *Proc. Am. Assoc. Cancer Res.* **1988**, *29*, 286.

(12) (a) Jackman, A. L.; Thornton, T. J.; O'Connor, B. M.; Bishop, J. A. M.; Bisset, G.; Calvert, A. H.; Hughes, L. R.; Oldfield, J.; Wardleworth, J. M.; Barker, A. J.; Marsham, P. R. In *Chemistry and Biology of Pteridines 1989, Pteridine and Folic Acid Derivatives*; Curtius, H. Ch., Ghisla, S., Blau, N., Eds.; de Gruyter: Berlin, 1990; p 1076. (b) Marsham, P. R.; Jackman, A. L.; Hughes, L. R.; Thornton, T. J.; Bisset, G. M. F.; Oldfield, J.; O'Connor, B. M.; Bishop, J. A. M.; Calvert, A. H. *J. Med. Chem.* **1990**, *33*, 3072.

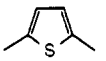
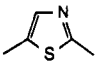
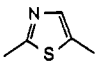
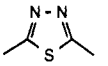
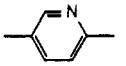
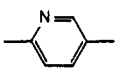
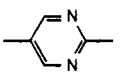
(13) Roberts, E. C.; Shealy, Y. F. *J. Med. Chem.* **1971**, *14*, 125.

(14) Roberts, E. C.; Shealy, Y. F. *J. Med. Chem.* **1972**, *15*, 1310.

(15) Roberts, E. C.; Shealy, Y. F. *J. Heterocycl. Chem.* **1974**, *11*, 547.

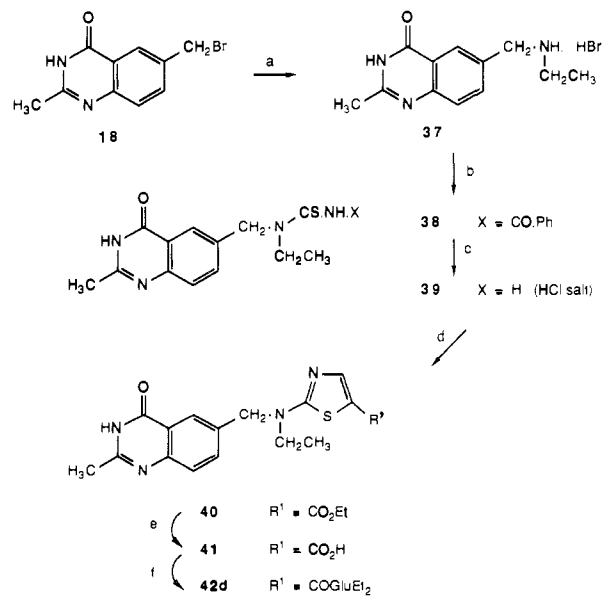
(16) Gurina, S. L.; Pushkareva, Z. V.; Volkova, N. V.; Andreeva, N. A.; Zubova, T. E. *Bioorg. Chem.* **1977**, *3*, 1133; *Chem. Abstr.* **1977**, *87*, 184936*k*.

Table I. Preparation of Heterocyclic (Aminobenzoyl)glutamate Diesters and Derived Antifolate Diesters

compd	starting material	method (temp, °C)	% yield	derived antifolate diester	method (temp, °C)	% yield	Ar	R
22a	21	C (50)	54	**23a**	D (60)	15		$CH_2C{\equiv}CH$
21	**20**	B (70)	82	**23b**	D (25)[a]	58		H
22c	21	C (70)	48	**23c**	D (80)	22		CH_3
	28c	I (20)	58					
22d	21	C (60)	26	**23d**	D (60)	77		CH_2CH_3
22e	21	C (70)	45	**23e**	D (80)	40		$(CH_2)_2CH_3$
22f	**28f**	I (20)	63	**23f**	D (90)[a]	80		$CH_2CH{=}CH_2$
22g	**28g**	I (20)	61	**23g**	M (80)[a]	32		$(CH_2)_2F$
22h	21	C (120)[a]	38	**23h**	D (90)[a]	31		$(CH_2)_2OAc$
22i	21	C (120)[a]	91	**23i**	D (90)[a]	17		$(CH_2)_3OAc$
30[b]	**29**[b]	C (90)[b]	66	**23j**[b]	D (100)[a,b]	29		CH_2CN
33a	32	C (77)	49	**35a**	M (90)	47		$CH_2C{\equiv}CH$
32	31	L (80)	92	**35b**	M (85)	50		H
33c	32	O (60)	29	**35c**	M (90)	76		CH_3
33d	32	O (70)	27	**35d**	M (90)	57		CH_2CH_3
33f	32	C (100)	46	**35f**[c]	P (100)	63		$CH_2CH{=}CH_2$
33g	32	C (120)[d]	28	**35g**	M (90)	33		$(CH_2)_2F$
33h	32	C (150)[e]	35	**35h**[c]	P (120)	46		$(CH_2)_2OAc$
33i	32	C (90)	21	**35i**[c]	P (120)	44		$(CH_2)_3OAc$
				42d	R (20)	48		CH_2CH_3
				48d	R (20)	78		CH_2CH_3
54a[g]	**53a**[f]	V (0)	~100[g]	**55a**	M (100)	17		$CH_2C{\equiv}CH$
54c[g]	**53c**[f]	V (0)	~100[g]	**55c**	M (75)	65		CH_3
54d[h]	**53d**[f]	V (0)	64[h]	**55d**	M (70)	45		CH_2CH_3
54f[h]	**53f**[f]	V (0)	67[h]	**55f**[c]	P (100)	38		$CH_2CH{=}CH_2$
54g[h]	**53g**[f]	V (0)	69[h]	**55g**[c]	P (100)	61		$(CH_2)_2F$
58c[h]	57	W (60)	**93**[h]	**59c**	M (90)	34		CH_3
				66c	R (20)	60		CH_3

[a] Reaction was performed in 1-methylpyrrolidinone. [b] Di-*tert*-butyl ester. [c] Protected as the 3-[(pivaloyloxy)methyl] derivative (X = CH_2OCO-*t*-Bu in 35 and 55). [d] Reaction was performed in *N,N*-dimethylacetamide in a sealed tube. [e] Reaction was performed in *N,N*-dimethylacetamide. [f] *tert*-Butoxycarbonyl derivative. [g] Isolated and reacted as the crude trifluoroacetate salt. [h] Isolated as the free base by treatment of the trifluoroacetate salt with a mixture of aqueous $NaHCO_3$ and EtOAc.

Scheme IV[a]

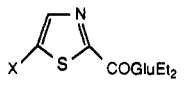


[a] (a) Ethylamine, MeCN; (b) benzoyl isothiocyanate, Et_3N, acetone; (c) concentrated HCl, 2-propanol; (d) $OHC{\cdot}C(Cl)CO_2Et$; (e) 1 N aqueous sodium hydroxide (method Q); (f) diethyl glutamate hydrochloride, DPPA, Et_3N, CH_2Cl_2 (method R).

approach was the more versatile as the whole range of N^{10} substituents could be incorporated in this way, but it suffered from the production of varying amounts of the unwanted *N,N*-dialkyl derivatives of **22** at the alkylation stage. This problem was overcome by the alternative approach (Scheme II) illustrated by the preparation of the *N*-methyl intermediate **22c**. Here, dialkylation was avoided by forming BOC-protected aminothiophene **25** and alkylating this to **26c** with iodomethane using NaH as base (method G). Carboxylic acid **27c** was formed by lithiation (*n*-BuLi-THF) of **26c** followed by the addition of CO_2 (method H). The acid chloride of **27c** underwent coupling (method K) to diethyl L-glutamate to give **28c**, which was treated with CF_3CO_2H to effect removal of the BOC protecting group (method I). This latter approach, however, cannot be used when the N^{10} substituent itself is susceptible to reaction with organolithium reagents. The synthesis of N^{10}-cyanomethyl derivative **7j** (Scheme III) required the glutamic acid moiety to be protected as the di-*tert*-butyl ester and to be hydrolyzed under acidic conditions (CF_3CO_2H) since this particular N^{10} substituent is known to be readily hydrolyzed by alkali to the amide.[12]

The syntheses of the 2-carbonylthiazole analogues **8a–i** were conducted essentially by the sequence in Scheme I starting from 5-nitrothiazole-2-carboxylic acid.[17] This was condensed via the acid chloride with diethyl L-glutamate (method K) and product **31** was reduced by Fe–HOAc (method L) to primary amine **32**. Sequential alkylation of **32** with the appropriate alkyl halide and then with

(17) Strehlke, P. *Chem. Ber.* **1973**, *106*, 721.

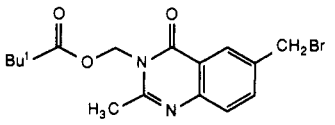


31 X = NO_2

32 X = NH_2

33a-l X = NHR (See Table I for identities of R)

34 X = $N(CH_3)_2$



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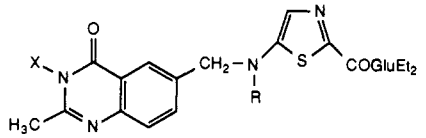
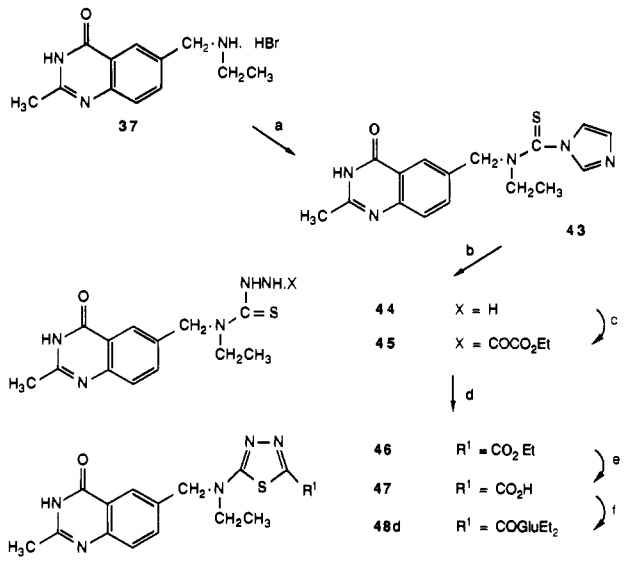


35a-t See Table I for identities of R and X.

(bromomethyl)quinazolinone **18** (method M) or its N^3-[(pivaloyloxy)methyl] derivative **36**[12] (method P) afforded antifolate diesters **35a–i** (see Table I for details). Final deprotection to the thiazole-containing antifolate diacids **8a–i** was accomplished by saponification in aqueous EtOH (method N, Table II). One example, **9d**, of a thiazole 5-carbonyl-linked analogue was prepared (Scheme IV). The strategy here was to build the thiazole ring onto 6-[(ethylamino)methyl]quinazolinone (**37**) in order to define unambiguously the required regioisomer. Starting amine **37** was readily prepared as the hydrobromide salt by treating (bromomethyl)quinazolinone **18** with anhydrous ethylamine in CH_3CN. Reaction of **37** with benzoyl isothiocyanate in the presence of Et_3N according to the method of Hartmann and Reuther[18] gave the *N*-benzoylthiourea derivative **38**. On acid hydrolysis, **38** yielded the corresponding thiourea **39**, which readily underwent condensation with ethyl 2-chloro-2-formylacetate[19] to afford the key thiazole-5-carboxylic ester **40** although in low yield (22%). This was saponified (method Q) and the derived carboxylic acid **41** was coupled via the in situ generated azide (method R) with diethyl L-glutamate. The resulting antifolate diester **42d** was hydrolyzed according to method N to yield **9d**. The synthesis of thiadiazole analogue **10d** also started from **37** (Scheme V). In this case **37** was elaborated via **43** into thiosemicarbazide derivative **44**, which was acylated to **45** with ethyl oxalyl chloride. Cyclization of **45** to key thiadiazole ester **46** was achieved by exposure to methanesulfonic acid in refluxing toluene. The final conversion of **46** into **10d** was accomplished by saponification to **47** followed by incorporation of glutamic acid using methods R and N.

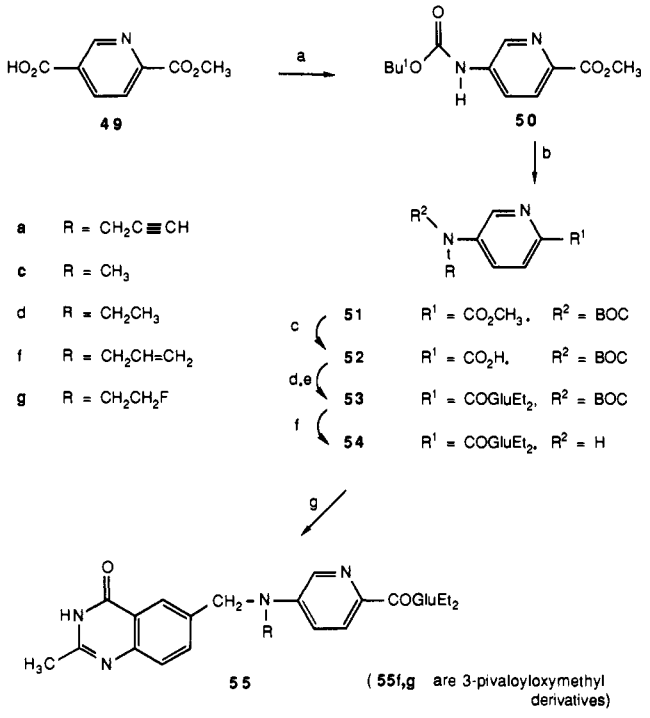
The BOC-protected amino ester **50**[20] proved a very convenient synthon for the 2′-aza analogues **11** (Scheme VI) in that it permitted the introduction of the N^{10} substituent in high yields at an early stage simply by treatment with NaH in *N*,*N*-dimethylformamide (DMF) followed by the appropriate alkyl bromide or iodide (method S). Alkylation products **51** were saponified and acids **52** were coupled via the acid chlorides to diethyl L-glutamate. At this stage the BOC protecting group was removed (CF_3CO_2H, 0 °C, method V) to give [5-(alkylamino)-picolinoyl]glutamate diesters **54** required for completion of the synthesis of the 2′-aza antifolate analogues **11a,c,-d,f,g** by our standard procedures (methods M, P, and N). The proposed strategy for the synthesis of compounds of the 3′-aza series **12** involved nucleophilic displacement at

Scheme V[a]



[a] (a) 1,1′-Thiocarbonyldiimidazole, Et_2N, CH_2Cl_2; (b) hydrazine hydrate, EtOH, reflux; (c) ethyl oxalyl chloride, Et_3N, DMF, 0–5 °C; (d) methanesulfonic acid, toluene, reflux; (e) 1 N aqueous sodium hydroxide, EtOH; (f) method R.

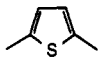
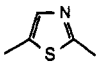
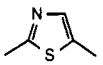
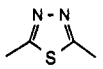
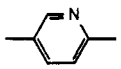
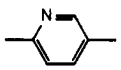
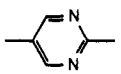
Scheme VI[a]



[a] (a) Diphenyl phosphorazidate, Et_3N, *t*-BuOH, reflux; (b) sodium hydride, DMF, RBr or RI (method S); (c) 1 N aqueous sodium hydroxide, EtOH (method T); (d) oxalyl chloride, Et_3N, DMF, CH_2Cl_2; (e) diethyl glutamate hydrochloride, Et_3N, CH_2Cl_2 (method U); (f) CF_3CO_2H (method V); (g) method M or P.

(18) Hartmann, von H.; Reuther, I. *J. Prakt. Chem.* **1973**, *315*, 144.
(19) Dornow, A.; Boberg, F.; Schurer, L. *Arch. Pharm. (Weinheim)* **1953**, *286*, 494.
(20) The method for the preparation of **50** was developed by Mr. R. I. Dowell, ICI Pharmaceuticals. See the Experimental Section for details.

Table II. Preparation and in Vitro Activity of Antifolate Diacids 7–13

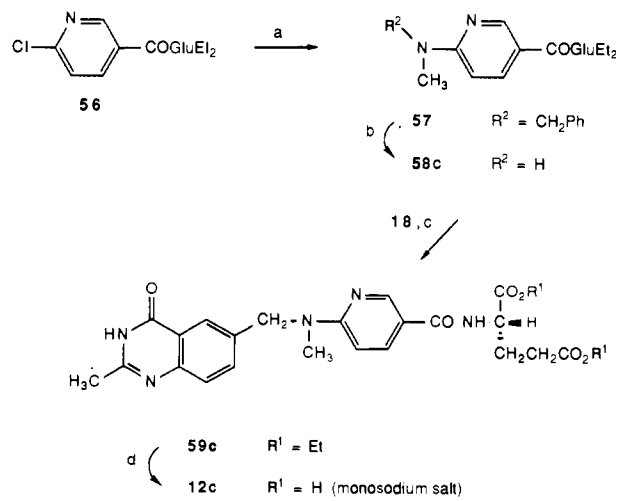
compd	Ar	R	method	% yield	mp, °C	formula[a]	mass spectra (FAB), *m/z*	IC_{50}, μM: inhibn of TS	IC_{50}, μM: inhibn of L1210 cell growth in culture
2a[b]		$CH_2C{\equiv}CH$			165	$C_{25}H_{24}N_4O_6{\cdot}2H_2O$		0.04	0.09
2c[b]		CH_3			254–257	$C_{23}H_{24}N_4O_6{\cdot}0.75H_2O$		0.25	0.11
2d[b]		CH_2CH_3			221–225	$C_{24}H_{26}N_4O_6{\cdot}0.5H_2O$		0.26	0.36
7a		$CH_2C{\equiv}CH$	E	42	215–225	$C_{23}H_{22}N_4O_6S{\cdot}0.7H_2O$	483[c]	0.44	0.06
7b		H	E	39	183–189[e]	$C_{20}H_{20}N_4O_6S{\cdot}3H_2O$	443[d]	24.78	3.00
7c		CH_3	E	41	180–184	$C_{21}H_{22}N_4O_6S{\cdot}H_2O$	457[d]	0.67	0.007
7d		CH_2CH_3	E	44	162–167	$C_{22}H_{24}N_4O_6S{\cdot}0.75H_2O$	473[c]	0.58	0.016
7e		$(CH_2)_2CH_3$	E	61	184–185	$C_{23}H_{26}N_4O_6S{\cdot}2.1H_2O$[f]	487[c]	1.85	0.40
7f		$CH_2CH{=}CH_2$	E	31	144–148[e,g]	$C_{23}H_{24}N_4O_6S{\cdot}1.5H_2O$	483[d]	1.68	0.058
7g		$(CH_2)_2F$	E	22	157–161	$C_{22}H_{23}FN_4O_6S{\cdot}0.75H_2O$	491[c]	0.55	0.10
7h		$(CH_2)_2OH$	E	42	157–161	$C_{22}H_{24}N_4O_7S{\cdot}H_2O$	487[d]	1.20	0.10
7i		$(CH_2)_3OH$	E	50	149–153	$C_{23}H_{26}N_4O_7S{\cdot}1.25H_2O$	501[d]	3.36	3.00
7j		CH_2CN	J[h]	48	125–130	$C_{22}H_{21}N_5O_6S{\cdot}1.5CF_3CO_2H$	482[d]	3.08	0.20
8a		$CH_2C{\equiv}CH$	N	70	148–152	$C_{22}H_{21}N_5O_6S{\cdot}0.3H_2O$	482[d]	0.23	0.008
8b		H	N	60	198–201	$C_{19}H_{19}N_5O_6S{\cdot}H_2O$	444[d]	7.12	0.28
8c		CH_3	N	93	120–125[e,i]	$C_{20}H_{21}N_5O_6S{\cdot}0.5H_2O$	458[d]	0.42	0.006
8d		CH_2CH_3	N	87	125–128[g]	$C_{21}H_{23}N_5O_6S{\cdot}0.7H_2O$	472[d]	0.23	0.008
8f		$CH_2CH{=}CH_2$	N[j]	80	147–153	$C_{22}H_{23}N_5O_6S{\cdot}1.3H_2O$	484[d]	1.07	0.013
8g		$(CH_2)_2F$	N	85	232–235	$C_{21}H_{22}FN_5O_6S{\cdot}1.5H_2O$	490[d]	0.19	0.02
8h		$(CH_2)_2OH$	N[j]	32	175–178[k]	$C_{21}H_{23}N_5O_7S{\cdot}1.75H_2O$	488[d]	0.42	0.02
8i		$(CH_2)_3OH$	N[j]	68	210–214[e,i]	$C_{22}H_{25}N_5O_7S{\cdot}0.5H_2O$	502[d]	1.74	0.06
9d		CH_2CH_3	N	75	110–115	$C_{21}H_{23}N_5O_6S{\cdot}0.5H_2O$	472[d]	0.76	0.90
10d		CH_2CH_3	N	57	236–238	$C_{20}H_{22}N_6O_6S$	475[c]	0.94	2.40
11a		$CH_2C{\equiv}CH$	N	84	250–251[e]	$C_{24}H_{23}N_5O_6{\cdot}0.8H_2O$	476[d]	0.04	0.06
11c		CH_3	N	62	205–208	$C_{22}H_{23}N_5O_6{\cdot}H_2O$	452[d]	0.35	0.16
11d		CH_2CH_3	N	74	230–233	$C_{23}H_{25}N_5O_6{\cdot}0.25H_2O$	466[d]	0.15	0.64
11f		$CH_2CH{=}CH_2$	N[j]	86	238–239[e]	$C_{24}H_{25}N_5O_6{\cdot}0.5H_2O$	478[d]	0.76	0.13
11g		$(CH_2)_2F$	N[j]	79	242–243	$C_{23}H_{24}N_5O_6F$	484[d]	0.16	0.14
12c		CH_3	N[m]	31	205–210	$C_{22}H_{22}N_5NaO_6{\cdot}2.5H_2O$[n]	452[d]	4.25	1.60
13c		CH_3	N	64	181–185	$C_{21}H_{22}N_6O_6{\cdot}H_2O$	455[c]	2.34	1.40

[a] Anal. C, H, N except where stated otherwise. [b] See ref 10. [c] $[MH]^+$. [d] $[M-H]^-$. [e] Decomposes at this temperature. [f] H: calcd, 5.75; found, 5.2. [g] Softens >90 °C. [h] Prepared from the di-*tert*-butyl ester. [i] Softens >110 °C. [j] The starting antifolate diethyl ester was protected as the 3-[(pivaloyloxy)methyl] derivative. [k] Softens >165 °C. [l] Softens >185 °C. [m] Purified by desalting on Sepabeads. Isolated as the hydrated monosodium salt. [n] H: calcd, 5.2; found, 4.7.

C^6 of the (6-chloronicotinoyl)glutamate diester **56**. Although **56** reacted smoothly with *N*-benzylmethylamine in a model reaction at 100 °C in 1-methyl-2-pyrrolidinone (NMP) to give **57** (Scheme VII), [(ethylamino)methyl]-quinazolinone **37** gave no reaction at all even under more forcing conditions. On the other hand an attempt to prepare **58c** by treatment of **56** with ethanolic methylamine at 100 °C in a sealed tube resulted in the formation of the bis-amide of the glutamate moiety with no displacement of the C^6-chlorine atom by the methylamino group. However the required intermediate **58c** was prepared by catalytic hydrogenolysis of **57** and the synthesis of N^{10}-methyl-3′-azaantifolate analogue **12c** was completed by the standard method.

One example (**13c**) of an analogue in the 2′,6′-diaza series (i.e. the N^{10}-methyl pyrimidine modification) was prepared (Scheme VIII) starting from 5-amino-2-(methylthio)pyrimidine (**60**).[21] This was alkylated with the POM-protected bromomethyl compound **36** and the resulting **61** was N-methylated to **62** by aqueous formaldehyde in the presence of $NaCNBH_3$. Oxidation of **62** by *m*-chloroperbenzoic acid (MCPBA) to the corresponding sulfone **63** was followed by treatment with NaCN at 100 °C, which effected the simultaneous insertion of the nitrile function and removal of the N^3-POM protecting group to afford **64**. Brief sa-

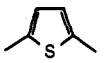
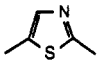
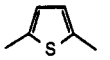
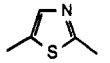
Scheme VII[a]



[a] (a) *N*-Benzylmethylamine, NMP, 100 °C; (b) H_2, CF_3CO_2H, EtOH, 60 °C; (c) method M; (d) method N.

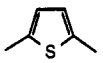
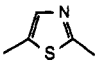
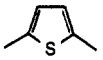
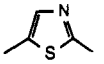
(21) Krchnak, V.; Arnold, Z. *Collect. Czech. Chem. Commun.* 1975, *40*, 1396.

Table III. Preparation of Antifolate Diesters 69–72

starting bromo compd	starting amine	antifolate diester	method (temp, °C)	% yield	X	Ar	R
67	**22c**	**69c**	D[a] (90)	71	F		CH_3
67	**22d**	**69d**	D[a] (80)	33	F		CH_2CH_3
67	**22g**	**69g**	D[a] (90)	10	F		$(CH_2)_2F$
67	**33d**	**70d**	D[b] (105)	48	F		CH_2CH_3
68	**22c**	**71c**	D[b] (80)	18	OAc		CH_3
68	**22d**	**71d**	D[b] (80)	18	OAc		CH_2CH_3
68	**33c**	**72c**	D (80)	27	OAc		CH_3

[a] Reaction was performed in 1-methylpyrrolidinone. [b] Reaction was performed in *N,N*-dimethylacetamide.

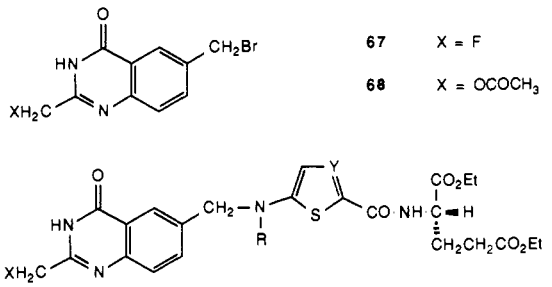
Table IV. Preparation and in Vitro Activity of Antifolate Diacids 14–17

compd	X	Ar	R	method	% yield	mp, °C	formula[a]	mass spectra, m/z $[M-H]^-$	IC_{50}, μM: inhibn of TS	IC_{50}, μM: inhibn of L1210 cell growth in culture
14c	F		CH_3	E	97	115[b,c]	$C_{21}H_{21}FN_4O_8S\cdot H_2O$	475	1.36	0.016
14d	F		CH_2CH_3	E	83	130[b,d]	$C_{21}H_{23}FN_4O_6S\cdot 1.5H_2O$	489	0.93	0.11
14g	F		$(CH_2)_2F$	E	85	155–160[b]	$C_{22}H_{22}F_2N_4O_6S\cdot 1.3H_2O$	507	1.07	0.67
15d	F		CH_2CH_3	E	89	140–145[b]	$C_{21}H_{22}FN_5O_6S\cdot 0.7H_2O$	490	0.22	0.10
16c	OH		CH_3	N	52	220–223[b]	$C_{21}H_{22}N_4O_7S\cdot H_2O$	473	1.36	0.78
16d	OH		CH_2CH_3	N	50	148–151[b]	$C_{22}H_{24}N_4O_7S\cdot H_2O$	487	1.03	2.70
17c	OH		CH_3	N	71	201–205[b]	$C_{20}H_{21}FN_5O_7S\cdot H_2O$	474	0.49	0.30

[a] Anal. C, H, N. [b] Decomposes at this temperature. [c] Softens >98 °C. [d] Softens >100 °C.

ponification of **64** at 100 °C gave the corresponding carboxylic acid **65**. A standard sequence (methods R, N) completed the synthesis of **13c**.

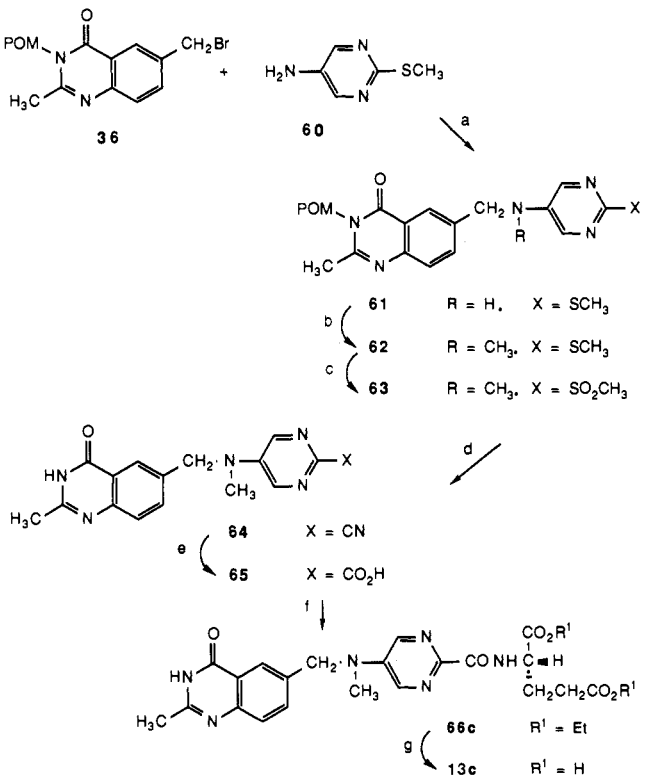
As an extension of this work some analogues of the thiophene- and thiazole-linked antifolates were prepared with C^2-fluoromethyl and C^2-hydroxymethyl substituents (Table IV). These were all elaborated from the 6-(bromomethyl)quinazolinone precursors **67**[10] and **68**[10] by the methods summarized in Tables III and IV.



Biological Evaluation

The novel quinazoline antifolate diacids were tested as inhibitors of TS partially purified from L1210 mouse leukemia cells that overproduce TS due to amplification of the TS gene.[22] The partial purification and assay method used in this study were as previously described and used a (±)-5,10-methylenetetrahydrofolic acid concentration of 200 μM.[22,23] The TS inhibitor **1** was included in each assay as a positive control ($IC_{50} \cong 20$ nM). The compounds were also tested for their inhibition of the growth of L1210 cells in culture,[24] and the results again

Scheme VIII[a]



[a] (a) 2,6-Lutidine, DMF, 90 °C; (b) HCHO, $NaCNBH_3$, HOAc, aqueous MeCN; (c) MCPBA, CH_2Cl_2; (d) NaCN, DMF, 100 °C; (e) 2 N aqueous NaOH, PrOH; (f) method R; (g) method N.

(22) Jackman, A. L.; Alison, D. L.; Calvert, A. H.; Harrap, K. R. *Cancer Res.* **1986**, *46*, 2810.
(23) Sikora, E.; Jackman, A. L.; Newell, D. R.; Calvert, A. H. *Biochem. Pharmacol.* **1988**, *37*, 4047.

were expressed as the concentration required to inhibit cell growth by 50% (IC_{50}). These results are collected in Tables II and IV.

Results and Discussion

The IC_{50} values of these new heterocycle-linked quinazoline antifolates for the inhibition of partially purified L1210 TS and for growth inhibition of L1210 cells are shown in Tables II and IV. The replacement of the benzene ring of **2a** by a thiophene (**7a**) resulted in a 10-fold drop in inhibitory activity against the isolated TS enzyme. On the other hand, the two compounds have similar growth inhibitory potencies against L1210 cells. In this thiophene series varying the N^{10}-propargyl group to a methyl (**7c**) and ethyl (**7d**) does not cause the drop in activity against the enzyme that was seen in the benzene series.[10] Moreover the N^{10}-methyl and N^{10}-ethyl thiophene analogues **7c,d** are considerably more growth inhibitory than the N^{10}-propargyl parent 7a, a complete reversal of the order of potencies in the benzene series **2a,c,d**. Other substituents at N^{10} [e.g. allyl (**7f**), fluoroethyl (**7g**), and hydroxyethyl (7h)] are well-tolerated by the enzyme and give good levels of growth inhibition. Substitution by a ring nitrogen atom in the 2′-position of 7a, to give thiazole derivative **8a**, increases enzyme inhibition 2-fold and growth inhibition by a factor of 10. In this thiazole series also the N^{10}-methyl (**8c**) and N^{10}-ethyl (**8d**) analogues are at least as potent as the N^{10}-propargyl compound **8a** against both TS and L1210 cells, and again other N^{10}-substituents are well-tolerated. An isomeric thiazole (**9d**) with the ring nitrogen in the 3′-position was also prepared but this proved to be 50–100-fold less growth inhibitory than the corresponding compounds **7d** and **8d** in the thiophene and 2′-azathiazole series despite showing only a slight drop in activity against TS. The thiadiazole modification (**10d**) proved to be even more detrimental to growth inhibition. In parallel with our earlier observations in the related benzoyl series,[9,10] a hydrogen substituent on N^{10} (i.e. **7b** and **8b**) gives relatively poor inhibition of TS. However, whereas in the benzoyl series the N^{10}-hydrogen compounds are relatively good inhibitors of cell growth ($\approx N^{10}$-propargyl), **7b** and **8b** are ca. 2 orders of magnitude less potent than the N^{10}-substituted compounds.

The isosteric modification of benzene to pyridine in which the ring nitrogen is in the $C^{2'}$ position (i.e. 11) has virtually no effect on either TS inhibitory potency or cytotoxicity. Here again, propargyl is the optimum N^{10}-substituent against both parameters. Two examples, one (**12c**) of the isomeric 3′-aza series and one (**13c**) of the 2′,6′-diaza (pyrimidine) series were prepared, for synthetic ease, with the N^{10}-methyl substituent. These modifications cause a 10-fold drop in potency compared with that of **2c** against both parameters and these series were consequently not investigated further.

The C^2-fluoromethyl (**14**, **15**) and C^2-hydroxymethyl (**16**, **17**) derivatives were only slightly less potent inhibitors of the enzyme than the corresponding C^2-methyl compounds but in general were considerably less growth inhibitory. These results are in accord with those produced by the same modifications in the *p*-aminobenzoyl series.[10]

It is clear that substitution of a nitrogen atom in the ring ortho to the carbonyl group is favored over the alternative meta arrangement in both the five- and six-membered ring cases (e.g. **8d** vs **9d** and **11c** vs **12c**). A parallel situation was observed when we compared the effect of 2′- and 3′-fluorine substituents on the benzene ring.[12] An explanation consistent with these results is that increased electron density ortho to the N^{10}-link causes an unfavorable interaction either directly with the enzyme or through modification of the conformation of the bridge region of the molecule. On the other hand a 2′-ring nitrogen atom (i.e. 8 and 11) has no detrimental interactions and in some cases may even stabilize a preferred conformation through the formation of a hydrogen bond with the amidic N–H atom. However when a second ring nitrogen atom (i.e. in the pyrimidine **13c**) is introduced ortho to the carbonyl group a repulsive interaction between the lone pairs of electrons on the carbonyl oxygen and one of the ring nitrogen atoms cannot be avoided and this presumably forces the amide unit out of the plane of the ring into a disfavored conformation for interaction with the enzyme.

Replacement of the benzene ring by a thiophene or thiazole ring decreases TS inhibition by 5–10-fold when the N^{10}-substituent is propargyl but has no significant effect when this substituent is methyl or ethyl. These observations can be rationalized in terms of the differences in geometry imposed around the N^{10}-substituents by the five- and six-membered ring *p*-aminobenzoate isosteres when the inhibitors are optimally bound in the enzyme. Recent X-ray crystallographic studies[25,26] of the ternary complex of 1, 5-fluoro-2′-deoxyuridylate and *Escherichia coli* TS (which has considerable homology with murine and human TS in the active site region) has identified the key binding modes of the quinazoline and glutamate moieties. They also indicate that the propargyl group fits into a specific solvent-lined pocket, thus suggesting a reason for the enhanced binding of this particular N^{10}-substituent relative to smaller alkyl groups. Since the angle subtended by the para substituents in a benzene ring is 180° and that of a 2,5-disubstituted thiophene or thiazole is considerably lower (~152°),[27] differences in N^{10}-substituent-enzyme interactions would be expected. The loss of enzyme activity observed with the N^{10}-propargyl analogues in the five-membered ring series is consistent with either a reduction of a specific enzyme–inhibitor binding interaction or a reduced desolvation potential in areas of the ternary complex available to the propargyl group in the ternary complex but not to the methyl or ethyl groups.

The thiophene and thiazole analogues are considerably more growth inhibitory than the corresponding benzene ring compounds in spite of their modest TS inhibitory potencies. However TS remains the sole locus of action since the growth inhibition of each analogue (at $10 \times IC_{50}$ values) can be completely prevented by 10 μM thymidine. On the other hand the cytotoxicity of methotrexate (MTX, whose primary locus of action is DHFR) to L1210 cells is not prevented by thymidine alone.[28] Moreover cross resistance was seen to the thiophenes and thiazoles in the TS overproducing (200-fold) W1L2:C1 variant of the W1L2 human lymphoblastoid cell line which has an acquired resistance to **2a** (i.e. ICI 198583) due to amplification of

(24) Jones, T. R.; Calvert, A. H.; Jackman, A. L.; Eakin, M. A.; Smithers, M. J.; Betteridge, R. F.; Newell, D. R.; Hayter, A. J.; Stocker, A.; Harland, S. J.; Davies, L. C.; Harrap, K. R. *J. Med. Chem.* **1985**, *28*, 1468.

(25) Matthews, D. A.; Appelt, K.; Oatley, S. J.; Xuong, Ng. H. *J. Mol. Biol.* **1990**, *214*, 923.

(26) Montfort, W. R.; Perry, K. M.; Fauman, E. B.; Finer-Moore, J. S.; Maley, G. F.; Hardy, L.; Maley, F.; Stroud, R. M. *Biochemistry* **1990**, *29*, 6964.

(27) (a) Bak, B.; Christensen, D.; Hansen-Nygaard, L.; Rastrup-Andersen, J. *J. Mol. Spectrosc.* **1961**, *7*, 58. (b) Hansen-Nygaard, L.; Asmussen, E.; Høg, J. H.; Maheshwari, R. C.; Nielsen, C. H.; Petersen, I. B.; Rastrup-Andersen, J.; Soerensen, C. O. *J. Mol. Struct.* **1971**, *9*, 222.

(28) Jackman, A. L.; Taylor, G. A.; O'Connor, B. M.; Bishop, J. A.; Moran, R. G.; Calvert, A. H. *Cancer Res.* **1990**, *50*, 5212.

the TS gene.[29] This W1L2:C1 variant is sensitive to MTX.[29] The enhanced growth inhibitory properties of these compounds must therefore be explained by effects on other contributory parameters. A number of these compounds have been tested as substrates for folylpolyglutamate synthetase (FPGS) and it was concluded that the N^{10}-substituted thiophene and thiazole analogues were some of the most potent substrates described for this enzyme.[29] A relationship was apparent between the potencies of these analogues in growth-inhibition assays and both their uptake via the reduced folate carrier and their FPGS substrate activities.[29,30]

Thus from the results reported here we conclude that the thiophene and thiazole analogues **7c,d** and **8a,c,d,f** stand out as being some of the most potent cytotoxic folate-based TS inhibitors so far observed. Extensive biological evaluation of these and other analogues in in vitro and in vivo systems has led to our choice of **7c** (the N^{10}-methyl, thiophene analogue, ICI D1694) as a development drug for clinical study. ICI D1694 (**7c**) is a mixed noncompetitive inhibitor of isolated L1210 TS with a K_i of 62 nM.[29] This 20-fold loss in TS inhibitory potency when compared with that of **1** is not reflected in poorer growth inhibition. Indeed **7c** is 600-fold more potent in inhibiting the growth of murine L1210 cells or human W1L2 cells.[29] Two factors appear to account for this. Firstly **7c** unlike **1** is transported via the reduced folate carrier.[29] Secondly **7c** is an excellent substrate for FPGS (K_m = 1.3 μM)[29,30] and the synthetic polyglutamates[31] of **7c** are up to 2 orders of magnitude more potent than the parent compound as inhibitors of TS.[30] Furthermore **7c** has good activity in a number of human xenograft tumor models[29,32] and the toxicities observed are to hematological tissues in mice.[33] The dose-limiting nephrotoxicity of **1** in mice was not observed with this compound.[33]

Experimental Section

The General Procedures used were described in the earlier paper[9] in this series.

Diethyl *N*-(5-Nitro-2-thenoyl)-L-glutamate (20). Method A. Et_3N (130 mL, 0.93 mol) was added to a stirred solution of diethyl L-glutamate hydrochloride (55.0 g, 0.23 mol) in CH_2Cl_2 (1.15 L) at 5 °C (ice–salt bath) under argon to form a white precipitate (Et_3N·HCl). After 1 h a solution of 5-nitro-2-thenoyl chloride[34] (34.5 g, 0.18 mol) in CH_2Cl_2 (500 mL) was added over 30 min while the temperature was kept below 20 °C. The reaction mixture was allowed to warm to 20 °C over 1 h and filtered. The filtrate was washed with aqueous $NaHCO_3$ (3 × 500 mL), dried, and evaporated to dryness. The crude brown oil was dissolved in EtOAc and percolated through a bed of Kieselgel 60 (500 g), eluting with EtOAc. Fractions containing the pure product by TLC were pooled and evaporated to afford an orange oil: 54.0 g (84%); NMR ($CDCl_3$) δ 1.25, 1.3 (2 t, 6 H, 2 OCH_2CH_3), 2.2 (m, 2 H, $CHCH_2CH_2CO_2Et$), 2.5 (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), 7.5 (d, 1 H, thiophene 3-H), 7.6 (d, 1 H, CONH), 7.85 (d, 1 H, thiophene 4-H).

Diethyl *N*-(5-Amino-2-thenoyl)-L-glutamate (21). Method B. Iron powder (12.3 g, 0.22 g-atom) was covered with 2 N HCl and kept for 10 min with occasional swirling. The iron was filtered off in a sinter, washed sequentially with H_2O and acetone, and dried for 1 h at 0.1 mmHg. The activated iron powder and $FeSO_4·7H_2O$ (4.74 g, 17.0 mmol) were added to a vigorously stirred solution of nitro compound **20** (6.10 g, 17.0 mmol) in MeOH (75 mL) and H_2O (25 mL). The resulting mixture was stirred for 5 h at 70 °C, cooled, and filtered through Celite, with washing of the filter cake with MeOH. The MeOH was evaporated; the oily residue was diluted with H_2O to 100 mL and extracted with EtOAc (3 × 75 mL). The combined EtOAc solutions were washed with brine and dried, and the solvent was evaporated. The crude brown oil was purified by chromatography using a gradient of 50–75% v/v EtOAc in hexane as eluent. The product was isolated as a brown gum: 4.60 g (82%); NMR ($CDCl_3$) δ 1.25, 1.3 (2 t, 6 H, 2 OCH_2CH_3), 2.2 (m, 2 H, $CHCH_2CH_2CO_2Et$), 2.45 (t 2 H, $CHCH_2CH_2CO_2Et$), 3.6 (br s, 2 H, NH_2), 4.1, 4.2 (2 q, 4 H, 2 OCH_2CH_3), 4.7 (m 1 H, CH), 6.1 (d, 1 H, thiophene 4-H), 6.6 (d, 1 H, CONH), 7.2 (d, 1 H, thiophene 3-H).

Diethyl *N*-[5-(Prop-2-ynylamino)-2-thenoyl]-L-glutamate (22a). Method C. A mixture of **21** (1.0 g, 3.05 mmol), 2,6-lutidine (0.54 mL, 4.65 mmol), and propargyl bromide (0.50 mL of an 80% v/v solution in toluene, 4.49 mmol) in DMF (25 mL) was stirred for 24 h at 50 °C under argon. The cooled reaction mixture was partitioned between EtOAc (3 × 50 mL) and H_2O (25 mL). The organic phase was washed with brine, dried, and evaporated to dryness. The residue was purified by chromatography using a gradient of 0–30% v/v EtOAc in CH_2Cl_2 as eluent to yield a golden oil: 600 mg (54%); NMR ($CDCl_3$) δ 1.25, 1.3 (2 t, 6 H, 2 OCH_2CH_3), 2.25 (m, 2 H, $CHCH_2CH_2CO_2Et$), 2.3 (t, 1 H, C≡CH), 2.45 (t, 2 H, $CHCH_2CH_2CO_2Et$), 3.95 (d, 2 H, $CH_2C{\equiv}C$), 4.1, 4.25 (2 q, 4 H, 2 OCH_2CH_3), 4.55 (t, 1 H, NH), 4.75 (m, 1 H, CH), 6.05 (d, 1 H, thiophene 4-H), 6.6 (d, 1 H, CONH), 7.25 (d, 1 H, thiophene 3-H).

The procedure was repeated, reacting **21** and **32** with the appropriate alkyl halides, to give the intermediates **22c,d,e,h,i** and **33a,f–i** (Table I).

Diethyl *N*-[5-[*N*-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]-2-thenoyl]-L-glutamate (23a). Method D. A mixture of **22a** (1.50 g, 41. mmol), bromomethyl compound **18**[10] (1.10 g, 4.35 mmol), and 2,6-lutidine (480 μL, 4.1 mmol) in DMF (25 mL) was stirred for 18 h at 60 °C under argon. The cooled mixture was evaporated to dryness below 40 °C and the residue was purified by chromatography using 1% MeOH in EtOAc as eluent to give a gum: 334 mg (15%); NMR ($CDCl_3$) δ 1.2, 1.3 (2 t, 6 H, 2 OCH_2CH_3), 2.2 (m, 2 H, $CHCH_2CH_2CO_2Et$), 2.3 (t, 1 H, C≡CH), 2.45 (t, 2 H, $CHCH_2CH_2CO_2Et$), 2.6 (s, 3 H, CH_3), 4.0 (d, 2 H, $CH_2C{\equiv}C$), 4.1, 4.2 (2 q, 4 H, 2 OCH_2CH_3), 4.6 (br s, 2 H, $ArCH_2N<$), 4.75 (m, 1 H, CH), 6.1 (d, 1 H, thiophene 4-H), 6.65 (d, 1 H, CONH), 7.3 (d, 1 H, thiophene 3-H), 7.65 (d, 1 H, quinazoline 8-H), 7.75 (dd, 1 H, quinazoline 7-H), 8.2 (d, 1 H, quinazoline 5-H).

The procedure was repeated with the appropriate amines to yield antifolate diesters **23b–f,h–j**, **69c,d,g**, **70d**, **71c,d**, and **72c** (Tables I and III).

***N*-[5-[*N*-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]-2-thenoyl]-L-glutamic Acid (7a). Method E.** Diester **23a** (334 mg, 0.62 mmol) was stirred for 2 h under argon in 1 N aqueous NaOH (6.2 mL, 6.2 mmol). The resulting solution was filtered and brought to pH 3.0 with 2 N aqueous HCl. The precipitate was collected by filtration and washed several times with H_2O until the filtrate was free of chloride ion ($AgNO_3$ test). The damp product was vacuum dried to yield an amorphous solid: 128 mg (42%); mp 215–225 °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), 4.2 (d, 2 H, $CH_2C{\equiv}C$), 4.3 (m, 1 H, CH), 4.65 (br s, 2 H, $ArCH_2N<$), 6.15 (d, 1 H, thiophene 4-H), 7.55 (d, 1 H, quinazoline 8-H), 7.6 (d, 1 H, thiophene 3-H), 7.75 (dd, 1 H, quinazoline 7-H), 8.05 (d, 1 H, quinazoline 5-H), 8.15 (d, 1 H, CONH); MS (FAB) m/z 483 $[MH]^+$. Anal. ($C_{23}H_{22}N_4O_6S·0.75\ H_2O$) C, H, N.

The procedure was repeated with the appropriate diethyl esters to yield antifolates **7b–i**, **14c,d,g**, and **15d** (Tables II and IV). Some of the compounds gave poor melting points but all had correct elemental analyses (C, H, N) for the formulae listed in the tables, and NMR and mass spectra were consistent with the assigned structures.

(29) Jackman, A. L.; Marsham, P. R.; Moran, R. G.; Kimbell, R.; O'Connor, B. M.; Hughes, L. R.; Calvert, A. H. *Adv. Enzyme Regul.* In press.
(30) Jackman, A. L.; Taylor, G. A.; Bishop, J. A.; O'Connor, B. M.; Bisset, G.; Hughes, L. R.; Moran, R. G.; Calvert, A. H. *Proc. Am. Assoc. Cancer Res.* **1990**, *31*, 342.
(31) Bisset, G. M. F.; Pawelczak, K.; Jackman, A. L.; Dix Perkin, E. E.; Calvert, A. H.; Hughes, L. R. Manuscript in preparation.
(32) Stephens, T. C.; Calvete, J. A.; Janes, D.; Waterman, S. E.; Valcaccia, B. E.; Hughes, L. R.; Calvert, A. H. *Proc. Am. Assoc. Cancer Res.* **1990**, *31*, 342.
(33) Jodrell, D. I.; Newell, D. R.; Calvete, J. A.; Stephens, T. C.; Calvert, A. H. *Proc. Am. Assoc. Cancer Res.* **1990**, *31*, 341.
(34) Rinkes, I. J. *Recl. Trav. Chim. Pays-Bas* **1933**, *52*, 538.

2-[*N*-(*tert*-Butoxycarbonyl)amino]thiophene (25). Method F. Et_3N (325 mL, 2.34 mol) was added dropwise to a stirred, cooled solution of 2-thiophenecarboxylic acid (300 g, 2.34 mol) in *t*-BuOH (2.5 L) at such a rate as to maintain the temperature at 20–25 °C. Diphenyl phosphorazidate (DPPA) (517 mL, 2.4 mol) was then added and the mixture was stirred under reflux for 12 h. The cooled yellow solution was poured into ice-cold H_2O (7 L). The resulting off-white precipitate was filtered off, washed with H_2O, and vacuum dried at 35 °C: 431 g (92%); mp 147–148 °C; NMR ($CDCl_3$) δ 1.5 (s, 9 H, *t*-Bu), 6.5 (dd, 1 H, thiophene 3-H), 6.8 (m, 2 H, thiophene 4-H and 5-H), 6.9 (br, 1 H, CONH). Anal. ($C_9H_{13}NO_2S$) C, H, N.

2-[*N*-(*tert*-Butoxycarbonyl)-*N*-methylamino]thiophene (26c). Method G. A solution of **25** (100 g, 0.502 mol) in DMF (300 mL) was added dropwise to a stirred cooled (ice–salt bath) suspension of NaH (23.1 g of a 55% dispersion in oil, 0.53 mol) in DMF (300 mL) under argon at such a rate as the temperature did not exceed 5 °C. After a further 30 min at 0–5 °C, MeI (31.4 mL, 0.50 mol) was added dropwise below 15 °C. Stirring was continued for 16 h at 20 °C. The reaction was quenched with H_2O (2 L) and extracted with Et_2O (4 × 1.5 L). The combined Et_2O solutions were washed with H_2O and brine, dried, and evaporated. The resulting yellow oil (123 g) was chromatographed with 1:1 v/v hexane/CH_2Cl_2 as eluent to provide an oil: 83.3 g (78%); NMR ($CDCl_3$) δ 1.55 (s, 9 H, *t*-Bu), 3.35 (s, 3 H, NCH_3), 6.5 (dd, 1 H, thiophene 3-H), 6.85 (m, 2 H, thiophene 4-H and 5-H).

5-[*N*-(*tert*-Butoxycarbonyl)-*N*-methylamino]-2-thiophenecarboxylic Acid (27c). Method H. A stirred solution of *i*-Pr_2NH (30.0 mL, 0.21 mol) in THF (200 mL) under argon at −78 °C was treated dropwise over 30 min with *n*-BuLi (119 mL of a 1.6 M solution in hexane, 0.19 mol) while the temperature was maintained below −50 °C. The mixture was stirred for 30 min at −78 °C and then treated dropwise below −60 °C with a solution of **26c** (40.8 g, 0.19 mol) in THF (200 mL). After a further 30 min at −78 °C, crushed solid CO_2 (200 g, 4.54 mol) was added in small portions with careful exclusion of moisture while the temperature was kept below −50 °C. The reaction mixture was allowed to warm slowly to 20 °C and stirred overnight at this temperature. The mixture was poured into H_2O (2 L) and brought to pH 5.0 by the addition of powdered citric acid. The precipitated off-white solid was isolated by filtration, washed with H_2O, and vacuum dried: 39.8 g (81%); mp 210–211 °C (dec); NMR ($CDCl_3$ + Me_2SO-d_6) δ 1.55 (s, 9 H, *t*-Bu), 3.4 (s, 3 H, NCH_3), 6.5 (d, 1 H, thiophene 4-H), 7.6 (d, 1 H, thiophene 3-H). Anal. ($C_{11}H_{15}NO_4S$) C, H, N.

Diethyl *N*-[5-(Methylamino)-2-thenoyl]-L-glutamate (22c). Method I. Acid **27c** (39.0 g, 0.152 mol) was condensed with diethyl L-glutamate hydrochloride (36.15 g, 0.152 mol) according to method K below. Crude glutamate derivative **28c** (74 g) was dissolved with stirring in CF_3CO_2H (350 mL). After 16 h the CF_3CO_2H was evaporated and the residue was partitioned between aqueous $NaHCO_3$ (1 L) and CH_2Cl_2 (3 × 500 mL). The combined CH_2Cl_2 solutions were dried and concentrated to an oil. Purification was achieved by chromatography using 10% v/v EtOAc in CH_2Cl_2 as eluent. Product **22c** was isolated as a brown viscous oil: 33.35 g (58%); NMR ($CDCl_3$) δ 1.2, 1.25 (2 t, 6 H, 2 OCH_2CH_3), 2.1 (m, 2 H, $CHCH_2CH_2CO_2Et$), 2.35 (t, 2 H, $CHCH_2CH_2CO_2Et$), 2.85 (s, 3 H, NCH_3), 4.05, 4.15 (2 q, 4 H, 2 OCH_2CH_3), 4.65 (m, 1 H, CH), 5.85 (d, 1 H, thiophene 4-H), 6.4 (d, 1 H, CONH), 7.15 (d, 1 H, thiophene 3-H).

***N*-[5-[*N*-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-(cyanomethyl)amino]-2-thenoyl]-L-glutamic Acid (7j). Method J.** Diester **23j** (prepared from di-*tert*-butyl L-glutamate by methods A–D; 500 mg, 0.84 mmol) was dissolved with stirring in CF_3CO_2H (5 mL). After 10 min the CF_3CO_2H was evaporated under vacuum. The resulting brown gum was triturated with Et_2O to give a solid which was purified on a column of HP20 SS resin using a gradient of 5–60% v/v MeOH in H_2O (+0.2% CF_3CO_2H) as eluent. Fractions containing pure **7j** by HPLC were combined, and the MeOH was evaporated below 30 °C on a rotary evaporator. The aqueous residue was lyophilized to a pale yellow solid which was further dried at 80 °C (0.1 mmHg) for 6 h: 263 mg (48%); mp 125–130 °C; NMR (Me_2SO-d_6) δ 1.95 (m, 2 H, $CHCH_2CH_2CO_2H$), 2.3 (t, 2 H, $CHCH_2CH_2CO_2H$), 2.45 (s, 3 H, CH_3), 4.3 (m, 1 H, CH), 4.7 (2 br s, 4 H, CH_2CN and $ArCH_2N<$), 6.3 (d, 1 H, thiophene 4-H), 7.65 (2 d, 2 H, quinazoline 8-H and thiophene 3-H), 7.8 (dd, 1 H, quinazoline 7-H), 8.1 (d, 1 H, quinazoline 5-H), 8.3 (d, 1 H, CONH); MS (FAB) *m/z* 482 $[M - H]^-$. Anal. ($C_{22}H_{21}N_5O_6S \cdot 1.5CF_3CO_2H$) C, H, N.

Diethyl *N*-[(5-Nitrothiazol-2-yl)carbonyl]-L-glutamate (31). Method K. Oxalyl chloride (39.3 mL, 0.45 mol) was added over 30 min to a stirred mixture of 5-nitrothiazole-2-carboxylic acid[17] (44.75 g, 0.26 mol) and DMF (60 mL) in CH_2Cl_2 (300 mL). After stirring for a further 30 min the solution was evaporated to dryness below 30 °C and the crude acid chloride, a sticky solid, was dried for 1 h under vacuum (0.1 mmHg).

Et_3N (89.5 mL, 0.64 mol) was added to a stirred solution of diethyl L-glutamate hydrochloride (100.0 g, 0.42 mol) in CH_2Cl_2 (700 mL) under argon. The reaction mixture was cooled (ice bath) to 10 °C. The above crude acid chloride was dissolved in CH_2Cl_2 (300 mL) and added to the stirred reaction mixture at such a rate as to keep the temperature below 15 °C. Stirring was continued overnight and the resulting dark green mixture was washed with H_2O (2 × 300 mL). The aqueous washings were extracted with CH_2Cl_2 (500 mL). The combined organic solutions were dried and evaporated to a black residue which was purified by chromatography using a gradient of 0–5% v/v EtOAc in CH_2Cl_2 as eluent. The product (73.4 g, 79%) was isolated as a brown gum: NMR (Me_2SO-d_6) δ 1.15, 1.2 (2 t, 6 H, 2 OCH_2CH_3), 2.15 (m, 2 H, $CHCH_2CH_2CO_2Et$), 2.4 (t, 2 H, $CHCH_2CH_2CO_2Et$), 4.05, 4.15 (2 q, 4 H, 2 OCH_2CH_3), 4.5 (m, 1 H, CH), 9.0 (s, 1 H, thiazole 4-H), 9.55 (d, 1 H, CONH).

Diethyl *N*-[(5-Aminothiazol-2-yl)carbonyl]-L-glutamate (32). Method L. Activated iron powder (see method B; 275 g) was added in portions over 30 min to a vigorously stirred solution of **31** (73.3 g, 0.204 mol) in HOAc (750 mL) at 80 °C. At the end of the addition the mixture was cooled and filtered through Celite. The iron residues on the Celite were washed with CH_2Cl_2 (750 mL) and H_2O (500 mL). The filtrates were combined and the phases were separated. The aqueous phase was washed with a second portion of CH_2Cl_2. The combined CH_2Cl_2 solutions were washed with H_2O, dried, and evaporated to give a brown oil. Purification was achieved by chromatography using a gradient of 0–25% v/v EtOAc in CH_2Cl_2 as eluent. The product (40.0 g, 60%) was isolated as a light brown oil: NMR (Me_2SO-d_6) δ 1.15, 1.2 (2 t, 6 H, 2 OCH_2CH_3), 2.05 (m, 2 H, $CHCH_2CH_2CO_2Et$), 2.35 (t, 2 H, $CHCH_2CH_2CO_2Et$), 4.05, 4.1 (2 q, 4 H, 2 OCH_2CH_3), 4.4 (m, 1 H, CH), 6.45 (br s, 2 H, NH_2), 6.85 (s, 1 H, thiazole 4-H), 8.35 (d, 1 H, CONH).

Diethyl *N*-[[5-[*N*-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]thiazol-2-yl]carbonyl]-L-glutamate (35a). Method M. A mixture of **33a** (223 mg, 0.607 mmol), bromomethyl compound **18** (239 mg, 0.945 mmol), and powdered $CaCO_3$ (126 mg, 1.26 mmol) in DMF (1.5 mL) was stirred for 2.5 h at 90 °C under argon. The cooled mixture was filtered and the filter cake was washed with DMF (2 mL). The combined filtrates were evaporated to dryness. The crude product was purified by chromatography using a gradient of 0–5% v/v EtOH in CH_2Cl_2 as eluent to give a hard yellow foam: 154 mg (47%); NMR (Me_2SO-d_6) δ 1.15, 1.2 (2 t, 6 H, 2 OCH_2CH_3), 2.05 (m, 2 H, $CHCH_2CH_2CO_2Et$), 2.35 (t, 2 H, $CHCH_2CH_2CO_2Et$), 2.35 (s, 3 H, CH_3), 3.35 (t, 1 H, C≡CH), 4.05, 4.1 (2 q, 4 H, 2 OCH_2CH_3), 4.3 (d, 2 H, $CH_2C{\equiv}C$), 4.4 (m, 1 H, CH), 4.7 (br s, 2 H, $ArCH_2N<$), 7.2 (s, 1 H, thiazole 4-H), 7.55 (d, 1 H, quinazoline 8-H), 7.75 (dd, 1 H, quinazoline 7-H), 8.05 (d, 1 H, quinazoline 5-H), 8.6 (d, 1 H, CONH).

The procedure was repeated with the appropriate amines to yield the antifolate diesters 35b–**d**,**g**, **55a**,**c**,**d** and **59c** (Table I).

***N*-[[5-[*N*-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]thiazol-2-yl]carbonyl]-L-glutamic Acid (8a). Method N.** Diester **35a** (145 mg, 0.27 mmol) was stirred for 4 h under argon in a mixture of 1 N aqueous NaOH (1.65 mL, 1.65 mmol), EtOH (3 mL), and H_2O (3 mL). The resulting solution was evaporated below 30 °C to ca. 2 mL, 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 repeated cycles of aqueous suspension–centrifugation–decantation until the supernatant was free of chloride ion. The damp product was vacuum dried to give a pale buff amorphous solid: 91 mg (70%); mp 148–152 °C; NMR (Me_2SO-d_6) δ 2.05 (m, 2 H, $CHCH_2CH_2CO_2H$), 2.25 (t, 2 H,

$CHCH_2CH_2CO_2H$), 2.4 (s, 3 H, CH_3), 3.25 (t, 1 H, C≡CH), 4.3 (d, 2 H, $CH_2C{\equiv}C$), 4.35 (m, 1 H, CH), 4.7 (br s, 2 H, $ArCH_2N<$), 7.15 (d, 1 H, thiazole 4-H), 7.6 (d, 1 H, quinazoline 8-H), 7.8 (dd, 1 H, quinazoline 7-H), 8.1 (d, 1 H, quinazoline 5-H), 8.6 (d, 1 H, CONH); MS (FAB) m/z 482 $[M - H]^-$. Anal. ($C_{22}H_{21}N_5O_6S \cdot 0.3H_2O$) C, H, N.

The procedure was repeated with the appropriate diethyl esters to yield antifolates **8b–d,f–i**, **9d**, **10d**, 11a,c,d,f,g, **12c**, **13c**, **16c,d**, and **17c** (Tables II and IV). Some of the compounds gave poor melting points but all had correct elemental analyses (C, H, N) for the formulae listed in the tables, and NMR and mass spectra were consistent with the assigned structures.

Diethyl *N*-[[5-(Methylamino)thiazol-2-yl]carbonyl]-L-glutamate (33c). Method O. A mixture of **32** (11.87 g, 0.036 mol) and MeI (10 mL, 0.16 mol) in DMF (33 mL) was stirred for 1 h at 60 °C under argon. The cooled mixture was diluted with saturated aqueous $NaHCO_3$ (50 mL) and extracted with EtOAc (100 mL). The EtOAc solution was washed with water, dried, and evaporated to dryness. The crude dark brown oil was purified by chromatography using a gradient of 0–10% v/v EtOAc in CH_2Cl_2 as eluent. The first product obtained was diethyl *N*-[[5-(dimethylamino)thiazol-2-yl]carbonyl]-L-glutamate (**34**) as a brown gum: 841 mg (21%); NMR (Me_2SO-d_6) δ 1.15, 1.2 (2 t, 6 H, 2 OCH_2CH_3), 2.05 (m, 2 H, $CHCH_2CH_2CO_2Et$), 2.4 (t, 2 H, $CHCH_2CH_2CO_2Et$), 3.0 (s, 6 H, $N(CH_3)_2$), 4.05, 4.1 (2 q, 4 H, 2 OCH_2CH_3), 4.4 (m, 1 H, CH), 6.95 (s, 1 H, thiazole 4-H), 8.5 (d, 1 H, CONH). Anal. ($C_{15}H_{23}N_3O_6S \cdot 0.5H_2O$) C, H, N. The second product obtained was the required methylamino compound **33c** as a golden gum: 1.08 g (29%); NMR (Me_2SO-d_6) δ 1.15, 1.2 (2 t, 6 H, 2 OCH_2CH_3), 2.05 (m, 2 H, $CHCH_2CH_2CO_2Et$), 2.4 (t, 2 H, $CHCH_2CH_2CO_2Et$), 2.8 (br s, 3 H, NCH_3), 4.05, 4.1 (2 q, 4 H, 2 OCH_2CH_3), 4.4 (m, 1 H, CH), 6.85 (s, 1 H, thiazole 4-H), 8.4 (d, 1 H, CONH). Anal. ($C_{14}H_{21}N_3O_6S \cdot 0.75H_2O$) C, H, N, S.

The procedure was repeated with EtI as the alkylating agent to afford **33d** (27%).

Diethyl *N*-[[5-[*N*-[[3,4-Dihydro-2-methyl-4-oxo-3-[(pivaloyloxy)methyl]-6-quinazolinyl]methyl]-*N*-prop-2-enylamino]thiazol-2-yl]carbonyl]-L-glutamate (35f). Method P. A mixture of **33f** (2.16 g, 5.86 mmol), bromomethyl compound **36**[12] (2.58 g, 7.04 mmol) and 2,6-lutidine (0.82 mL, 7.04 mmol) in DMA (10 mL) was stirred for 5 h at 100 °C under argon. The cooled mixture was evaporated to dryness and the residue was purified by chromatography using a gradient of 0–20% v/v EtOAc in CH_2Cl_2 as eluent. The product was isolated as a foam: 2.44 g (63%); NMR (Me_2SO-d_6) δ 1.13 (s, 9 H, *t*-Bu), 1.15 (t, 6 H, 2 OCH_2CH_3), 2.05 (m, 2 H, $CHCH_2CH_2CO_2Et$), 2.35 (t, 2 H, $CHCH_2CH_2CO_2Et$), 2.6 (s, 3 H, CH_3), 4.05 (m, 6 H, $NCH_2CH{=}CH_2$ and 2 OCH_2CH_3), 4.4 (m, 1 H, CH), 4.7 (br s, 2 H, $ArCH_2N<$), 5.25 (m, 2 H, $CH{=}CH_2$), 5.85 (m, 1 H, $CH{=}CH_2$), 6.05 (s, 2 H, OCH_2N), 7.05 (s, 1 H, thiazole 4-H), 7.6 (d, 1 H, quinazoline 8-H), 7.8 (dd, 1 H, quinazoline 7-H), 8.05 (d, 1 H, quinazoline 5-H), 8.5 (d, 1 H, CONH). Anal. ($C_{32}H_{41}N_5O_8S \cdot 0.75H_2O$) C, H, N.

The procedure was repeated with the appropriate amines to give the (pivaloyloxy)methyl-protected antifolate diesters **35h,i** and **55f,g** (Table I).

***N*-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-ethylamine Hydrobromide (37).** Powdered **18** (10.0 g, 39.5 mmol) was added in portions over 10 min to a stirred solution of anhydrous $EtNH_2$ (8.0 mL, 0.12 mol) in MeCN (300 mL). Stirring was continued overnight and the reaction mixture was evaporated to dryness. The residue was dissolved in H_2O (100 mL). The resulting solution was filtered through Celite to remove a small amount of insoluble material and the filtrate was evaporated to dryness. Trituration with acetone afforded an amorphous off-white solid which was filtered off and dried under vacuum: 8.84 g (75%); mp 298 °C; NMR (Me_2SO-d_6) δ 1.3 (t 3 H, NCH_2CH_3), 2.4 (s, 3 H, CH_3), 3.05 (q, 2 H, NCH_2CH_3), 4.3 (br s, 2 H, $ArCH_2N<$), 7.7 (d, 1 H, quinazoline 8-H), 8.0 (dd, 1 H, quinazoline 7-H), 8.3 (d, 1 H, quinazoline 5-H), 9.0 (br s, 1 H, >NH). Anal. ($C_{12}H_{15}N_3O \cdot HBr$) C, H, N.

***N*-Benzoyl-*N'*-[(3,4-dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N'*-ethylthiourea (38).** Benzoyl isothiocyanate (2.75 mL, 20.5 mmol) was added over 1 min to a stirred mixture of **37** (6.1 g, 20.47 mmol) and Et_3N (3.1 mL, 22.2 mmol) in anhydrous acetone (25 mL). An exotherm to 42 °C was observed. The mixture was stirred for 1 h under reflux and poured rapidly with stirring into H_2O (250 mL). After standing overnight at 4 °C the precipitated light brown solid was filtered off, allowed to dry, washed with hot toluene, and vacuum dried: 6.54 g (84%); mp 200–202 °C. Anal. ($C_{20}H_{20}N_4O_2S$) H, N S; C: calcd, 63.1; found, 62.5.

***N*-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-ethylthiourea Hydrochloride (39).** A mixture of **38** (6.11 g, 16.1 mmol), concentrated HCl (80 mL), and *i*-PrOH (48 mL) was stirred for 1 h at 100 °C under argon. The cooled mixture was evaporated to dryness, the residue redissolved in *i*-PrOH (48 mL) and reevaporated. The solid product was washed with EtOAc and vacuum dried to yield impure **39**: 5.32 g; MS (EI) m/z 277 $[MH]^+$, 218 $[MH - HCN]^+$. Anal. ($C_{13}H_{16}N_4OS \cdot 1.25HCl$) C, H; N: calcd, 17.4; found, 16.7. This material was used directly in the preparation of **40**.

Ethyl 2-[*N*-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-ethylamino]thiazole-5-carboxylate (40). A solution of **39** (4.67 g, 16.9 mmol) and ethyl 2-chloro-2-formylacetate[19] (2.55 g, 16.9 mmol) in DMF (25 mL) was heated at 100 °C for 1 h under argon. The DMF was evaporated and the residue was partitioned between CH_2Cl_2 (2 × 50 mL) and aqueous $NaHCO_3$ (50 mL). The combined CH_2Cl_2 solutions were washed with H_2O, dried, and evaporated to dryness. The crude product was triturated with EtOAc to give a buff solid which was filtered off and vacuum dried: 1.37 g (22%); mp 183 °C; NMR (Me_2SO-d_6) δ 1.2 (2 t, 6 H, $>NCH_2CH_3$ and OCH_2CH_3), 2.35 (s, 3 H, CH_3), 3.6 (q, 2 H, $>NCH_2CH_3$), 4.2 (q, 2 H, OCH_2CH_3), 4.85 (s, 2 H, $ArCH_2N<$), 7.55 (d, 1 H, quinazoline 8-H), 7.7 (dd, 1 H, quinazoline 7-H), 7.85 (s, 1 H, thiazole 4-H), 8.0 (d, 1 H, quinazoline 5-H). Anal. ($C_{18}H_{20}N_4O_3S \cdot 0.25H_2O$) C, H, N.

2-[*N*-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-ethylamino]thiazole-5-carboxylic Acid Hemihydrate (41). Method Q. Ethyl ester **40** (1.30 g, 3.50 mmol) was stirred in 1 N aqueous NaOH (10.5 mL, 10.5 mmol) for 1 h at 50 °C under argon. The resulting solution was cooled to 0 °C and brought to pH 4.0 with 2 N aqueous HCl. The gummy precipitate was washed twice with H_2O and vacuum dried to give a buff amorphous solid (1.05 g, 85%) which softened above 170 °C and decomposed at 185 °C. Anal. ($C_{16}H_{16}N_4O_3S \cdot 0.5H_2O$) C, H, N.

Diethyl *N*-[[2-[*N*-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-ethylamino]thiazol-5-yl]carbonyl]-L-glutamate (42d). Method R. A solution of acid **41** hemihydrate (1.10 g, 3.12 mmol) and diethyl L-glutamate hydrochloride (843 mg, 3.52 mmol) in DMF (10 mL) was stirred at 0 °C during the dropwise addition over 15 min of DPPA (0.76 mL, 3.52 mmol) followed by Et_3N (1.15 mL, 10.8 mmol) again over 15 min. The reaction mixture was then stirred at room temperature for 40 h and partitioned between CH_2Cl_2 (2 × 50 mL) and ice-cold H_2O (100 mL). The combined CH_2Cl_2 solutions were washed with H_2O, dried, and evaporated to dryness. The crude product was purified by chromatography using a gradient of 0–5% v/v EtOH in CH_2Cl_2 as eluent to give a gum: 792 mg (48%); NMR (Me_2SO-d_6) δ 1.2 (2 q and t, 9 H, 2 OCH_2CH_3 and $>NCH_2CH_3$), 2.0 (m, 2 H, $CHCH_2CH_2CO_2Et$), 2.35 (s, 3 H, CH_3), 2.4 (t, 2 H, $CHCH_2CH_2CO_2Et$), 3.55 (q, 2 H, NCH_2CH_3), 4.05, 4.1 (2 q, 4 H, 2 OCH_2CH_3), 4.35 (m, 1 H, CH), 4.85 (br s, 2 H, $ArCH_2N<$), 7.55 (d, 1 H, quinazoline 8-H), 7.7 (dd, 1 H, quinazoline 7-H), 7.9 (s, 1 H, thiazole 4-H), 8.0 (d, 1 H, quinazoline 5-H), 8.4 (d, 1 H, CONH). Anal. ($C_{25}H_{31}N_5O_6S \cdot 0.25H_2O$) C, H, N.

1-[[*N*-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-ethylamino](thiocarbonyl)]imidazole (43). 1,1'-Thiocarbonyldiimidazole (5.38 g, 30.2 mmol) was added to a stirred mixture of **37** (9.00 g, 30.2 mmol) and Et_3N (4.6 mL, 33.2 mmol) in CH_2Cl_2 (150 mL). The initial yellow suspension rapidly dissolved to give a red solution. After 16 h the precipitated yellow solid was filtered off, washed with CH_2Cl_2, dried in air, washed with H_2O, and vacuum dried: 6.92 g (70%); mp 205 °C. Anal. ($C_{16}H_{17}N_5OS \cdot 0.4H_2O$) C, H, N. Evaporation of the filtrate and trituration of the residue with EtOAc gave a further 2.19 g (22%) of **43**.

4-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-4-ethyl-3-thiosemicarbazide Hemihydrate (44). A mixture of **43** (9.07 g, 27.74 mmol) and hydrazine hydrate (1.48 mL, 30.5 mmol) in EtOH (125 mL) was stirred for 2 h under reflux and

allowed to cool overnight. The precipitated solid was filtered off, washed with EtOH, dried overnight in air, and vacuum dried: 5.30 g (64%); mp 182 °C. Anal. ($C_{13}H_{17}N_5OS\cdot0.5H_2O$) C, H, N.

Ethyl 5-[*N*-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-ethylamino]-1,3,4-thiadiazole-2-carboxylate (46). Ethyl oxalyl chloride (2.30 mL, 20.6 mmol) was added dropwise over 15 min to a stirred solution of **44** (4.59 g, 15.3 mmol) and Et_3N (3.30 mL, 23.7 mmol) in DMF (35 mL) at 0–5 °C. After 1 h the solvent was evaporated below 30 °C and the residue was partitioned between H_2O (50 mL) and CH_2Cl_2 (6 × 30 mL). The combined CH_2Cl_2 solutions were dried and evaporated to yield crude **45** (6.60 g) which was dissolved in hot toluene (200 mL). Methanesulfonic acid (1.65 mL, 25.5 mmol) was added over 5 min to the stirred solution which was then heated under reflux for 45 min. The reaction mixture was cooled to 0 °C and the toluene supernatant was decanted from the gummy precipitate. This was stirred rapidly with a mixture of H_2O and CH_2Cl_2 while the aqueous layer was adjusted to pH 8.0 by the addition of powdered $NaHCO_3$. The mixture was extracted twice with CH_2Cl_2. The combined organic extracts were dried and evaporated to a brown gum. Purification was achieved by chromatography using a gradient of 0–5% EtOH in CH_2Cl_2 as eluent to give **46**: 3.55 g (61%); mp 175 °C. Anal. ($C_{17}H_{19}N_5O_3S\cdot0.5H_2O$) C, H, N.

5-[*N*-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-ethylamino]-1,3,4-thiadiazole-2-carboxylic Acid Hydrate (47). Ethyl ester **46** (373 mg, 0.98 mmol) was stirred for 2 h under argon in a mixture of 1 N aqueous NaOH (3.0 mL, 3.0 mmol) and EtOH (5 mL). The resulting solution was evaporated below 30 °C to ca. 2 mL, washed in a centrifuge tube, and acidified to pH 3.0 with 2 N aqueous HCl with ice-bath cooling. The gummy precipitate was isolated by centrifugation, washed twice with H_2O, and vacuum dried to a hard foam: 305 mg (86%); NMR (Me_2SO-d_6) δ 1.2 (t, 3 H, $>NCH_2CH_3$), 2.35 (s, 3 H, CH_3), 3.55 (q, 2 H, $>NCH_2CH_3$), 4.8 (s, 2 H, $ArCH_2N<$), 7.55 (d, 1 H, quinazoline 8-H), 7.7 (dd, 1 H, quinazoline 7-H), 8.0 (d, 1 H, quinazoline 5-H); MS (FAB) *m/z* 344 $[M - H]^-$, 300 $[M - H - CO_2]^-$. Anal. ($C_{15}H_{15}N_5O_3S\cdot H_2O$) C, H, N.

Methyl 5-[*N*-(*tert*-Butoxycarbonyl)amino]picolinate (50).[20] A solution of 6-(methoxycarbonyl)nicotinic acid (**49**)[35] (50.0 g, 0.28 mol), DPPA (59.6 mL, 0.28 mol), and Et_3N (38.5 mL, 0.28 mol) in *t*-BuOH (500 mL) was stirred for 3.5 h under reflux. The solvent was evaporated to give a yellow oil which was dissolved in EtOAc (700 mL). This solution was washed successively with 5% aqueous citric acid, H_2O, aqueous $NaHCO_3$, brine (150 mL of each) and dried. Evaporation of the EtOAc and trituration of the residue with toluene yielded a pale yellow solid: 38.8 g (55%); mp 145–146 °C; NMR (Me_2SO-d_6) δ 1.5 (s, 9 H, *t*-Bu), 3.85 (s, 3 H, OCH_3), 8.0 (d, 1 H, pyridine 3-H), 8.05 (dd, 1 H, pyridine 4-H), 8.7 (d, 1 H, pyridine 6-H), 9.95 (br s, 1 H, NH). Anal. ($C_{12}H_{16}N_2O_4$) C, H, N.

Methyl 5-[*N*-(*tert*-Butoxycarbonyl)-*N*-prop-2-ynylamino]picolinate (51a). Method S. Methyl ester **50** (4.00 g, 15.87 mmol) was added in portions over 30 min to a stirred suspension of NaH (727 mg of a 55% dispersion in oil, 16.7 mmol) in DMF (40 mL) under argon. After the H_2 gas evolution had ceased, propargyl bromide (1.94 mL of an 80% w/w solution in toluene, 17.46 mmol) was added dropwise below 30 °C (cold-water bath). Stirring was continued for 2 h and the reaction mixture was partitioned between EtOAc (3 × 60 mL) and H_2O (150 mL). The combined EtOAc solutions were washed with H_2O, dried, and evaporated to a buff solid which was washed with hexane and vacuum dried: 3.94 g (86%); mp 107–108 °C; NMR ($CDCl_3$) δ 1.5 (s, 9 H, *t*-Bu), 2.2 (t, 1 H, C≡CH), 4.0 (s, 3 H, OCH_3), 4.4 (d, 2 H, $>NCH_2C{\equiv}C$), 7.8 (dd, 1 H, pyridine 4-H), 8.1 (d, 1 H, pyridine 3-H), 8.7 (d, 1 H, pyridine 6-H). Anal. ($C_{15}H_{18}N_2O_4\cdot0.5H_2O$) C, N; H: calcd, 6.3; found, 5.8.

5-[*N*-(*tert*-Butoxycarbonyl)-*N*-prop-2-ynylamino]picolinic Acid (52a). Method T. Methyl ester **51a** (3.94 g, 13.59 mmol) was stirred for 16 h under argon in a mixture of 1 N aqueous NaOH (27 mL, 27 mmol), H_2O (70 mL), and EtOH (50 mL). The resulting solution was evaporated below 30 °C to ca. 25 mL and acidified to pH 4.0 with 2 N aqueous HCl. The precipitated solid was filtered off, washed with H_2O and vacuum dried: 3.15 g (84%); mp 130–131 °C. Anal. ($C_{14}H_{16}N_2O_4$) C, H; N: calcd, 10.1; found, 9.0.

Diethyl *N*-[5-[*N*-(*tert*-Butoxycarbonyl)-*N*-prop-2-ynylamino]picolinyl]-L-glutamate (53a). Method U. Et_3N (2.35 mL, 16.85 mmol) was added to a stirred solution of acid **52a** (3.11 g, 11.3 mmol) and DMF (1.0 mL) in CH_2Cl_2 (20 mL). Oxalyl chloride (1.5 mL, 16.8 mmol) was added dropwise to give a deep red solution. After 30 min the solvent was evaporated; the crude acid chloride was dried for 30 min under vacuum (0.1 mmHg) and redissolved in CH_2Cl_2 (20 mL). This solution was added dropwise to a stirred, cooled solution of diethyl L-glutamate hydrochloride (2.96 g, 12.35 mmol) and Et_3N (4.7 mL, 33.7 mmol) in CH_2Cl_2 (40 mL) while the temperature was kept below 15 °C. Stirring was continued for 16 h and the solution was sequentially washed with aqueous 2 N HCl, aqueous $NaHCO_3$, and brine. The aqueous layers were back-extracted with CH_2Cl_2 (40 mL). The combined CH_2Cl_2 solutions were dried and evaporated to dryness. Chromatography of the crude product using a gradient of 0–2% v/v EtOH in CH_2Cl_2 afforded a gum: 4.73 g (91%); NMR ($CDCl_3$) δ 1.2, 1.3 (2 t, 6 H, 2 OCH_2CH_3), 1.5 (s, 9 H, *t*-Bu), 2.15 (m, 2 H, $CHCH_2CH_2CO_2Et$), 2.3 (t, 1 H, C≡CH), 2.4 (t, 2 H, $CHCH_2CH_2CO_2Et$), 4.1, 4.25 (2 q, 4 H, 2 OCH_2CH_3), 4.45 (d, 2 H, $>NCH_2C{\equiv}C$), 4.8 (m, 1 H, CH), 7.8 (dd, 1 H, pyridine 4-H), 8.15 (d, 1 H, pyridine 3-H), 8.4 (d, 1 H, CONH), 8.6 (d, 1 H, pyridine 6-H).

Diethyl *N*-[5-[*N*-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]picolinyl]-L-glutamate (55a). Method V. *tert*-Butoxycarbonyl derivative **53a** (4.73 g, 10.26 mmol) was dissolved in ice-cold CF_3CO_2H (50 mL). After 30 min HPLC indicated complete removal of the *tert*-butoxycarbonyl-protecting group. The solution was evaporated to dryness and the residue was further dried by azeotropic rotary evaporation in the presence of toluene. The crude gummy **54a** CF_3CO_2H salt (3.33 g) was reacted with the bromomethyl compound **18** according to method M to afford **55a** as a gum: 840 mg (17%); NMR (Me_2SO-d_6) δ 1.15, 1.2 (2 t, 6 H, 2 OCH_2CH_3), 2.1 (m, 2 H, $CHCH_2CH_2CO_2Et$), 2.35 (t, 2 H, $CHCH_2CH_2CO_2Et$), 2.35 (s, 3 H, CH_3), 3.25 (t, 1 H, C≡CH), 4.0, 4.1 (2 q, 4 H, 2 OCH_2CH_3), 4.45 (d, 2 H, $CH_2C{\equiv}C$), 4.5 (m, 1 H, CH), 4.85 (br s, 2 H, $ArCH_2N<$), 7.3 (dd, 1 H, pyridine, 4-H), 7.55 (d, 1 H, quinazoline 8-H), 7.7 (dd, 1 H, quinazoline 7-H), 7.85 (d, 1 H, pyridine 3-H), 8.0 (d, 1 H, quinazoline 5-H), 8.2 (d, 1 H, pyridine 6-H), 8.5 (d, 1 H, CONH).

Diethyl *N*-(6-Chloronicotinoyl)-L-glutamate (56). 6-Chloronicotinic acid (8.27 g, 52.5 mmol) was converted according to method K to **56**, an oil: 16.97 g (94%); NMR ($CDCl_3$) δ 1.25, 1.35 (2 t, 6 H, 2 OCH_2CH_3), 2.2 (m, 2 H, $CHCH_2CH_2CO_2Et$), 2.35 (t, 2 H, $CHCH_2CH_2CO_2Et$), 4.15, 4.25 (2 q, 4 H, 2 OCH_2CH_3), 4.75 (m, 1 H, CH), 7.4 (d, 1 H, pyridine 5-H), 7.55 (d, 1 H, CONH), 8.1 (dd, 1 H, pyridine 4-H), 8.85 (d, 1 H, pyridine 2-H). Anal. ($C_{15}H_{19}ClN_2O_5$) C, H, N.

Diethyl *N*-[6-(*N*-Benzyl-*N*-methylamino)nicotinoyl]-L-glutamate (57). *N*-Benzylmethylamine (0.50 mL, 3.85 mmol) was stirred in a solution of **56** (526 mg, 1.54 mmol) in NMP (2 mL) at 100 °C for 16 h under argon. The solvent was removed by rotary evaporation at 40 °C (0.1 mmHg). The residue was partitioned between EtOAc (2 × 25 mL) and aqueous $NaHCO_3$ (10 mL). The EtOAc solution was dried and evaporated to a yellow oil: 572 mg (87%); NMR (Me_2SO-d_6) δ 1.15, 1.2 (2 t, 6 H, 2 OCH_2CH_3), 2.05 (m, 2 H, $CHCH_2CH_2CO_2Et$), 2.4 (t, 2 H, $CHCH_2CH_2CO_2Et$), 3.1 (s, 3 H, $>NCH_3$), 4.05, 4.1 (2 q, 4 H, 2 OCH_2CH_3), 4.4 (m, 1 H, CH), 4.85 (s, 2 H, $PhCH_2N<$), 6.7 (d, 1 H, pyridine 5-H), 7.25 (m, 5 H, Ph), 7.95 (dd, 1 H, pyridine 4-H), 8.4 (d, 1 H, CONH), 8.6 (d, 1 H, pyridine 2-H).

Diethyl *N*-[6-(Methylamino)nicotinoyl]-L-glutamate (58c). Method W. A solution of **57** (300 mg, 0.70 mmol) in EtOH (2 mL) and CF_3CO_2H (1 mL) was stirred with 10% Pd–C (50 mg) at 60 °C for 2 h in an atmosphere of H_2. The cooled reaction mixture was filtered through Celite and the filter pad was washed well with EtOH. The combined EtOH filtrates were evaporated to dryness, and the residue was partitioned between EtOAc and aqueous $NaHCO_3$. Evaporation of the dried EtOAc solution afforded a gum: 220 mg (93%); NMR (Me_2SO-d_6) δ 1.15, 1.2 (2 t, 6 H, 2 OCH_2CH_3), 2.05 (m, 2 H, $CHCH_2CH_2CO_2Et$), 2.4 (t, 2

(35) Isagawa, K.; Kawai, M.; Fushizaki, Y. *Nippon Kaguku Zasshi* **1967**, *88*, 553; *Chem. Abstr.* 1968, *68*, 68840*h*.

H, $CHCH_2CH_2CO_2Et$), 2.8 (d, 3 H, $NHCH_3$), 4.05, 4.1 (2 q, 4 H, 2 OCH_2CH_3), 4.4 (m, 1 H, CH), 6.45 (d, 1 H, pyridine 5-H), 7.0 (q, 1 H, NH), 7.85 (dd, 1 H, pyridine 4-H), 8.3 (d, 1 H, CONH), 8.55 (d, 1 H, pyridine 2-H).

N-[6-[N-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-N-methylamino]nicotinoyl]-L-glutamic Acid Monosodium Salt (12c). The amino compound **58c** was converted according to method M into diethyl ester **59c** (94 mg, 0.185 mmol) which was hydrolyzed according to method N. Acidification of the aqueous solution to pH 4.0 produced no precipitate. The resulting solution was therefore applied to a column of Sepabeads (10 mL) which was eluted with H_2O until the eluent was free of chloride ions, then with a gradient of 10–30% v/v MeCN in H_2O. Fractions containing the product by HPLC were pooled, the MeCN was removed by rotary evaporation and the aqueous solution was lyophilized to a fluffy white solid: 30 mg (31%); mp 205–210 °C; NMR (Me_2SO-d_6) δ 2.15 (m, 2 H, $CHCH_2CH_2CO_2H$), 2.4 (t, 2 H, $CHCH_2CH_2CO_2H$), 2.45 (s, 3 H, CH_3), 3.1 (s, 3 H, NCH_3), 4.3 (m, 1 H, CH), 5.0 (s, 2 H, $ArCH_2N<$), 6.7 (d, 1 H, pyridine 5-H), 7.5 (d, 1 H, quinazoline 8-H), 7.6 (dd, 1 H, quinazoline 7-H), 7.9 (d, 1 H, quinazoline 5-H), 7.95 (dd, 1 H, pyridine 4-H), 7.95 (d, 1 H, CONH), 8.6 (d, 1 H, pyridine 2-H); MS (FAB) m/z 452 $[M - H]^-$. Anal. ($C_{22}H_{22}N_5O_6 \cdot 2.5H_2O$) C, N; H: calcd, 5.2; found, 4.7.

5-[N-[[3,4-Dihydro-2-methyl-4-oxo-3-[(pivaloyloxy)methyl]-6-quinazolinyl]methyl]amino]-2-(methylthio)pyrimidine (61). A mixture of bromomethyl compound **36** (22.9 g, 62.4 mmol), 5-amino-2-(methylthio)pyrimidine (**60**)[21] (6.77 g, 48.0 mmol), and 2,6-lutidine (8.14 mL, 70 mmol) in DMF (70 mL) was stirred for 2 h at 90 °C under argon. The cooled mixture was evaporated to dryness and the residue was partitioned between EtOAc (2 × 300 mL) and H_2O (300 mL). The combined EtOAc solutions were washed with H_2O, dried, and evaporated. The crude product was purified by chromatography using a gradient of 0–70% v/v EtOAc in CH_2Cl_2 as eluent to give a buff foam: 11.91 g (58%); NMR (Me_2SO-d_6) δ 1.15 (s, 9 H, t-Bu), 2.4 (s, 3 H, CH_3), 2.6 (s, 3 H, SCH_3), 4.45 (br d, 2 H, CH_2NH), 6.0 (s, 2 H, OCH_2N), 6.65 (br t, 1 H, CH_2NH), 7.6 (d, 1 H, quinazoline 8-H), 7.8 (dd, 1 H, quinazoline 7-H), 8.05 (s, 2 H, pyrimidine 4-H and 6-H), 8.1 (d, 1 H, quinazoline 5-H).

5-[N-[[3,4-Dihydro-2-methyl-4-oxo-3-[(pivaloyloxy)methyl]-6-quinazolinyl]methyl]-N-methylamino]-2-(methylthio)pyrimidine (62). HCHO (37% w/v aqueous solution, 2.70 mL, 32.8 mmol) and $NaCNBH_3$ (647 mg, 10.3 mmol) were added to a stirred solution of **61** (2.00 g, 4.7 mmol) in MeCN (33 mL) maintained at 20 °C (H_2O bath). Glacial HOAc (2.80 mL, 46.8 mmol) was added dropwise during 5 min. Stirring was continued for 75 min and the MeCN was evaporated below 30 °C. The residue was partitioned between CH_2Cl_2 (3 × 30 mL) and H_2O (30 mL). The combined CH_2Cl_2 solutions were evaporated to dryness, and the crude product was purified by chromatography using a gradient of 0–5% v/v EtOAc in CH_2Cl_2 as eluent to yield a yellow foam: 1.31 g (64%); NMR (Me_2SO-d_6) δ 1.15 (s, 9 H, t-Bu), 2.45 (s, 3 H, CH_3), 2.6 (s, 3 H, SCH_3), 3.1 (s, 3 H, NCH_3), 4.7 (s, 2 H, $ArCH_2N<$), 6.05 (s, 2 H, OCH_2N), 7.6 (d, 1 H, quinazoline 8-H), 7.7 (dd, 1 H, quinazoline 7-H), 7.95 (d, 1 H, quinazoline 5-H), 8.25 (s, 2 H, pyrimidine 4-H and 6-H); MS (FAB) m/z 442 $[MH]^+$.

2-Cyano-5-[N-[(3,4-dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-N-methylamino]pyrimidine (64). MCPBA (2.62 g of 50% w/w, 7.54 mmol) was added in portions over 90 min to a stirred solution of **62** (1.31 g, 2.97 mmol) in CH_2Cl_2 (50 mL). After a further 30 min the solution was washed with aqueous $NaHCO_3$, dried, and evaporated to dryness to give crude sulfone **63** (1.4 g) as a golden gum. This was dissolved in DMF (20 mL) and stirred with NaCN (1.47 g, 30 mmol) for 3 h at 100 °C under argon. The mixture was cooled and the DMF was removed by rotary evaporation. The residue was partitioned between EtOAc (2 × 50 mL) and H_2O (50 mL). The combined EtOAc solutions were dried and evaporated to a brown amorphous solid: 310 mg (34% from **62**); NMR (Me_2SO-d_6) δ 2.35 (s, 3 H, CH_3), 3.2 (s, 3 H, NCH_3), 4.9 (s, 2 H, $ArCH_2N<$), 7.55 (d, 1 H, quinazoline 8-H), 7.65 (dd, 1 H, quinazoline 7-H), 7.9 (d, 1 H, quinazoline 5-H), 8.4 (s, 2 H, pyrimidine 4-H and 6-H).

5-[N-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-N-methylamino]pyrimidine-2-carboxylic Acid 0.8-Hydrate (65). Nitrile **64** (310 mg, 1.01 mmol) was stirred for 15 min at 100 °C in a mixture of 1 N aqueous NaOH (3.0 mL, 3.0 mmol) and n-PrOH (3.0 mL). The reaction mixture was evaporated to dryness. The residue was dissolved in H_2O (2 mL), the solution was filtered and acidified to pH 3.0 with 2 N aqueous HCl. The precipitated pale buff solid was filtered off, washed with H_2O, and vacuum dried: 190 mg (56%); mp 287–288.5 °C; NMR (Me_2SO-d_6) δ 2.35 (s, 3 H, CH_3), 3.2 (s, 3 H, NCH_3), 4.9 (s, 2 H, $ArCH_2N<$), 7.55 (d, 1 H, quinazoline 8-H), 7.65 (dd, 1 H, quinazoline 7-H), 7.9 (d, 1 H, quinazoline 5-H), 8.4 (s, 2 H, pyrimidine 4-H and 6-H), 12.2 (br s, 1 H, quinazoline 3-H). Anal. ($C_{16}H_{15}N_5O_3 \cdot 0.8H_2O$) C, H; N: calcd, 20.6; found, 19.9.

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Registry No. **7a**, 112887-99-7; **7b**, 132462-57-8; **7c**, 112887-68-0; **7d**, 112887-69-1; **7e**, 112888-00-3; **7f**, 132462-58-9; **7g**, 132462-59-0; **7h**, 132462-60-3; **7i**, 132462-61-4; **7j** (free base), 132462-62-5; **7j**·TFA, 132462-63-6; **8a**, 132462-64-7; **8b**, 132462-65-8; **8c**, 132462-66-9; **8d**, 132462-67-0; **98f**, 132462-68-1; **8g**, 132462-69-2; **8h**, 132462-70-5; **8i**, 132462-71-6; **9d**, 112888-37-6; **10d**, 132462-72-7; **11a**, 112887-70-4; **11c**, 112888-01-4; **11d**, 132462-73-8; **11f**, 132462-74-9; **11g**, 132462-75-0; **12c** (free acid), 112888-02-5; **12c**·Na, 132462-76-1; **13c**, 132462-77-2; **14c**, 132462-78-3; **14d**, 132462-79-4; **14g**, 132491-06-6; **15d**, 132462-80-7; **16c**, 112888-35-4; **16d**, 112888-36-5; **17c**, 132462-81-8; **18**, 112888-43-4; **19**, 39978-57-9; **20**, 106585-64-2; **21**, 106585-63-1; **22a**, 106585-72-2; **22c**, 112889-02-8; **22d**, 112914-38-2; **22e**, 132462-82-9; **22f**, 132462-83-0; **22g**, 132462-84-1; **22h**, 132462-85-2; **22i**, 132462-86-3; **23a**, 132463-00-4; **23b**, 132463-01-5; **23c**, 132463-02-6; **23d**, 132463-03-7; **23e**, 132463-04-8; **23f**, 132463-05-9; **23g**, 132463-06-0; **23h**, 132463-07-1; **23i**, 132463-08-2; **23j**, 132463-22-0; **24**, 527-72-0; **25**, 56267-50-6; **26c**, 132463-32-2; **27c**, 131052-68-1; **28c**, 132463-33-3; **28f**, 132463-34-4; **28g**, 132463-35-5; **29**, 132463-36-6; **30**, 132462-99-8; **31**, 119063-91-1; **32**, 119063-92-2; **33a**, 132462-87-4; **33c**, 119063-89-7; **33d**, 132462-88-5; **33f**, 132462-89-6; **33g**, 132462-90-9; **33h**, 132462-91-0; **33i**, 132462-92-1; **34**, 119063-93-3; **35a**, 132463-09-3; **35b**, 132463-10-6; **35c**, 132463-11-7; **35d**, 132463-12-8; **35f**, 132463-23-1; **35g**, 132463-13-9; **35h**, 132463-24-2; **35i**, 132463-25-3; **36**, 112888-39-8; **37**, 132463-37-7; **38**, 132463-38-8; **39**, 132463-39-9; **40**, 112889-06-2; **41**, 112889-03-9; **42d**, 132463-14-0; **43**, 132463-40-2; **44**, 132463-41-3; **45**, 132463-42-4; **46**, 132463-43-5; **47**, 132463-44-6; **48d**, 132463-15-1; **49**, 17874-76-9; **50**, 131052-40-9; **51a**, 112888-59-2; **52a**, 132463-45-7; **53a**, 132463-46-8; **53c**, 112888-58-1; **53d**, 132463-47-9; **53f**, 132463-48-0; **53g**, 132463-49-1; **54a**, 132462-93-2; **54c**, 132462-94-3; **54d**, 132462-95-4; **54f**, 132462-96-5; **54g**, 132462-97-6; **55a**, 132463-16-2; **55c**, 132491-07-7; **55d**, 132463-17-3; **55f**, 132463-18-4; **55g**, 132463-19-5; **56**, 132463-50-4; **57**, 112888-61-6; **58c**, 132462-98-7; **59c**, 132463-20-8; **60**, 42382-46-7; **61**, 132463-51-5; **62**, 132463-52-6; **63**, 132463-53-7; **64**, 132463-54-8; **65**, 132463-55-9; **66c**, 132463-21-9; **67**, 112888-46-7; **68**, 112889-01-7; **69c**, 132463-26-4; **69d**, 132463-27-5; **69g**, 132491-08-8; **70d**, 132463-28-6; **71c**, 132463-29-7; **71d**, 132463-30-0; **-72c**, 132463-31-1; TS, 9031-61-2; H-Glu(OEt)-OEt·HCl, 1118-89-4; $HC{\equiv}CCH_2Br$, 106-96-7; $BrCH_2CN$, 590-17-0; PhCONCS, 532-55-8; OHCCHClCOOEt, 33142-21-1; EtOCOCOCl, 4755-77-5; $MeNHCH_2Ph$, 103-67-3; 5-nitrothiazole-2-carboxylic acid, 39920-61-1; 1,1′-thiocarbonyldiimidazole, 6160-65-2; 6-chloronicotinic acid, 5326-23-8.