to produce less severe steric clashes and so to be the more effective inhibitor.

To our knowledge there has so far been no detailed model presented for the binding of reversible lyase inhibitors to the enzyme. The model developed here permits the rationalization of the lyase inhibition data without the postulation of features of the binding site not evident from the substrate structure, i.e. a second hydrophobic pocket. That the binding of the ester moiety does not involve a pocket related to that postulated for aromatase inhibition is supported in part by the observation that, for chiral esters (menthyl, borneyl and isopinocampheyl), the enantiomer that was more inhibitory against aromatase was the less inhibitory against lyase.[14] This suggests that the hydrophobic pockets are quite different in the two enzymes. The positioning of the pyridyl group was based on the relationship between oxidation site, postulated heme position and pyridyl orientation developed for aromatase inhibition. The model requires torsion angle β to take a value of about 60°. Such an angle is readily accessible to **1** and the *R* enantiomer of **2**, and only slightly less so for (*S*)-**2**. The root of the increased lyase inhibitory potential of **2** is therefore hypothesized as stemming largely from the ability of the methyl group to substitute either for C(16) or for C(20) of a steroid substrate. The observation that the dimethylated derivative is a poorer lyase inhibitor than racemic **2** suggests that in fact the mimicry of only one of these two sites is favorable. However, at this stage the model does not provide any predictions as to which of these it will be and so as to which of the two enantiomers of **2** will be the best lyase inhibitor.

The results presented here provide further support for the model of aromatase inhibition that has previously been developed[5] and which has already been used predictively with success.[12] The model presented here for lyase inhibition can only be regarded as preliminary since its validation will require the examination of a broader range of structurally diverse lyase inhibitors. However, it is clear that it provides enough detail to suggest avenues of structural modification which should lead to both more potent and more selective inhibitors of lyase based on the pyridylacetate framework.

Acknowledgment. This work was supported by the Cancer Research Campaign. We are grateful to Dr. R. McCague for the crystals of 1 and to him and Dr. M. Jarman and M. Rowlands for discussions.

Registry No. 1, 129175-15-1; (*S*)-2, 129175-16-2; (*R*)-2, 129175-18-4; 3, 129175-17-3; steroid 17-20-lyase, 9044-50-2; aromatase, 9039-48-9.

Supplementary Material Available: Tables of crystallographic data for compound 3 including temperature factors, H-atom coordinates, and bond lengths and angles (4 pages). Ordering information is given on any current masthead page.

Quinazoline Antifolate Thymidylate Synthase Inhibitors: Alkyl, Substituted Alkyl, and Aryl Substituents in the C2 Position

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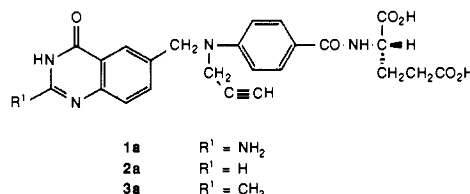
Modification of the potent thymidylate synthase (TS) inhibitor *N*-[4-[*N*-[(2-amino-3,4-dihydro-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoyl]-L-glutamic acid (**1a**) has led to the synthesis of quinazoline antifolates bearing alkyl, substituted alkyl, and aryl substituents at C2. In general the synthetic route involved the coupling of the appropriate diethyl *N*-[4-(alkylamino)benzoyl]-L-glutamate with a C2-substituted 6-(bromomethyl)-3,4-dihydro-4-oxoquinazoline followed by deprotection using mild alkali. Good enzyme inhibition and cytotoxicity were found with compounds containing small nonpolar groups in the C2 position with the 2-desamino-2-methyl analogue **3a** being the most potent. Larger C2 substituents were tolerated by the enzyme, but cytotoxicity was reduced. Highly potent series were followed up by the synthesis of a number of analogues in which the N10 substituent was varied. In this manner a number of interesting TS inhibitors have been prepared. Although none of these was more potent than **1a** against the isolated enzyme, over half of the compounds prepared were more potent as cytotoxic agents against L1210 cells in culture. The potential of such compounds as useful antitumor agents was further enhanced by the finding that the improved aqueous solubilities of compounds such as **3a** over **1a** were reflected in vivo in that **3a** was at least 5 times less toxic to mice than **1a**.

The potent antifolate thymidylate synthase (TS) inhibitor *N*-[4-[*N*-[(2-amino-3,4-dihydro-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoyl]-L-glutamic acid (**1a**)[1,2] has shown encouraging antitumor activity against breast and ovarian cancers[3,4] and hepatomas[5] in recent clinical trials. However unacceptable liver and kidney toxicities have prevented its widespread use.[6] The hypothesis has been that these toxicities are the result of the physicochemical properties of **1a**, in particular its



poor water solubility, rather than being intrinsic to this

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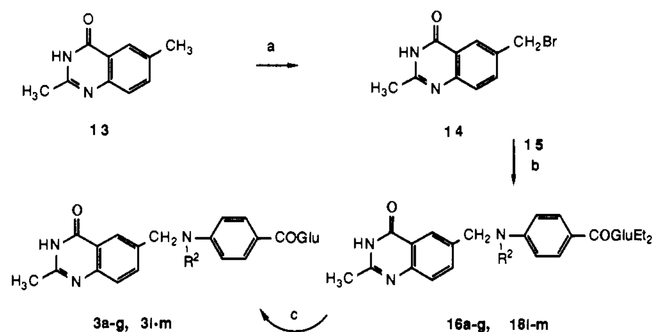
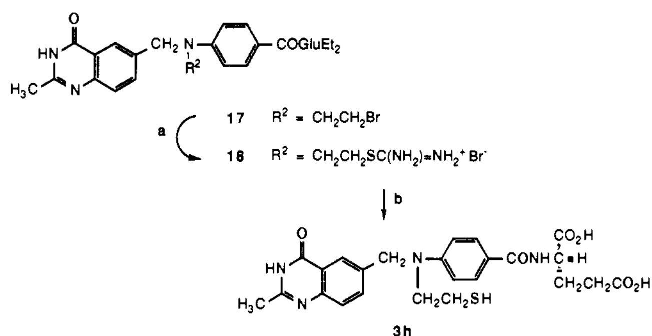
Scheme I[a]



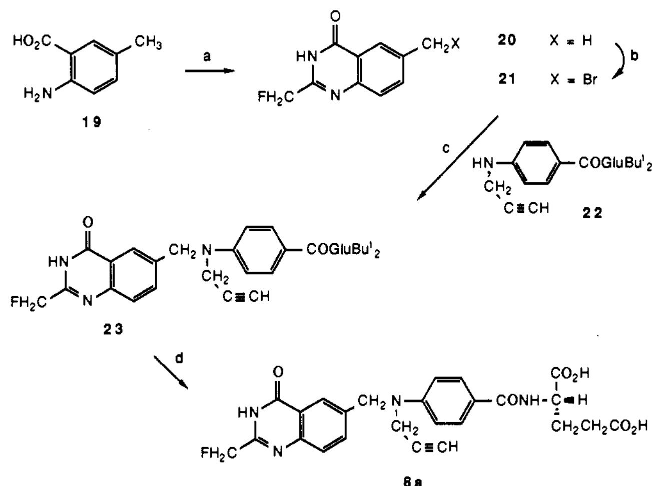
[a] (a) NBS, $(PhCOO)_2$, $CHCl_3$ (method A); (b) 2,6-lutidine, DMF, 80 °C (method B); (c) 1 N aqueous NaOH, EtOH (method C).

Scheme II[a]



[a] (a) Thiourea, DMA, 100 °C; (b) 1 N aqueous NaOH, EtOH.

Scheme III[a]



[a] (a) $FH_2CC(OEt)$=NH·HCl, NaOEt, EtOH; (b) NBS, $(PhCOO)_2$, CCl_4; (c) 2,6-lutidine, DMF, 60 °C; (d) CF_3CO_2H, $CHCl_3$.

type of TS inhibitor. Hence, in the effort to find a replacement clinical candidate, compounds have been sought that, while retaining good TS inhibition, display improved aqueous solubilities. In the search for such agents work was concentrated initially on modifications to the quinazoline portion of the molecule and in particular on replacement of the C2-amino group of 1a. The discovery that the more soluble C2-desamino analogue 2a was less toxic to the liver and kidneys of rodents, while retaining good TS inhibition,[7,8] has prompted a detailed study of C2 modifications. In this paper work is described relating to the synthesis and in vitro activity of a series of quinazoline antifolates bearing alkyl, substituted alkyl, and aryl substituents in the C2 position.[9] The related study of groups on C2 attached via a heteroatom has been reported earlier.[10]

Chemistry

The majority of the C2-methyl compounds described in Table I were prepared by the route shown in Scheme I. Thus the (bromomethyl)quinazolinone **14** was condensed with the (aminobenzoyl)glutamate diesters **15a–g,i–m** with use of 2,6-lutidine to scavenge HBr (method B) to give the antifolate diesters **16**, which were subsequently hydrolyzed (method C) with 1 N aqueous NaOH to the diacids **3a–g,i–m**. The amines used either were known compounds or were prepared by alkylation of the primary amine **15b** using the appropriate alkyl halide. The N10-mercaptoethyl derivative **3h** was prepared by converting the bromoethyl diester **17** to the corresponding isothiouronium salt **18** (Scheme II), which on saponification simultaneously liberated the thiol functionality and hydrolyzed the diester to the required diacid.

The compounds **4a,b**, **5a**, **7a**, and **12a** were also synthesized by methods B and C (see Scheme I) using, in place of **14**, the appropriate C2-substituted 6-(bromomethyl)quinazolinones **31–33** and **37**, which were in turn derived from the quinazolinones **24–26** and **30** by bromination with *N*-bromosuccinimide (NBS) in CCl_4 (method G). The 2-(fluoromethyl)quinazolinone **20** required for the synthesis of **6a,c,d,f** was prepared by the condensation of ethyl fluoroacetimidate with 2-amino-5-methylbenzoic acid (Scheme III). After bromination with NBS to **21**, condensation with the amine **22** gave the di-*tert*-butyl ester **23**. The choice of these acid labile esters was made in this case to prevent any possibility of hydrolysis of the fluoromethyl group under the usual basic conditions used to hydrolyze diethyl esters. Thus treatment of the coupled di-*tert*-butyl esters (e.g. **23**) with CF_3CO_2H in $CHCl_3$ gave the CF_3CO_2H salt of the required antifolate diacids **6a,c,d,f**. The required di-*tert*-butyl esters were prepared from the known[11] di-*tert*-butyl(4-aminobenzoyl)-L-glutamate by analogous routes to those used for the preparation of the corresponding diethyl esters.[12-14]

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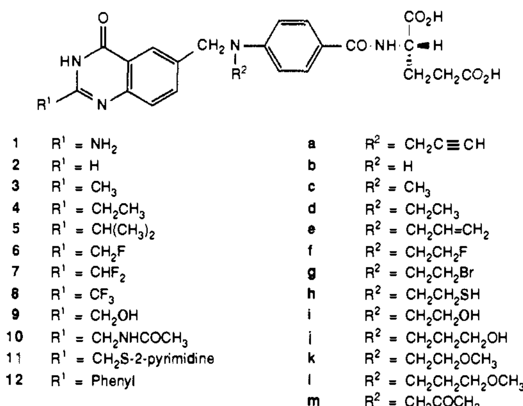
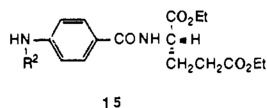
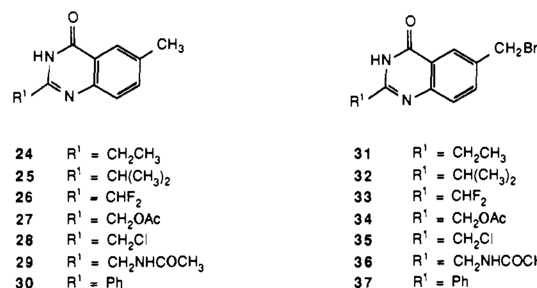


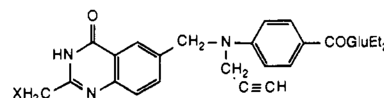
See Table I for compounds prepared.



15

a [1]	$R^2 = CH_2C{\equiv}CH$	g	$R^2 = CH_2CH_2Br$
b [26]	$R^2 = H$	i [10]	$R^2 = CH_2CH_2OAc$
c [12]	$R^2 = CH_3$	j [14]	$R^2 = CH_2CH_2CH_2OAc$
d [13]	$R^2 = CH_2CH_3$	k	$R^2 = CH_2CH_2OCH_3$
e [1]	$R^2 = CH_2CH{=}CH_2$	l	$R^2 = CH_2CH_2CH_2OCH_3$
f [14]	$R^2 = CH_2CH_2F$	m	$R^2 = CH_2COCH_3$





38 $X = OCOCH_3$
43 $X = Cl$
44 X = S-2-pyrimidinyl

The hydroxyl group in compound **9a** (and in the corresponding N10 variants shown in Table I) was carried through the synthesis protected as the acetate and was released in the final base-catalyzed hydrolysis of the triester **38** to the diacid. The starting bromomethyl compound **34** was prepared (NBS, method G) from the corresponding known 6-methyl derivative.[15] In the preparation of the C2-trifluoromethyl antifolate **8a**, it was necessary, because of the poor solubility of the quinazolinone **39**, to protect the N3 position as the (pivaloyloxy)methyl derivative **40** prior to bromination of the 6-methyl group to give **41** (Scheme IV). Again the protecting group was removed in the final saponification step.

The known[15] 2-(chloromethyl)quinazolinone **28** was used as the starting material for the synthesis of both the 2-acetamidomethyl (**10a**) and 2-[(2-pyrimidinylthio)methyl] (**11a**) antifolates. Thus treatment of **28** with ammonia followed by acetylation with acetic anhydride gave **29**. Subsequent bromination, coupling, and hydrolysis (in an analogous sequence to that shown in Scheme I) led to **10a**. Alternatively, direct bromination of **28** to **35** followed by coupling with the amine **15a** led specifically to the required 2-chloromethyl intermediate **43**. Replacement of the chlorine atom in **43** by the 2-pyrimidinylthio moiety was effected with the sodium salt of 2-mercaptopyrimidine. Subsequent hydrolysis gave the diacid **11a**.

Biological Evaluation

The antifolate diacids listed in Table I were tested as inhibitors of TS partially purified from L1210 mouse leukaemia cells that overproduce TS due to amplification of the TS gene.[16] The partial purification and assay method used in this study was as previously described and used a (±)-5,10-methylenetetrahydrofolic acid concentration of 200 μM.[16,17] The results were expressed as IC_{50} values (concentration required to inhibit control enzyme activity by 50%). The TS inhibitor **1a** was included in each assay as a positive control ($IC_{50} \simeq 20$ nM). The compounds were also tested for their inhibition of the growth of L1210 cells in culture,[14] and the results again were expressed as the concentration required to inhibit cell growth by 50% (IC_{50} values). The analogues **1a**–**3a** were further tested as inhibitors of rat liver dihydrofolate reductase (DHFR) by the method described earlier.[14]

Results and Discussion

The IC_{50} values for the inhibition of partially purified L1210 TS and for growth inhibition of L1210 cells are shown in Table I. Replacement of the C2-amino group of **1a** by methyl (to give **3a**) led to approximately a 2-fold loss in enzyme-inhibitory activity but a 40-fold improvement in cytotoxicity to L1210 cells. The prevention of the cytotoxicity of **3a** by thymidine alone has shown that inhibition of TS is its locus of action.[18] Interestingly **3a** is a very poor inhibitor of DHFR (see Table II). Thus replacement of the C2-amino by a methyl group leads to a 2-fold drop in TS inhibition but a >67-fold drop in activity against DHFR.

The exciting activity of **3a** led to the synthesis of a series of C2-methyl compounds modified at N10 (Table I). In general the structure–activity relationships for TS inhibition mirrored that seen with the C2-amino series,[14] with the propargyl compound **3a** being the most potent. However, the cytotoxicity of a number of other analogues (e.g. **3b,c,e,g**) was comparable to that of **3a** despite lower potency against the enzyme.

The C2-methyl analogues **3h,k,l,m** contain N10 substituents previously undescribed in quinazoline antifolates. Conversion of the primary hydroxyl groups of **3i** and **3j** to the corresponding methyl ethers **3k** and **3l** decreases enzyme inhibition by some 20–30-fold and L1210 growth inhibitory potency by at least 100-fold. Since **3k** and **3j** have N10 substituents of similar size, it would appear that steric bulk in this region of the enzyme is only tolerated if the substituent is capable of donating a hydrogen bond. The increased steric bulk of the N10-mercaptoethyl substituent in **3h** causes a 3.5-fold decrease in enzyme affinity compared to the N10-hydroxyethyl substituent in **3i**. Moreover the virtual lack of L1210 growth inhibition of

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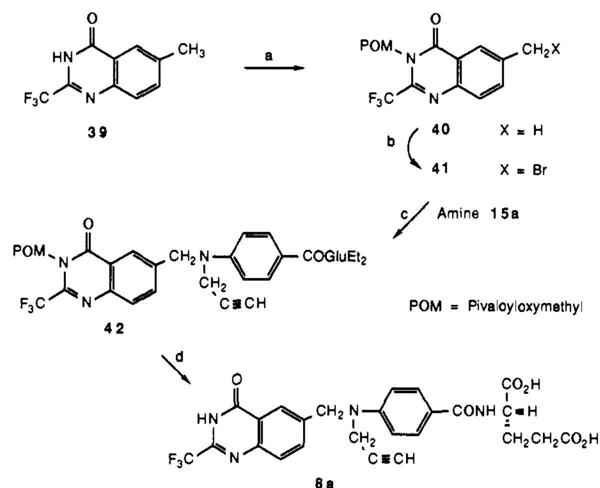
Table I. Preparation and in Vitro Activities of Antifolate Diacids

compd	R^1	R^2	method	yield, %	mp, °C	formula[a]	inhibn of TS: IC_{50}, μM	inhibn of L1210 cell growth in culture: IC_{50}, μM
1a	NH_2	$CH_2C{\equiv}CH$	*b*		232–235	$C_{24}H_{23}N_5O_6$	0.02	3.40
2a	H	$CH_2C{\equiv}CH$	*c*		170–173	$C_{24}H_{22}N_4O_6{\cdot}H_2O$	0.16	0.40
3a	CH_3	$CH_2C{\equiv}CH$	A, B, C	53	165[d]	$C_{25}H_{24}N_4O_6{\cdot}2H_2O$	0.04	0.09
3b	CH_3	H	A, B, C	85	197–201	$C_{22}H_{22}N_4O_6{\cdot}H_2O$	4.50	0.07
3c	CH_3	CH_3	A, B, C	81	254–257	$C_{23}H_{24}N_4O_6{\cdot}0.75H_2O$	0.30	0.11
3d	CH_3	CH_2CH_3	A, B, C	78	221–225	$C_{24}H_{26}N_4O_6{\cdot}0.5H_2O$	0.17	0.36
3e	CH_3	$CH_2CH{=}CH_2$	A, B, C	41	188[e]	$C_{25}H_{26}N_4O_6{\cdot}1.5H_2O$	0.48	0.17
3f	CH_3	$(CH_2)_2F$	A, B, C	90	207–210	$C_{24}H_{25}FN_4O_6{\cdot}1.25H_2O$	0.24	0.40
3g	CH_3	$(CH_2)_2Br$	A, B, C	66	213–215	$C_{24}H_{25}BrN_4O_6$[f]	1.30	0.82
3h	CH_3	$(CH_2)_2SH$	A, B, F	30	160[d]	$C_{24}H_{26}N_4O_6S{\cdot}2H_2O$	1.76	>100
3i	CH_3	$(CH_2)_2OH$	A, B,[g] C	56	>300	$C_{24}H_{26}N_4O_7{\cdot}1.5H_2O$	0.50	0.24
3j	CH_3	$(CH_2)_3OH$	A, B, C	39	300[e]	$C_{25}H_{28}N_4O_7{\cdot}H_2O$	0.54	1.24
3k	CH_3	$(CH_2)_2OCH_3$	A, B,[g] C	68	248[e]	$C_{25}H_{28}N_4O_7{\cdot}H_2O$	16.26	40.0
3l	CH_3	$(CH_2)_3OCH_3$	A, B,[g] C	64	260[e]	$C_{26}H_{30}N_4O_7{\cdot}0.5H_2O$	12.92	>100
3m	CH_3	CH_2COCH_3	A, B,[g] C	63	155–157	$C_{25}H_{26}N_4O_7{\cdot}H_2O$	25.0	1.20
4a	CH_2CH_3	$CH_2C{\equiv}CH$	G, B, C	33	150–157	$C_{26}H_{26}N_4O_6{\cdot}0.5H_2O$	0.14	2.50
4b	CH_2CH_3	H	G, B, C	38	156–166	$C_{23}H_{24}N_4O_6{\cdot}2H_2O$[h]	20.0	3.20
5a	$CH(CH_3)_2$	$CH_2C{\equiv}CH$	G, B, C	28	148–150	$C_{27}H_{28}N_4O_6{\cdot}2.5H_2O$[i]	0.62	48.0
6a	CH_2F	$CH_2C{\equiv}CH$	G, H	56	126–131	$C_{25}H_{23}FN_4O_6{\cdot}CF_3CO_2H{\cdot}0.5H_2O$	0.10	0.37
6c	CH_2F	CH_3	G, H	31	160–165	$C_{23}H_{23}FN_4O_6{\cdot}0.75CF_3CO_2H$	2.92	0.003
6d	CH_2F	CH_2CH_2	G, H	21	162–167	$C_{24}H_{25}FN_4O_6{\cdot}0.5CF_3CO_2H{\cdot}0.5H_2O$	0.37	0.70
6f	CH_2F	$(CH_2)_2F$	G, H	24	190–196	$C_{24}H_{24}F_2N_4O_6{\cdot}CF_3CO_2H{\cdot}H_2O$	0.34	1.20
7a	CHF_2	$CH_2C{\equiv}CH$	G, B, C	63	135–140	$C_{25}H_{22}F_2N_4O_6{\cdot}H_2O$	0.58	24
8a	CF_3	$CH_2C{\equiv}CH$	J	40	110–115	$C_{25}H_{21}F_3N_4O_6{\cdot}3H_2O$[j]	5.70	>100
9a	CH_2OH	$CH_2C{\equiv}CH$	G, I	55	137–143	$C_{25}H_{24}N_4O_7{\cdot}2H_2O$	0.10	5.00
9c	CH_2OH	CH_3	G, I	23	194–197	$C_{23}H_{24}N_4O_7{\cdot}1.5H_2O$[k]	0.64	13.0
9d	CH_2OH	CH_2CH_3	G, I	61	140–150	$C_{24}H_{26}N_4O_7{\cdot}H_2O$	0.26	16.0
9e	CH_2OH	$CH_2CH{=}CH_2$	G, I	15	150–160	$C_{25}H_{26}N_4O_7{\cdot}H_2O$	0.92	6.60
9f	CH_2OH	$(CH_2)_2F$	G, I	33	215–222	$C_{24}H_{25}FN_4O_7{\cdot}0.5H_2O$	0.22	20.0
9i	CH_2OH	$(CH_2)_2OH$	G, I	28	150–155	$C_{24}H_{26}N_4O_8{\cdot}1.5H_2O$[l]	0.78	14.5
10a	$CH_2NHCOCH_3$	$CH_2C{\equiv}CH$	G, B, C	51	229–240	$C_{27}H_{29}N_5O_7{\cdot}H_2O$	0.48	125
11a	CH_2S-2-pyrimidine	$CH_2C{\equiv}CH$	G, B, K	87	151–153	$C_{29}H_{26}N_6O_6S{\cdot}H_2O$	0.24	100
12a	Phenyl	$CH_2C{\equiv}CH$	G, B, C	61	170–171	$C_{30}H_{26}N_4O_6{\cdot}(CH_3)_2CO{\cdot}0.5H_2O$[m]	0.22	>100

[a] Anal. C, H, N except where stated otherwise. [b] Reference 1. [c] Reference 7. [d] Sinters above this temperature but does not have a discrete melting point. [e] Decomposes at this temperature. [f] C: calcd, 52.9; found, 53.4. [g] For preparation of the required amine, see the Experimental Section. [h] H: calcd, 5.7; found, 5.1. [i] N: calcd, 10.2; found, 9.5. [j] H: calcd, 4.6; found, 3.8. [k] N: calcd, 11.3; found, 10.8. [l] N: calcd, 10.7; found, 10.2. [m] Solidification of the product was induced by trituration with acetone. The NMR spectrum indicated the presence of 1 mol of acetone.

Scheme IV[a]



[a] (a) NaH, chloromethyl pivalate, DMF; (b) NBS, $(PhCOO)_2$, CCl_4; (c) $CaCO_3$, DMF, 50 °C; (d) 1 N aqueous NaOH.

3h may well be due to the inability of this compound to penetrate cell membranes since the thiol function is capable of forming a covalent bond to protein. The poor TS inhibition of the N10-(2-oxopropyl) analogue **3m** is in line with previous experience[14] which suggests that a polar carbonyl group in this location was disfavored. The reason for the potent cytotoxicity of this analogue is not clear, but other alternative loci of action have not been ruled out.

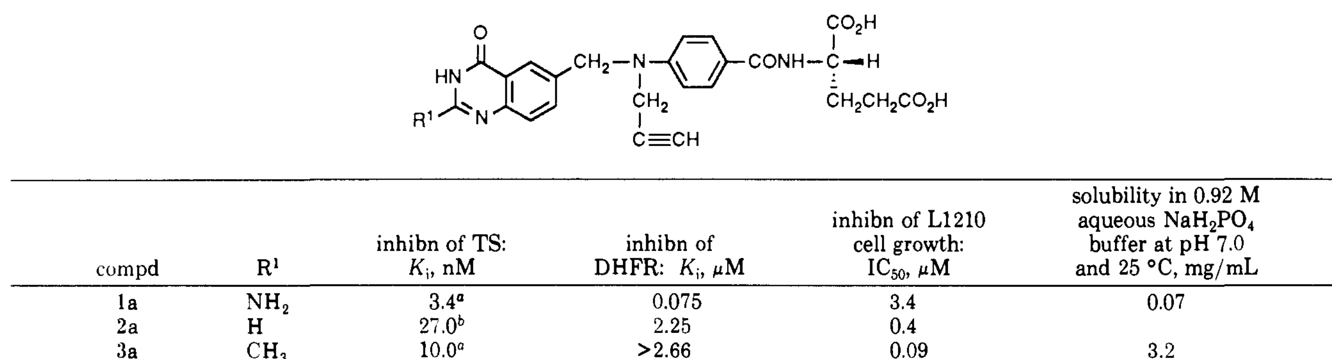
Homologation of the C2-methyl group to give the C2-ethylquinazolinone **4a** lowers enzyme inhibition marginally but leads to a 30-fold drop in cytotoxicity. This is accentuated in the C2-isopropyl **5a** and C2-phenyl **12a** analogues, as well as the substituted methyl compounds **10a** and **11a**. In each case poor cytotoxicity was seen despite reasonable enzyme inhibition. In general the enzyme seems able to tolerate a range of bulky substituents of different electronic character, but cytotoxicity is diminished whenever the C2 substituent is much larger than methyl.

The C2-fluoromethyl analogues **6a**,**c**,**d**,**f** and the hydroxymethyl analogues **9a**,**c–f**,**i** exhibit only marginally lower enzyme inhibition when compared to the corresponding C2-methyl compounds. The cytotoxicity of these C2-fluoromethyl derivatives against L1210 cells is slightly (up to 5-fold) lower than the corresponding C2-methyl analogues, whereas the hydroxymethyl analogues are considerably less potent (up to 100-fold). The exception is **6c**, which is less potent against the enzyme but more cytotoxic in cell culture.

Successive replacement of the hydrogen atoms of the C2-fluoromethyl substituent in **6a** by fluorines (to give **7a** and **8a**) led to a loss in enzyme inhibition of some 6- and 60-fold while cytotoxicity decreased by 65- and >270-fold, respectively. The large drop in activity in moving to the C2-trifluoromethyl derivative **8a** is not consistent with the small increase in bulk that this change brings about. However, measurements of the pK_a values of the parent systems[19] have demonstrated that changes in the electronic

(19) The pK_a values for the parent quinazolinones (Table III) were determined by Dr. J. J. Morris using the method described in: Albert, A.; Serjeant, E. P. *The Determination of Ionization Constants*, 3rd ed.; Chapman and Hall: London and New York, 1984; Chapter 4.

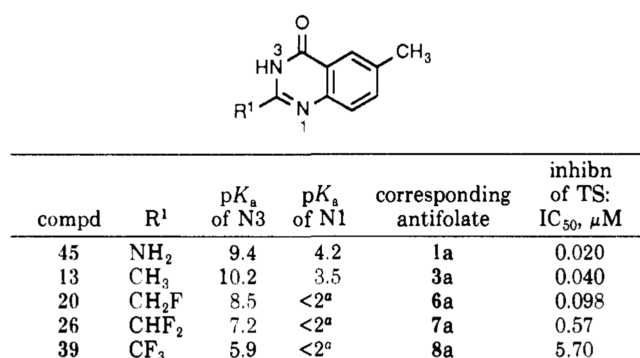
Table II



compd	R^1	inhibn of TS: K_i, nM	inhibn of DHFR: K_i, μM	inhibn of L1210 cell growth: IC_{50}, μM	solubility in 0.92 M aqueous NaH_2PO_4 buffer at pH 7.0 and 25 °C, mg/mL
1a	NH_2	3.4[a]	0.075	3.4	0.07
2a	H	27.0[b]	2.25	0.4	
3a	CH_3	10.0[a]	>2.66	0.09	3.2

[a] Reference 18. [b] Reference 8.

Table III



compd	R^1	pK_a of N3	pK_a of N1	corresponding antifolate	inhibn of TS: IC_{50}, μM
45	NH_2	9.4	4.2	**1a**	0.020
13	CH_3	10.2	3.5	**3a**	0.040
20	CH_2F	8.5	<2[a]	**6a**	0.098
26	CHF_2	7.2	<2[a]	**7a**	0.57
39	CF_3	5.9	<2[a]	**8a**	5.70

[a] Too low to measure accurately.

character of the C2 substituent have a profound effect on the properties of the quinazoline ring system (Table III). Thus while the C2-methyl quinazolinone 13 has similar acidic and basic pK_a values (and hence presumably hydrogen bonding characteristics) to the C2-amino compound, the C2-trifluoromethylquinazolinone is ionized at physiological pH and in addition the N1-nitrogen atom is very nonbasic. These differences are probably responsible for the relatively poor activity of 8a and to a lesser extent 7a.

The poor correlation between the TS inhibition and the cytotoxicity of these antifolates is likely to be due to differences in intracellular concentrations caused by the variation in transport into cells and metabolism within the cells to form γ-polyglutamate derivatives. It has already been shown that the polyglutamates of 1a, 2a, and **3a** are up to 200 times more potent as TS inhibitors than the parent compounds.[20-22] Hence compounds that are readily metabolized to form polyglutamates may be more potent in cell culture than would be expected from their activities against the isolated enzyme.

Solubility measurements[23] (Table II) showed that **3a** did indeed have a higher aqueous solubility than 1a. The analogue **3a** is not toxic to the liver and kidneys of mice at 500 mg/kg iv whereas 1a resulted in significant toxicity to these organs at 100 mg/kg[18] although the two compounds had identical AUC's.[24] Against the L1210:ICR tumor **3a** was found to be 10-fold more potent than **1a**, curing 90% of the mice at 5 mg/kg daily × 5 days.[18] These results reinforce the contention that the toxicity associated with **1a** is related to the poor aqueous solubility of the compound.

In conclusion, compounds have been synthesized in which the C2-amino moiety of the potent TS inhibitor 1a has been replaced by alkyl, substituted alkyl, and aryl groups. Favorable modifications have been followed up by the exploration of alternative N10 substituents. In vitro testing using the isolated enzyme has shown that TS can accommodate both bulky and polar substituents in the C2-position of the quinazoline nucleus. In contrast the cytotoxicity was markedly lower in such compounds. However, a number of TS inhibitors have been prepared that are more potent cytotoxic agents than the clinically tested agent **1a**. In addition, in mice **3a** shows a greater than 50-fold improvement over 1a in therapeutic index. The greater cytotoxic potency and lower toxicity of **3a** compared to **1a** suggest that the former, or a related compound, may have considerable potential as an antitumor agent in the clinic.

Experimental Section

General Procedures. All experiments were carried out under an inert atmosphere and at room temperature unless otherwise stated. The standard work-up procedure involved pouring into H_2O, extracting with the named solvent, washing the organic extracts with H_2O and saturated brine, drying over $MgSO_4$ or Na_2SO_4, and evaporating under vacuum. *N,N*-Dimethylformamide (DMF) and *N,N*-dimethylacetamide (DMA) were purified by azeotropic distillation at 10 mmHg. Flash chromatography was carried out on Merck Kieselgel 60 (Art. 9385). The purities of compounds for test were assessed by analytical HPLC on a Hichrom S5ODS1 Spherisorb Column System set to run isocratically with 60–70% MeOH + 0.2% CF_3CO_2H in H_2O as eluent. TLC was performed on precoated silica gel plates (Merck Art. 5715), and the resulting chromatograms were visualized under UV light at 254 nm. Melting points were determined on a Kofler Block or with a Büchi melting point apparatus and are uncorrected. The ^1H NMR spectra were determined in Me_2SO-d_6 solution (unless stated otherwise) on a Bruker AM 200 (200MHz) spectrometer. Chemical shifts are expressed in units of δ (ppm), and peak multiplicities are expressed as follows: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; br s, broad singlet; m, multiplet. Fast atom bombardment (FAB) mass spectra were determined with a VG MS9 spectrometer and Finnigan Incos data system, using Me_2SO as the solvent and glycerol as the matrix. With the appropriate mode either positive or negative ion data could be collected. NMR and mass spectra were run on all isolated intermediates and final products and are consistent with the proposed structures.

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(22) Bisset, G.; Pawelczak, K.; Jackman, A. L.; Dix Perkin, E. E.; Calvert, A. H.; Hughes, L. R. *Br. J. Cancer* **1989**, *60*, 501.
(23) These values were obtained by Dr. J. J. Morris.
(24) 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.

6-(Bromomethyl)-3,4-dihydro-2-methyl-4-oxoquinazoline (14). Method A. A mixture of 3,4-dihydro-2,6-dimethyl-4-oxoquinazoline (13)[25] (5.0 g, 28.7 mmol), NBS (5.3 g, 29.8 mmol), and benzoyl peroxide (100 mg) in $CHCl_3$ (600 mL) was stirred at 50 °C for 6 h while being illuminated with a 275-W infrared reflector lamp. The mixture was cooled, and the precipitated solid was filtered off, washed with $CHCl_3$ (2 × 50 mL), and vacuum dried to give 14 as an off-white solid: 5.1 g (69%); mp >320 °C; NMR δ 2.36 (s, 3 H, CH_3), 4.85 (s, 2 H, CH_2Br), 7.57 (d, 1 H, J = 8 Hz, quinazoline 8-H), 7.83 (dd, 1 H, J = 8 and 2 Hz, quinazoline 7-H), 8.15 (d, 1 H, J = 2 Hz, quinazoline 5-H). Anal. ($C_{10}H_9BrN_2O$) C, H, N; Br: calcd, 31.6; found, 30.3.

Diethyl *N*-[4-[*N*-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-methylamino]benzoyl]-L-glutamate (16c). Method B. A solution of 14 (5.1 g, 20.2 mmol), diethyl *N*-[4-(methylamino)benzoyl]-L-glutamate (**15c**)[12] (6.77 g, 20.2 mmol), and 2,6-lutidine (7 mL) in DMF (40 mL) was stirred at 80 °C for 18 h. The mixture was cooled and poured into H_2O. Standard workup with EtOAc gave a gum that was purified by chromatography using EtOAc as eluent to give the diester **16c** as a gum: 4.2 g (41%); NMR δ 1.25 (2 t, 6 H, J = 7 Hz, 2 OCH_2CH_3), 2.10 (m, 2 H, $CHCH_2CH_2CO_2Et$), 2.35 (s, 3 H, CH_3), 2.46 (t, 2 H, J = 6.5 Hz, $CHCH_2CH_2CO_2Et$), 3.20 (s, 3 H, NCH_3), 4.10, 4.15 (2 q, 4 H, J = 7 Hz, 2 OCH_2CH_3), 4.53 (m, 1 H, CH), 4.86 (br s, 2 H, $ArCH_2N$<), 6.82 (d, 2 H, J = 8 Hz, 3'-H and 5'-H), 7.55 (d, 1 H, J = 9 Hz, quinazoline 8-H), 7.65 (dd, 1 H, J = 9 and 2 Hz, quinazoline 7-H), 7.77 (d, 2 H, J = 8 Hz, 2'-H and 6'-H), 7.85 (d, 1 H, J = 2 Hz, quinazoline 5-H), 8.47 (d, 1 H, J = 6.5 Hz, CONH).

The procedure was repeated with the appropriate amines to yield the diethyl esters of the antifolates **3a–g,i–m**, **4a,b**, **5a**, **7a**, **12a** and the diester **43**. All of these diesters were purified by chromatography to give gums or amorphous solids that were homogeneous by HPLC.

***N*-[4-[*N*-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-methylamino]benzoyl]-L-glutamic Acid (3c). Method C.** The diester **16c** (4.1 g, 8.1 mmol) was stirred for 3 h in a mixture of 1 N aqueous NaOH (24.3 mL, 24.3 mmol) and EtOH (25 mL). The solvent was evaporated under vacuum below 30 °C and the residue was dissolved in H_2O (20 mL). The solution was filtered and acidified to pH 2.0 with 2 N aqueous HCl. The precipitated solid was isolated by centrifugation and washed successively with H_2O (2 × 20 mL), Et_2O (20 mL), and acetone (20 mL). The product was vacuum dried to give an amorphous off-white solid: 3.1 g (83%); mp 254–257 °C; NMR δ 2.00 (m, 2 H, $CHCH_2CH_2CO_2H$), 2.35 (br t, 2 H, $CHCH_2CH_2CO_2H$), 2.35 (s, 3 H, CH_3), 3.12 (s, 3 H, NCH_3), 4.38 (m, 1 H, CH), 4.78 (s, 2 H, $ArCH_2N$<), 6.77 (d, 2 H, J = 9 Hz, 3'-H and 5'-H), 7.53 (d, 1 H, J = 9 Hz, quinazoline 8-H), 7.62 (dd, 1 H, J = 9 and 2 Hz, quinazoline 7-H), 7.73 (d, 2 H, J = 9 Hz, 2'-H and 6'-H), 7.88 (d, 1 H, J = 2 Hz, quinazoline 5-H), 8.15 (d, 1 H, J = 7 Hz, CONH), 12.2 (s, 1 H, quinazoline 3-H); MS (FAB) m/e 453 $[MH]^+$. Anal. ($C_{23}H_{24}N_4O_6 \cdot 0.75H_2O$) C, H, N.

The procedure was repeated with the appropriate diethyl esters to yield the antifolates **3a–g,i–m**, **4a,b**, **5a**, **7a**, and **12a** (Table I). All compounds had NMR and mass spectra consistent with the assigned structures.

Diethyl *N*-[4-[(2-Methoxyethyl)amino]benzoyl]-L-glutamate (15k). Method D. A mixture of diethyl (4-aminobenzoyl)-L-glutamate 15b[26] (2.10 g, 6.5 mmol), 2-methoxyethyl bromide (1.0 g, 7.2 mmol), and powdered $CaCO_3$ (650 mg, 6.5 mmol) in DMA (25 mL) was stirred for 48 h at 100 °C. The standard workup followed by purification of the crude product by chromatography using 10% v/v acetone in $CHCl_3$ as eluent gave a gum: 1.48 g (60%).

Diethyl *N*-[4-[(3-Methoxypropyl)amino]benzoyl]-L-glutamate (15l). The alkylation of 15b (2.10 g, 6.5 mmol) with 3-methoxypropyl bromide according to method D afforded a gum: 1.34 g (52%).

Diethyl *N*-[4-[(2-Oxopropyl)amino]benzoyl]-L-glutamate (15m). Method E. A mixture of 15b (3.22 g, 10 mmol), chloroacetone (1.61 mL, 20 mmol), and 2,6-lutidine (2.33 mL, 20 mmol) in DMF (15 mL) was stirred at 100 °C for 2 h. The standard workup with EtOAc followed by purification by chromatography using 20% v/v EtOAc in CH_2Cl_2 as eluent gave an amorphous solid: 2.05 g (54%); NMR δ 1.06 (2 t, 6 H, J = 7 Hz, 2 OCH_2CH_3), 2.10 (m, 2 H, $CHCH_2CH_2CO_2Et$), 2.13 (s, 3 H, $COCH_3$), 2.42 (t, 2 H, J = 7 Hz, $CHCH_2CH_2CO_2Et$), 4.04 (d, 2 H, J = 6 Hz, $COCH_2N$), 4.07 (2 q, 4 H, J = 7 Hz, 2 OCH_2CH_3), 4.40 (m, 1 H, CH), 6.38 (t, 1 H, J = 6 Hz, NHAr), 6.60 (d, 2 H, J = 8 Hz, 3'-H and 5'-H), 7.66 (d, 2 H, J = 8 Hz, 2'-H and 6'-H), 8.24 (d, 1 H, J = 7 Hz, CONH).

Diethyl *N*-[4-[(2-Bromoethyl)amino]benzoyl]-L-glutamate (15g). The alkylation of 15b (2.0 g, 6.2 mmol) at 90 °C for 17 h according to method E afforded a gum: 310 mg (12%); NMR δ 1.16 (2 t, 6 H, J = 8 Hz, 2 OCH_2CH_3), 2.03 (m, 2 H, $CHCH_2CH_2CO_2Et$), 2.42 (t, 2 H, J = 7.5 Hz, $CHCH_2CH_2CO_2Et$), 3.55 (m, 4 H, $BrCH_2CH_2N$), 4.05, 4.10 (2 q, 4 H, J = 8 Hz, 2 OCH_2CH_3), 4.38 (m, 1 H, CH), 6.62 (d, 2 H, J = 8 Hz, 3'-H and 5'-H), 7.66 (d, 2 H, J = 8 Hz, 2'-H and 6'-H), 8.24 (d, 1 H, J = 7 Hz, CONH).

***N*-[4-[*N*-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-(2-mercaptoethyl)amino]benzoyl]-L-glutamic Acid (3h). Method F.** The N10-bromoethyl antifolate diester 17 was prepared according to method B from 14 (190 mg, 0.77 mmol) and **15g** (300 mg, 0.70 mmol): 160 mg (38%) of a golden gum; NMR δ 1.05 (2 t, 6 H, J = 6.5 Hz, 2 OCH_2CH_3), 2.04 (m, 2 H, $CHCH_2CH_2CO_2Et$), 2.35 (s, 3 H, CH_3), 2.40 (t, 2 H, J = 7 Hz, $CHCH_2CH_2CO_2Et$), 3.69 (br t, 2 H, J = 6.5 Hz, CH_2Br), 3.96 (br t, 2 H, J = 6.5 Hz, CH_2N<), 4.04, 4.08 (2 q, 4 H, J = 6.5 Hz, 2 OCH_2CH_3), 4.39 (m, 1 H, CH), 4.85 (br s, 2 H, $ArCH_2N$<), 6.77 (d, 2 H, J = 8 Hz, 3'-H and 5'-H), 7.54 (d, 1 H, J = 7 Hz, quinazoline 8-H), 7.64 (dd, 1 H, J = 7 and 2 Hz, quinazoline 7-H), 7.70 (d, 2 H, J = 8 Hz, 2'-H and 6'-H), 7.87 (d, 1 H, J = 2 Hz, quinazoline 5-H), 8.30 (d, 1 H, J = 6.5 Hz, CONH).

A mixture of 17 (100 mg, 0.166 mmol) and thiourea (14 mg, 0.18 mmol) in DMA (5 mL) was stirred at 100 °C for 17 h. The solvent was evaporated under vacuum and the gummy residue was triturated with EtOAc. The resulting solid crude thiouronium salt 18 (71 mg) was stirred for 3 h in a mixture of 1 N aqueous NaOH (1.0 mL, 1.0 mmol), H_2O (10 mL), and EtOH (10 mL). The solvent was evaporated below 30 °C and the resulting gum was dissolved in H_2O (5 mL). The solution was filtered and brought to pH 3.0 with aqueous 2 N HCl. The off-white solid precipitate was washed with H_2O (2 × 5 mL) and vacuum dried to give **3h**: 25 mg (30% from 17); mp 160 °C; NMR δ 2.00 (m, 2 H, $CHCH_2CH_2CO_2H$), 2.32 (br t, 2 H, $CHCH_2CH_2CO_2H$), 2.33 (s, 3 H, CH_3), 3.00 (br t, 2 H, CH_2SH), 3.80 (br t, 2 H, NCH_2CH_2S), 4.35 (m, 1 H, CH), 4.76 (s, 2 H, $ArCH_2N$<), 6.79 (d, 2 H, J = 9 Hz, 3'-H and 5'-H), 7.53 (d, 1 H, J = 9 Hz, quinazoline 8-H), 7.65 (dd, 1 H, J = 9 and 2 Hz, quinazoline 7-H), 7.71 (d, 2 H, J = 9 Hz, 2'-H and 6'-H), 7.88 (d, 1 H, J = 2 Hz, quinazoline 5-H), 8.16 (d, 1 H, J = 7 Hz, CONH), 12.10 (s, 1 H, quinazoline 3-H); MS (FAB) m/e 497 $[M - H]^-$. Anal. ($C_{24}H_{26}N_4O_6S \cdot 2H_2O$) C, N; H: calcd, 5.6; found, 5.0.

3,4-Dihydro-2-(fluoromethyl)-6-methyl-4-oxoquinazoline (20). HCl gas was bubbled through a solution of fluoroacetonitrile (50 g, 0.85 mol) in Et_2O (750 mL) and absolute EtOH (40 mL) at 0 °C for 45 min to give a saturated solution. The reaction mixture was allowed to warm to room temperature overnight. The white solid precipitate of ethyl 2-fluoroacetimidate hydrochloride was filtered off, washed with Et_2O, and dried under vacuum: 101.2 g (72%).

The freshly prepared ethyl 2-fluoroacetimidate hydrochloride (100 g, 0.70 mol) was added to a stirred solution of NaOEt which had been prepared in situ by the addition of Na metal (22.0 g, 0.96 g-atom) to absolute EtOH (600 mL). After 45 min, 2-amino-5-methylbenzoic acid (17)[26] (100 g, 0.66 mol) was added and the mixture was stirred for 60 h. The off-white precipitate was filtered off, washed with H_2O (2 × 200 mL), and dried under vacuum over P_2O_5: 85.5 g (67%); mp 229–230 °C; NMR δ 2.47 (s, 3 H, CH_3), 5.30 (d, 2 H, J = 47 Hz, CH_2F), 7.60 (d, 1 H, J = 8 Hz, quinazoline 8-H), 7.65 (dd, 1 H, J = 8 and 1.5 Hz, quinazoline 7-H), 7.93 (d, 1 H, J = 1.5 Hz, quinazoline 5-H); MS (CI) m/e 193 $[MH]^+$. Anal. ($C_{10}H_9FN_2O \cdot 0.6H_2O$) C, H, F, N.

6-(Bromomethyl)-3,4-dihydro-2-(fluoromethyl)-4-oxoquinazoline (21). Method G. A suspension of finely powdered **20** (8.0 g, 0.042 mol) in CCl_4 (300 mL) was stirred vigorously with

(25) Sen, A. B.; Gupta, J. K. *J. Indian Chem. Soc.* **1962**, *31*, 369.
(26) Aldrich Chemical Co.

NBS (7.0 g, 0.04 mol) and benzoyl peroxide (300 mg) for 8 h under reflux and a 275-W infrared reflector lamp. On cooling, the white solid was filtered off, washed with H_2O and Et_2O and vacuum dried: 9.35 g of a mixture of 90% **21** and 10% starting material **20**: NMR δ 4.87 (s, 2 H, CH_2Br), 5.30 (d, 2 H, J = 45 Hz, CH_2F), 7.68 (d, 1 H, J = 2.5 Hz, quinazoline 8-H), 7.87 (dd, 1 H, J = 7.5 and 2 Hz, quinazoline 7-H), 8.20 (d, 1 H, J = 2 Hz, quinazoline 5-H).

The procedure was repeated with the appropriate 2-substituted-6-methyl-4-oxoquinazolines **24**,[27] **25**,[28] **26**, **27**,[15] **28**,[15] **29**, and **30**[29] to give the corresponding bromomethyl derivatives **31–37**.

2-(Difluoromethyl)-3,4-dihydro-6-methyl-4-oxoquinazoline (26). This was prepared from 2-amino-5-methylbenzamide and difluoroacetic acid by the method described in ref 30 and was used without purification: NMR δ 2.50 (s, 3 H, CH_3), 6.55 (t, 1 H, J = 52 Hz, CHF_2), 7.64 (m, 2 H, quinazoline 7-H and 8-H), 8.08 (br s, 1 H, quinazoline 5-H).

2-(Acetamidomethyl)-3,4-dihydro-6-methyl-4-oxoquinazoline (29). A suspension of 2-(chloromethyl)-3,4-dihydro-6-methyl-4-oxoquinazoline (**28**)[15] (2.00 g, 9.6 mmol) in concentrated aqueous NH_4OH (500 mL) was stirred for 20 h. The NH_3 was boiled off on a steam bath and the H_2O was evaporated under vacuum. Final traces of H_2O were removed by azeotropic rotary evaporation in the presence of added toluene. The residue was stirred under reflux in Ac_2O (20 mL) for 30 min. The solvent was removed under vacuum and the residue was purified by chromatography to give a gum: 650 mg (30%); NMR δ 2.40 (s, 3 H, CH_3), 2.44 (s, 3 H, CH_3), 4.80 (br s, 2 H, CH_2NH), 7.43 (d, 1 H, J = 7 Hz, quinazoline 8-H), 7.57 (dd, 1 H, J = 7 and 2 Hz, quinazoline 7-H), 7.88 (d, 1 H, J = 2 Hz, quinazoline 5-H), 12.27 (br s, 1 H, quinazoline 3-H).

***N*-[4-[*N*-[[3,4-Dihydro-2-(fluoromethyl)-4-oxo-6-quinazolinyl]methyl]-*N*-prop-2-ynylamino]benzoyl]-L-glutamic Acid (6a). Method H.** Di-*tert*-butyl *N*-[4-(prop-2-ynylamino)benzoyl]-L-glutamate (**22**) was prepared as a gum from di-*tert*-butyl *N*-(4-aminobenzoyl)-L-glutamate[11] using the method described[1] for the preparation of the diethyl ester **15a**.

A mixture of the bromomethyl compound **21** (620 mg, 2.3 mmol), the amine **22** (1.20 g, 2.3 mmol), and 2,6-lutidine (1.60 mL, 14 mmol) in DMF (20 mL) was stirred at 60 °C for 18 h. The solvent was removed under vacuum and the resulting oil was purified by chromatography using 33% v/v EtOAc in CH_2Cl_2 as eluent to give the diester **23** as a gum: 650 mg (37%). This was dissolved in a mixture of CF_3CO_2H (2 mL) and $CHCl_3$ (6 mL). After 4 h the solution was poured into Et_2O (40 mL) and the mixture was stirred for 10 min. The precipitated buff solid was filtered from the solution, washed with Et_2O (3 × 10 mL), and dried under vacuum to give **6a** as the trifluoroacetate salt hemihydrate: 300 mg (56%); mp 126–131 °C; NMR δ 2.00 (m, 2 H, $CHCH_2CH_2CO_2H$), 2.30 (t, 2 H, J = 6.5 Hz, $CHCH_2CH_2CO_2H$), 3.18 (t, 1 H, J = 2 Hz, C≡CH), 4.15 (m, 1 H, CH), 4.15 (d, 2 H, J = 2 Hz, CH_2C≡C), 4.80 (s, 2 H, $ArCH_2N$<), 5.27 (d, 2 H, J = 47 Hz, CH_2F), 6.84 (d, 2 H, J = 9 Hz, 3′-H and 5′-H), 7.66 (d, 1 H, J = 9 Hz, quinazoline 8-H), 7.75 (dd, 1 H, J = 9 and 2 Hz, quinazoline 7-H), 7.75 (d, 2 H, J = 9 Hz, 2′-H and 6′-H), 8.04 (d, 1 H, J = 2 Hz, quinazoline 5-H), 8.21 (d, 1 H, J = 8 Hz, CONH); MS (FAB) *m/e* 493 $[M - H]^-$. Anal. ($C_{25}H_{23}FN_4O_6{\cdot}CF_3CO_2H{\cdot}0.5H_2O$) C, H, N.

The procedure was repeated with the appropriate amines in place of **22** to yield the antifolate diacids **6c,d,f**.

***N*-[4-[*N*-[[3,4-Dihydro-2-(hydroxymethyl)-4-oxo-6-quinazolinyl]methyl]-*N*-prop-2-ynylamino]benzoyl]-L-glutamic Acid (9a). Method I.** A mixture of the bromomethyl compound **34** (1.50 g, 4.8 mmol), the amine **15a** (1.70 g, 4.8 mmol), and powdered $CaCO_3$ (1.90 g, 19.3 mmol) in DMF (50 mL) was stirred at 50 °C for 24 h. The cooled mixture was filtered through Celite and the solvent was evaporated under vacuum. The resulting oil was purified by chromatography using EtOAc as eluent to give **38** as a gum: 1.30 g (46%); NMR δ 1.14, 1.18 (2 t, 6 H, J = 6.5 Hz, 2 OCH_2CH_3), 2.05 (m, 2 H, $CHCH_2CH_2CO_2Et$), 2.12 (s, 3 H, $OCOCH_3$), 2.40 (t, 2 H, J = 7 Hz, $CHCH_2CH_2CO_2Et$), 3.26 (t, 1 H, J = 2 Hz, C≡CH), 4.04, 4.09 (2 q, 4 H, J = 6.5 Hz, 2 OCH_2CH_3), 4.34 (d, 2 H, J = 2 Hz, CH_2C≡C), 4.40 (m, 1 H, CH), 4.80 (br s, 2 H, $ArCH_2N$<), 4.94 (s, 2 H, CH_2OAc), 6.85 (d, 2 H, J = 8 Hz, 3′-H and 5′-H), 7.60 (d, 1 H, J = 7 Hz, quinazoline 8-H), 7.73 (d, 2 H, J = 8 Hz, 2′-H and 6′-H), 7.73 (dd, 1 H, J = 7 and 2 Hz, quinazoline 7-H), 8.01 (d, 1 H, J = 2 Hz, quinazoline 5-H), 8.32 (d, 1 H, J = 7 Hz, CONH).

The triester **38** (1.29 g, 2.18 mmol) was dissolved in 1 N aqueous NaOH (7.2 mL, 7.2 mmol). After 4 h the solution was filtered into a centrifuge tube and acidified to pH 3.0 with 2 N aqueous HCl. The precipitated off-white solid **9a** was isolated by centrifugation, washed with H_2O, and dried under vacuum: 650 mg (55%); mp 137–143 °C; NMR δ 2.00 (m, 2 H, $CHCH_2CH_2CO_2H$), 2.32 (t, 2 H, J = 7 Hz, $CHCH_2CH_2CO_2H$), 3.18 (br s, 1 H, C≡CH), 4.35 (br s, 2 H, CH_2C≡C), 4.38 (m, 1 H, CH), 4.43 (s, 2 H, CH_2OH), 4.80 (s, 2 H, $ArCH_2N$<), 6.85 (d, 2 H, J = 9 Hz, 3′-H and 5′-H), 7.62 (d, 1 H, J = 9 Hz, quinazoline 8-H), 7.75 (dd, 1 H, J = 9 and 2 Hz, quinazoline 7-H), 7.75 (d, 2 H, J = 9 Hz, 2′-H and 6′-H), 8.03 (d, 1 H, J = 2 Hz, quinazoline 5-H), 8.22 (d, 1 H, J = 7 Hz, CONH); MS (FAB) *m/e* 491 $[M - H]^-$. Anal. ($C_{25}H_{24}N_4O_7{\cdot}2H_2O$) C, H, N.

3,4-Dihydro-6-methyl-3-[(pivaloyloxy)methyl]-2-(trifluoromethyl)-4-oxoquinazoline (40). NaH (1.06 g of a 50% dispersion in oil, 22 mmol) was added to a solution of 3,4-dihydro-6-methyl-2-(trifluoromethyl)-4-oxoquinazoline (**39**)[31] (4.20 g, 18.4 mmol) in DMF (40 mL) and the mixture was stirred for 1 h. Chloromethyl pivalate (2.53 g, 22 mmol) was added and the stirring was continued for a further 17 h. The standard workup with Et_2O followed by purification by chromatography gave **40** as an oil: 2.87 g (46%); NMR δ 1.22 (s, 9 H, *t*-Bu), 2.58 (s, 3 H, CH_3), 6.36 (s, 2 H, OCH_2N), 7.80 (dd, 1 H, J = 7 and 2 Hz, quinazoline 7-H), 8.05 (d, 1 H, J = 2 Hz, quinazoline 5-H), 8.08 (d, 1 H, J = 7 Hz, quinazoline 8-H); MS (EI) *m/e* 342 $[M]^+$.

***N*-[4-[*N*-[[3,4-Dihydro-4-oxo-2-(trifluoromethyl)-6-quinazolinyl]methyl]-*N*-prop-2-ynylamino]benzoyl]-L-glutamic Acid (8a). Method J.** A mixture of **40** (2.86 g, 8.36 mmol), NBS (1.50 g, 8.40 mmol), and benzoyl peroxide (100 mg) in CCl_4 (100 mL) was stirred under reflux for 3 h with simultaneous illumination by a 275-W infrared reflector lamp. The mixture was cooled and filtered through Florisil. The Florisil was washed with CCl_4 (250 mL), and the combined filtrates were evaporated to give the bromomethyl compound **41** as an oil (1.30 g, 37%), which was used without purification.

A mixture of **41** (1.30 g, 3.1 mmol), the amine **15a** (1.10 g, 3.1 mmol), and powdered $CaCO_3$ (1.24 g, 12.4 mmol) in DMF (50 mL) was stirred for 17 h at 50 °C. The cooled mixture was filtered through Celite and the filtrate was evaporated to dryness below 30 °C. The crude product was purified by chromatography using 15% v/v EtOAc in CH_2Cl_2 as eluent to give the 2-(trifluoromethyl)quinazoline antifolate diester **42** as a clear gum: 1.20 g (57%). This diester was stirred for 4 h in a mixture of EtOH (5 mL) and 1 N aqueous NaOH (5.2 mL, 5.2 mmol). The solvent was evaporated under vacuum and the residue was dissolved in H_2O (10 mL). This solution was filtered and acidified to pH 3.0 with 2 N aqueous HCl. The precipitated solid was isolated by centrifugation, washed with H_2O, and vacuum dried to yield **8a**: 400 mg (40%); mp 110–115 °C; NMR δ 2.00 (m, 2 H, $CHCH_2CH_2CO_2H$), 2.34 (t, 2 H, J = 7 Hz, $CHCH_2CH_2CO_2H$), 3.18 (br s, 1 H, C≡CH), 4.35 (m, 1 H, CH), 4.38 (br s, 2 H, CH_2C≡C), 4.86 (br s, 2 H, $ArCH_2N$<), 6.84 (d, 2 H, J = 9 Hz, 3′-H and 5′-H), 7.75 (m, 4 H, 2′-H and 6′-H, quinazoline 7-H and 8-H), 8.10 (br s, 1 H, quinazoline 5-H), 8.20 (d, 1 H, J = 7 Hz, CONH); MS (FAB) *m/e* found, 531.1467 $[MH]^+$; calcd, 531.1491. Anal. ($C_{25}H_{21}F_3N_4O_6{\cdot}3H_2O$) C, N; H: calcd, 4.6; found, 3.8; F: calcd, 9.7; found, 9.1.

***N*-[4-[*N*-[[3,4-Dihydro-4-oxo-2-[(2-pyrimidinylthio)methyl]-6-quinazolinyl]methyl]-*N*-prop-2-ynylamino]benzoyl]-L-glutamic Acid (11a). Method K.** Condensation of 6-(bromomethyl)-2-(chloromethyl)-3,4-dihydro-4-oxoquinazoline (**35**)[15] (4.0 g, 13.9 mmol) and the amine **15a** (5.0 g, 13.9 mmol) according to method B gave diethyl *N*-[4-[*N*-[[2-(chloro-

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methyl)-3,4-dihydro-4-oxo-6-quinazolinyl]methyl]-*N*-prop-2-ynylamino]benzoyl]-L-glutamate (**43**) as a gum: 4.2 g (53%); NMR δ 1.15, (2 t, 6 H, J = 6.5 Hz, 2 OCH_2CH_3), 2.04 (m, 2 H, $CHCH_2CH_2CO_2Et$), 2.40 (t, 2 H, J = 7 Hz, $CHCH_2CH_2CO_2Et$), 3.18 (t, 1 H, J = 1.5 Hz, C≡CH), 4.04, 4.08 (2 q, 4 H, J = 6.5 Hz, 2 OCH_2CH_3), 4.39 (d, 2 H, J = 1.5 Hz, CH_2C≡C), 4.40 (m, 1 H, CH), 4.56 (br s, 2 H, CH_2Cl), 4.82 (br s, 2 H, $ArCH_2N$<), 6.85 (d, 2 H, J = 9 Hz, 3'-H and 5'-H), 7.61 (d, 1 H, J = 8 Hz, quinazoline 8-H), 7.75 (d, 2 H, J = 9 Hz, 2'-H and 6'-H), 7.75 (dd, 1 H, J = 8 and 2 Hz, quinazoline 7-H), 8.03 (d, 1 H, J = 2 Hz, quinazoline 5-H), 8.34 (d, 1 H, J = 6.5 Hz, CONH).

Powdered 2-mercaptopyrimidine (110 mg, 0.98 mmol) was added to a stirred suspension of NaH (47 mg of a 50% dispersion in oil, 0.98 mmol) in DMF (10 mL). After 30 min a solution of the chloromethyl compound **43** (560 mg, 0.98 mmol) in DMF (5 mL) was added and the mixture was stirred for a further 17 h. The standard workup with EtOAc followed by purification of the crude product by chromatography using EtOAc as eluent afforded 470 mg (75%) of the 2-pyrimidinylthio derivative **44** as a gum. This product was stirred for 2 h in a mixture of EtOH (5 mL) and 1 N aqueous NaOH (6.9 mL, 6.9 mmol). The reaction was worked up according to method C to give the off-white solid **11a**: 320 mg (87%); mp 143–147 °C; NMR δ 2.00 (m, 2 H, $CHCH_2CH_2CO_2H$), 2.35 (t, 2 H, J = 7 Hz, $CHCH_2CH_2CO_2H$), 3.18 (t, 1 H, J = 2 Hz, C≡CH), 4.35 (m, 1 H, CH), 4.35 (d, 2 H, J = 2 Hz, CH_2C≡C), 4.41 (s, 2 H, CH_2S), 4.78 (br s, 2 H, $ArCH_2N$<), 6.85 (d, 2 H, J = 9 Hz, 3'-H and 5'-H), 7.25 (t, 1 H, J = 5 Hz, pyrimidine 5-H), 7.53 (d, 1 H, J = 9 Hz, quinazoline 8-H), 7.70 (m, 3 H, 2'-H, 6'-H, and quinazoline 7-H), 8.00 (d, 1 H, J = 2 Hz, quinazoline 5-H), 8.34 (d, 1 H, J = 7 Hz, CONH), 8.65 (d, 2 H, J = 5 Hz, pyrimidine 4-H and 6-H); MS (FAB) m/e 587 $[MH]^+$. Anal. ($C_{29}H_{26}N_6O_6S{\cdot}H_2O$) C, H, N.

Quinazoline Antifolate Thymidylate Synthase Inhibitors: 2'-Fluoro-N^{10}-propargyl-5,8-dideazafolic Acid and Derivatives with Modifications in the C2 Position

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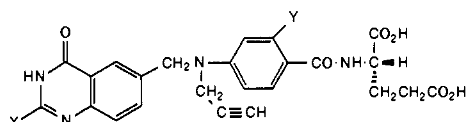
The synthesis of 2'-fluoro-10-propargyl-5,8-dideazafolic acid and its 2-desamino, 2-desamino-2-hydroxymethyl, and 2-desamino-2-methoxy analogues is described. In general the synthetic route involved the coupling of diethyl *N*-[2-fluoro-4-(prop-2-ynylamino)benzoyl]-L-glutamate (**15**) with the appropriate 6-(bromomethyl)quinazoline followed by deprotection with mild alkali. These four compounds together with the 2-desamino-2-methyl analogue were tested for their activity against L1210 thymidylate synthase (TS). They were also examined for their inhibition of the growth of the L1210 cell line and of two mutant L1210 cell lines, the L1210:R7A that overproduces dihydrofolate reductase (DHFR) and the L1210:1565 that has impaired uptake of reduced folates. Compared with their non-fluorinated parent compounds, the 2'-fluoro analogues were all ~2-fold more potent as TS inhibitors. Similarly, they also showed improved inhibition of L1210 cell growth (1.5–5-fold), and this activity was prevented by co-incubation with thymidine. All had retained or improved activity against both the L1210:R7A and L1210:1565 cell lines.

Introduction

The discovery that the quinazoline-based analogue of folic acid N^{10}-propargyl-5,8-dideazafolic acid (**1**)[1] was a



	X	Y
1	X = NH_2	Y = H
2	X = NH_2	Y = F
3	X = H	Y = H
4	X = H	Y = F
5	X = CH_3	Y = H
6	X = CH_3	Y = F
7	X = CH_2OH	Y = H
8	X = CH_2OH	Y = F
9	X = OCH_3	Y = H
10	X = OCH_3	Y = F

potent inhibitor of thymidylate synthase (TS, EC 2.1.1.45) and had experimental antitumor activity[1-4] led to several clinical studies in man.[5-10] Significant activity was observed in some tumor types, particularly ovarian, breast, and liver cancer. However, this drug was withdrawn from clinical study because of its nephrotoxicity and unpredictable hepatotoxicity. The relative insolubility of **1** at

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