close to sites Ia and IIa here.

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Registry No. Tamoxifen, 10540-29-1; tamoxifen *N,N*-diethyl derivative, 97818-91-2; tamoxifen piperazino derivative, 97818-85-4; tamoxifen pyrrolidino derivative, 15917-44-9; tamoxifen 4-iodo derivative, 116057-66-0; tamoxifen 4-hydroxy derivative, 65213-48-1; tamoxifen *c*-methyl derivative, 15917-50-7.

Quinoline Antifolate Thymidylate Synthase Inhibitors: Variation of the C2- and C4-Substituents

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Modifications to the bicyclic ring system 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 (1, CB3717) have led to the synthesis of a series of quinoline antifolates bearing a variety of substituents at the C2 and C4 positions. In general the synthetic route involved the coupling of the appropriate diethyl *N*-[4-(prop-2-ynylamino)benzoyl]-L-glutamate with a disubstituted 6-(bromomethyl)quinoline followed by deprotection using mild alkali. The compounds were tested as inhibitors of partially purified L1210 TS. As a measure of cytotoxicity, the compounds were tested for their inhibition of the growth of L1210 cells in culture. Good enzyme inhibition and cytotoxicity were found for compounds containing chloro, amino, or methyl substituents at the C2 position with chloro or bromo substituents at C4. The effect on enzyme inhibition of varying the N10 substituent of **2h** was similar to that observed in the quinazolinone-containing antifolates, indicating that the quinoline compounds may be interacting with the enzyme in a similar way to the quinazolinones. Also, the introduction of a 2'-fluoro substituent into the benzoyl ring of several of the quinoline antifolates led to an increase in both TS inhibition and the inhibition of L1210 cell growth. These data demonstrate that the N3-H of the pyrimidine ring of the quinazolinone antifolates is not required for binding to TS if appropriate substituents are placed at the C2 and C4 positions of the bicyclic ring system.

Introduction

The 2-aminoquinazoline antifolate *N*-[4-[*N*-[(2-amino-3,4-dihydro-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoyl]-L-glutamic acid (CB3717, 1) is a potent inhibitor ($K_i \approx 3$ nM) of both murine and human thymidylate synthase (TS, EC 2.1.1.45) and shows antitumor activity in some animal models.[1-7] Phase I/II clinical studies with 1 demonstrated responses in patients, particularly those with breast, ovarian, and liver cancer.[8-14] However, the compound caused unacceptable side effects, in particular, unpredictable liver and dose–limiting life–threatening kidney toxicities. Studies indicated that at least the nephrotoxicity of the compound was due to the physicochemical properties of the compound, especially poor aqueous solubility, rather than an intrinsic property of antifolate based TS inhibitors.[15,16]

Several studies have been carried out in which the effects of modifications to various parts of 1 were investigated.[17-28] In particular, the consequences on in vitro TS inhibition and tumor cell growth inhibition of changing the C2-amino group of the bicycle have been reported.[18-21,23] Replacement of this functional group of 1 with a hydrogen[18,20,27] or methyl[19,21] substituent led to compounds with greater aqueous solubility, a lack of liver and kidney toxicity in mice, and enhanced efficacy as inhibitors of tumor cell growth in vitro. Subsequently a large number of compounds with a variety of C2 substituents have been synthesized and evaluated as novel cancer cell growth inhibitors.[23] The effects of modifications to the N10 substituent[28] and the *p*-aminobenzoyl ring[22,25,26] have also been studied.

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‡ Institute of Cancer Research.

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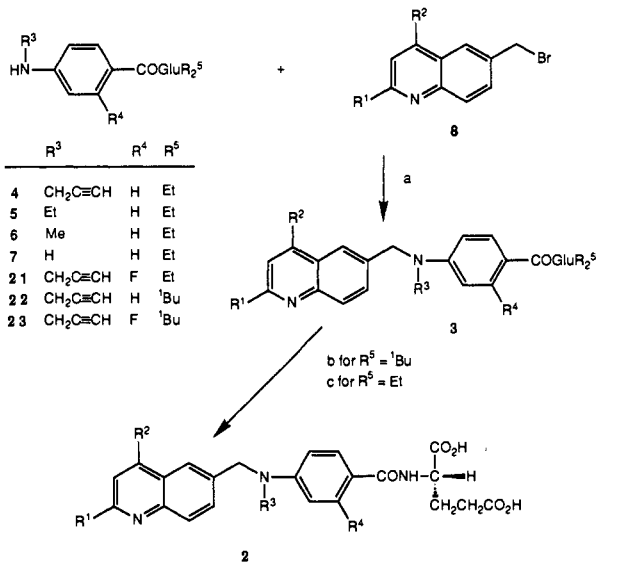
Scheme I[a,b]

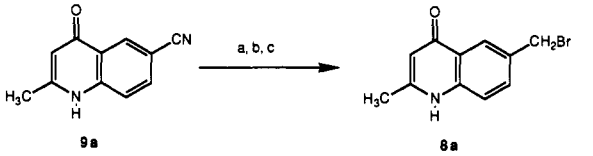


	R3	R4	R5
4	$CH_2C{\equiv}CH$	H	Et
5	Et	H	Et
6	Me	H	Et
7	H	H	Et
21	$CH_2C{\equiv}CH$	F	Et
22	$CH_2C{\equiv}CH$	H	tBu
23	$CH_2C{\equiv}CH$	F	tBu

[a] See Table I for values of R^1 and R^2. [b] Reagents: (a) 2,6-lutidine, DMF, 70 °C; (b) TFA, 20 °C; (c) EtOH, 1 M aqueous NaOH, 20 °C.

It was our aim to make more extensive modifications to the bicyclic ring system of 1 to develop a novel series of

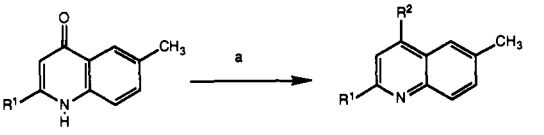
Scheme II[a]



9a 8a

[a] Reagents: (a) Raney nickel, HCO_2H, 100 °C; (b) $NaBH_4$, EtOH; (c) PBr_3, 38 °C.

Scheme III[a]

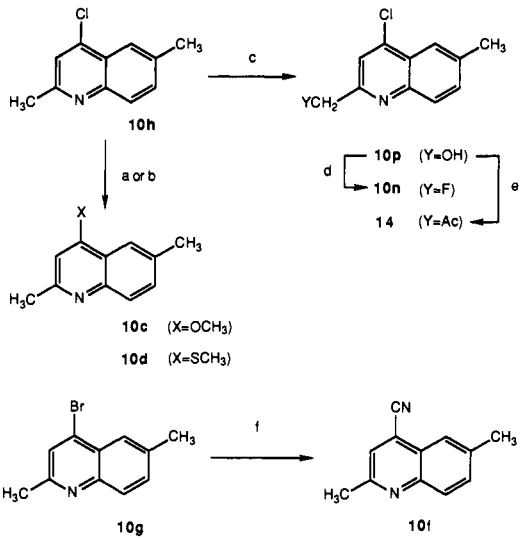


	R1
11	CH_3
12	CH_2CH_3
13	CF_3

	R1	R2
10h	CH_3	Cl
10g	CH_3	Br
10m	CH_2CH_3	Cl
10o	CF_3	Cl

[a] Reagents: $POCl_3$ or $POBr_3$, *N,N*-dimethylaniline, $PhCH_3$, 110 °C.

Scheme IV[a]



[a] Reagents: (a) $NaOCH_3$, MeOH, 60 °C; (b) $NaSCH_3$, MeCN, 70 °C; (c) (1) *m*-CPBA, $CHCl_3$, (2) Ac_2O, 80 °C, (3) 10% aqueous HCl, 100 °C; (d) Et_2NSF_3, CH_2Cl_2, −70 °C; (e) Ac_2O, py, 60 °C; (f) CuCN, DMF, 140 °C.

TS inhibitors without the quinazoline ring. In particular, by radically altering the hydrogen-bonding characteristics

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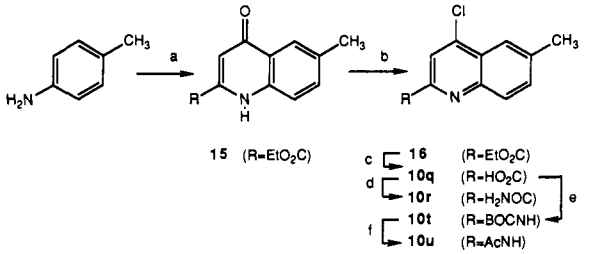
Scheme V[a]



[a] Reagents: (a) diethyl acetylenedicarboxylate; (b) $POCl_3$, *N,N*-dimethylaniline, $PhCH_3$, 110 °C; (c) 2 M aqueous NaOH, EtOH; (d) (1) $SOCl_2$, 80 °C, (2) NH_3, H_2O, MeCN; (e) diphenyl phosphorazidate, Et_3N, ^tBuOH, 100 °C; (f) (1) TFA, (2) Ac_2O.

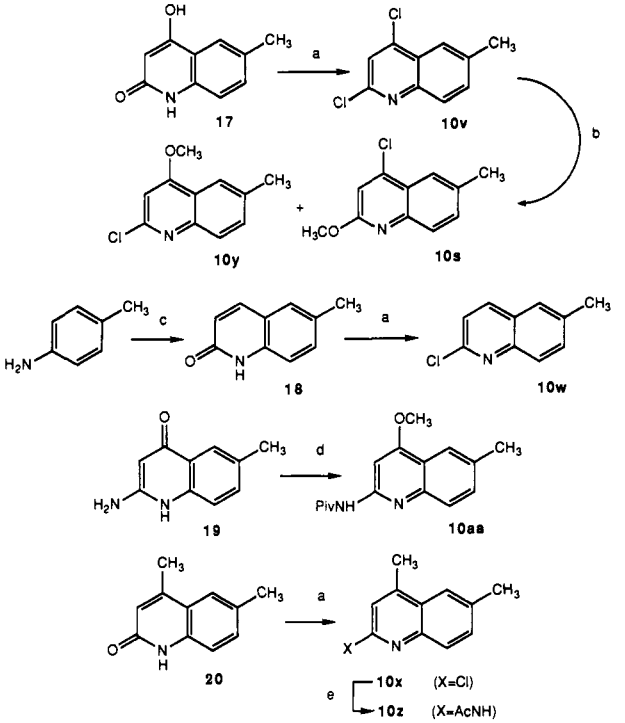
of the bicycle, it was anticipated that modulation of the physicochemical properties of the compounds, such as solubility, could be achieved. However, it was not clear whether such compounds would be potent inhibitors of TS. Recently the synthesis and TS inhibition of a 3,5,8-trideazapteridine (quinoline) containing antifolate was reported.[17] The compound, 2-desamino-2-methyl-N^{10}-propargyl-3,5,8-trideazafolate, was found to be a poor inhibitor of human TS.[17] However, in this paper we describe the synthesis and biological activity of a series of TS inhibitors containing the quinoline ring system, examples of which are potent inhibitors of murine TS.

Chemistry

The quinoline antifolates **2a–ee** described in this paper were prepared by alkylation of the (*p*-aminobenzoyl)-glutamate esters **4**,[1] **5**,[29] **6**,[30] **7**,[31] **21**,[22] **22**[21] and **23**[32] with the appropriate 6-(bromomethyl)quinolines **8a–h** and **8l–aa** and saponification of the resulting diesters under either basic conditions for the diethyl esters (**3a–t,w–cc,ee**) or acidic conditions for the di-*tert*-butyl esters (**3u,v,dd**) (Scheme I). The 6-(bromomethyl)quinolines were generally derived from the corresponding 6-methylquinolines by radical benzylic bromination with *N*-bromosuccinimide. 2,6-Dimethylquinoline and 2,4,6-trimethylquinoline, obtained commercially,[31] were found to brominate selectively at the 6-methyl position. 6-(Bromomethyl)-4-hydroxy-2-methylquinoline (**8a**) could not be prepared by NBS bromination of the corresponding 6-methyl compound because of competing ring bromination at the 3-position. Therefore **8a** was synthesized from the corresponding nitrile **9a**[33] by reduction with Raney nickel to give the intermediate aldehyde, further reduction with $NaBH_4$ to the benzylic alcohol, and bromination with PBr_3 (Scheme II).

The 4-hydroxyquinolines **11–13** were prepared from *p*-toluidine and β-keto esters by a literature procedure[34] and converted to the 4-haloquinolines **10h,g,m,o** by the appropriate phosphorus oxyhalide (Scheme III). The 4-chloro substituent of **10h** was readily displaced by

Scheme VI[a]



[a] Reagents: (a) $POCl_3$, *N,N*-dimethylaniline, $PhCH_3$, 110 °C; (b) $NaOCH_3$, MeOH, 65 °C; (c) (1) cinnamoyl chloride, py, CH_2Cl_2, (2) $AlCl_3$, 100 °C; (d) (1) $TsOCH_3$, 120 °C, (2) pivaloyl chloride, Et_3N, DMF; (e) (1) NH_3 gas, PhOH, 140 °C, (2) Ac_2O.

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Table I.[a] Preparation and in Vitro Activities of Antifolate Diacids

compd	quinoline precursor	R^1	R^2	R^3	R^4	method[b]	% yield	mp, °C	formula[c]	IC_{50}, μM: inhibn of TS	IC_{50}, μM: inhibn of L1210 cell growth in culture
1[d]										0.02	3.4
2a	**8a**	CH_3	OH	$CH_2C{\equiv}CH$	H	E,A,B	90	235–237	$C_{26}H_{25}N_3O_6{\cdot}1.5H_2O$	6.3	>100
2b	**8b**	CH_3	H	$CH_2C{\equiv}CH$	H	D,A,B	64	103–108	$C_{26}H_{25}N_3O_5{\cdot}1.75H_2O$[e]	7.9	>100
2c	**8c**	CH_3	OCH_3	$CH_2C{\equiv}CH$	H	F,D,A,B	62	178–182	$C_{27}H_{27}N_3O_6{\cdot}2H_2O$	6.0	>100
2d	**8d**	CH_3	SCH_3	$CH_2C{\equiv}CH$	H	C,G,D,A,B	61	105–112	$C_{27}H_{27}N_3O_5S{\cdot}2H_2O$	8.9	100
2e	**8e**	CH_3	CH_3	$CH_2C{\equiv}CH$	H	C,D,A,B	53	118–120	$C_{27}H_{27}N_3O_5{\cdot}2.6H_2O$	0.8	>100
2f	**8f**	CH_3	CN	$CH_2C{\equiv}CH$	H	C,H,D,A,B	68	133–139	$C_{27}H_{24}N_4O_5{\cdot}H_2O$	1.5	75
2g	**8g**	CH_3	Br	$CH_2C{\equiv}CH$	H	C,D,A,B	65	135–140	$C_{26}H_{24}BrN_3O_5{\cdot}1.5H_2O$	0.22	14
2h	**8h**	CH_3	Cl	$CH_2C{\equiv}CH$	H	C,D,A,B	91	130–135	$C_{26}H_{24}ClN_3O_5{\cdot}H_2O$	0.25	8
2i	**8h**	CH_3	Cl	H	H	C,D,A,B	87	148–153	$C_{23}H_{22}ClN_3O_5{\cdot}1.5H_2O$	38	>100
2j	**8h**	CH_3	Cl	CH_3	H	C,D,A,B	94	180–182	$C_{24}H_{24}ClN_3O_5{\cdot}1.5H_2O$	2.3	24
2k	**8h**	CH_3	Cl	CH_2CH_3	H	C,D,A,B	92	128–130	$C_{25}H_{26}ClN_3O_5{\cdot}0.75H_2O$	0.74	23
2l	**8l**	H	Cl	CH_2CH_3	H	D,A,B	23	114–120	$C_{24}H_{24}ClN_3O_5{\cdot}HCO_2H$[f]	1.13	30
2m	**8m**	CH_2CH_3	Cl	$CH_2C{\equiv}CH$	H	C,D,A,B	90	104–110	$C_{27}H_{26}ClN_3O_5{\cdot}H_2O$	0.99	>100
2n	**8n**	CH_2F	Cl	$CH_2C{\equiv}CH$	H	I,J,D,A,B	76	97–106	$C_{26}H_{23}ClFN_3O_5{\cdot}2H_2O$	0.62	>100
2o	**8o**	CF_3	Cl	$CH_2C{\equiv}CH$	H	C,D,A,B	77	90–94	$C_{26}H_{21}ClF_3N_3O_5{\cdot}0.5H_2O$	0.15	>100
2p	**8p**	CH_2OH	Cl	CH_2CH_3	H	K,D,A,B	89	119–125	$C_{25}H_{26}ClN_3O_6{\cdot}0.75H_2O$	1.05	>100
2q	**8q**	CO_2H	Cl	$CH_2C{\equiv}CH$	H	C,D,A,B	83	145–150	$C_{26}H_{22}ClN_3O_7{\cdot}H_2O$	31	>100
2r	**8r**	$CONH_2$	Cl	$CH_2C{\equiv}CH$	H	B,L,D,A,B	60	140–145	$C_{26}H_{23}ClN_4O_6{\cdot}0.5H_2O$	1.9	>100
2s	**8s**	OCH_3	Cl	$CH_2C{\equiv}CH$	H	C,F,D,A,B	54	132–134	$C_{26}H_{24}ClN_3O_6{\cdot}H_2O$	9.0	>100
2t	**8t**	NH_2	Cl	$CH_2C{\equiv}CH$	H	M,D,A,N	52	177–179	$C_{25}H_{23}ClN_4O_5{\cdot}1.5H_2O$	0.096	11
2u	**8u**	$NHCOCH_3$	Cl	$CH_2C{\equiv}CH$	H	O,D,A,S	51	160–165	$C_{27}H_{25}ClN_4O_6{\cdot}0.8CF_3CO_2H$	1.2	>100
2v	**8v**	Cl	Cl	$CH_2C{\equiv}CH$	H	C,D,A,S	65	110–117	$C_{25}H_{21}Cl_2N_3O_5{\cdot}0.5H_2O$	0.064	8
2w	**8w**	Cl	H	$CH_2C{\equiv}CH$	H	P,C,D,A,B	52	114–120	$C_{25}H_{22}ClN_3O_5{\cdot}1.1H_2O$	2.6	>100
2x	**8x**	Cl	CH_3	$CH_2C{\equiv}CH$	H	C,D,A,B	77	120–126	$C_{26}H_{24}ClN_3O_5{\cdot}H_2O$	0.12	>100
2y	**8y**	Cl	OCH_3	$CH_2C{\equiv}CH$	H	C,F,D,A,B	76	85–90	$C_{26}H_{24}ClN_3O_6{\cdot}2.25H_2O$[g]	0.15	*h*
2z	**8z**	NH_2	CH_3	$CH_2C{\equiv}CH$	H	Q,D,A,B	55	205–210	$C_{26}H_{26}N_4O_5{\cdot}1.25H_2O$	0.35	4
2aa	**8aa**	NH_2	OCH_3	CH_2CH_3	H	R,D,A,B	93	200–206	$C_{25}H_{26}N_4O_6{\cdot}2H_2O$	1.9	100
2bb	**8h**	CH_3	Cl	$CH_2C{\equiv}CH$	F	C,D,A,B	61	115–120	$C_{26}H_{23}ClFN_3O_5{\cdot}2H_2O$	0.066	0.42
2cc	**8g**	CH_3	Br	$CH_2C{\equiv}CH$	F	C,D,A,B	65	121–125	$C_{26}H_{23}BrFN_3O_5{\cdot}1.5H_2O$	0.096	1.0
2dd	**8h**	Cl	Cl	$CH_2C{\equiv}CH$	F	C,D,A,S	72	88–94	$C_{25}H_{20}Cl_2FN_3O_5{\cdot}H_2O$	0.023	1.0
2ee	**8h**	NH_2	Cl	$CH_2C{\equiv}CH$	F	Q,D,A,B	56	165–170	$C_{25}H_{22}ClFN_4O_5{\cdot}HCl{\cdot}0.5H_2O$[i]	0.048	2.9

[a] See Scheme I for positions of substituents R^1–R^4. [b] See Experimental Section for lettered methods. [c] Anal. C, H, N except where stated otherwise. [d] See ref 21. [e] N: calcd, 8.6; found, 8.0. [f] 2l was purified by flash chromatography on silica gel eluting with 1% HCO_2H in EtOAc as eluant. The NMR spectrum indicated the presence of 1.0 mol HCO_2H. [g] H: calcd, 5.2; found 4.6. [h] Insufficient solubility in test medium. [i] N: calcd, 10.0; found 9.3.

NaOMe or NaSMe to give **10c** and **10d** respectively. The 4-bromo substituent of **10g** was displaced with CuCN to give the nitrile **10f** (Scheme IV). The 2-methyl group of **10h** was oxidized to a hydroxymethyl substituent by the sequence of N-oxidation with *m*-CPBA, acetylation with rearrangement, and hydrolysis to give **10p** (Scheme IV). The fluoromethyl compound **10n** was subsequently prepared from **10p** by reaction with Et_2NSF_3 (Scheme IV).

The synthesis of the 2-carboxyquinoline **10q** was achieved by a literature procedure[35] involving the reaction of *p*-toluidine with diethyl acetylenedicarboxylate to give the ester **15**, chlorination with $POCl_3$ to give the 4-chloro compound **16**, and hydrolysis of the ester group. The resulting acid **10q** was converted into amide **10r** via the acid chloride of **10q** and rearranged to the *t*-BOC-protected amine **10t** with diphenyl phosphorazidate in *t*-BuOH. Compound **10t** was subsequently deprotected and acetylated to give the amide **10u** (Scheme V).

2,4-Dichloro-6-methylquinoline (**10v**), prepared from the corresponding 4-hydroxycarbostyril **17**,[36] was heated with NaOMe in MeOH to give a mixture of the monomethyl ethers **10y** and **10s**, which were separated by chromatography (Scheme VI). 2-Chloro-6-methylquinoline **10w** was prepared from *p*-toluidine by the sequence of condensation with cinnamoyl chloride, cyclization and elimination of benzene with $AlCl_3$[37] to give 6-methylcarbostyril (**18**), and chlorination with $POCl_3$. 2-Chloro-4,6-dimethylquinoline (**10x**), synthesized similarly from the corresponding carbostyril **20** was converted into the 2-acetylamino derivative **10z** with ammonia gas in molten phenol and subsequent acetylation. The precursor (**10aa**) to the 2-amino-4-methoxyquinoline analogue **2aa** was prepared from 2-amino-4-hydroxy-6-methylquinoline (**19**)[38] by O-methylation with methyl tosylate and acylation with pivaloyl chloride.

Biological Evaluation

The antifolate diacids were tested as inhibitors of partially purified TS from L1210 mouse leukemia cells that overproduce TS due to amplification of the TS gene.[39]

(35) Heindel, N. D.; Bechara, I. S.; Kennewell, P. D.; Molnar, J.; Ohmacht, C. J.; Lemke, S. M.; Lemke, T. F. Cyclisation of Aniline-Acetylenedicarboxylate Adducts. A Modified Conrad-Limpach Method for the Synthesis of Potential Antimalarials. *J. Med. Chem.* **1968**, *11*, 1218–1221.

(36) Zeigler, E.; Fortaita, H. G.; Kappe, T. Synthesen von Heterocyclen, 92. Mitt.: Uber die Synthese Substituierter Indigoderivate. *Monatsh. Chem.* **1967**, *98*, 324–328.

(37) Johnston, K. M.; Luker, R. M.; Williams, G. H. Friedel Crafts Cyclisations. Part III. Synthesis of Derivatives of 2(1H)-Quinolone (Carbostyril) by Aluminum Chloride-Catalysed Cycloeliminations of Cinnamanilide and Related Compounds. *J. Chem. Soc. Perkin Trans 1* **1972**, 1648–1652.

(38) Kadin, S. B.; Lamphere, C. H. A Convenient Synthesis of 2-Amino-4-hydroxyquinolines. *Synthesis* **1977**, 500–501.

(39) Jackman, A. L.; Alison, D. L.; Calvert, A. H.; Harrap, K. R. Increased Thymidylate Synthase in L1210 Cells Possessing Acquired Resistance to N^{10}-Propargyl-5,8-dideazafolic Acid (CB3717): Development, Characterisation, and Cross-Resistance Studies. *Cancer Res.* **1986**, *46*, 2810–2815.

The partial purification and assay methods used in this study were as previously described and used a (±)-5,10-methylenetetrahydrofolic acid concentration of 200 μM.[39,40] The TS inhibitor 1 was included in each assay as a positive control (IC_{50} = 20 nM). The compounds were also tested for their inhibition of the growth of L1210 cells in culture[28] and the results expressed as the concentration required to inhibit cell growth by 50% (IC_{50}). 1 was included in each assay as an internal control (IC_{50} = 3.4 μM). Both the TS and L1210 tests were performed at five inhibitor concentrations, each in duplicate. The results are collected in Table I.

Results and Discussion

Replacement of the nitrogen atom at the 3-position of 2-methylquinazolinone with carbon results in a 4-quinolinone ring system. The quinolinone-containing folate **2a** was synthesized and found to be a modest inhibitor of TS and of L1210 cell growth in vitro (Table I). However, this result is not unexpected since replacement of the N3 nitrogen atom of the quinazolinone with carbon radically alters the hydrogen-bonding properties of the bicycle. In the predominating aqueous solution tautomer[41] the N1 hydrogen-bond acceptor of the quinazoline is transformed into a donor in the quinoline and the donor at the 3-position of the quinazoline is eliminated. The ability to act as a hydrogen-bond acceptor was restored to the N1 nitrogen by replacing systematically the carbonyl oxygen at C4 with a variety of substituents that are incapable of tautomerization (Table I).

Methylation of the C4 oxygen or replacement with hydrogen did not give compounds with improved levels of TS inhibition or L1210 cell growth inhibition. However, substitution at C4 with a methyl, cyano, or halogen group led to compounds with improved enzyme inhibition. In particular the chloroquinoline **2h** is 20 times more potent than **2a** and only 10 times less potent than 1 as a TS inhibitor. In addition, it is moderately potent as an inhibitor of the growth of L1210 cells and the human-derived cell line W1-L2 (IC_{50} = 0.44 μM). Coadministration of 10 μM thymidine in the L1210 cell assay abolished the inhibition of cell growth by **2h** (at 10 times the IC_{50} value), demonstrating that TS inhibition is the sole locus of action of this compound.

The effect of varying the N10 substituent on TS inhibition in several series of quinazolinone inhibitors has been well-defined.[28] The 4-chloro-2-methylquinoline folate was chosen to explore the SAR at the N10 position in the quinoline series. The N10 hydrogen compound **2i** possessed very modest activity, while the potency increased in the order methyl < ethyl < propargyl (Table I). This is in line with the results obtained for the quinazolinone antifolates.[17] These data provide some evidence that the quinolines may bind to TS in an analogous manner to the quinazolinones.

A selection of 2-substituted 4-chloroquinoline antifolates were synthesized to define the optimal functionality at the 2-position (Table I). Replacement of the C2 methyl of **2h** with chloro (**2v**) or amino (**2t**) groups led to an enhancement of TS inhibition. The 2,4-dichloroquinoline antifolate **2v** is 4 times more potent than the corresponding 2-methyl-4-chloro compound **2h**. Unfortunately neither compound **2v** nor **2t** expresses its improved enzyme inhibition by increased L1210 cell growth inhibition. The other analogues were all less active than the C2-methyl compound **2h**. The placement of a carboxyl group at the C2 position of the quinoline is particularly detrimental to activity. Clearly the enzyme does not tolerate a negative charge in this region. The other compounds demonstrate a trend toward decreasing enzyme inhibition with increasing steric bulk at C2.

The promising activities of the 2-chloro- and 2-aminoquinoline compounds **2v** and **2t** were pursued further by the synthesis of a series of 4-substituted-2-aminoquinolines and -2-chloroquinolines (Table I). Although the enzyme tolerated the C4 chlorine of **2v** being replaced with methyl or methoxy substituents in both the 2-chloro- or 2-aminoquinolines, the C4 hydrogen substituted analogue **2w** was significantly less potent. The 2-amino-4-methylquinoline **2z** was found to inhibit L1210 cell growth while the 2-chloro-4-methyl analogue **2x** did not.

It has been established in several series of quinazolinone TS inhibitors that the placement of fluorine atoms in the *p*-aminobenzoyl ring can enhance both TS and L1210 cell growth inhibition.[22] In particular, a 2′-fluorine is very beneficial. This feature was incorporated into several 2-substituted-4-haloquinolines (Table I). In each case the TS inhibitory potency of the fluorinated analogue was improved 3- or 4-fold compared with the desfluoro compound. For example, the 2,4-dichloro compound **2dd** has an in vitro potency comparable with that of 1 with respect to its TS inhibition and is 3-fold more potent as an inhibitor of L1210 cell growth.

The poor aqueous solubility of 1 is probably due to strong intramolecular hydrogen bonding of the 2-aminoquinazolinone ring system. Compounds containing the quinoline ring system are unable to interact strongly in this way and would be expected to be more soluble. Indeed, solubility measurements[42] have shown that the quinoline analogue **2k** has an aqueous solubility (13.1 mg/mL, pH 6.72) significantly higher than 1 (0.06 mg/mL, pH 6.78). It is anticipated that any toxicity of 1 due to its poor solubility would be reduced or eliminated in the quinoline series.

In conclusion, compounds have been synthesized in which the quinazoline ring, typical of most folate-based TS inhibitors such as 1, has been replaced by a quinoline ring. This structural modification has led to the development of a series of novel antifolates, representatives of which are very potent inhibitors of TS in vitro. The effect of substitution of the C2 and C4 positions of the quinoline ring on potency has been investigated. Electron withdrawing substituents such as chlorine appear to be optimal at the C4 position. Both chloro and amino substituents are particularly favored at C2. In addition, it has been established that several quinoline antifolates are comparable to or better than 1 as inhibitors of the growth of L1210 cells in vitro. However, it is clear from the enzyme inhibition and cell growth inhibition data presented in this paper that there is not a straightforward correlation between these two important factors. This phenomenon is also found within the quinazoline antifolate series of TS inhibitors. It is generally accepted in the quinazolinone series that the extents of polyglutamation and active transport into cells have profound effects on intracellular

(40) Sikora, E.; Jackman, A. L.; Newell, D. R.; Calvert, A. H. Formation and Retention and Biological Activity of N^{10}-Propargyl-5,8-dideazafolic Acid (CB3717) Polyglutamates in L1210 Cells in vitro. *Biochem. Pharmacol.* **1988**, *37*, 4047–4054.

(41) Tucker, G. F.; Irvin, J. L. Apparent Ionisation Exponents of 4-Hydroxyquinoline, 4-Methoxyquinoline and N-Methyl-4-quinolone; Evaluation of Lactam-Lactim Tautomerisation. *J. Am. Chem. Soc.* **1951**, *73*, 1923–1929.

(42) Solubility values were determined in 0.2 M aqueous NaH_2PO_4 by Dr. J. J. Morris.

levels of the antifolates.[6,22] Evidence for the importance of these mechanisms in the quinoline series and the further exploration of the structural features important for TS inhibition in the quinoline series will be reported elsewhere.

Experimental Section

General Procedures. All experiments were carried out under an atmosphere of argon and at room temperature unless otherwise stated. *N,N*-Dimethylformamide (DMF) was purified by distillation onto molecular sieves at 10 mmHg. Flash chromatography was performed on Merck Kieselgel 60 (art. 9385). TLC was carried out on precoated silica gel plates (Merck Art. 5715) and the resulting chromatograms visualized under UV light (254 nm). The purity of products was assessed by HPLC analysis on a Hichrom S50DS1 Spherisorb Column system set to run isocratically with 60–70% methanol and 0.2% trifluoroacetic acid in water as eluant. Melting points were determined on a Koffler block apparatus and are uncorrected. The proton NMR spectra were determined on a Bruker AM 200 (200 MHz) spectrometer. Chemical shifts are expressed in units of δ (ppm) and peak multiplicities are designated as follows: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; q, quartet; br s, broad singlet; m, multiplet. Fast atom bombardment (FAB) mass spectra were determined with a VG MS9 spectrometer and a Finnigan Incos data system using DMSO as the solvent and glycerol as the matrix. NMR and mass spectra were obtained for all final products and intermediates and are consistent with the proposed structures.

Diethyl *N*-[4-[*N*-[(4-Chloro-2-methylquinolin-6-yl)methyl]-*N*-prop-2-ynylamino]-2-fluorobenzoyl]-L-glutamate (3bb). Method A. A mixture of 8h (4.67 g, 17.3 mmol), diethyl *N*-[2-fluoro-4-(prop-2-ynylamino)benzoyl]-L-glutamate (21) (6.56 g 17.4 mmol),[22] and 2,6-lutidine (2.05 mL, 17.6 mmol) in DMF (30 mL) was stirred at 70 °C for 18 h. The mixture was cooled, poured into H_2O (300 mL), and extracted with EtOAc (3 × 200 mL). The combined extracts were evaporated to give a dark oil which was purified by chromatography using Et_2O as eluant to give the diester **3bb** as a gum: 2.04 g (21%); NMR δ ($CDCl_3$) 1.23 (t, J = 6 Hz, 3 H, CH_2CH_3), 1.30 (t, J = 6.5 Hz, 3 H, CH_2CH_3), 2.12 (m, 1 H, CH_2CO_2Et), 2.32 (t, J = 1.5 Hz, 1 H, C≡CH), 2.43 (m, 3 H, $CH_2CH_2CO_2Et$), 2.76 (s, 3 H, CH_3), 4.09 (q, J = 6.5 Hz, 2 H, CH_2CH_3) 4.16 (d, J = 1.5 Hz, 2 H, CH_2C≡CH), 4.22 (q, J = 6.5 Hz, 2 H, CH_2CH_3), 4.83 (m, 3 H, CH_2N and NHCH), 6.55 (dd, J = 13.5 and 2 Hz, 1 H, Ar 3′-H), 6.72 (dd, J = 10.5 and 2 Hz, 1 H, Ar 5′-H), 7.19 (m, 1 H, NH), 7.43 (s, 1 H, Ar 3-H), 7.65 (dd, J = 8.5 and 1.5 Hz, 1 H, Ar 7-H), 7.98 (t, J = 8.5 Hz, Ar 6′-H), 8.06 (m, 2 H, Ar 5- and 8-H); MS (CI) m/z 568 $[M + H]^+$.

This procedure was repeated with the appropriate amines and bromides to yield the diesters **3a–aa,cc–ee**. All the diesters were purified by chromatography to give gums or amorphous solids that were homogeneous by TLC. All compounds had NMR and mass spectra consistent with the assigned structures.

***N*-[4-[*N*-[(4-Chloro-2-methylquinolin-6-yl)methyl]-*N*-prop-2-ynylamino]-2-fluorobenzoyl]-L-glutamic Acid (2bb). Method B.** The diester **3bb** (2.04 g, 3.59 mmol) was stirred for 2.5 h in a mixture of 1 N aqueous NaOH (21.6 mL, 21.6 mmol) and EtOH (40 mL). The EtOH was evaporated and the resulting aqueous solution was filtered and acidified to pH 3 and 2 N HCl. The resulting precipitate was isolated by filtration, washed with H_2O (50 mL) and Et_2O (50 mL) and dried to give **2bb** as a white amorphous solid: 1.12 g (61%); mp 115–120 °C; NMR δ (Me_2SO-d_6) 2.0 (m, 2 H, CH_2), 2.32 (t, J = 7.5 Hz, 2 H, CH_2CO_2H), 2.64 (s, 3 H, CH_3), 3.28 (br s, 1 H, C≡CH), 4.37 (m, 1 H, NHCH), 4.41 (d, J = 1.5 Hz, 2 H, CH_2C≡CH), 4.93 (s, 2 H, CH_2N), 6.65 (dd, J = 1.5 and 15 Hz, 1 H, Ar), 6.72 (dd, J = 1.5 and 8 Hz, 1 H, Ar), 7.56 (t, J = 9 Hz, 1 H, Ar), 7.67 (s, 1 H, Ar), 7.75 (dd, J = 1.5 and 8 Hz, 1 H, Ar), 7.93 (m, 1 H, NH), 7.97 (d, J = 8 Hz, 1 H, Ar) 8.08 (d, J = 1.5 Hz, 1 H, Ar); MS (FAB) m/z 510 $[M - H]^-$. Anal. ($C_{26}H_{23}ClFN_3O_5 \cdot 2H_2O$), C, H, N.

This procedure was repeated with diesters **3a–s,u,w–y,cc,ee** to yield the antifolates **2a–s,u,w–y,cc,ee** as amorphous solids. For the diesters **3z** and **3aa** the procedure was modified by heating the compounds in ethanolic base at 65 °C for 18 h to give **2z** and **2aa** as amorphous solids. All compounds had correct elemental analyses (C, H, N) for the formulae listed unless otherwise stated (Table I) and NMR and mass spectra consistent with the assigned structures.

4-Chloro-2,6-dimethylquinoline (10h). Method C. A mixture of 11[43] (20 g, 116 mmol) *N,N*-dimethylaniline (27.97 g, 231 mmol), and $POCl_3$ (14.18 g, 92.4 mmol) in toluene (200 mL) was stirred at 90 °C for 3.5 h. The mixture was poured into ice water (100 mL) and extracted with $CHCl_3$ (4 × 100 mL). The combined extracts were evaporated to give a dark oil which was purified by chromatography using 20% v/v EtOAc in hexane as eluent. The product (**10h**) was isolated as a white solid: 18.9 g (85%); NMR δ ($CDCl_3$) 2.55 (s, 3 H, CH_3), 2.70 (s, 3 H, CH_3), 7.31 (s, 1 H, Ar 3-H), 7.52 (dd, J = 9 and 1.5 Hz, Ar 7-H), 7.90 (d, J = 9 Hz, 1 H, Ar 8-H), 7.93 (d, J = 1.5 Hz, 1 H, Ar 5-H); MS (CI) m/z 192 $[M + H]^+$. Anal. ($C_{11}H_{10}ClN$) C, H, N.

This procedure was repeated with quinolones **12**, **13**, **15**, **17**, **18**, and **20** to yield chloroquinolines **10m,o**, **16**, and **10v–x**. The bromoquinoline **10g** was prepared from 11 by this procedure using $POBr_3$ in place of $POCl_3$. All compounds had NMR and mass spectra consistent with the assigned structures.

6-(Bromomethyl)-4-chloro-2-methylquinoline (8h). Method D. A mixture of 10h (6.0 g, 31.3 mmol), NBS (5.96 g, 33.5 mmol) and benzoyl peroxide (0.151 g, 0.550 mmol) in CCl_4 (120 mL) was stirred at 77 °C and illuminated by a 250-W tungsten light bulb for 3 h. The mixture was filtered and the filtrate evaporated to give an orange solid which was purified by chromatography using 20% v/v EtOAc in hexane as eluant. The product **8h** was isolated as a white solid: 4.7 g (56%); mp 111–113 °C; NMR δ ($CDCl_3$) 2.64 (s, 3 H, CH_3), 4.60 (s, 2 H, CH_2), 7.32 (s, 1 H, Ar 3-H), 7.64 (dd, J = 9 and 2 Hz, 1 H, Ar 7-H), 7.91 (d, J = 9 Hz, 1 H, Ar 8-H), 8.07 (d, J = 2 Hz, 1 H, 6-H).

This procedure was repeated with quinolines **10b–h,l–o**, **14**, **16**, **10r–aa** to give (bromomethyl)quinolines **8b–h,l–o,p,q,r–aa**. All compounds had NMR and mass spectra consistent with the assigned structures.

6-(Bromomethyl)-4-hydroxy-2-methylquinoline (8a). Method E. A mixture of 6-cyano-2-methyl-4-quinolone (**9a**[33]) (9.2 g, 50 mmol) and Raney nickel (10 g) in 75% HCO_2H (150 mL) was heated at reflux for 3 h. The mixture was cooled, neutralized with saturated aqueous $NaHCO_3$, and filtered. The solid residue was washed with warm methanol (3 × 50 mL). The combined filtrates were extracted with EtOAc (3 × 50 mL). The combined extracts were washed with H_2O (100 mL), dried (Na_2SO_4), filtered, and evaporated to give 6-formyl-2-methyl-4-quinolone (9.3 g, 99%) as a fawn solid.

A mixture of 6-formyl-2-methyl-4-quinolone (9.3 g, 49.7 mmol) and $NaBH_4$ (3.8 g, 100 mmol) in EtOH (100 mL) was stirred for 18 h under argon. Water (50 mL) was added and the solution adjusted to pH 6 with 1 N HCl, the solvents were evaporated, methanol (50 mL) was added, and the mixture was filtered. The filtrate was evaporated to give 6-(hydroxymethyl)-2-methyl-4-quinolone as an off-white solid (8.1 g, 86%), mp 249–252 °C.

A mixture of 6-(hydroxymethyl)-2-methyl-4-quinolone (8.1 g, 42.9 mmol) and PBr_3 (11.8 g, 43.5 mmol) in Et_2O (150 mL) was heated under reflux for 2 h. The mixture was cooled, poured into H_2O (100 mL), and the resulting solid **8a** (10.6 g, 98%) isolated by filtration: mp 190–200 °C; NMR δ (Me_2SO) 2.48 (s, 3 H, CH_3), 4.90 (s, 2 H, CH_2), 6.35 (s, 1 H, Ar 3-H), 7.72 (m, 2 H, Ar 7-H, 8-H), 8.20 (d, J = 2.0 Hz, 1 H, Ar 5-H), 12.84 (br s, 1 H, NH); MS (CI) m/z 174 $[M + H]^+$.

2,6-Dimethyl-4-methoxyquinoline (10c). Method F. A mixture of 10h (0.50 g, 2.61 mmol) and NaOMe (0.92 g, 17.0 mmol) in MeOH (10 mL) was heated at reflux for 17 h. The mixture was cooled, the solvent evaporated, and the residue purified by chromatography using 5% v/v MeOH in CH_2Cl_2 as eluant. The product **10c** was isolated as a white solid: 0.29 g (59%); NMR δ ($CDCl_3$) 2.51 (s, 3 H, CH_3), 2.70 (s, 3 H, CH_3), 4.01 (s, 3 H, OCH_3), 6.60 (s, 1 H, Ar 3-H), 7.48 (dd, J = 9 and 2 Hz, 1 H, Ar 7-H), 7.86 (d, J = 9 Hz, 1 H, Ar 8-H), 7.91 (br s, 1 H, Ar 5-H); MS (CI) m/z 188 $[M + H]^+$.

The procedure was repeated with **10v** heating at reflux for 1 h to yield a mixture of **10s** and **10y**, which were separated by chromatography using CH_2Cl_2 as eluant.

2,6-Dimethyl-4-methylthioquinoline (10d). Method G. A mixture of 10h (0.50 g, 2.61 mmol) and NaSMe (0.55 g, 7.86 mmol) in CH_3CN (20 mL) was heated at reflux for 15 h. The mixture

(43) Prepared by the general method described in ref 31.

was cooled, poured into H_2O (50 mL), and extracted with EtOAc (2 × 50 mL). The organic layers were dried ($MgSO_4$) and evaporated to yield **10d** (0.52 g, 98%) as a white solid: NMR δ (Me_2SO-d_6) 2.57 (s, 3 H, CH_3), 2.60 (s, 3 H, CH_3), 3.21 (s, 3 H, SCH_3), 7.11 (s, 1 H, Ar 3-H), 7.47 (dd, J = 9 and 2 Hz, Ar 7-H), 7.66 (br s, 1 H, Ar 5-H), 7.73 (d, J = 9 Hz, 1 H, Ar 8-H); MS (CI) m/z 204 $[M + H]^+$.

4-Cyano-2,6-dimethylquinoline (10f). Method H. A mixture of **10g** (1.70 g, 7.20 mmol) and CuCN (1.64 g, 18.3 mmol) in DMF (25 mL) was heated at 140 °C for 6 h. The mixture was poured into ice water (40 mL) and ethylenediamine (1 mL) and extracted with EtOAc (4 × 100 mL). The combined extracts were evaporated, and the residue was purified by chromatography using 20% EtOAc in hexane as eluant. The product **10f** was isolated as a yellow solid: 0.60 g (46%); mp 113–115 °C; NMR δ ($CDCl_3$) 2.60 (s, 3 H, CH_3), 2.80 (s, 3 H, CH_3), 7.54 (s, 1 H, Ar 3-H), 7.60 (dd, J = 2 and 9 Hz, Ar 7-H), 7.90 (d, J = 2 Hz, 1 H, Ar 6-H), 7.94 (d, J = 9 Hz, 1 H, Ar 8-H); MS (CI) m/z $[183]^+$.

4-Chloro-2-(hydroxymethyl)-6-methylquinoline (10p). Method I. To a solution of **10h** (4.0 g, 20.9 mmol) in $CHCl_3$ (50 mL) was added dropwise a solution of *m*-chloroperbenzoic acid (6.75 g, 39.1 mmol) in $CHCl_3$ (50 mL). The mixture was stirred for 18 h, washed with 10% aqueous Na_2CO_3 (2 × 50 mL) and water (50 mL), and evaporated. A mixture of the residue, purified by chromatography using EtOAc as eluant, and acetic anhydride (30 mL) was stirred at 80 °C for 2 h, the solvent evaporated, and a mixture of the residue and 10% HCl (25 mL) heated at reflux for 30 min. The resulting mixture was cooled, basified to pH 14 with 2 N NaOH, and extracted with EtOAc (3 × 50 mL). The combined extracts were evaporated, and the residue was purified by chromatography using 60% v/v EtOAc in hexane as eluant. The product **10p** was isolated as a white solid: 2.00 g (46%); mp 147–148 °C; NMR δ ($CDCl_3$) 2.57 (s, 3 H, CH_3), 4.86 (s, 2 H, CH_2), 7.36 (s, 1 H, Ar 3-H), 7.56 (dd, J = 9 and 1.5 Hz, 1 H, Ar 7-H), 7.94 (br, s, 1 H, Ar 5-H), 7.95 (d, J = 9 Hz, 1 H, Ar 8-H); MS (CI) m/z 208 $[M + H]^+$.

4-Chloro-2-(fluoromethyl)-6-methylquinoline (10n). Method J. To a solution of Et_2NSF_3 (1.20 g, 7.42 mmol) in CH_2Cl_2 (10 mL) at −70 °C was added dropwise a solution of **10p** (1.0 g, 4.82 mmol) in CH_2Cl_2 (30 mL). The mixture was warmed to 20 °C and washed with H_2O, and the solvent evaporated. The residue was purified by chromatography using CH_2Cl_2 as eluant. The product **10n** was obtained as a white solid (0.28 g, 28%) and without further characterization converted to **8n** using method D: 0.27 g (69%); NMR δ ($CDCl_3$) 4.70 (s, 2 H, CH_2Br), 5.62 (d, J = 47 Hz, 1 H, CH_2F), 7.72 (d, J = 1 Hz, 1 H, Ar 3-H), 7.81 (dd, J = 2 and 8.5 Hz, 1 H, Ar 7-H), 8.05 (d, J = 8.5 Hz, 1 H, Ar 8-H), 8.22 (d, J = 2 Hz, 1 H, Ar 5-H); MS (CI) m/z 288 $[M + H]^+$.

2-(Acetoxymethyl)-4-chloro-6-methylquinoline (14). Method K. A mixture of **10p** (1.00 g, 4.82 mmol), Ac_2O (0.91 mL, 9.66 mmol), and pyridine (0.78 mL, 9.66 mmol) in EtOAc (20 mL) was stirred at 60 °C for 18 h. The mixture was cooled, poured into H_2O (50 mL), and extracted with EtOAc (3 × 20 mL). The organic extracts were combined and evaporated, and the residue was purified by chromatography using 25% v/v EtOAc in hexane as eluant. The product **14** was isolated as a yellow solid: 0.58 g (48%); NMR δ ($CDCl_3$) 2.17 (s, 3 H, CH_3), 2.56 (s, 3 H, CH_3), 5.29 (s, 2 H, CH_2), 7.48 (s, 1 H, Ar 3-H), 7.54 (dd, J = 9 and 1.5 Hz, 1 H, Ar 7-H), 7.91 (br s, 1 H, Ar 5-H), 7.93 (d, J = 9 Hz, H, Ar 8-H). MS (CI) m/z 250 $[M + H]^+$.

4-Chloro-6-methylquinoline-2-carboxamide (10r). Method L. A mixture of **10q**[35] (1.00 g, 4.51 mmol) and $SOCl_2$ (16.3 g, 137 mmol) was heated at 80 °C for 1.5 h. The excess $SOCl_2$ was evaporated and the residue, dissolved in CH_3CN (25 mL), was added to aqueous NH_3 solution (specific gravity = 0.88, 25 mL) and stirred for 1 h. The white precipitate was isolated by filtration, washed (H_2O), and dried to yield **10r** as an off-white solid: 0.8 g (80%); NMR δ ($CDCl_3$) 2.38 (s, 3 H, CH_3), 6.63 (br s, 1 H, NH_2), 7.42 (dd, J = 9 and 1.5 Hz, 1 H, Ar 7-H), 7.71 (br s, 1 H, NH_2), 7.79 (m, 2 H, Ar 5-H and 8-H), 8.07 (s, 1 H, Ar 3-H); MS (CI) m/z 221 $[M + H]^+$.

2-[(*tert*-Butoxycarbonyl)amino]-4-chloro-6-methylquinoline (10t). Method M. A mixture of **10q**[35] (1.30 g, 5.87 mmol), *t*-BuOH (30 mL), diphenyl phosphorazidate (1.66 g, 6.00 mmol), and Et_3N (1.6 mL) in DMF (40 mL) was heated at 100 °C for 7 h. The mixture was cooled, evaporated, and purified by chromatography using CH_2Cl_2 as eluant. The product **10t** was isolated as an off-white solid: 1.12 g (68%); NMR δ ($CDCl_3$) 1.54 (s, 9 H, $C(CH_3)_3$), 2.54 (s, 3 H, CH_3), 7.47 (dd, J = 9 and 1.5 Hz, 1 H, Ar 7-H), 7.69 (m, 2 H, NH and Ar 8-H), 7.87 (br s, 1 H, Ar 5-H), 8.33 (s, 1 H, Ar 3-H); MS (CI) m/z 293 $[M + H]^+$.

***N*-[4-[*N*-[(2-Amino-4-chloroquinolin-6-yl)methyl]-*N*-prop-2-ynylamino]benzoyl]-L-glutamic Acid (2t). Method N.** A mixture of **3t** (0.22 g, 0.345 mmol) and CF_3CO_2H (5.23 g, 45.4 mmol) was stirred at 20 °C for 30 min under argon, the solvent evaporated, and the residue purified by chromatography using Et_2O then 5% v/v CH_3OH in Et_2O as eluant. A mixture of the resulting material and 1 N NaOH solution (1.40 mL, 1.40 mmol) in EtOH (7 mL) was stirred for 2.5 h, and the solvent evaporated and acidified to pH 3 with 2 N HCl. The precipitate was isolated by filtration, washed with H_2O, and dried to give **2t** as a white amorphous solid: 60 mg (35%); mp 177–179 °C; NMR δ (Me_2SO-d_6) 1.85–2.20 (m, 2 H, CH_2), 2.32 (t, J = 6.5 Hz, 2 H, CH_2CO_2H), 3.20 (s, 1 H, C≡CH), 4.28–4.45 (m, 3 H, NHC*H* and $CH_2C{\equiv}C$), 4.79 (s, 2 H, CH_2N), 6.86 (d, J = 8 Hz, 2 H, Ar), 6.91 (br s, 2 H, NH_2), 6.99 (s, 1 H, Ar 3-H), 7.5–7.6 (m, 2 H, Ar 7-H, 8-H), 7.75 (d, J = 8 Hz, 2 H, Ar), 7.86 (s, 1 H, Ar 5-H), 8.21 (d, J = 10 Hz, 1 H, CONH); MS (FAB) m/z 493 $[M - H]^-$. Anal. ($C_{25}H_{23}ClN_4O_5 \cdot 1.5H_2O$), C, H, N.

2-Acetamido-4-chloro-6-methylquinoline (10u). Method O. A mixture of **10t** (1.04 g, 3.71 mmol) and CF_3CO_2H (15 mL) was stirred for 30 min. The excess CF_3CO_2H was evaporated, and the residue dissolved in Ac_2O (10.8 g, 106 mmol) and stirred for 3 h. The excess Ac_2O was evaporated and the residue purified by chromatography using increasingly polar mixtures of CH_2Cl_2 and EtOAc as eluant to give **10u** (0.78 g, 90%): NMR δ (Me_2SO-d_6) 2.11 (s, 3 H, CH_3), 2.50 (s, 3 H, CH_3), 7.56 (dd, J = 9 and 1.5 Hz, 1 H, Ar 7-H), 7.71 (d, J = 9 Hz, 1 H, Ar 8-H), 7.78 (d, J = 1.5 Hz, 1 H, Ar 5-H), 8.40 (s, 1 H, Ar 3-H), 10.90 (br s, 1 H, NH); MS (CI) m/z 235 $[M + H]^+$.

6-Methyl-2-quinolone (18). Method P. A solution of cinnamoyl chloride (18.88 g, 113 mmol) in CH_2Cl_2 (100 mL) was added to a solution of *p*-toluidine (12.14 g, 113 mmol) and pyridine (8.94 g, 113 mmol) in CH_2Cl_2 (400 mL) at 0 °C. The mixture was stirred at 0 °C for 3 h, washed with H_2O (2 × 100 mL), 2 N HCl (2 × 100 mL), and H_2O (2 × 100 mL), and evaporated. A mixture of the resulting solid and $AlCl_3$ (16.2 g, 122 mmol) was heated at 100 °C for 1 h and poured into ice water (200 mL). The resulting precipitate was isolated by filtration, washed with 2 N HCl (100 mL) and H_2O (100 mL), and dried to give **18** as a white solid: 4.35 g (24%); NMR δ (Me_2SO-d_6) 2.37 (s, 3 H, CH_3), 6.52 (d, J = 10 Hz, 1 H, Ar), 7.2–7.5 (m, 3 H, Ar), 7.83 (d, J = 10 Hz, 1 H, Ar), 8.44 (br s, 1 H, NH); MS (CI) m/z 160 $[M + H]^+$.

2-Acetamido-4,6-dimethylquinoline (10z). Method Q. A mixture of **10x** (3.8 g, 19.8 mmol) and phenol (14 g, 149 mmol) was heated at 140 °C with NH_3 gas bubbling through the mixture for 3 h. The mixture was dissolved in KOH solution (10% w/v) and extracted with EtOAc (3 × 50 mL). The organic extracts were washed (1 N NaOH), dried ($MgSO_4$), and evaporated. The residue, purified by chromatography using 12% v/v MeOH in EtOAc as eluant, and Ac_2O (10 mL) were stirred together for 3 h and evaporated. The residue was washed with hexane (3 × 25 mL) to give **10z**: 0.37 g (8%); NMR δ ($CDCl_3$) 2.24 (s, 3 H, CH_3), 2.55 (s, 3 H, CH_3), 2.70 (s, 3 H, CH_3), 7.49 (dd, J = 8 and 1.5 Hz, 1 H, Ar 7-H), 7.69 (d, J = 1.5 Hz, 1 H, Ar 5-H), 7.70 (d, J = 8 Hz, 1 H, Ar 8-H), 8.22 (s, 1 H, Ar 3-H), 8.30 (br s, 1 H, NH); MS (CI) m/z 215 $[M + H]^+$.

4-Methoxy-6-methyl-2-(pivaloylamino)quinoline (10aa). Method R. A mixture of **19**[38] (3.1 g, 17.8 mmol) and methyl *p*-toluenesulfonate (3.32 g, 19.5 mmol) was heated at 120 °C for 30 min and left to stand at 20 °C for 18 h. The resulting solid was triturated with 2 N NaOH (100 mL) and isolated by filtration to give 2.13 g of solid. A mixture of the solid (1.0 g, 5.32 mmol) pivaloyl chloride (0.64 g, 5.31 mmol) and Et_3N (0.617 g, 6.10 mmol) in DMF (17 mL) was stirred for 18 h, poured into water (100 mL), and extracted with EtOAc (3 × 50 mL). The extracts were washed with H_2O (50 mL), dried (Na_2SO_4), and evaporated. The resulting solid was purified by chromatography using 25% v/v EtOAc in hexane as eluant to give **10aa**: 0.535 g (37%); mp 75–76 °C; NMR δ ($CDCl_3$) 1.33 (s, 9 H, $(CH_3)_3$), 2.46 (s, 3 H, CH_3), 4.02 (s, 3 H, OCH_3), 7.45 (dd, J = 8.0 and 2 Hz, 1 H, Ar 7-H), 7.67 (d, J = 8.0 Hz, 1 H, Ar 8-H), 7.88 (br s, 1 H, Ar 5-H), 7.99 (s, 1 H, Ar 3-H),

8.85 (br s, 1 H, NH); MS (CI) m/z 273 $[M + H]^+$.

***N*-[4-[*N*-[(2,4-Dichloroquinolin-6-yl)methyl]-*N*-prop-2-ynylamino]-2-fluorobenzoyl]-L-glutamic Acid (2dd). Method S.** The diester **3dd** (0.18 g, 0.279 mmol) was stirred for 15 min in trifluoroacetic acid (1.8 mL). The trifluoroacetic acid was evaporated and the residue dissolved in 1 N aqueous NaOH (5 mL), filtered, and acidified to pH 3 with 2 N HCl. The resulting precipitate was isolated by filtration, washed with H_2O (20 mL), and dried to give **2dd** as a white amorphous solid: 107 mg (72%); mp 88–94 °C; NMR δ (Me_2SO-d_6) 2.00 (m, 2 H, CH_2), 2.31 (t, J = 7.5 Hz, 2 H, CH_2CO_2H), 3.28 (br s, 1 H, C≡CH), 4.37 (m, 1 H, NHC*H*), 4.41 (d, J = 1.5 Hz, 2 H, $CH_2C{\equiv}CH$), 4.96 (s, 2 H, CH_2N), 6.65 (m, 2 H, Ar 3′-H and 5′-H), 7.54 (t, J = 9 Hz, 1 H, Ar 6′-H), 7.85 (dd, J = 8.5 and 1.5 Hz, 1 H, Ar 7-H), 7.93 (s, 1 H, Ar 3-H), 8.01 (d, J = 8.5 Hz, 1 H, Ar 8-H), 8.12 (d, J = 1.5 Hz, 1 H, Ar 5-H); MS (FAB) m/z 530 $[M - H]^-$. Anal. ($C_{25}H_{20}Cl_2FN_3O_5 \cdot H_2O$) C, H, N.

This procedure was repeated with diesters **3u** and **3v** to give **2u** and **2v** as amorphous solids. The compounds had correct elemental analyses (C, H, N) for the formulae listed (Table I) and NMR and mass spectra consistent with the assigned structures.

Registry No. 1, 76849-19-9; **2a**, 123637-02-5; **2b**, 123636-84-0; **2c**, 123637-07-0; **2d**, 123637-10-5; **2e**, 123636-86-2; **2f**, 123637-00-3; **2g**, 123636-98-6; **2h**, 123636-89-5; **2i**, 141848-56-8; **2j**, 123636-91-9; **2k**, 123636-90-8; **2l**, 123636-88-4; **2m**, 123636-97-5; **2n**, 123637-13-8; **2o**, 123636-71-5; **2p**, 123637-06-9; **2q**, 141848-57-9; **2r**, 123637-11-6; **2s**, 123637-05-8; **2t**, 123636-76-0; **2u**, 123637-26-3; **2v**, 123636-69-1; **2w**, 123637-16-1; **2x**, 123637-09-2; **2y**, 123636-72-6; **2z**, 123651-23-0; **2aa**, 123637-22-9; **2bb**, 123636-66-8; **2cc**, 123636-67-9; **2dd**, 123636-65-7; **2ee**, 123636-77-1; **3t**, 141848-58-0; **3bb**, 123637-34-3; **3dd**, 141848-59-1; **4**, 76858-72-5; **5**, 70280-71-6; **6**, 2378-95-2; **7**, 13726-52-8; **8a**, 123637-49-0; **8b**, 141848-60-4; **8e**, 141848-61-5; **8g**, 123637-46-7; **8h**, 123637-33-2; **8l**, 141848-62-6; **8m**, 123637-43-4; **8n**, 123637-71-8; **8o**, 123637-51-4; **8q**, 141848-63-7; **8s**, 123637-54-7; **8v**, 123651-25-2; **8w**, 123637-77-4; **8x**, 123637-60-5; **8v**, 123637-55-8; **9a**, 123638-03-9; **10c**, 75896-58-1; **10d**, 123637-61-6; **10f**, 123637-47-8; **10g**, 123637-45-6; **10h**, 6270-08-2; **10n**, 123637-70-7; **10p**, 123637-58-1; **10q**, 123637-66-1; **10r**, 123637-63-8; **10t**, 123651-26-3; **10u**, 123637-82-1; **10x**, 3913-18-6; **10z**, 123637-72-9; **10aa**, 123637-85-4; **11**, 25428-07-3; **12**, 123637-44-5; **13**, 123638-04-0; **14**, 141848-64-8; **15**, 90033-68-4; **17**, 1677-44-7; **18**, 4053-34-3; **19**, 123638-05-1; **20**, 23947-37-7; **21**, 106585-57-3; **22**, 112888-47-8; **23**, 123637-80-9; thymidylate synthase, 9031-61-2; 6-(hydroxymethyl)-2-methyl-4-quinolone, 123651-24-1; cinnamoyl chloride, 102-92-1; *p*-toluidine, 106-49-0; pivaolyl chloride, 3282-30-2.

Inhibition of Collagenase by Aranciamycin and Aranciamycin Derivatives

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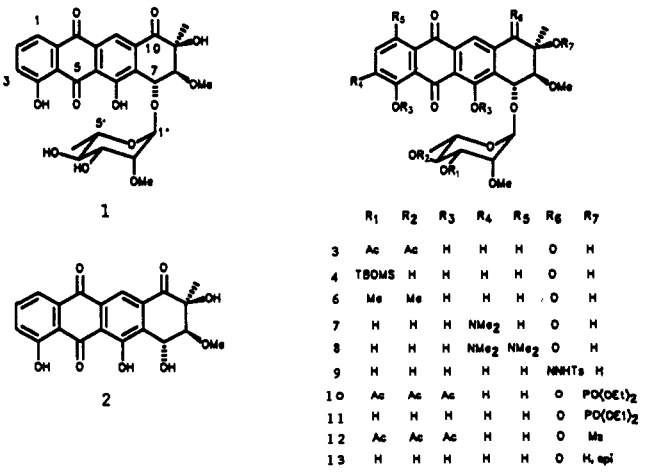
Aranciamycin (1), an anthracycline antibiotic, was found to be an inhibitor of *Clostridium histolyticum* collagenase, with an $IC_{50} = 3.7 \times 10^{-7}$ M. Elastase and trypsin were not inhibited at concentrations $\leq 10^{-6}$ M. A number of aranciamycin derivatives **2–13** were prepared and tested for collagenase inhibition. While loss of activity was found for derivatives modified in the sugar ring or rings B and D of the aglycone, increased potency was found when the tertiary alcohol at C-9 was esterified. All compounds **1–13** were found to inhibit DNA synthesis of Yoshida sarcoma tumor cells.

Introduction

Collagenase, the metalloprotease that cleaves collagen, plays an important role in the organism by "tidying up" dead or defective connective tissue.[1] However uncontrolled high levels of collagenase are suspected to be a major destructive instrument of several diseases such as arthritis[2] or tumor metastasis.[3] Thus, there is an increasing interest in collagenase inhibitors as therapeutic agents.[4]

The classes of compounds that have been found to inhibit collagenase include mostly peptide analogs,[5] but recently tetracyclines[6] and anthraquinones[7] have been found to be moderate inhibitors.

Scheme I



In this paper we report the inhibition of collagenase by the naturally occuring antibiotic aranciamycin (**1**, Scheme I), the preparation of a number of derivatives of **1**, and our biological findings regarding these derivatives.

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