Quinazoline Antifolate Thymidylate Synthase Inhibitors: Nitrogen, Oxygen, Sulfur, and Chlorine Substituents in the C2 Position

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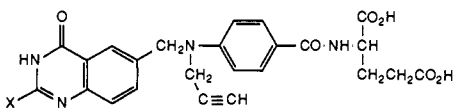
The synthesis of 16 new N^{10}-propargylquinazoline antifolates with methylamino, ethylamino, (2-aminoethyl)amino, [2-(dimethylamino)ethyl]amino, (2-hydroxyethyl)amino, (carboxymethyl)amino, dimethylamino, imidazol-1-yl, methoxy, ethoxy, phenoxy, 2-methoxyethoxy, 2-hydroxyethoxy, mercapto, methylthio, and chloro substituents at C2 is described. In general, the synthetic route involved the coupling of diethyl *N*-[4-(prop-2-ynylamino)benzoyl]-L-glutamate (**5a**) with 6-(bromomethyl)-2-chloro-3,4-dihydro-4-oxoquinazoline in *N*,*N*-dimethylformamide with calcium carbonate as the base, displacement of the C2-chloro substituent with nitrogen and sulfur nucleophiles, and deprotecton using mild alkali. The C2-ether analogues were most conveniently prepared by coupling **5a** with 6-(bromomethyl)-2,4-diakoxy(or diphenoxy)quinazolines. In this series the final deprotection step with aqueous alkali gave simultaneous selective hydrolysis of the C4-alkoxy or C4-phenoxy substituent. The compounds were tested as inhibitors of partially purified L1210 thymidylate synthase (TS). As a measure of cytotoxicity, they were examined for their inhibition of the growth of L1210 cells in culture. The C2-methoxy analogue **11a** was equivalent to the previously described tight binding TS inhibitor N^{10}-propargyl-5,8-dideazafolic acid (CB3717, ICI 155387, **1a**) against the TS enzyme and exhibited enhanced potency in culture. The C^2-methoxy substituent also gave a 110-fold enhancement in aqueous solubility relative to the C2-amine. These results suggest that **11a** will be an interesting compound for further study as a potential antitumor agent in vivo. A further series of 2-methoxyquinazoline antifolates with modified alkyl substituents at N^{10} is also described. None of these analogues equalled the activity of **11a**. Thus the propargyl group appears to be the optimum N^{10} substituent in both 2-amino- and 2-methoxyquinazoline antifolates.

The quinazoline-based antifolate N^{10}-propargyl-5,8-dideazafolic acid (**1a**)[1,2] is a potent inhibitor of the enzyme thymidylate synthase (TS, EC 2.1.1.45). Its antitumor



1a, X = NH_2
1b, X = H

activity in vitro and in vivo has been shown to result from inhibition of TS alone with no complicating action at any other locus.[3-5] On the basis of these properties **1a** was selected for clinical study. In phase I/II studies responses were observed in patients with a variety of tumor types, particularly in breast,[6] ovarian,[6,7] and hepatocellular[8] carcinomas. Despite this initial promise, the use of the compound in the clinic is likely to be limited by renal and hepatic toxicity.[6,7,9] In mice it has been demonstrated[9,10] that **1a** accumulates in both the liver and kidney, and this accumulation, in the latter at least, is probably responsible for the toxicity observed. Indeed the solubility in urine is only 0.04 mg/mL at pH 7.[9] Thus modifications to the molecule that lead to increased aqueous solubility could be expected to give compounds having lower toxicity to these key organs. Providing the antitumor potency can be maintained, this could give rise to a more acceptable clinical agent. A search for an analogue of **1a** having an improved therapeutic ratio was therefore undertaken. Modifications to the N^{10}-substituent,[11] the benzoyl ring,[12] and the amino moiety[13] have failed to produce compounds more active than **1a** as inhibitors of TS or cell growth.

Attention was turned to the C2 region of the molecule, for despite the large number of folic acid analogues made as potential anticancer agents there has been little study of the requirement for the C2-amino group. This is a potential weak hydrogen bond donor and as such may contribute to the lack of solubility of **1a** through intermolecular forces in the solid state. In following this argument a major advance was made when the highly water soluble 2-desamino analogue **1b** was synthesized.[14] Despite only an 8-fold loss in TS inhibitory activity, a 10-fold improvement in L1210 cytotoxicity was observed. Of

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(1) Jones, T. R.; Calvert, A. H.; Jackman, A. L.; Brown, S. J.; Jones, M.; Harrap, K. R. *Eur. J. Cancer* **1981**, *17*, 11.
(2) Synonyms: ICI 155387; CB3717; NSC 327182; *N*-[4-[*N*-[(2-amino-4-hydroxy-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoyl]-L-glutamic acid.
(3) Jackson, R. C.; Jackman, A. L.; Calvert, A. H. *Biochem. Pharmacol.* **1983**, *32*, 3783.
(4) Jackman, A. L.; Calvert, A. H.; Taylor, G. A.; Harrap, K. R. In *The Control of Tumour Growth and its Biological Bases*; Davis, W., Maltoni, C., Tanneberger, S., Eds.; Akademie-Verlag: Berlin, 1983; p 404.
(5) Jackman, A. L.; Taylor, G. A.; Calvert, A. H.; Harrap, K. R. *Biochem. Pharmacol.* **1984**, *33*, 3269.
(6) Calvert, A. H.; Alison, D. L.; Harland, S. J.; Robinson, B. A.; Jackman, A. L.; Jones, T. R.; Newell, D. R.; Siddik, Z. H.; Wiltshaw, E.; McElwain, T. J.; Smith, I. E.; Harrap, K. R. *J. Clin. Oncol.* **1986**, *4*, 1245.
(7) Calvert, A. H.; Newell, D. R.; Jackman, A. L.; Gumbrell, L. A.; Sikora, E.; Grzelakowska-Sztabert, B.; Bishop, J. A. M.; Judson, I. R.; Harland, S. J.; Harrap, K. R. *NCI Monogr.* **1987**, *5*, 213.
(8) Bassendine, M. F.; Curtin, N. J.; Loose, H.; Harris, A. L.; James, D. F. *J. Hepatol.* **1987**, *4*, 349.
(9) Newell, D. R.; Siddik, Z. H.; Calvert, A. H.; Jackman, A. L.; Alison, D. L.; McGhee, K. G.; Harrap, K. R. *Proc. Am. Assoc. Cancer Res.* **1982**, *23*, 181.
(10) Newell, D. R.; Alison, D. L.; Calvert, A. H.; Harrap, K. R.; Jarman, M.; Jones, T. R.; Manteuffel-Cymborowska, M.; O'Connor, P. *Cancer Treatment Rep.* **1986**, *70*, 971.
(11) Jones, T. R.; Calvert, A. H.; Jackman, A. L.; Eakin, M. A.; Smithers, M. J.; Betteridge, R. F.; Newell, D. R.; Hayter, A. J.; Stocker, A.; Harland, S. J.; Davies, L. C.; Harrap, K. R. *J. Med. Chem.* **1985**, *28*, 1468.
(12) Jones, T. R.; Smithers, M. J.; Taylor, M. A.; Jackman, A. L.; Calvert, A. H.; Harland, S. J.; Harrap, K. R. *J. Med. Chem.* **1986**, *29*, 468.
(13) Jones, T. R.; Smithers, M. J.; Betteridge, R. F.; Taylor, M. A.; Jackman, A. L.; Calvert, A. H.; Davies, L. C.; Harrap, K. R. *J. Med. Chem.* **1986**, *29*, 1114.
(14) Jones, T. R.; Jackman, A. L.; Thornton, T. J.; Flinn, A.; O'Connor, B. *Proc. Am. Assoc. Cancer Res.* **1987**, *28*, 276.

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Table I. Preparation of Antifolate Diesters 7 (Method A)

compd	starting amine	C2-substituent	% yield
7a	methylamine (33% in EtOH)	$NHCH_3$	83
7b	ethylamine	$NHCH_2CH_3$	61
7c	ethylenediamine	$NHCH_2CH_2NH_2$	38
7d	*N,N*-dimethylethylenediamine	$NHCH_2CH_2N(CH_3)_2$	56
7e	ethanolamine	$NHCH_2CH_2OH$	82[a]
7f	glycine ethyl ester hydrochloride	$NHCH_2CO_2Et$	68
7g	dimethylamine (33% in EtOH)	$N(CH_3)_2$	85[a]
7h	imidazole	imidazol-1-yl	96

[a] The product was purified by trituration with 25% MeCN in H_2O.

Table II. Preparation of Antifolate Diacids 2, 17, and 18 (Method B)

compd	C2-substituent	% yield	mp, °C	formula[a]	mass spectra, *m/z* [M − H]	inhibn of TS (IRP)[b]	inhibn of L1210 cell growth in culture: IC_{50}, μM
1a[c]	NH_2	81	232–235	$C_{24}H_{23}N_5O_6$		1.0	3.4
2a	$NHCH_3$	95	208–211	$C_{25}H_{25}N_5O_6 \cdot 2.5H_2O$[f]	490	9.2	24.0
2b	$NHCH_2CH_3$	72	174–188	$C_{26}H_{27}N_5O_6 \cdot 2.2H_2O$	504	15.0	>100
2c	$NHCH_2CH_2NH_2$	79	>240	$C_{26}H_{28}N_6O_6 \cdot 4H_2O$[g]	519	23.0	97.0
2d	$NHCH_2CH_2N(CH_3)_2$	27	192–195	$C_{28}H_{32}N_6O_6 \cdot 2.6H_2O$	547	260.9	>100
2e	$NHCH_2CH_2OH$	62	182–185	$C_{26}H_{27}N_5O_7 \cdot 2.5H_2O$[h]	520	9.5	47.0
2f	$NHCH_2CO_2H$	23[d]	191–196	$C_{26}H_{25}N_5O_8 \cdot 2H_2O$[i]	534	60.0	>100
23g	$N(CH_3)_2$	61	185–187.5	$C_{26}H_{27}N_5O_6 \cdot 1.5H_2O$	504	115.4	>100
2h	imidazol-1-yl	69[d]	155[e]	$C_{27}H_{24}N_6O_6 \cdot H_2O$	527	217.4	>100
17a	SH	92[d]	161–166	$C_{24}H_{22}N_4O_6S \cdot H_2O$	493	26.3	60.0
17b	SCH_3	94	157–163	$C_{25}H_{24}N_4O_6S \cdot 0.75H_2O$	507	5.8	13.0
18	Cl	95	150[e]	$C_{24}H_{21}ClN_4O_6 \cdot 1.33H_2O$	495	10.0	67.0

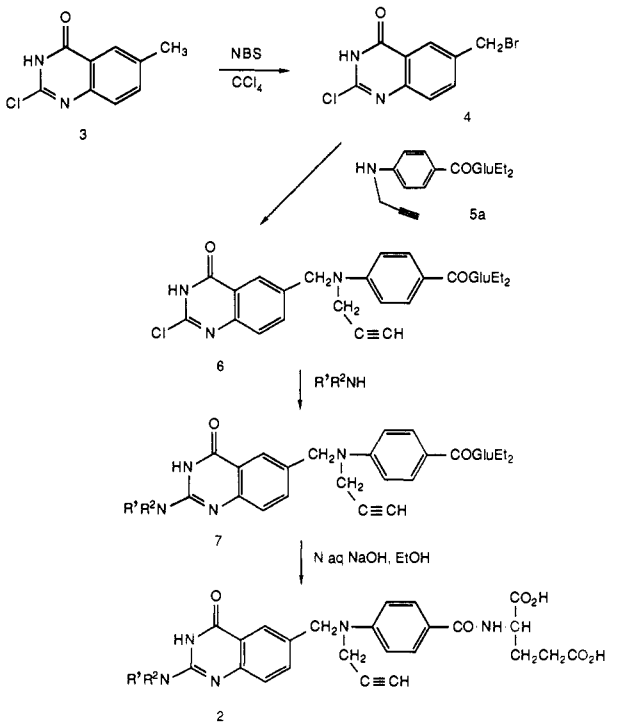
[a] Anal. C, H, N except where stated otherwise. [b] IRP = inverse relative potency, defined as I_{50} (compound)/I_{50} (1a) determined in the same test. [c] See ref 1. [d] Reaction carried out at 40 °C. [e] Sinters above this temperature but does not give a discrete melting point. [f] H: calcd, 5.6; found, 4.9. [g] H: calcd, 6.1; found, 5.5. [h] H: calcd, 5.7; found, 5.0. [i] H: calcd, 5.0; found, 4.5.

particular note was the lack of renal and hepatic toxicity in mice.[15] This observation of improved cytotoxicity in the C2-desamino derivatives stimulated us to undertake a systematic study of new substituents in the C2 position of the quinazoline ring with the aim of finding analogues of **1a** that combine similar or greater potency against the TS enzyme with increased aqueous solubility in anticipation of lower toxicity. In this paper we describe the synthesis and biological activity of a novel series of analogues of **1a** containing nitrogen, oxygen, sulfur, and chlorine substituents in this position of the quinazoline ring.

Chemistry

The series of compounds (**2a–h**) having secondary and tertiary amino functionality at C2 were synthesized by the procedure outlined in Scheme I. The strategy involved the key 2-chloroquinazoline folate diester **6**, which readily reacted with a variety of amine nucleophiles to insert the required 2-alkylamino groups. This 2-chloro intermediate was conveniently prepared in 50% overall yield by the bromination of 2-chloro-3,4-dihydro-4-oxo-6-methylquinazoline (**3**)[16] using *N*-bromosuccinimide (NBS) followed by alkylation of diethyl *N*-[*p*-(*N*-propargylamino)benzoyl]glutamate (**5a**)[1] with the resulting bromo derivative **4**. Treatment of **6** with the amines listed in Table I afforded the desired antifolate diesters **7a–h**. In this reaction high yields were achieved and unwanted amide byproducts were avoided when 1-methyl-2-pyrrolidinone at 100 °C was used as solvent. Of the amines tried in this reaction, only glycine failed to react with **6**. However, glycine ethyl ester hydrochloride reacted smoothly under the standard conditions to yield the triethyl ester precursor **7f** to the 2-[(carboxymethyl)amino]quinazoline antifolate **2f**. This observation suggests that protonation at N1 promotes the nucleophilic displacement of the C2-chlorine atom. Deprotection of the diesters was accomplished in the final step by treatment with aqueous alkali (Table II).

Scheme I[a]



[a] See Table II for values of R^1R^2.

In the preparation of the series of analogues bearing a C2-methoxy substituent, it was decided to elaborate the molecule as a 2,4-dimethoxyquinazoline to give the suitably protected diethyl ester precursors **10a–h** (Scheme II). Bromination of **8**[17] (NBS) was followed by amination of

(15) Jackman, A. L.; Newell, D. R.; Taylor, G. A.; O'Connor, B.; Hughes, L. R.; Calvert, A. H. *Proc. Am. Assoc. Cancer Res.* **1987**, *28*, 271.
(16) Bindra, J. S. U.S. Patent 4085213, 1985.

Scheme II

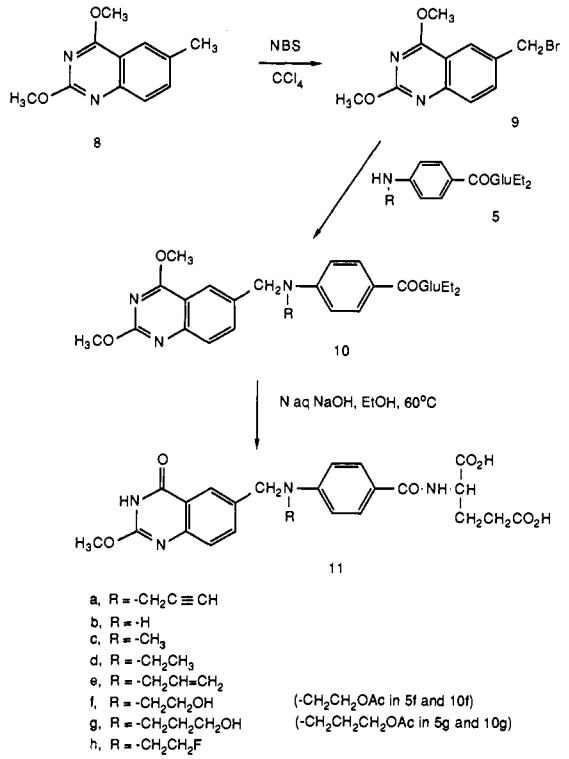
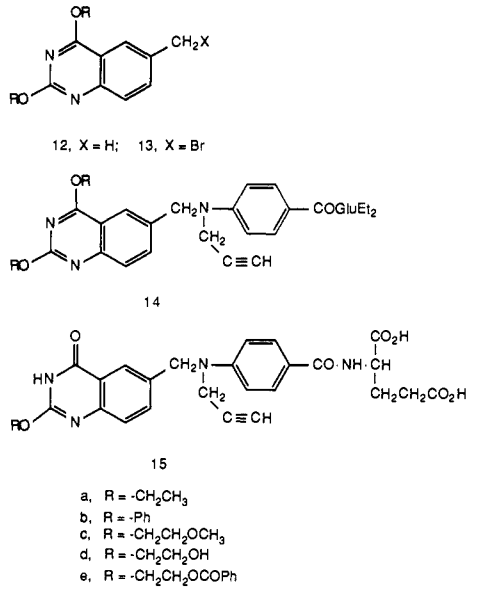


Table III. Preparation of Dimethoxyantifolate Diesters 10 (Method C)

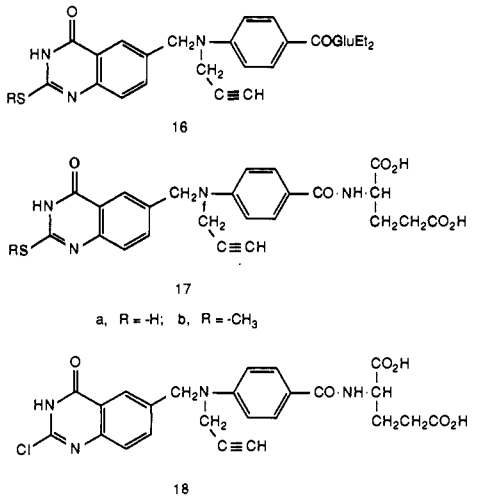
compd	N10-substit	aniline starting material	no. of equiv of 9	% yield
10a	$CH_2C{\equiv}CH$	**5a**[a]	1.20	29
10b	H	**5b**[b]	1.10	23
10c	CH_3	**5c**[c]	1.04	51
10d	CH_2CH_3	**5d**[d]	1.04	32
10e	$CH_2CH{=}CH_2$	**5e**[a]	1.04	86
10f	$(CH_2)_2OCOCH_3$	**5f**	1.03	47
10g	$(CH_2)_3OCOCH_3$	**5g**[e]	1.11	41
10h	$(CH_2)_2F$	**5h**[e]	1.04	31

[a] See ref 1. [b] Aldrich Chemical Co. [c] Fu, S-C.J.; Reiner, M.; Loo, T. L. *J. Org. Chem.* **1965**, *30*, 1277. [d] Montgomery, J. A.; Piper, J. R.; Elliot, R. D.; Temple, C.; Roberts, E. C.; Shealy, Y. F. *J. Med. Chem.* **1979**, *22*, 862. [e] See ref 10.

the resulting bromide **9** with a series of N-substituted diethyl (4-aminobenzoyl)glutamates[11] to afford the 2,4-dimethoxy diethyl esters **10a–h** (Table III). It is well established that 2,4-dialkoxyquinazolines can be selectively hydrolyzed by alkali to give the 2-alkoxy-4-hydroxy derivative,[18] and this property was utilized to effect complete deprotection of **10a–h** in one step (8 equiv of NaOH at 60 °C) to the desired 2-methoxyquinazoline antifolates (**11a–h**). The same approach was used in the synthesis of the other C2-ether analogues **15a–d** via the quinazoline 2,4-diether intermediates **14a–d**. In the case of **15d** the 2-hydroxyethoxy substituent was introduced by treating 2,4-dichloro-6-methylquinazoline with sodium 2-hydroxyethoxide in DMF. It was then necessary to protect the free hydroxy groups in **12d** as the benzoate esters before completing the elaboration of the molecule to **14e**. The benzoic acid generated upon alkaline hydrolysis of **14e** could be readily removed from the product **15d** by trituration with MeCN.



Introduction of sulfur at C2 was accomplished by exposure of **6** to thiourea in the presence of formic acid. The resulting thiol diethyl ester **16a** was hydrolyzed to the 2-mercaptoquinazoline antifolate diacid **17a**. Methylation of **16a** (MeI–NH_4OH) followed by alkaline hydrolysis yielded the corresponding 2-(methyl thioether) **16b** (Table II). Finally, **6** itself could be hydrolyzed by aqueous NaOH under carefully controlled conditions to give the 2-chloroquinazoline antifolate **18** (Table II).



Biological Evaluation

The diacids **2a–h**, **11a–h**, **15a–d**, **17a,b**, and **18** were tested as inhibitors of TS partially purified from L1210 mouse leukemia cells that overproduce TS due to amplification of the TS gene.[19] 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.[19,20] **1a** was included in each assay as a positive control ($I_{50} \simeq 20$ nM). The ratio of I_{50}'s (defined as an inverse relative potency, IRP) could then be compared. The compounds were also tested for their inhibition of the growth of L1210 cells in culture,[11] and the results again were expressed as the concentration required to inhibit cell growth by 50% (IC_{50}). These results are

(17) Mead Johnson & Co., British Patent 920019, 1963.
(18) Lange, N. A.; Sheibley, F. E. *J. Am. Chem. Soc.* **1933**, *55*, 1188.
(19) Jackman, A. L.; Alison, D. L.; Calvert, A. H.; Harrap, K. R. *Cancer Res.* **1986**, *46*, 2810.
(20) Sikora, E.; Jackman, A. L.; Newell, D. R.; Calvert, A. H. *Biochem. Pharmacol.* **1988**, *37*, 4047.

Table IV. Preparation of Antifolate Diacids 11 and 15 (Method D)

compd	C2-substit	N10-substit	% yield	mp, °C	formula[a]	mass spectra, m/z $[M - H]^-$	inhibn of TS (IRP)	inhibn of L1210 cell growth in culture: IC_{50}, μM
11a	OCH_3	$CH_2C{\equiv}CH$	88	155–165	$C_{25}H_{24}N_4O_7{\cdot}H_2O$	491	1.4	1.9
11b	OCH_3	H	25	150–160	$C_{22}H_{22}N_4O_7{\cdot}1.2H_2O$	453	311.8	6.4
11c	OCH_3	CH_3	83	240–245	$C_{23}H_{24}N_4O_7{\cdot}2.5H_2O$	467	11.6	7.0
11d	OCH_3	CH_2CH_3	64	140–146	$C_{24}H_{26}N_4O_7{\cdot}H_2O$	481	6.6	12.0
11e	OCH_3	$CH_2CH{=}CH_2$	75	130–134	$C_{25}H_{26}N_4O_7{\cdot}H_2O$	493	17.1	16.0
11f	OCH_3	$(CH_2)_2OH$	17[b]	150–175	$C_{24}H_{26}N_4O_8{\cdot}1.25H_2O{\cdot}$ 0.3MeCN	497	13.8	7.3
11g	OCH_3	$(CH_2)_3OH$	63	145–155	$C_{25}H_{28}N_4O_8{\cdot}1.5H_2O$	511	38.1	80.0
11h	OCH_3	$(CH_2)_2F$	80	141–145	$C_{24}H_{25}FN_4O_7{\cdot}1.25H_2O$	499	7.5	17.0
15a	OCH_2CH_3	$CH_2C{\equiv}CH$	53	134–136	$C_{26}H_{26}N_4O_7{\cdot}2H_2O$[c]	467	7.3	>100
15b	OPh	$CH_2C{\equiv}CH$	57	159–164	$C_{30}H_{26}N_4O_7{\cdot}3H_2O$[d]	553	35.2	>100
15c	$OCH_2CH_2OCH_3$	$CH_2C{\equiv}CH$	87	134–140	$C_{27}H_{28}N_4O_8{\cdot}H_2O$	535	4.2	100.0
15d	OCH_2CH_2OH	$CH_2C{\equiv}CH$	21	145–149	$C_{26}H_{26}N_4O_8{\cdot}2H_2O$[e]	521	5.5	>100

[a] Anal. C, H, N except where stated otherwise. [b] Solidification of the product was induced by trituration with MeCN. The NMR spectrum indicated the presence of 0.3 mol of MeCN. [c] H: calcd, 5.5; found 4.9. [d] H: calcd, 5.2; found, 4.4. [e] H: calcd, 5.3; found, 4.8.

collected in Tables II and IV.

Results and Discussion

The values of the inverse relative potency for the inhibition of partially purified L1210 TS and the IC_{50} values for growth inhibition of L1210 cells are shown in Tables II and IV. The replacement of the C2-amino group by a variety of secondary and tertiary amines has resulted in compounds of reduced potency against both parameters. Loss of activity is particularly marked in the tertiary amine **2g**, the imidazole derivative **2h**, and the amino acid **2f**. Even in the simple secondary amines the activities rapidly dwindle as the size of the alkyl group increases from methyl to ethyl. The incorporation of a hydroxyl group into the secondary amine to give **2e** has some benefit in terms of potency against both the enzyme and cells, but this is not maintained when the hydroxyl is replaced by the amino group in **2c**. As can be seen from Table IV, the methoxy group (in **11a**) can replace the C2-amine without appreciable loss of enzyme activity. Indeed a slight improvement in activity against L1210 cells is seen. As was the case in the C2-amino series,[10] a propargyl substituent on N^{10} appears to be optimum in the C2-methoxy series. The methyl (**11c**), ethyl (**11d**), and 2-hydroxyethyl (**11f**) analogues are also quite cytotoxic despite their reduced potency against the isolated enzyme. That the enzyme has a low tolerance of a hydrogen substituent at N^{10} is reinforced by the relatively poor activity of **11b**. The cytotoxic activity of **11b** is unlikely to be due to its activity against dihydrofolate reductase (EC 1.5.1.4, DHFR, $K_i \simeq 0.6$ μM)[21] but could possibly result from improved transport into the cells or from enhanced polyglutamation within the cells. Larger ether groups at C2, although still active enzyme inhibitors, are less well tolerated by the enzyme and are much poorer cytotoxic agents. The C2-methylthio derivative **17b** also appears to fall into this series, being intermediate in both TS and L1210 activity between the methoxy and ethoxy analogues, reflecting the relative volumes of these three substituents. The reduced activity of the C2-thiol **17a** presumably results from a predominance of the 2-thione tautomer in aqueous solution. A chlorine substituent at C2 (**18**) is also quite well tolerated by the enzyme despite its high lipophilicity. The overall conclusion therefore is that the C2-amino group is not absolutely essential for activity as a TS inhibitor. The enzyme can accommodate a wide range of substituents in this position, but cytotoxicity is severely limited by the size of this group. The solubility of the C2-methoxy analogue **11a** in aqueous sodium phosphate has been determined

Table V

compd	solvent: 0.01 M aqueous NaH_2PO_4 initially at pH 7 — solubility, mg/mL	0.01 M aqueous NaH_2PO_4 initially at pH 7 — pH attained at saturation	0.20 M aqueous NaH_2PO_4 initially at pH 7 — solubility, mg/mL	0.20 M aqueous NaH_2PO_4 initially at pH 7 — pH attained at saturation
1a	0.05	5.76	0.06	6.78
11a	1.2	4.93	6.6	5.67

(Table V)[22] and as expected is many times that of **1a**. Thus from the results reported here, we conclude that **11a** has comparable in vitro activity to **1a** and the enhanced aqueous solubility makes this an interesting compound for further study.

Experimental Section

General Procedures. All procedures were carried out at room temperature unless otherwise stated. *N,N*-Dimethylformamide (DMF) and *N,N*-dimethylacetamide (DMA) were purified by azeotropic distillation at 10 mmHg. EtOH was dried by distillation from sodium metal. MeCN (Fisons HPLC Grade) and CCl_4 (BDH Technical Grade) were used without further purification. Solutions in organic solvents were dried over anhydrous Na_2SO_4. Preparative chromatography was performed on Merck Kieselgel 60 (ART 9385 or ART 15111) packed in Corning glass HPLC columns. A flow rate of 10–30 mL/min was achieved with a Gilson 302 pump, and the eluent was passed through a Gilson 111 ultraviolet monitor set to detect at 254 nm. TLC was performed on precoated silica gel plates (Merck ART 5715), and the resulting chromatograms were visualized under UV light at 254 nm. Analytical HPLC was performed on a Hichrom S5ODS1 Spherisorb Column System set to run isocratically at 60% MeOH + 0.2% CF_3CO_2H in water for final antifolate diacids and 70% MeOH + 0.2g CF_3CO_2H for intermediates. Melting points were determined on a Kofler block or with a Büchi melting point apparatus and are uncorrected. Fast atom bombardment (FAB) mass spectra were determined with a VG MS9 spectrometer and Finnigan Incos data system, using DMSO as the solvent and glycerol as the matrix. The NMR spectra were determined on a Bruker AM200 (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; br s, broad singlet; m, multiplet.

Diethyl *N*-[4-[*N*-[(2-Chloro-3,4-dihydro-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoyl]-L-glutamate (6). A mixture of powdered 2-chloro-3,4-dihydro-4-oxo-6-methylquinazoline (**3**) (6.34 g, 32.6 mmol), *N*-bromosuccinimide (6.38 g, 35.8 mmol), and benzoyl peroxide (0.16 g, 0.65 mmol) in CCl_4 (250 mL) was stirred vigorously under reflux for 16 h. The cooled reaction mixture was filtered. The filter

(21) Jackman, A. L. Unpublished results.

(22) Solubility values were determined by Dr. J. J. Morris.

cake was washed with H_2O and dried in vacuo to give the crude bromomethyl compound 4 (7.98 g, 91%), which was used without purification.

A mixture of 4 (7.9 g, 28.9 mmol), diethyl *N*-[4-(prop-2-ynylamino)benzoyl]-L-glutamate (**5a**)[1] (7.34 g, 20.4 mmol), powdered $CaCO_3$ (2.45 g, 24.5 mmol), and DMF (30 mL) was stirred at 60 °C for 16 h under argon. The cooled reaction mixture was partitioned between EtOAc and H_2O. The organic phase was separated, washed with H_2O, dried, and evaporated to dryness. The crude oil was purified by chromatography eluting with CH_2Cl_2 followed by CH_2Cl_2–EtOAc (7:3 v/v). The product 6 (6.08 g, 54%) sintered above 150 °C and melted at 177 °C; NMR (Me_2SO-d_6) δ 1.04, 1.07 (2 t, 6 H, OCH_2CH_3), 2.05 (m, 2 H, $CHCH_2CH_2CO_2Et$), 2.4 (t, 2 H, $CHCH_2CH_2CO_2Et$), 3.2 (t, 1 H, C≡CH), 4.05, 4.1 (2 q, 4 H, OCH_2CH_3), 4.35 (br s, 2 H, $CH_2C{\equiv}C$), 4.8 (br s, 2 H, $ArCH_2N<$), 6.85 (d, 2 H, 2 ArH), 7.6 (d, 1 H, quinazoline 8-H), 7.7 (d, 2 H, 2 ArH), 7.75 (dd, 1 H, quinazoline 7-H), 8.0 (d, 1 H, quinazoline 5-H), 8.3 (d, 1 H, CONH). Anal. ($C_{28}H_{29}ClN_4O_6$) C, H, N.

Diethyl *N*-[4-[*N*-[[3,4-Dihydro-2-(methylamino)-4-oxo-6-quinazolinyl]methyl]-*N*-prop-2-ynylamino]benzoyl]-L-glutamate (7a). Method A. A mixture of 6 (0.55 g, 1 mmol), methylamine (0.60 mL of a 33 wt % solution in alcohol), and 1-methyl-2-pyrrolidinone (10 mL) was stirred at 100 °C in a stoppered flask. After 5 h the absence of 6 was demonstrated by HPLC. The reaction mixture was cooled and partitioned between EtOAc and water. The organic phase was separated, washed twice with water, dried, and evaporated to dryness. The crude product was purified by chromatography using a gradient of 0–10% v/v 2-propanol in CH_2Cl_2 as eluent. The product (0.50 g, 91%) was isolated as a gum: NMR (Me_2SO-d_6) δ 1.2 (t, 6 H, 2 OCH_2CH_3), 2.05 (m, 2 H, $CHCH_2CH_2CO_2Et$), 2.4 (t, 2 H, $CHCH_2CH_2CO_2Et$), 2.85 (d, 3 H CH_3NH), 3.2 (t, 1 H, C≡CH), 4.1 (q, 4 H, 2 OCH_2CH_3), 4.2–4.5 (m, 3 H, CH + $CH_2C{\equiv}C$), 4.65 (s, 2 H, $CH_2N<$) 6.2 (br s, 1 H, CH_3NH), 6.85 (d, 2 H, 2 ArH), 7.25 (d, 1 H, quinazoline 8-H), 7.5 (dd, 1 H, quinazoline 7-H), 7.75 (d, 2 H, 2 ArH), 7.8 (d, 1 H, quinazoline 5-H), 8.35 (d, 1 H, CONH); MS (FAB), *m/z* 548 $[MH]^+$.

The procedure was repeated with the appropriate amines to yield the antifolate diesters **7b–h** (Table I).

***N*-[4-[*N*-[[3,4-Dihydro-2-(methylamino)-4-oxo-6-quinazolinyl]methyl]-*N*-prop-2-ynylamino]benzoyl]-L-glutamic Acid (2a). Method B.** The diester **7a** (0.470 g, 0.86 mmol) was stirred for 4 h under argon in a mixture of 1 N aqueous NaOH (4.3 mL, 4.3 mmol), EtOH (40 mL), and H_2O (10 mL). The resulting solution was evaporated below 30 °C to ca. 10 mL, filtered into a centrifuge tube, and brought to pH 4.0 with 1 N aqueous HCl. The precipitate was isolated by centrifugation and freed from inorganic ions by repeated cycles of aqueous suspension–centrifugation–decantation until the supernatant was free of chloride ion ($AgNO_3$ test). The damp product was freeze-dried to give an amorphous solid: 0.48 g (95%); mp 208–211 °C; NMR (Me_2SO-d_6) δ 2.0 (m, 2 H, $CHCH_2CH_2CO_2H$), 2.35 (t, 2 H, $CHCH_2CH_2CO_2H$), 2.9 (d, 3 H, CH_3NH), 3.2 (t, 1 H, C≡CH), 4.3 (d, 2 H, $CH_2C{\equiv}C$), 4.4 (m, 1 H, CH), 4.7 (s, 2 H, CH_2N), 6.4 (br s, 1 H, CH_3NH), 6.9 (d, 2 H, 2 ArH), 7.3 (d, 1 H, quinazoline 8-H), 7.5 (dd, 1 H, quinazoline 7-H), 7.75 (d, 2 H, 2 ArH), 7.8 (d, 1 H, quinazoline 5-H), 8.7 (d, 1 H, CONH); MS (FAB), *m/z* 490 $[M - H]^-$. Anal. ($C_{25}H_{25}N_5O_6 \cdot 2.5H_2O$) C, H, N.

The procedure was repeated with the appropriate diethyl esters **7b–h** and **16a,b** to yield the antifolates **2b–h** and **17a,b** (Table II). Some of the compounds gave poor melting points but all had correct elemental analyses (C, H, N) for the formulae listed in the table and NMR spectra consistent with the assigned structures.

Diethyl *N*-[4-[(2-Acetoxyethyl)amino]benzoyl]-L-glutamate (5f). A mixture of the amine **5b** (12.88 g, 40 mmol), 2-bromoethyl acetate (5.07 mL, 46 mmol), and 2,6-lutidine (4.65 mL, 40 mmol) in DMA (50 mL) was stirred for 16 h at 100 °C. The cooled reaction mixture was poured into 2 N aqueous H_2SO_4 (190 mL) and extracted with EtOAc (3 × 100 mL). The combined organic solution was washed twice with brine, dried, and concentrated to a golden oil. Purification was achieved by chromatography using a gradient of 0–25% v/v EtOAc in CH_2Cl_2 as eluent. The product (6.98 g, 43%) melted at 76–77 °C; NMR ($CDCl_3$) δ 1.20, 1.35 (2 t, 6 H, 2 OCH_2CH_3), 2.08 (s, 3 H, $COCH_3$), 2.25 (m, 2 H, $CHCH_2CH_2CO_2Et$), 2.45 (t, 2 H, $CHCH_2CH_2CO_2Et$), 3.46 (t, 2 H, $ArNHCH_2$), 4.10 (t, 2 H, CH_2OAc), 4.24, 4.28 (2 q, 4 H, 2 CH_2O), 4.80 (m, 1 H, CH), 6.62 (d, 2 H, 2 ArH), 6.80 (d, 1 H, CONH), 7.70 (d, 2 H, 2 ArH). Anal. ($C_{20}H_{28}N_2O_7$) C, H, N.

6-(Bromomethyl)-2,4-dimethoxyquinazoline (9). A mixture of 2,4-dimethoxy-6-methylquinazoline (**8**)[17] (8.2 g, 40.2 mmol), *N*-bromosuccinimide (7.9 g, 44.2 mmol), and benzoyl peroxide (0.19 g, 0.78 mmol) in CCl_4 (200 mL) was refluxed for 2 h and allowed to cool to room temperature. The precipitated solid was filtered off and the filtrate was evaporated to dryness to yield a white solid: 11.4 g (100%); mp 138–143 °C. Anal. ($C_{11}H_{11}BrN_2O_2$) C, H, N, Br.

Diethyl *N*-[4-[*N*-[(2,4-Dimethoxy-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoyl]-L-glutamate (10a). Method C. A mixture of the bromo compound **9** (4.00 g, 14.1 mmol), the amine **5a** (4.24 g, 11.8 mmol), and 2,6-lutidine (6.86 mL, 59 mmol) in DMF (25 mL) was stirred under argon at 70 °C. After 4 h a second portion of **9** (2.0 g, 7 mmol) was added and stirring was continued for a further 2 h at 70 °C. The cooled reaction mixture was partitioned between EtOAc (350 mL) and 1 N aqueous H_2SO_4 (60 mL). The organic solution was washed several times with brine until the washings were neutral, dried, and concentrated to a gum. Purification was achieved by chromatography using a gradient of 0–20% v/v EtOAc in CH_2Cl_2 as eluent. The product (1.93 g, 29%) was isolated as a foam: NMR (Me_2SO-d_6) δ 1.2 (t, 6 H, 2 OCH_2CH_3), 2.05 (m, 2 H, $CHCH_2CH_2CO_2Et$), 2.4 (t, 2 H, $CHCH_2CH_2CO_2Et$), 3.2 (t, 1 H, C≡CH), 3.95 (s, 3 H, OCH_3), 4.05 (q, 4 H, 2 OCH_2CH_3), 4.1 (s, 3 H, OCH_3), 4.35 (br s, 2 H, $CH_2C{\equiv}C$), 4.5 (m, 1 H, CH), 4.8 (br s, 2 H, $ArCH_2<$), 6.85 (d, 2 H, 2 ArH), 7.65–7.8 (m, 4 H, 2 ArH + quinazoline 7-H and 8-H), 7.95 (d, 1 H, quinazoline 5-H), 8.35 (d, 1 H, CONH).

The procedure was repeated with the amines **5b–h** to yield the 2,4-dimethoxyantifolate diesters **10b–h** (Table III).

***N*-[4-[*N*-[(3,4-Dihydro-2-methoxy-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoyl]-L-glutamic Acid (11a). Method D.** The diester **10a** (1.93 g, 3.43 mmol) was stirred in a mixture of 1 N aqueous NaOH (27.5 mL), EtOH (75 mL), and H_2O (15 mL) for 14.5 h at 60 °C. The resulting solution was cooled and evaporated below 30 °C to ca. 20 mL, filtered into a centrifuge tube, and brought to pH 4.0 with 1 N aqueous HCl. The precipitate was isolated by centrifugation and freed from inorganic ions by repeated cycles of aqueous suspension–centrifugation–decantation. The damp product was freeze-dried to give an amorphous solid: 1.55 g (88%); NMR (Me_2SO-d_6) δ 2.0 (m, 2 H, $CHCH_2CH_2CO_2H$), 2.35 (t, 2 H, $CHCH_2CH_2CO_2H$), 3.2 (t, 1 H, C≡CH), 3.95 (s, 3 H, OCH_3), 4.3 (br s, 2 H, $CH_2C{\equiv}C$), 4.35 (m, 1 H, CH), 4.75 (br s, 2 H, $ArCH_2N<$), 6.85 (d, 2 H, 2 ArH), 7.45 (d, 1 H, quinazoline 8-H), 7.6 (dd, 1 H, quinazoline 7-H), 7.75 (d, 2 H, 2 ArH), 7.9 (d, 1 H, quinazoline 5-H), 8.2 (d, 1 H, CONH); MS (FAB), *m/z* 491 $[M - H]^-$. Anal. $C_{25}H_{24}N_4O_7 \cdot H_2O$) C, H, N.

The procedure was repeated with the appropriate diethyl esters **10b–h** and **14a–c,e** to yield the antifolates **11b–h** and **15a–d** (Table IV).

All of the compounds gave poor melting points but had correct elemental analyses (C, H, N) unless otherwise stated for the formulae listed in the table and NMR spectra consistent with the assigned structures.

2,4-Diethoxy-6-methylquinazoline (12a). Powdered 2,4-dichloro-6-methylquinazoline[23] (6.0 g, 14 mmol) was added to a solution of sodium ethoxide (6.25 g, 92 mmol) in anhydrous EtOH (200 mL). The mixture was stirred 2 h under reflux and filtered while hot, and the filtrate was evaporated to dryness. The residue was partitioned between EtOAc (2 × 100 mL) and water (50 mL). The organic solution was washed with brine, dried, and evaporated to dryness. Chromatography with CH_2Cl_2 as eluent gave a pale yellow crystalline solid: 4.82 g (74%); mp 60–62 °C. Anal. ($C_{13}H_{16}N_2O_2$) C, H, N.

Diethyl *N*-[4-[*N*-[(2,4-Diethoxy-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoyl]-L-glutamate (14a). A mixture of **12a** (4.82 g, 20.8 mmol), *N*-bromosuccinimide (3.89

(23) Oakes, V.; Rydon, H. N.; Undheim, K. *J. Chem. Soc.* **1962**, 4678.

g, 21.8 mmol), and benzoyl peroxide (0.10 g, 0.41 mmol) in CCl_4 (100 mL) was refluxed for 2 h. The warm solution was filtered and the filtrate was evaporated to dryness to give the crude bromo derivative **13a** (6.75 g), which was used without purification.

A mixture of **13a** (1.09 g, 2.5 mmol), amine **5a** (0.90 g, 2.5 mmol), and powdered $CaCO_3$ (0.75 g, 7.5 mmol) in DMA (10 mL) was stirred 16 h under argon. The reaction mixture was filtered and the filtrate was evaporated to dryness. The orange gummy residue was purified by chromatography using a gradient of 0–10% EtOAc in CH_2Cl_2 as eluent to give **14a** (0.74 g, 50%) as a gum: NMR (Me_2SO-d_6) δ 1.2 (2 t, 6 H, 2 $CO_2CH_2CH_3$), 1.4 (2 t, 6 H, 2 OCH_2CH_3), 2.05 (m, 2 H, $CHCH_2CH_2CO_2Et$), 2.4 (t, 2 H, $CHCH_2CH_2CO_2Et$), 3.2 (t, 1 H, C≡CH), 4.05, 4.1 (2 q, 4 H, 2 $CO_2CH_2CH_2$), 4.35 (br s, 2 H, $CH_2C{\equiv}C$), 4.4 (m, 1 H, $CHCH_2CH_2CO_2Et$), 4.4, 4.55 (2 q, 4 H, 2 OCH_2CH_3), 4.8 (br s, 2 H, $ArCH_2N<$), 6.85 (d, 2 H, 2 ArH), 7.6–7.8 (m, 4 H, 2 ArH + quinazoline 7-H and 8-H), 7.95 (d, 1 H, quinazoline 5-H), 8.3 (d, 1 H, CONH).

2,4-Diphenoxy-6-methylquinazoline (12b). Molten phenol (30 g) was stirred at 80 °C during the addition of sodium metal (0.80 g). Stirring was continued until all the sodium had dissolved and the temperature was raised to 120 °C. Powdered 2,4-dichloro-6-methylquinazoline (3.2 g, 15 mmol) was added and the temperature was raised to 180 °C and maintained for 1 h. The hot mixture was poured with stirring into water (200 mL), followed by the addition of 10 N aqueous sodium hydroxide (5 mL). The solution was then adjusted to pH 6 by the addition of HOAc. The resulting precipitate was filtered off and washed well with water. Purification was achieved by chromatography, eluting with CH_2Cl_2 to yield **12b** (4.57 g, 93%), an off-white crystalline solid: mp 184–185 °C. Anal. ($C_{21}H_{16}N_2O_2$) C, H, N.

Diethyl *N*-[4-[*N*-[(2,4-Diphenoxy-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoyl]-L-glutamate (14b). A mixture of **12b** (1.0 g, 3.05 mmol), *N*-bromosuccinimide (0.60 g, 3.37 mmol), and benzoyl peroxide (25 mg, 0.10 mmol) in CCl_4 (25 mL) was refluxed for 1.5 h. The warm solution was filtered and the filtrate was evaporated to dryness to give the crude bromo derivative **13b** (1.45 g), which was used without purification.

A mixture of **13b** (1.39 g, 3.4 mmol), amine **5a** (0.86 g, 2.4 mmol), and 2,6-lutidine (1.40 mL, 12 mmol) in DMF (10 mL) was stirred for 16 h at 60 °C. The cooled reaction mixture was partitioned between 1 N aqueous H_2SO_4 (70 mL) and EtOAc (100 mL). The organic solution was washed with brine (5 × 20 mL), dried, and evaporated to dryness. The orange gummy residue was purified by chromatography with a gradient of 0–10% v/v EtOAc in CH_2Cl_2 to give **14b** (0.95 g, 58%) as a gum: NMR (Me_2SO-d_6) δ 1.2 (t, 6 H, 2 OCH_2CH_3), 2.05 (m, 2 H, $CHCH_2CH_2CO_2Et$), 2.4 (t, 2 H, $CHCH_2CH_2CO_2Et$), 3.2 (t, 1 H, C≡CH), 4.05 (q, 4 H, 2 OCH_2CH_3), 4.4 (br s, 2 H, $CH_2C{\equiv}C$), 4.4 (m, 1 H, $CHCH_2CH_2CO_2Et$), 4.9 (br s, 2 H, $ArCH_2N<$), 6.9 (d, 2 H, 2 ArH), 7.1–7.5 (m, 10 H, 2 OPh), 7.6 (d, 1 H, quinazoline 8-H), 7.75 (d, 2 H, 2 ArH), 7.85 (dd, 1 H, quinazoline 7-H), 8.25 (d, 1 H, quinazoline 5-H), 8.4 (d, 1 H, CONH).

Diethyl *N*-[4-[*N*-[[2,4-Bis(2-methoxyethoxy)-6-quinazolinyl]methyl]-*N*-prop-2-ynylamino]benzoyl]-L-glutamate (14c). This was prepared from 2,4-dichloro-6-methylquinazoline as for **14a** but with a solution of sodium in 2-methoxyethanol in place of ethanolic sodium ethoxide. The product **14c** had the following: NMR (Me_2SO-d_6) δ 1.2 (m, 6 H, 2 OCH_2CH_3), 2.05 (m, 2 H, $CHCH_2CH_2CO_2Et$), 2.4 (t, 2 H, $CHCH_2CH_2CO_2Et$), 3.2 (t, 1 H, C≡CH), 3.3, 3.35 (2 s, 6 H, 2 OCH_3), 3.75 (m, 4 H, 2 CH_2OCH_3), 4.0, 4.15 (2 q, 4 H, 2 OCH_2CH_3), 4.35 (br s, 2 H, $CH_2C{\equiv}C$), 4.4 (m, 1 H, $CHCH_2CH_2CO_2Et$), 4.5 4.65 (2 t, 4 H, 2 CH_2O quinazoline), 4.85 (br s, 2 H, $ArCH_2N<$), 6.85 (d, 2 H, 2 ArH), 7.65 (d, 1 H, quinazoline 8-H), 7.75 (d, 2 H, 2 ArH), 7.8 (dd, 1 H, quinazoline 7-H), 8.0 (d, 1 H, quinazoline 5-H), 8.35 (d, 1 H, CONH).

2,4-Bis[2-(benzoyloxy)ethoxy]-6-methylquinazoline (12e). Sodium hydride (2.4 g of a 50% oil dispersion, 50 mmol) was washed free of oil (hexane) under argon and treated dropwise with ethane-1,2-diol (30 mL). When the effervescence had subsided, a solution of 2,4-dichloro-6-methylquinazoline (2.06 g, 9.67 mmol) in DMF (5 mL) was added and the mixture was stirred 2 h at 100 °C. The cooled mixture was partitioned between EtOAc (3 × 200 mL) and H_2O (100 mL). The combined organic solutions were washed with brine, dried, and evaporated to dryness. The resulting gum (2.0 g) was dissolved in pyridine (20 mL) and treated dropwise at 20 °C (with cooling) with benzoyl chloride (1.9 mL, 15.6 mmol). The mixture was stirred for 16 h, poured into H_2O (100 mL), and extracted with CH_2Cl_2 (200 mL). The organic solution was washed with aqueous $NaHCO_3$ (100 mL) and water (50 mL), dried, and evaporated. Traces of pyridine were removed from the product by rotary evaporation in the presence of toluene. The resulting oil was chromatographed with 5% v/v EtOAc in CH_2Cl_2 as the eluent to provide an oil that crystallized on standing: 1.30 g (36%); mp 108–109 °C; NMR ($CDCl_3$) δ 2.5 (s, 3 H), CH_3), 4.8 (m, 8 H, OCH_2CH_2O), 7.3–8.1 (m, 13 H, aromatic protons). Anal. ($C_{27}H_{24}N_2O_6$) C, H, N.

Diethyl *N*-[4-[*N*-[[2,4-Bis[2-(benzoyloxy)ethoxy]-6-quinazolinyl]methyl]-*N*-prop-2-ynylamino]benzoyl]-L-glutamate (14e). A mixture of **12e** (1.30 g, 2.75 mmol), *N*-bromosuccinimide (0.59 g, 3.3 mmol), and benzoyl peroxide (0.027 g, 0.11 mmol) in CCl_4 (35 mL) was stirred for 2 h under reflux. The warm solution was filtered and the filtrate was evaporated to dryness. The resulting crude bromo compound (1.40 g), the amine **5a** (1.19 g, 3.3 mmol), and 2,6-lutidine (1.74 mL, 15 mmol) in DMF (6 mL) were stirred 16 h at 60 °C under argon. The cooled reaction mixture was partitioned between EtOAc (75 mL) and 1 N aqueous H_2SO_4 (50 mL). The organic solution was washed several times with brine until the washings were neutral, dried, and concentrated to a gum. Purification was achieved by chromatography using a gradient of 0–20% v/v EtOAc in CH_2Cl_2 as eluent. Evaporation of the appropriate fractions gave a solid: 0.30 g (15%); mp 43–45 °C. Anal. ($C_{46}H_{46}N_4O_{11}$) C, H, N.

Diethyl *N*-[4-[*N*-[(3,4-Dihydro-2-mercapto-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoyl]-L-glutamate (16a). A mixture of the chloro compound **6** (0.75 g, 1.36 mmol), thiourea (0.125 g, 1.63 mmol), and formic acid (50 μL) in EtOH (20 mL) was refluxed for 15 min. The reaction mixture was evaporated to dryness and the residue was dissolved in CH_2Cl_2. This solution was filtered and applied to a column of silica. Elution with 7:3 v/v CH_2Cl_2–EtOAc afforded **16a** (0.34 g, 45%); mp 92–94 °C. Anal. ($C_{28}H_{30}N_4O_6S{\cdot}0.5H_2O$) C, H, N.

Diethyl *N*-[4-[*N*-[[3,4-Dihydro-2-(methylthio)-4-oxo-6-quinazolinyl]methyl]-*N*-prop-2-ynylamino]benzoyl]-L-glutamate (16b). Aqueous ammonia (3.2 mL, sp gr 0.88) was added to a stirred suspension of the thiol **16a** (0.19 g, 0.345 mmol) in a mixture of EtOH (9.5 mL) and H_2O (12.8 mL). When complete solution had been achieved, MeI (0.13 mL) was added and stirring was continued for 1 h. The precipitated solid was filtered off, washed with 50% aqueous EtOH, and dried in vacuo: 0.16 g (81%); mp 230–233 °C. Anal. ($C_{29}H_{32}N_4O_6S{\cdot}0.25H_2O$) C, H, N.

***N*-[4-[*N*-[(2-Chloro-3,4-dihydro-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoyl]-L-glutamic Acid (18).** The diester **6** (0.055 g, 0.1 mmol) was stirred for 4 h under argon in a mixture of 1 N aqueous NaOH (0.4 mL, 0.4 mmol) and EtOH (8.0 mL). The resulting solution was evaporated to dryness at room temperature and the residue was dissolved in H_2O (2 mL). The solution was filtered into a centrifuge tube and brought to pH 4.0 with 1 N aqueous HCl. The precipitate was isolated by centrifugation and freed from inorganic ions by four cycles of aqueous suspension–centrifugation–decantation. The damp product was freeze-dried to give a colorless solid: 0.047 g (95%); NMR (Me_2SO-d_6) δ 2.0 (m, 2 H, $CHCH_2CH_2CO_2H$), 2.3 (t, 2 H, $CHCH_2CH_2CO_2H$), 3.2 (t, 1 H, C≡CH), 4.35 (m, 3 H, $CH_2C{\equiv}C$ + CH), 4.8 (br s, 2 H, CH_2N), 6.85 (d, 2 H, 2 ArH), 7.6 (d, 1 H, quinazoline 8-H), 7.75 (d, 2 H, quinazoline 7-H + dd, 1 H, quinazoline 5-H, 8.2 (d, 1 H, CONH); MS (FAB), m/z 495 [M − H]$^-$. Anal. ($C_{24}H_{21}ClN_4O_6{\cdot}1.33H_2O$) C, H, N, Cl.

Registry No. 1a, 76849-19-9; **2a**, 118141-90-5; **2b**, 118111-64-1; **2c**, 118111-65-2; **2d**, 118111-66-3; **2e**, 118111-67-4; **2f**, 118141-91-6; **2g**, 118111-68-5; **2h**, 118111-69-6; **3**, 62484-42-8; **4**, 118111-59-4; **5a**, 76858-72-5; **5b**, 13726-52-8; **5c**, 2378-95-2; **5d**, 70280-71-6; **5e**, 76858-71-4; **5f**, 107716-29-0; **5g**, 118111-86-7; **5h**, 97280-21-2; **6**, 112888-80-9; **7a**, 118111-60-7; **7b**, 118111-70-9; **7c**, 118111-71-0; **7d**, 118111-72-1; **7e**, 118111-73-2; **7f**, 118111-74-3; **7g**, 118111-75-4; **7h**, 118111-76-5; **8**, 92316-82-0; **9**, 112888-62-7; **10a**, 118111-61-8; **10b**, 118111-77-6; **10c**, 118111-78-7; **10d**, 118111-79-8; **10e**, 118111-80-1; **10f**, 118111-81-2; **10g**, 118111-82-3; **10h**, 118111-83-4; **11a**, 112887-72-6; **11b**, 112888-04-7; **11c**, 112888-03-6; **11d**,

112888-05-8; 11e, 112888-06-9; 11f, 112888-07-0; 11g, 112888-08-1; 11h, 112888-09-2; 12a, 112888-63-8; 12b, 112888-68-3; 12d, 112888-65-0; 12e, 112888-66-1; 13a, 112888-64-9; 13b, 112888-69-4; 13e, 112888-67-2; 14a, 118111-62-9; 14b, 118111-84-5; 14c, 118111-85-6; 14e, 118111-87-8; 15a, 112888-14-9; 15b, 112888-17-2; 15c, 112888-15-0; 15d, 112888-16-1; 16a, 118111-63-0; 16b, 112888-79-6; 17a, 112888-82-1; 17b, 112888-23-0; 18, 112887-93-1; methylamine, 74-89-5; 2-bromoethyl acetate, 927-68-4; 2,4-dichloro-6-methylquinazoline, 39576-82-4; phenol, 108-95-2; 2-methoxyethanol, 109-86-4; ethylamine, 75-04-7; ethylenediamine, 107-15-3; *N,N*-dimethylethylenediamine, 108-00-9; ethanolamine, 141-43-5; glycine ethyl ester hydrochloride, 623-33-6; dimethylamine, 124-40-3; imidazole, 288-32-4; thymidylate synthase, 9031-61-2.

2(1*H*)-Quinolinones with Cardiac Stimulant Activity. 2. Synthesis and Biological Activities of 6-(N-Linked, Five-Membered Heteroaryl) Derivatives

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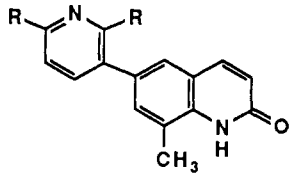
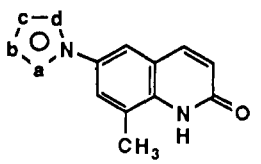
A series of 6-(N-linked, five-membered heteroaryl)-2(1*H*)-quinolinone derivatives was synthesized and evaluated for cardiotonic activity. Most compounds were prepared by sulfuric acid catalyzed cyclization of an *N*-(4-heteroarylphenyl)-3-ethoxypropenamide or by condensation of a 2-amino-5-heteroarylbenzaldehyde or -acetophenone derivative with the ylide derived from triethyl phosphonoacetate. In anesthetized dogs, 6-imidazol-1-yl-8-methyl-2(1*H*)-quinolinone (**3**; 25 μg/kg) produced a greater increase in cardiac contractility (percentage increase in d*P*/d*t* max) than alternative 6-(five-membered heteroaryl)-substituted analogues (**4–8**). Introduction of 4-methyl (**10**) or 2,4-dimethyl (**13**) substituents into the imidazole ring of **3** produced a marked increase in inotropic activity, and these compounds were some 10 and 5 times more potent than milrinone. Most of these quinolinones also displayed positive inotropic effects (decrease in QA interval) in conscious dogs after oral administration (0.0625–1 mg/kg) and in many cases (**3**, **5–7**, **9**, **11**, **13**, **16**) there was little difference in activities at both the 1- and 3-h time points. Compound **13** (62.5, 125, 250 μg/kg po) demonstrated dose-related cardiac stimulant activity which, in contrast to milrinone, was maintained over the whole 7-h test period. No changes in heart rate were detected at any dose level and compounds **3**, **9**, **10**, and **13** also displayed high selectivity for the stimulation of cardiac contractile force rather than heart rate in the Starling dog heart–lung preparation. Increases in d*P*/d*t* max of approximately 50% were accompanied by heart rate changes of less than 10 beats/minute. Physicochemical measurements gave a log *P* of 1.64 for **13** with pK_a values of 7.13 ± 0.04 and 11.5 ± 0.2 for the imidazole and quinolinone moieties, respectively. X-ray structural analysis of **13** showed the imidazole and quinolinone rings at 52° to one another in close agreement with the minimum-energy conformation (30°) suggested by PCILO calculations. 6-(2,4-Dimethylimidazol-1-yl)-8-methyl-2(1*H*)-quinolinone (**13**, UK-61,260) is currently undergoing phase II clinical evaluation in congestive heart failure patients.

In a previous paper,[1] the synthesis and cardiac stimulant activities of a series of 2(1*H*)-quinolinone derivatives incorporating six-membered, heteroaryl substituents were described. These structure–activity relationship (SAR) studies identified the quinolinone 6-position as the preferred location for the heteroaryl moiety and also demonstrated the beneficial effects of an 8-methyl substituent. Two compounds from this series (**1a**, **1b**) showed greater



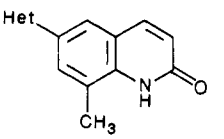
1a, R = H
1b, R = CH_3



2, a - d = N or C

intrinsic inotropic potency than milrinone and also improved duration of action after oral administration to conscious dogs. Moreover, **1a** and **1b** and related analogues had barely any effect on heart rate. In order to define the individual structural features that influence this favorable hemodynamic profile, a series of 8-methyl-2(1*H*)-quinolinones (**2**) incorporating various N-linked, five-membered heteroaryl moieties at the 6-position has been synthesized[2] and SARs for cardiac stimulant activity determined. Manipulation of the location and number of nitrogen atoms in these heteroaryl systems allows the electron-withdrawing capacity of the 6-substituent to be varied in a controlled manner and its effects on cardiac stimulant activity determined. Moreover, five-membered di- and triaza heterocycles are less susceptible to metabolic

Table I. Synthetic Routes and Physicochemical Data for 6-Heterocyclic 8-Methyl-2(1*H*)-quinolinone Derivatives



no.	Het	route	mp, °C	formula	anal.
3	imidazol-1-yl	A	259–262	$C_{13}H_{11}N_3O$	C, H, N
4	pyrazol-1-yl	A	229–231	$C_{13}H_{11}N_3O$	C, H, N
5	1,2,4-triazol-1-yl	A	318–321	$C_{12}H_{10}N_4O$	C, H, N
6	1,2,4-triazol-4-yl	A	365–369	$C_{12}H_{10}N_4O \cdot H_2O$	C,[a] H, N[b]
7	tetrazol-1-yl	A	267–268	$C_{11}H_9N_5O \cdot 0.5H_2O$	C, H, N
8	tetrazol-2-yl	B	264–266	$C_{11}H_9N_5O \cdot 0.25H_2O$	C, H, N

[a] C: calcd, 59.0; found, 59.7. [b] N: calcd, 23.0; found, 22.5.

(1) Alabaster, C. T.; Bell, A. S.; Campbell, S. F.; Ellis, P.; Henderson, C. G.; Roberts, D. A.; Ruddock, K. S.; Samuels, G. M. R.; Stefaniak, M. H. *J. Med. Chem.* **1988**, *31*, 2048.

(2) Campbell, S. F.; Roberts, D. A. European Patent 0166533, 1986; *Chem. Abstr.* **1986**, *105*, 115061z.

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