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Received July 28, 1997

Classical inhibitors of thymidylate synthase such as *N*¹⁰-propargyl-5,8-dideazafolic acid (**1**), *N*-(5-[*N*-(3,4-dihydro-2-methyl-4-oxoquinazolin-6-ylmethyl)-*N*-methylamino]-2-thenoyl)-L-glutamic acid (ZD1694, **2**) and *N*-[2-amino-4-oxo-3,4-dihydro(pyrrolo[2,3-*d*]pyrimidin-5-yl)ethylbenzoyl]-L-glutamic acid (LY231514, **3**) while potent, suffer from a number of potential disadvantages, such as impaired uptake due to an alteration of the active transport system required for their cellular uptake, as well as formation of long acting, non-effluxing polyglutamates *via* the action of folylpolyglutamate synthetase, which are responsible for toxicity. To overcome some of the disadvantages of classical inhibitors, there has been considerable interest in the synthesis and evaluation of nonclassical thymidylate synthase inhibitors, which could enter cells *via* passive diffusion. In an attempt to elucidate the role of saturation of the B-ring of non-classical, quinazoline antifolate inhibitors of thymidylate synthase, analogues **7-17** were designed. Analogues **13-17** which contain a methyl group at the 7-position, were synthesized in an attempt to align the methyl group in an orientation which allows interaction with tryptophan-80 in the active site of thymidylate synthase. The synthesis of these analogues was achieved *via* the reaction of guanidine with the appropriately substituted cyclohexanone-ketoester. These ketoesters were in turn synthesized *via* a Michael addition of the appropriate thiophenol with 2-carbethoxycyclohexen-1-one or 5-methyl-2-carbethoxycyclohexen-1-one to afford a mixture of diastereomers. The most inhibitory compound was the 3,4-dichloro, 7-methyl derivative **17** which inhibited the *Escherichia coli* and *Pneumocystis carinii* thymidylate synthase 50% at 5×10^{-5} M. Our results confirm the importance of the 7-CH₃ group and electron withdrawing groups on the aromatic side chain for thymidylate synthase inhibition.

J. Heterocyclic Chem., **34**, 1669 (1997).

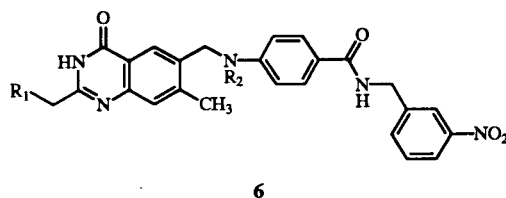
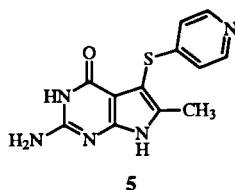
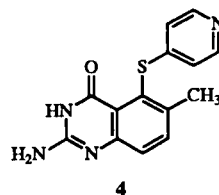
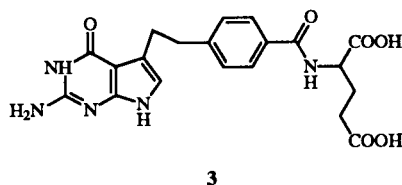
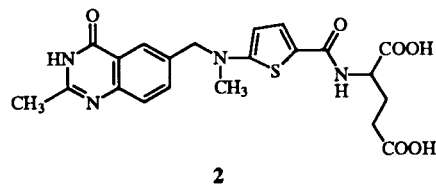
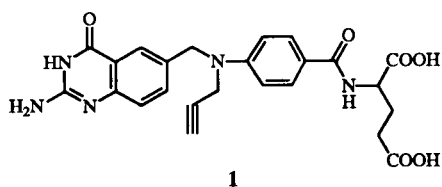
Thymidylate synthase catalyzes the conversion of deoxyuridine monophosphate to thymidine monophosphate by a reductive methylation using 5,10-methylene-tetrahydrofolate as the reductant [2]. This rate limiting step is the exclusive *de novo* source of thymidine for DNA synthesis. Without providing an exogenous source of thymidine, inhibiting thymidylate synthase activity leads to "thymineless death" [3,4].

Several antifolates that act by inhibiting thymidylate synthase have been developed as antitumor agents. These compounds bind either to the pyrimidine binding site or the cofactor binding site. Significant efforts have focused on the design of analogues which bind to the folate cofactor site. Notable among these are the quinazolines, *N*¹⁰-propargyl-5,8-dideazafolate (**1**) [5], and its third generation analogue *N*-{5-[*N*-(3,4-dihydro-2-methyl-4-oxo-quinazolin-6-yl-methyl)-*N*-methylamino]-2-thenoyl}-L-glutamic acid (ZD1694, Tomudex, **2**) [6], which has been approved for use in Europe as an antitumor agent. Taylor *et al.* [7] have reported a B-ring contracted analogue, *N*-[2-amino-4-oxo-3,4-dihydro-(pyrrolo[2,3-*d*]pyrimidin-5-yl)ethylbenzoyl]-L-glutamic acid (LY231514, **3**) which is currently undergoing clinical trials as an antitumor agent.

Classical antifolates, when used as antitumor agents, suffer from complications such as renal and hepatic toxicity [8,9]. Further, these analogues need the active trans-

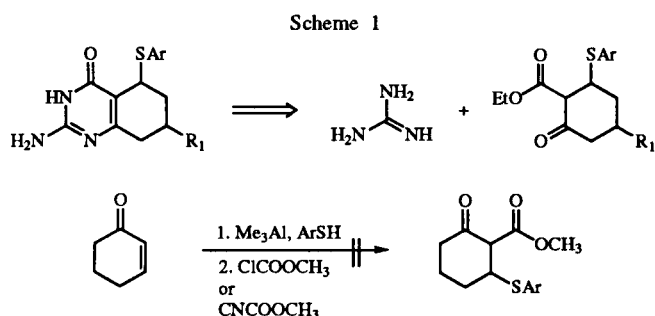
port system to cross cell membranes. Impairment of this transport mechanism can lead to drug resistance [10-12]. In addition the antitumor activities of classical thymidylate synthase inhibitors are, in part, determined by their ability to function as substrates of the enzyme folylpolyglutamate synthetase [13-16]. Polyglutamylation produces long-acting, noneffluxing poly- γ -glutamates of classical antifolates that leads to high intracellular concentrations of these antifolates. Further, polyglutamylation, in some instances, leads to a significant increase in inhibitory activity against thymidylate synthase as compared to monoglutamates [14-17]. Both **1** and **2**, when converted to their pentaglutamate forms, are 60-fold [6] and 130-fold [7] more potent than their monoglutamates, respectively. Though polyglutamylation for several classical antifolates is necessary for cytotoxicity to tumor cells, it has been implicated in adverse effects on normal tissues due to retention of poly- γ -glutamate metabolites [18]. The problem of resistance in tumors due to decreased or altered folylpolyglutamate synthase, is also a potential limitation in the clinical use of classical antifolates [19-22].

In view of the drawbacks of classical antifolates as thymidylate synthase inhibitors, there has recently been considerable interest in lipophilic, nonclassical antifolates as thymidylate synthase inhibitors which lack the L-glutamic acid side chain found in classical antifolates thus allowing for passive uptake of these inhibitors, independent of the



folate transport system(s). Weber *et al.* [23] synthesized a series of 5-arylthio substituted quinazolines which lacked the glutamic acid side chain. In these analogues, the hydrophobic cavity in thymidylate synthase which normally binds the *para*-aminobenzoyl side chain was accessed via the 5-position rather than the 6-position. Weber *et al.* [23] proposed an interaction of the 6-methyl moiety with a tryptophan residue (Trp 80) in the active site of *Escherichia coli* thymidylate synthase, providing for additional potency. One of the analogues, 2-methyl-4-oxo-6-mercapto(4-pyridyl)quinazoline (4, AG337) was an extremely potent inhibitor of human thymidylate synthase with a K_i of 0.015 μM , and is currently undergoing clinical evaluation as an antitumor agent. Gangjee *et al.* [24] have recently reported a 5-mecapto-pyridylpyrrolo[2,3-*d*]pyrimidine (5) as a nonclassical thymidylate synthase inhibitor, and found it to have significant human thymidylate synthase inhibition ($\text{IC}_{50} = 0.042 \mu\text{M}$). This compound also exhibited selectivity for human versus bacterial thymidylate synthase, indicating a species difference in thymidylate synthase from different sources. Gangjee *et al.* [25] have extended this methodology further to the synthesis of other lipophilic thymidylate synthase inhibitors and have found compounds superior to 1 and 2 as inhibitors of human thymidylate synthase. Recently, Jackman *et al.*, [26] reported a series of 6,7-disubstituted-quinazolines 6 as thymidylate synthase inhibitors. These analogues, though classical antifolates, had a 7-methyl group, which provided for a substantial increase in potency. This suggested that there is sufficient space in the active site of thymidylate synthase to accommodate a methyl group at the 7-position of the quinazolines. On the basis of these results, we initiated a study aimed at determining the importance of

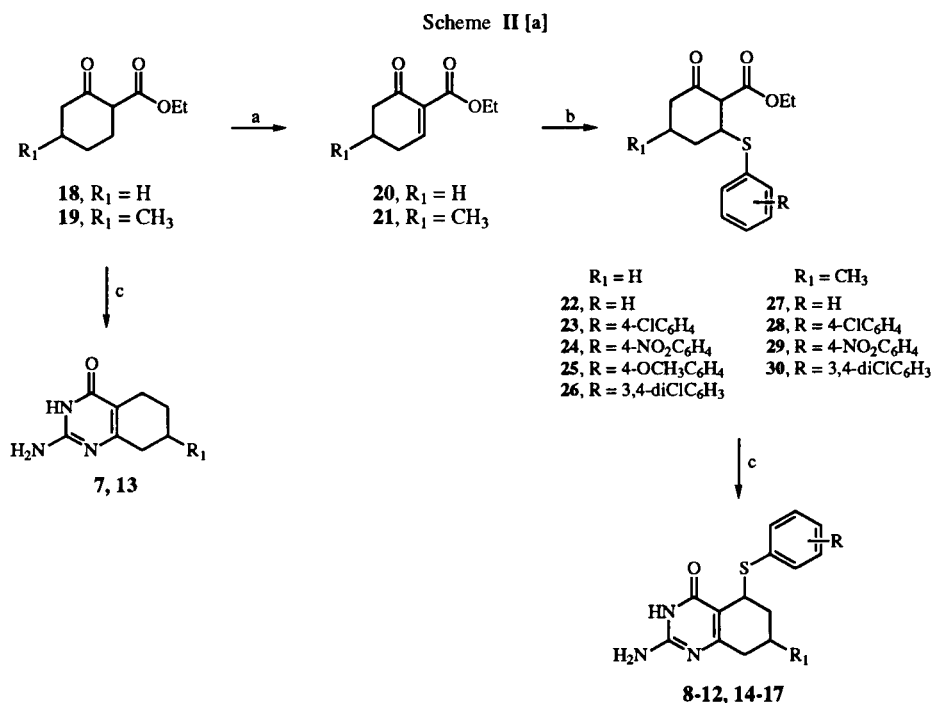
the coplanar nature (quinazolines versus tetrahydroquinazolines) of the B-ring of nonclassical antifolates on thymidylate synthase inhibition. Nair *et al.*, [27] have attempted such a comparison in the classical series (*N*-10-propargyl-5,8-dideaza-5,6,7,8-tetrahydrofolic acid and 1), and have proposed an altered mode of binding of tetrahydroquinazolines to thymidylate synthase compared to the quinazolines. It was also of interest to explore the effects of 7-methyl substituted tetrahydroquinazolines on thymidylate synthase inhibition, the first such study of its kind. With these objectives in mind, we synthesized analogues 7-17. We chose mainly electron withdrawing substituents on the phenyl ring since Weber *et al.* [23] as well as work from our own laboratory [25], has shown that such substituents provide for more potent inhibition of thymidylate synthase. This could be attributed to the diminution of the magnitude and an alteration of the direction of the dipole-dipole interaction between thymidylate synthase and the substituted phenyl ring of the inhibitor [28]. An added advantage of these lipophilic, thymidylate synthase inhibitors would be their potential use in the treatment of opportunistic infections with *Pneumocystis carinii* and *Toxoplasma gondii*, which are the principal causes of death in patients with the acquired immunodeficiency syndrome. The lack of the active transport system(s) in these organisms required for the uptake of classical antifolates, precludes the use of classical antifolates in the treatment of infections with these organisms. It was reasoned that since a difference in inhibitory potency exists between thymidylate synthase from different sources, nonclassical, lipophilic inhibitors of thymidylate synthase, which enter the cells via passive diffusion, may also provide for selective inhibition of bacterial and/or fungal thymidylate synthase.



The initial strategy employed for the synthesis of the target analogues is shown in Scheme I. A facile reaction between a suitably substituted biselectrophile and guanidine was envisaged, which would provide the desired analogues in a single step. β -Ketoesters have been shown to react regiospecifically with guanidine to give 2-amino-4-oxotetrahydroquinazolines [29]. For the syntheses of the appropriately substituted β -ketoesters, the initial approach was to carry out a trimethylaluminum-mediated three component coupling reaction involving the Michael addition of the appropriate thiophenols and 2-cyclohexen-1-one, followed by trapping of the intermediate trimethylaluminum enolate with chloroethylformate. Accordingly, trimethylaluminum was added at 0° to thiophenol to generate the dimethylaluminum thiolate, to which was then added 2-cyclohexen-1-one. Analysis (tlc) after 3 hours indicated the absence of 2-cyclohexen-1-one, and the appearance of a new product. The reaction was diluted

with tetrahydrofuran and chloroethyl formate was added. However, in spite of the use of a variety of different conditions, successful quenching of the aluminum enolate with chloroethyl formate was unsuccessful, possibly due to competing *O*-acylation with chloroethyl formate or due to the decreased electrophilicity and hence reactivity of esters to an aluminum enolate [30]. In an attempt to circumvent the problem of competing *O*-acylation, methyl cyanoformate (Manders reagent) which provides for selective *C*-acylation [31] was used instead of chloroethyl formate in the three component approach. However, the desired derivative remained elusive due to the lack of reactivity of the ester towards aluminum enolates. When *tert*-butyllithium was used in place of trimethylaluminum, followed by the addition of cyclohexenone and Manders reagent, the desired trisubstituted derivative was obtained in poor yield (15-20%).

Since trapping of the intermediate enolate presented the major obstacle to the synthesis of these analogues *via* this methodology, an alternate approach was utilized to obtain the desired bis-electrophiles as depicted in Scheme II. This involved the initial synthesis of the α,β -unsaturated ketoester, 2-carbethoxycyclohexen-1-one **20** [32], *via* oxidation of 2-carbethoxycyclohexanone **18**, by the method of Liotta *et al.* [33]. The presence of the cyclohexenone was confirmed by the appearance of the vinylic proton as a triplet at δ 7.24 ppm in the ^1H nmr spectra. A similar oxidation with 5-methyl-2-carbethoxycyclohexanone **19** afforded 5-methyl-2-carbethoxycyclohexen-1-one **21**.



[a] Reagents: (a) i. Pyridine/ PhSeCl ; ii. H_2O_2 ; (b) ArSH , Et_3N , CH_2Cl_2 , 0° ; (c) Guanidine carbonate, EtOH , reflux

Once the carbethoxycyclohexenones were obtained, 1,4-addition with various thiophenols was performed utilizing one of three methods:

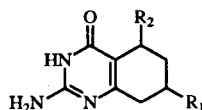
a. ArSH, CH₂Cl₂; b. ArSH, *n*-BuLi, THF, 0°; c. ArSH, Et₃N, CH₂Cl₂, 0° [34].

Method c afforded the best yields (55-60%) of the desired trisubstituted ketoester. The ¹H nmr of the mixture of stereoisomers in each case indicated the absence of the vinylic proton. Repeated attempts at purifying the substituted cyclohexanones **22-30** resulted in recovery of the starting unsaturated ketoester derivatives **20** or **21**, due to facile elimination of the β-thiophenyl moiety. Hence these compounds were identified by their ¹H nmr and were used in their crude form for further transformations. Initial attempts at cyclization of the substituted ketoesters with freshly generated guanidine (guanidine hydrochloride and sodium ethoxide) did not afford the desired product, presumably due

to elimination of the β-thiophenyl moiety in the presence of trace amounts of base (sodium ethoxide). In order to circumvent this, the cyclization was performed using guanidine carbonate, which afforded the target analogues in 25-30% yield. The ¹H nmr of the products in deuterated dimethylsulfoxide indicated the presence of the N3-H as a broad singlet at δ 10.69-11.01, which was exchangeable with deuterium oxide. Surprisingly, in the case of analogues **14-17**, no evidence of diastereomers was observed in the ¹H nmr. This was unusual since the ¹H nmr of all the ketoesters synthesized, **22-30**, showed the presence of diastereomers as indicated by characteristic multiple resonances for the methyl group as well as for the C3-H. The origin for this stereochemical preference during the cyclization is unclear and could be due to the 1,3-diaxial strain between the thiophenyl substituent and the methyl moiety of the ketoesters **27-30**, which dictates the mode of cyclization in a stereospecific manner, with only the sterically less demanding diastereomer participating in the cyclization with guanidine. No attempt was made to elucidate the configuration of the stereogenic centers of analogues **8-17**.

All the analogues were evaluated [36,37] against thymidylate synthase from human, *Lactobacillus casei*, *Escherichia coli*, and *Pneumocystis carinii* and the results are shown in Table II. There is a trend toward greater inhibition of the human, *Escherichia coli* and *Pneumocystis carinii* thymidylate synthases in the 7-CH₃ series which is further enhanced as electron withdrawing groups are added to aromatic side chain, the most inhibitory compound being **17** the 7-CH₃, 3,4-dichloro derivative. Compared to the quinazoline analogues these tetrahydroquinazoline compounds were less potent against thymidylate synthase. This suggests that either the planar nature of the B-ring of 6-6 ring fused analogues is extremely important for the inhibition of thymidylate synthase, and/or that the thymidylate synthase inhibitory activity resides in the alternate stereoisomer(s) than those obtained in this study. These results along with observations reported in the pyrrolo[2,3-*d*]pyrimidine series [35] suggest that minor structural changes can completely alter the biological activities of 2-amino-4-oxo antifolates.

Table I



Compound	R ₁	R ₂
7	H	H
8	H	SC ₆ H ₅
9	H	S(4-ClC ₆ H ₄)
10	H	S(4-NO ₂ C ₆ H ₄)
11	H	S(4-OCH ₃ C ₆ H ₄)
12	H	S(3,4-diClC ₆ H ₃)
13	CH ₃	H
14	CH ₃	SC ₆ H ₅
15	CH ₃	S(4-ClC ₆ H ₄)
16	CH ₃	S(4-NO ₂ C ₆ H ₄)
17	CH ₃	S(3,4-diClC ₆ H ₃)

Table II

Inhibitory Concentrations of Analogues **7-17** as Inhibitors of Thymidylate Synthase from Human, *L. casei*, *E. coli*, and *P. carinii* [36,37]

Compound	Human	Percent Inhibition at 5 × 10 ⁻⁵ M			
		<i>L. casei</i>	<i>E. coli</i>	<i>P. carinii</i>	
7	0	0	0	0	
8	0	0	14	0	
9	0	15	30	13	
10	0	15	15	0	
11	0	0	30	0	
12	22	15	0	18	
13 [a]	0	0	0	0	
14 [a]	27	0	0	0	
15 [a]	25	20	15	20	
16 [a]	25	10	22	33	
17 [a]	43	0	50	50	

[a] Represents a single diastereomer.

EXPERIMENTAL

All glassware was meticulously washed with chromic acid, dried in a hot-air oven for 48 hours and then cooled under nitrogen, prior to use. Melting points were determined on a Fisher-Johns melting point apparatus or a Mel Temp apparatus and are uncorrected. Infrared spectra (ir) were recorded with a Perkin Elmer Model 1430, in Nujol mulls. Nuclear magnetic resonance spectra for proton (¹H nmr) were recorded on a Bruker WH-300 (300 MHz). The data was accumulated by 16K size with 0.5 second delay time and 70° tip angle with internal standard tetra-

methylsilane; s = singlet, br s = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet. Thin layer chromatography was performed on silica gel plates with fluorescent indicator and were visualized with light at 254 nm and 366 nm, unless indicated otherwise. Column chromatography was performed with 230-400 mesh silica gel purchased from Aldrich Chemical Company, Milwaukee, Wisconsin. Elution was performed using a gradient, and 10 ml fractions were collected, unless mentioned otherwise. All anhydrous solvents were purchased from Aldrich Chemical Company and were used without further purification. Samples for micro analysis were dried *in vacuo* over phosphorous pentoxide at 70° or 110°. Fractional moles of water and/or organic solvents found in some analytical samples could not be prevented, despite 24-48 hours of drying *in vacuo*, and were confirmed by their presence in the ^1H nmr spectrum. Microanalysis were performed by Atlantic Microlabs, Norcross, Georgia.

2-Carboethoxycyclohexen-1-one (20).

To a solution of phenylselenenyl chloride (1.18 g, 6.17 mmoles) and pyridine (0.51 g, 6.65 mmoles) in 25 ml of dichloromethane, at 0°, was added 2-carboethoxycyclohexanone **18** (1.0 g, 5.88 moles) and the reaction stirred under nitrogen for 4 hours. Analysis (tlc, hexanes:ethyl acetate 1:1) indicated the presence of a new product ($R_f = 0.8$). The solution was washed with 5 x 25 ml of 1N hydrochloric acid and cooled again to 0°. Hydrogen peroxide (30%) (4 x 0.45 ml) was added, in four separate portions at 15 minute intervals. The color of the reaction changed from a light yellow solution to a milky white suspension (additional amounts of hydrogen peroxide were added when necessary, to ensure completion of reaction). Analysis (tlc, hexanes:ethyl acetate 1:1) indicated the product as a single spot ($R_f = 0.20$). The reaction mixture was quenched with ~ 2-3 ml of water, washed with saturated sodium bicarbonate, and the organic layer dried over magnesium sulfate and evaporated to afford **20** as a yellow oil, (0.98 g, 99%); ^1H nmr (deuteriochloroform): δ 0.88 (t, 3 H, OCH_2CH_3), 1.60 (m, 2 H, CH_2), 2.10 (m, 4 H, CH_2), 3.81 (q, 2 H, OCH_2CH_3), 7.24 (t, 1 H, CH).

5-Methyl-2-carboethoxycyclohexen-1-one (21).

To a solution of phenylselenenyl chloride (2.17 g, 1.05 equivalents) and pyridine (0.94 g, 1.1 equivalents) in dichloromethane at 0°, was added 5-methyl-2-carboethoxycyclohexanone (2.0 g) and the reaction stirred under nitrogen for 5 hours. The reaction mixture was washed with 1 N hydrochloric acid, and then cooled to 0°. Hydrogen peroxide (30%) (4 x 1 ml) was added in four portions, 15 minutes apart and the reaction stirred for 10 minutes after the last addition. Water (5 ml) was added, and the reaction washed with (2 x 30 ml) of saturated sodium bicarbonate, the organic layer dried over magnesium sulfate and evaporated to afford **21** (mixture of stereoisomers) as a colorless oil, (1.45 g, 74%); tlc [hexanes:ethyl acetate (1:1) $R_f = 0.31$]; ^1H nmr (dimethyl- d_6 sulfoxide): δ 0.95 (d, 3 H, CH_3), 1.15 (t, 3 H, $\text{COOCH}_2\text{CH}_3$), 2.15 (m, 1 H, CH(CH_3)), 2.51 (m, 1 H, CH), 2.57 (m, 1 H, CH), 4.14 (m, 4 H, CH_2 , $\text{COOCH}_2\text{CH}_3$), 7.57 (dd, 1 H, C=CH).

General Procedure for the 1,4-Addition of Thiophenol and Substituted Thiophenols with 2-Carboethoxycyclohexen-1-one and 2-Carboethoxy-5-methylcyclohexen-1-one.

To a solution of 2-carboethoxycyclohexen-1-one **20** or 2-carboethoxy-5-methylcyclohexen-1-one **21** (1.00 equivalent) in 15 ml of dichloromethane at 0°, was added thiophenol (1.00 equiv-

alent) followed by triethylamine (0.1 ml). The reaction was stirred at 0° for 3 hours. Analysis (tlc, hexanes:ethyl acetate 1:1) indicated the absence of the starting cyclohexenone. The reaction mixture was washed with 1 N hydrochloric acid, and the organic layer dried over magnesium sulfate and evaporated to afford the adducts as a mixture of stereoisomers, which were not purified further and used for subsequent transformations.

3-Thiophenyl-2-carboethoxycyclohexanone (22).

This compound was synthesized from 2-carboethoxycyclohexen-1-one **20** (1.00 g, 5.95 mmoles), thiophenol (0.65 g, 5.95 mmoles) and triethylamine (0.1 ml) to afford **22** (mixture of stereoisomers) as a colorless oil. Analysis (tlc, hexanes:ethyl acetate 1:1) - $R_f = 0.68$ and 0.72; ^1H nmr (deuteriochloroform): δ 1.10-1.15 (m, 3 H, CH_3), 1.79-1.91 (m, 3 H), 2.01-2.25 (m, 3 H), 3.51 (m, 1 H, H-3), 3.92 (br s, 1 H, H-2), 4.09-4.19 (m, 2 H), 7.21-7.43 (m, 5 H).

3-(4'-Chlorothiophenyl)-2-carboethoxycyclohexanone (23).

This compound was synthesized from 2-carboethoxycyclohexen-1-one **20** (1.00 g, 5.95 mmoles), 4-chlorothiophenol (0.86 g, 5.95 mmoles) and triethylamine (0.1 ml) to afford **23** (mixture of stereoisomers) as a colorless oil; tlc (hexanes:ethyl acetate 1:1 - $R_f = 0.74$ and 0.80); ^1H nmr (dimethyl- d_6 sulfoxide): δ 1.12-1.19 (m, 3 H, CH_3), 1.57-1.65 (m, 3 H), 2.12-2.27 (m, 3 H), 3.81 (m, 1 H, H-3), 3.92 (br s, 1 H, H-2), 4.21-4.29 (m, 2 H, CH_2), 7.24 (m, 2H), 7.53 (m, 2H).

3-(4'-Nitrothiophenyl)-2-carboethoxycyclohexanone (24).

This compound was synthesized from 2-carboethoxycyclohexen-1-one **20** (1.00 g, 5.95 mmoles), 4-nitrothiophenol (0.84 g, 5.95 mmoles) and triethylamine (0.1 ml) to afford **24** (mixture of stereoisomers) as a yellow oil; tlc analysis (hexanes:ethyl acetate 1:1) - $R_f = 0.60$ and 0.70; ^1H nmr (dimethyl- d_6 sulfoxide): δ 1.23-1.27 (m, 3 H, CH_3), 1.51-1.60 (m, 3 H), 2.03-2.14 (m, 3 H), 3.91 (m, 1 H, H-3), 4.01 (br s, 1 H, H-2), 4.17-4.23 (m, 2 H, CH_2), 7.59 (m, 2H), 7.96 (m, 2H).

3-(4'-Methoxythiophenyl)-2-carboethoxycyclohexanone (25).

This compound was synthesized from 2-carboethoxycyclohexen-1-one **20** (1.00 g, 5.95 mmoles), 4-methoxythiophenol (0.86 g, 5.95 mmoles) and triethylamine (0.1 ml) to afford **25** (mixture of stereoisomers) as a colorless oil; tlc analysis (hexanes:ethyl acetates 5:2 - $R_f = 0.52$ and 0.58); ^1H nmr (dimethyl- d_6 sulfoxide): δ 1.17-1.28 (m, 3 H, CH_3), 1.62-1.72 (m, 3 H), 2.07-2.27 (m, 3 H), 3.41 (m, 1 H), 3.74 (m, 3 H, OCH_3), 3.91 (br s, 1 H), 4.15-4.22 (m, 2H, CH_2), 6.94 (m, 2H), 7.39 (m, 2H).

3-(3',4'-Dichlorothiophenyl)-2-carboethoxycyclohexanone (26).

This compound was synthesized from 2-carboethoxycyclohexen-1-one **20** (1.0 g, 5.95 mmoles), 3,4-dichlorothiophenol (1.10 g, 5.95 mmoles) and triethylamine (0.1 ml) to give **26** (mixture of stereoisomers) as a colorless oil; ^1H nmr (deuteriochloroform): δ 1.01-1.11 (overlapping t, 3 H, CH_3), 1.45-1.51 (m, 2 H, CH_2CH_2), 1.91-2.01 (m, 4 H), 3.94-4.05 (m, 4 H, CH_2 , CH), 6.89-6.95 (m, 1 H, 6'-H), 7.23-7.43 (m, 2 H, 2'-H and 5'-H).

5-Methyl-3-thiophenyl-2-carboethoxycyclohexanone (27).

This compound was synthesized from 2-carboethoxy-5-methylcyclohexen-1-one **21** (0.93 g, 5.1 mmoles), thiophenol (0.56 g, 5.1 mmoles) and triethylamine (0.1 ml) to afford **27** (mixture of stereoisomers) as a colorless oil; ^1H nmr (deuteriochloroform): δ 1.10-1.15 (m, 3 H, CH_3), 1.21-1.45 (m, 3 H), 1.71-1.82 (m, 1

H), 2.31-2.35 (m, 2 H), 3.51 (m, 2 H), 3.71 (m, 1 H), 3.97 (m, 1 H), 4.14-4.19 (m, 2 H), 7.28-7.40 (m, 5 H).

5-Methyl-3-(4'-chlorothiophenyl)-2-carbethoxycyclohexanone (28).

This compound was synthesized from 2-carbethoxy-5-methylcyclohexen-1-one **21** (0.93 g, 5.1 mmol), 4-chlorothiophenol (0.74 g, 5.1 mmol) and triethylamine (0.1 ml) to afford **28** (mixture of stereoisomers) as a yellow oil; ^1H nmr (dimethyl- d_6 sulfoxide): δ 1.12-1.19 (m, 6 H, CH_3), 1.63-1.65 (m, 1 H), 2.23-2.27 (m, 2 H), 3.52-3.55 (m, 2 H), 3.73 (m, 1 H), 3.92 (m, 1 H), 4.15-4.19 (m, 2 H, CH_2), 7.32 (m, 2 H), 7.39 (m, 2 H).

5-Methyl-3-(4'-nitrothiophenyl)-2-carbethoxycyclohexanone (29).

This compound was synthesized from 2-carbethoxy-5-methylcyclohexen-1-one **21** (1.02 g, 5.59 mmol), 4-nitrothiophenol (0.83 g, 5.59 mmol) and triethylamine (0.1 ml) to afford **29** (mixture of stereoisomers) as a yellow oil; ^1H nmr (dimethyl- d_6 sulfoxide): δ 1.14-1.15 (d, 3 H, CH_3), 1.29-1.37 (m, 3 H, CH_3), 1.46-1.52 (m, 1 H), 2.13-2.19 (m, 2 H), 3.55-3.61 (m, 2 H), 3.81 (m, 1 H), 3.97 (m, 1 H), 4.07-4.16 (m, 2 H, CH_2), 7.46 (m, 2 H), 7.89 (m, 2 H).

5-Methyl-3-(3',4'-dichlorothiophenyl)-2-carbethoxycyclohexanone (30).

This compound was synthesized from 5-methylcyclohexen-1-one **21** (1.02 g, 5.59 mmol), 3,4-dichlorothiophenol (1.00 g, 5.59 mmol) and triethylamine (0.1 ml) to afford **30** (mixture of stereoisomers) as a colorless oil; ^1H nmr (deuteriochloroform): δ 1.12-1.19 (overlapping t, 6 H, CH_3), 1.51-1.54 (m, 1 H), 2.21-2.25 (m, 2 H), 3.61-3.63 (m, 2 H), 3.89 (m, 1 H), 3.92 (m, 1 H), 3.99-4.11 (m, 2 H, CH_2), 6.90-6.99 (m, 1 H, 6'-H), 7.42-7.47 (m, 2 H, 2'-H and 5'-H).

2-Amino-4-oxo-5,6,7,8-tetrahydroquinazoline (7).

Freshly cut pieces of sodium metal (0.23 g, 10 mmol) were suspended in absolute ethanol and stirred for 30 minutes. At the end of this period, guanidine hydrochloride (1.0 g, 10 mmol) was added and the mixture stirred for 1 hour. This was followed by the addition of 2-carbethoxycyclohexanone (1.97 g, 10 mmol) and the mixture heated at reflux for 12 hours. A thick white precipitate formed which was filtered; tlc (chloroform:methanol 5:1) of this solid indicated it to be a single component ($R_f = 0.3$). The solid was washed with water to remove any unreacted guanidine and dried to afford **7** as a fluffy, white solid (1.64 g, 97%), mp 256-258°; ^1H nmr (dimethyl- d_6 sulfoxide): δ 1.61 (m, 4 H, CH_2), 2.18 (t, 2 H, CH_2), 2.29 (t, 2 H, CH_2), 6.51 (s, 2 H, 2-NH₂), 11.00 (s, 1 H, NH).

Anal. Calcd. for $\text{C}_8\text{H}_{11}\text{N}_3\text{O} \cdot 0.3\text{H}_2\text{O}$: C, 56.32; H, 6.85; N, 24.63. Found: C, 56.21; H, 6.63; N, 24.35.

2-Amino-4-oxo-7-methyl-5,6,7,8-tetrahydroquinazoline (13).

Freshly cut pieces of sodium metal (0.13 g, 5.65 mmol) were suspended in absolute ethanol and stirred for 30 minutes. At the end of this period, guanidine hydrochloride (1.0 g, 5.55 mmol) was added and the mixture stirred for 1 hour. This was followed by the addition of 5-methyl-2-carbethoxycyclohexanone (**21**) (0.94 g, 5.55 mmol) and the mixture heated at reflux for 12 hours. A thick white precipitate formed which was filtered. The tlc (chloroform:methanol 5:1) of this solid indicated it to be a single component ($R_f = 0.42$). The solid was washed with

water to rid of any guanidine and dried to afford pure 2-amino-4-oxo-7-methyl-5,6,7,8-tetrahydroquinazoline as a white solid, (1.64 g, 97%), mp = 256-259°; ^1H nmr (dimethyl- d_6 sulfoxide): δ 0.96 (d, 3 H, CH_3), 1.15 (m, 1 H), 1.71 (m, 2 H), 1.91-2.08 (m, 2 H), 2.32-2.37 (m, 2 H), 6.19 (s, 2 H, 2-NH₂), 10.69 (s, 1 H, NH).

Anal. Calcd. for $\text{C}_9\text{H}_{13}\text{N}_3\text{O} \cdot 0.5\text{H}_2\text{O}$: C, 57.43; H, 7.50; N, 22.32. Found: C, 57.11; H, 7.65; N, 22.47.

General Procedure for the Reaction of Guanidine Carbonate with Thiophenyl-substituted 2-Carbethoxycyclohexanone or Thiophenyl-substituted 2-Carbethoxy-5-methylcyclohexanone.

Guanidine carbonate (1 equivalent) was suspended in absolute ethanol and heated to reflux. At reflux, the appropriately substituted cyclohexanone (1.1 equivalents) was added and the reaction refluxed for 12 hours. At the end of this period, tlc analysis (chloroform:methanol 6:1) indicated the presence of a new product. The reaction was cooled to room temperature, 1.0 g of silica gel added, and the solvent evaporated to afford a dry plug which was eluted on a silica gel column with chloroform:methanol (5:1) to afford the desired compound.

2-Amino-4-oxo-5-(thiophenyl)-5,6,7,8-tetrahydroquinazoline (8).

This compound was synthesized from guanidine carbonate (0.72 g, 3.99 mmol) and 3-thiophenyl-2-carbethoxycyclohexanone (1.12 g, 4.03 mmol); tlc analysis (chloroform:methanol 6:1) indicated the presence of a new product ($R_f = 0.68$). Purification according to the general procedure described before afforded **8** as a white solid, (0.20 g, 18%), mp 283-285°; ^1H nmr (dimethyl- d_6 sulfoxide): δ 1.41-1.53 (m, 2 H), 1.81-2.4 (m, 4 H), 4.01-4.04 (m, 1 H), 6.5 (br s, 2 H, 2-NH₂), 7.23-7.54 (m, 5 H), 10.87 (br s, NH).

Anal. Calcd. for $\text{C}_{14}\text{H}_{15}\text{N}_3\text{OS} \cdot 0.6\text{H}_2\text{O}$: C, 59.17; H, 5.75; N, 14.79; S, 11.28. Found: C, 59.34; H, 5.91; N, 14.43; S, 11.19.

2-Amino-4-oxo-5-(4'-chlorothiophenyl)-5,6,7,8-tetrahydroquinazoline (9).

This compound was synthesized from guanidine carbonate (0.50 g, 2.77 mmol) and 3-(4'-chlorothiophenyl)-2-carbethoxycyclohexanone (0.30 g, 1.5 mmol); tlc analysis (chloroform:methanol 6:1) indicated the presence of a new product ($R_f = 0.76$). Purification according to the general procedure described above afforded **9** as a white solid; (0.12 g, 28%), mp 281-284°; ^1H nmr (dimethyl- d_6 sulfoxide): δ 1.41-1.49 (m, 3 H), 2.12-2.21 (m, 3 H), 3.41-3.43 (m, 1 H), 6.45 (br s, 2 H, NH₂), 7.38 (d, 2 H), 7.47 (d, 2 H), 10.80 (s, 1 H, NH).

Anal. Calcd. for $\text{C}_{14}\text{H}_{14}\text{N}_3\text{OSCl} \cdot 0.2\text{H}_2\text{O}$: C, 54.00; H, 4.66; N, 13.49; S, 10.30; Cl, 11.38. Found: C, 54.17; H, 4.95; N, 13.21; S, 10.43; Cl, 11.77.

2-Amino-4-oxo-5-(4'-nitrothiophenyl)-5,6,7,8-tetrahydroquinazoline (10).

This compound was synthesized from guanidine carbonate (0.70 g, 3.88 mmol) and 3-(4'-nitrothiophenyl)-2-carbethoxycyclohexanone (**24**). At the end of this period, tlc analysis (chloroform:methanol 6:1) indicated the presence of a new product ($R_f = 0.51$). Purification according to the general procedure described above afforded **10** as a yellow solid, (0.25 g, 20%), mp 264-266°; ^1H nmr (dimethyl- d_6 sulfoxide): δ 1.43-1.51 (m, 3 H), 2.41-2.57 (m, 3 H), 4.11 (m, 1 H), 6.61 (br s, 2H, NH₂), 7.53 (m, 2 H), 8.19 (m, 2 H), 10.98 (br s, NH).

Anal. Calcd. for $C_{14}H_{14}N_4SO_3 \cdot 0.7H_2O$: C, 50.81; H, 4.69; N, 16.93; S, 9.69. Found: C, 50.45; H, 4.92; N, 16.61; S, 9.33.

2-Amino-4-oxo-5-(4'-methoxythiophenyl)-5,6,7,8-tetrahydroquinazoline (11).

This compound was synthesized from guanidine carbonate (0.58 g, 3.21 mmoles), and 3-(4'-methoxythiophenyl)-2-carbethoxycyclohexanone (25) (1.0 g, 3.25 mmoles). At the end of this period, tlc analysis (chloroform:methanol 6:1) indicated the presence of a new product ($R_f = 0.63$). Purification according to the general procedure described above afforded 11, (0.21 g, 21%), mp 260-262°; 1H nmr (dimethyl- d_6 sulfoxide): δ 1.15-1.23 (m, 2 H), 1.59-1.75 (m, 2 H), 2.30-2.48 (m, 2 H), 3.71 (s, 3 H), 4.13 (m, 1 H), 6.39 (br s, 2 H, NH_2), 6.93 (d, 2 H), 7.45 (d, 2 H), 10.77 (br s, NH).

Anal. Calcd. for $C_{15}H_{17}N_3O_2S \cdot 0.3H_2O$: C, 58.35; H, 5.75; N, 13.61; S, 10.38. Found: C, 58.17; H, 5.42; N, 13.97; S, 10.57.

2-Amino-4-oxo-5-(3',4'-dichlorothiophenyl)-5,6,7,8-tetrahydroquinazoline (12).

This compound was synthesized from guanidine carbonate (0.26 g, 1.44 moles) and 4-(3',4'-dichlorothiophenyl)-2-carbethoxycyclohexanone (0.50 g, 1.44 mmoles). At the end of this period, tlc analysis (chloroform:methanol 6:1) indicated the presence of a new product ($R_f = 0.82$). Purification according to the general procedure described above afforded 12, (0.11 g, 23%), mp 289-292°; 1H nmr (dimethyl- d_6 sulfoxide): δ 1.51-1.62 (m, 4 H), 2.32-2.56 (m, 2 H), 4.12 (m, 1 H), 6.67 (br s, 2 H), 6.91 (m, 1 H, 6'-H), 7.34-7.41 (m, 2 H), 10.91 (br s, 1 H, NH).

Anal. Calcd. for $C_{14}H_{13}N_3OSCl_2 \cdot 0.7H_2O$: C, 47.39; H, 4.09; N, 11.84; S, 9.04; Cl, 19.98. Found: C, 47.51; H, 3.99; N, 11.48; S, 9.31; Cl, 20.32.

2-Amino-4-oxo-5-(thiophenyl)-7-methyl-5,6,7,8-tetrahydroquinazoline (14).

This compound was synthesized from guanidine carbonate (0.93 g, 5.16 mmoles) and 3-thiophenyl-2-carbethoxy-5-methylcyclohexanone (27) (1.5 g, 5.13 mmoles). Purification according to the general procedure described above afforded 14 as a white solid, (0.38 g, 26%), mp 282-285°; 1H nmr (dimethyl- d_6 sulfoxide): δ 1.13 (d, 3 H), 1.41-1.53 (m, 2 H), 1.87-2.2 (m, 1 H), 2.82-2.84 (m, 2 H), 4.12-4.14 (m, 1 H), 6.67 (br s, 2 H), 7.31-7.54 (m, 5 H), 10.92 (br s, NH).

Anal. Calcd. for $C_{15}H_{17}N_3OS \cdot 0.3H_2O$: C, 61.53; H, 6.06; N, 14.35; S, 10.95. Found: C, 61.21; H, 6.33; N, 14.67; S, 11.09.

2-Amino-4-oxo-5-(4'-chlorothiophenyl)-7-methyl-5,6,7,8-tetrahydroquinazoline (15).

This compound was synthesized from guanidine carbonate (0.55 g, 3.1 mmoles) and 3-(4'-chlorothiophenyl)-2-carbethoxy-5-methylcyclohexanone (28) (1.0 g, 3.1 mmoles). Purification according to the general procedure described above afforded 15 as a white solid, (0.22 g, 21%), mp 263-266°; 1H nmr (dimethyl- d_6 sulfoxide): δ 1.12-1.14 (d, 3 H, CH_3), 1.63-1.65 (m, 2 H), 2.23-2.27 (m, 3 H), 4.21-4.23 (m, 1 H), 6.51 (br s, 2 H, NH_2), 7.21 (d, 2 H), 7.45 (d, 2 H), 10.81 (s, 1 H).

Anal. Calcd. for $C_{14}H_{14}N_4OSCl \cdot 1.2H_2O$: C, 51.05; H, 5.02; N, 12.76; S, 9.73; Cl, 10.76. Found: C, 50.87; H, 4.81; N, 12.53; S, 9.61; Cl, 10.95.

2-Amino-4-oxo-5-(4'-nitrothiophenyl)-7-methyl-5,6,7,8-tetrahydroquinazoline (16).

This compound was synthesized from guanidine carbonate (0.48 g, 2.67 mmoles) and 3-(4'-nitrothiophenyl)-2-carbethoxy-5-methylcyclohexanone (29) (0.9 g, 2.67 mmoles). The reaction was cooled to room temperature, 1.0 g of silica gel added, and the solvent evaporated to afford a dry plug which was eluted on a silica gel column with chloroform:methanol (6:1) to afford 16 as a yellow solid, (0.27 g, 30%), mp 270-272°; 1H nmr (dimethyl- d_6 sulfoxide): δ 1.13-1.15 (d, 3 H), 1.49-1.54 (m, 2 H), 2.40-2.57 (m, 3 H), 4.02 (m, 1 H), 6.51 (br s, 2 H, NH_2), 7.59 (d, 2 H), 8.31 (d, 2 H), 11.01 (br s, NH).

Anal. Calcd. for $C_{15}H_{16}N_4O_3S \cdot 1.0H_2O$: C, 51.42; H, 5.18; N, 15.99; S, 9.15. Found: C, 51.05; H, 5.41; N, 15.81; S, 9.35.

2-Amino-4-oxo-5-(3',4'-dichlorothiophenyl)-7-methyl-5,6,7,8-tetrahydroquinazoline (17).

This compound was synthesized from guanidine carbonate (0.95 g, 5.27 mmoles) and 4-(3',4'-dichlorothiophenyl)-2-carbethoxy-5-methylcyclohexanone (30) (1.90 g, 5.26 mmoles). Purification according to the general procedure described above afforded 17 as a white solid, (0.54 g, 29%), mp 269-272°; 1H nmr (dimethyl- d_6 sulfoxide): δ 1.13-1.23 (d, 3 H), 1.50-1.61 (m, 2 H), 2.30-2.41 (m, 3 H), 4.11-4.15 (m, 1 H), 6.92 (br s, 2 H), 6.87 (m, 1 H, 6'-H), 7.21-7.41 (m, 2 H), 10.99 (br s, 1 H, NH).

Anal. Calcd. for $C_{15}H_{16}N_3OSCl_2 \cdot 0.6H_2O$: C, 48.95; H, 4.71; N, 11.42; S, 8.71; Cl, 19.26. Found: C, 48.61; H, 4.43; N, 11.15; S, 8.39; Cl, 19.04.

Acknowledgments.

This work was supported in part by NIH grant GM 40998 (AG) and CA 10914 (RLK). Human and *E. coli* TS were supplied by Dr. David Matthews, Agouron Pharmaceuticals, San Diego, CA. *Pneumocystis carinii* thymidylate synthase was supplied by Dr. D. V. Santi, University of California, San Francisco.

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