Design and Synthesis of Novel 6,7-Imidazotetrahydroquinoline Inhibitors of Thymidylate Synthase Using Iterative Protein Crystal Structure Analysis[1]

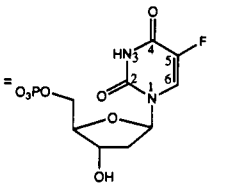
Siegfried H. Reich,* Mary Ann M. Fuhry, Dzuy Nguyen, Mark J. Pino, Katherine M. Welsh, Stephanie Webber, Cheryl A. Janson, Steven R. Jordan, David A. Matthews, Ward W. Smith, Charlotte A. Bartlett, Carol L. J. Booth, Steven M. Herrmann, Eleanor F. Howland, Cathy A. Morse, Robert W. Ward, and Jennifer White

Agouron Pharmaceuticals, Inc., 3565 General Atomics Court, San Diego, California 92121. *Received August 13, 1991*

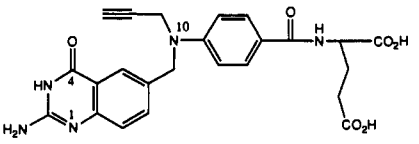
Antifolate inhibitors of thymidylate synthase (TS) have primarily been based on the structure of folic acid. This paper describes the identification and development of novel 6,7-imidazotetrahydroquinoline TS inhibitors by iterative ligand design, synthesis, and crystallographic analysis of protein–inhibitor complexes. Beginning with a high-resolution crystal structure of *E. coli* TS (TS, EC 2.1.1.45), an imidazotetrahydroquinoline inhibitor was designed de novo to occupy the folate binding pocket. Structural modifications of the initial compound **1h** ($K_i \approx 5$ μM human/*E. coli* TS) were then made on the basis of feedback from additional cocrystal structures and activity data. An amino group in the 2-position of the imidazole was found to increase the potency of the series by 1–2 orders of magnitude. Other substitutions on the imidazole ring (1-CH_3, 2-CH_3, 2-$NHCH_3$, 2-SCH_3) generally led to weaker inhibition. Additional improvements in activity were obtained by modification of the substituents on the tetrahydroquinoline nitrogen, bringing the K_i of three of the compounds below 15 nM against the human TS enzyme. The compounds were tested for cytotoxicity and were shown to inhibit the growth of three tumor cell lines in vitro.

Thymidylate synthase (TS) is the limiting enzyme in the metabolic pathway for the de novo synthesis of thymidylate (dTMP). The activity of this enzyme is particularly high in rapidly proliferating cells such as tumor cells and considerably lower in normal cells. The enzyme carries out a one-carbon transfer from the cofactor methylenetetrahydrofolate (mTHF) to the substrate deoxyuridylate monophosphate (dUMP). Since TS provides the sole de novo source of thymidylate, a required precursor in DNA biosynthesis, the enzyme is a target for cancer and other proliferative disease chemotherapy.[2] Inhibitors exist for both the folate cofactor mTHF and the pyrimidine substrate dUMP. The pyrimidine analogues 5-fluorouracil and 5-fluorodeoxyuridine are clinical agents which are metabolized to the active anti-tumor compound 5-fluoro-2′-deoxyuridylate (FdUMP).[3]

While inhibition of TS has been accomplished with both folate cofactor and nucleotide substrate analogues, folate-based inhibitors have certain therapeutic advantages. A folate inhibitor will not compete with elevated levels of dUMP arising through inhibition of TS. Also, most nucleotide-based inhibitors require prior metabolic activation providing additional loci for resistance. Folate analogues are free of toxicity problems involving incorporation of nucleotide-based inhibitors, such as 5-fluorouracil, into nucleic acids.[4] Cofactor analogues have for the most part been based structurally on folic acid.[5] The N^{10}-propargyldideazafolic acid compound, CB3717, is representative of this type of "classical" glutamate containing TS inhibitor.[6] As part of our ongoing effort to apply information obtained from a protein crystal structure to the design of novel inhibitors we report a new class of lipophilic TS inhibitor structurally unrelated to other known TS inhibitors.



5-Fluoro-2'-deoxyuridylate
(F-dUMP)



N-10-Propargyl-5,8-dideazafolate
(CB3717)

The detailed information contained in a protein crystal structure such as the morphology of the binding site, the location and orientation of hydrogen bonding groups, and the electrostatic potential surface can be used to aid the design and optimization of inhibitor leads. This allows the chemist to rely less on the structure of the natural ligand and more on the three-dimensional details of the binding site which ultimately dictate which type of compound will bind effectively. Many enzymes utilize folates as cofactors so that the information from a crystal structure has the added potential of increasing the selectivity for a particular folate binding enzyme, in this case TS.

Beginning with a high-resolution crystal structure of the ternary complex of *E. coli* TS, the known inhibitor CB3717, and the substrate analogue FdUMP, a novel imidazotetrahydroquinoline inhibitor was designed on a computer graphics terminal to occupy the folate binding site. The initial compound **1h** was synthesized and had

(1) A preliminary account of this work has been published: Appelt, K., et al. Design of Enzyme Inhibitors Using Iterative Protein Crystallographic Analysis. *J. Med. Chem.* **1991**, *34*, 1925–34.

(2) Jackman, A. L.; Jones, T. R.; Calvert, H. A. Thymidylate Synthase Inhibitors: Experimental and Clinical Aspects. *Experimental and Clinical Progress in Cancer Chemotherapy*; Muggia, F. M., Ed.; Martinus Nijhoff Publishers: Boston, 1985; pp 155–210. Santi, D. V.; Danenberg, P. V. Folates in Pyrimidine Nucleotide Biosynthesis. *Folates and Pterins*; Blakley, R. L., Benkovic, S. J., Eds.; John Wiley & Sons: New York, 1984; pp 345–398.

(3) Lewis, C. A.; Dunlap, R. B. Thymidylate Synthase and Its Interaction with 5-Fluoro-2′-deoxyuridylate. *Topics in Molecular Pharmacology*; Burgen, A. S. V., Roberts, G. C. K., Eds.; Elsevier: Amsterdam, 1981; pp 169–219.

(4) Myers, C. E. The Pharmacology of Fluoropyrimidines. *Pharmacol. Rev.* **1981**, *33*, 1–15.

(5) Hughes, W. T. Folate Antagonists as Therapeutic Agents; Sirotnak, F. M., Ed.; Academic Press: Orlando, 1984; Vol. 2, pp 1025–1033.

(6) Jones, T. R.; Calvert, A. H.; Jackman, A. L.; Brown, S. J.; Jones, M.; Harrap, K. R. A Potent Antitumour Quinazoline Inhibitor of Thymidylate Synthetase: Synthesis, Biological Properties and Therapeutic Results in Mice. *Eur. J. Cancer* **1981**, *17*, 11–19.

0022-2623/92/1835-0847$03.00/0

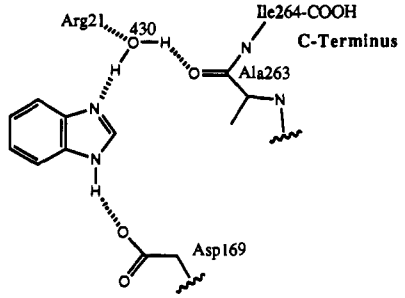


Figure 1. Hydrogen-bonding network made by the proposed benzimidazole system involving amino acid sidechains Arg21, Asp169, Ala263, and water molecule 430 in the active site of *E. coli* TS.

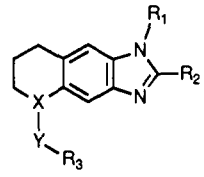
a K_i of ca. 5 μM against both the human and *E. coli* TS enzyme. Through the analysis of subsequent TS–inhibitor crystal structures modified inhibitors were designed and synthesized, resulting in compounds having K_is in the low-nanomolar range. These were also shown to inhibit the growth of three tumor cell lines in vitro.

Design of Inhibitors/TS Inhibition Studies

Our primary focus has been on the identification of anticancer agents based on the inhibition of TS. The human TS enzyme, therefore, becomes the logical choice for structure-based design. We initiated our design work, however, using the *E. coli* enzyme because it could be expressed in sufficient quantities required for crystallographic studies.[7] The crystal structure of the *E. coli* thymidylate synthase (TS) ternary complex containing FdUMP and CB3717 was determined to 2.3-Å resolution and has been previously described.[8] The inhibitor was removed from the complex model, leaving the substrate analogue FdUMP in place, which itself comprises the back of the folate binding pocket. Analysis of the folate binding site and in particular the H-bonding requirements near the C-terminus of the protein led to the concept of a benzimidazole substructure which was modeled to accept a hydrogen bond from ordered water molecule 430 and donate an H-bond to the carboxylate of Asp169 (Figure 1). Similar hydrogen bonding interactions are observed in the quinazoline inhibitor CB3717 from N1 and N3. The folate binding site is narrow and flat in this area but then widens considerably as it makes an acute angle toward Phe176 and the entrance of the active site.

To qualitatively measure the interaction energies between different functional groups and the protein, the empty active site was analyzed using the program GRID.[9] Areas interacting favorably with a methyl probe were identified particularly in the wider portion of the active site. A saturated ring was attached to the benzimidazole resulting in the 6,7-imidazotetrahydroquinoline system

Table I. Substituted Imidazotetrahydroquinoline Thymidylate Synthase Inhibitors



compd	X	Y	R_1	R_2	R_3
1h	N	CH_2	H	H	4-$(SO_2N(C_2H_4)_2NH)C_6H_4$
2h	N	CH_2	H	NH_2	4-$(SO_2N(C_2H_4)_2NH)C_6H_4$
2i	N	CH_2	H	NH_2	4-$(PhSO_2)C_6H_4$
2j	N	CH_2	H	NH_2	4-$((4\text{-}OHC_6H_4)SO_2)C_6H_4$
2k	N	CH_2	H	NH_2	4-$((4\text{-}OMeC_6H_4)SO_2)C_6H_4$
2l	N	CH_2	H	NH_2	6-$(HOCH_2)$B-naphthyl
2m	N	CH_2	H	NH_2	4-$(O(C_2H_4)_2NSO_2)C_6H_4$
3i	N	CH_2	H	SCH_3	4-$(PhSO_2)C_6H_4$
4i	N	CH_2	H	$NHCH_3$	4-$(PhSO_2)C_6H_4$
5k	N	CH_2	CH_3	NH_2	4-$((4\text{-}OMeC_6H_4)SO_2)C_6H_4$
6k	N	CH_2	H	CH_3	4-$((4\text{-}OMeC_6H_4)SO_2)C_6H_4$
7k	N	CH_2	CH_3	CH_3	4-$((4\text{-}OMeC_6H_4)SO_2)C_6H_4$
8	N	CH_2	H	NH_2	4-$(CONHC(CO_2H)\text{-}(C_2H_4CO_2H)$
9i[a]	CH	S	H	NH_2	4-$(PhSO_2)C_6H_4$

[a] A mixture of enantiomers.

reminiscent of the reduced cofactor tetrahydrofolate. This ring system was found to fill the wider portion of the active site well while allowing additional groups to be added to the tetrahydroquinoline nitrogen to interact with hydrophobic side chains in the active site entrance such as Phe176, Val262, and Ile79. Initially, a (piperazinylsulfonyl)benzyl group, derived from work on related novel TS inhibitors,[10] was modeled to fill this region and impart additional water solubility,[11] resulting in compound **1h** as our first test compound.

Compound **1h** was synthesized and found to have K_is of 4.9 and 7.7 μM against *E. coli* and human TS, respectively (Table I). A crystal structure of this new inhibitor complexed with the *E. coli* TS enzyme was obtained, providing a starting point for further optimization. Analysis of the cocrystal structure indicated that compound **1h** bound essentially as modeled. Continuous electron density between N1 of the ligand and water 430 and N3 of the ligand and Asp169 are indicative of hydrogen bonding (see Figure 2). The saturated ring of the tetrahydroquinoline makes good van der Waals contact with hydrophobic residues Ile79 and Trp80. A minor difference between the binding observed in the crystal structure and the modeled conformation was a slight rotation of about 20–30 degrees in the phenyl ring off the tetrahydroquinoline nitrogen (the torsion between the benzyl carbon and the phenyl ring). This mode of binding was observed with 1h and in most other complexes in this series. This rotation causes the phenyl ring to move away from an edge-on interaction with Phe176, typically observed in quinazoline-based inhibitors such as CB3717, to one that is more parallel being sandwiched between Phe176 and Leu172. Inspection of the cocrystal structure of compound 1h revealed that additional hydrogen bonding to the backbone carbonyl oxygen of Ala263 and the protein C-terminus might be realized by extending an amino group off the 2-position of the imidazole ring. The position of this modeled amino group was essentially the

(7) There is about a 70% identity between residues comprising the folate binding site in the human and *E. coli* enzyme. Work on the human TS structure is currently ongoing.

(8) Matthews, D. A.; Appelt, K.; Oatley, S. J.; Xuong, Ng. H. Crystal Structure of *Escherichia coli* Thymidylate Synthase Containing Bound 5-Fluoro-2′-deoxyuridylate and 10-Propargyl-5,8-dideazafolate. *J. Mol. Biol.* **1990**, *214*, 923–936. Matthews, D. A.; Villafranca, J. E.; Janson, C. A.; Smith, W. A.; Welsh, K.; Freer, S. Stereochemical Mechanism of Action for Thymidylate Synthase Based on the X-ray Structure of the Covalent Inhibitory Ternary Complex with 5-Fluoro-2′-deoxyuridylate and 5,10-Methylenetetrahydrofolate. *J. Mol. Biol.* **1990**, *214*, 937–948.

(9) Goodford, P. J. A Computational Procedure for Determining Energetically Favorable Binding Sites on Biologically Important Macromolecules. *J. Med. Chem.* **1985**, *28*, 849–857.

(10) Varney, M. D.; et al. *J. Med. Chem.*, in press.

(11) Solubility of the ligand in excess of its binding constant is desirable when testing a new inhibitor class to increase the chances of obtaining a cocrystal structure.

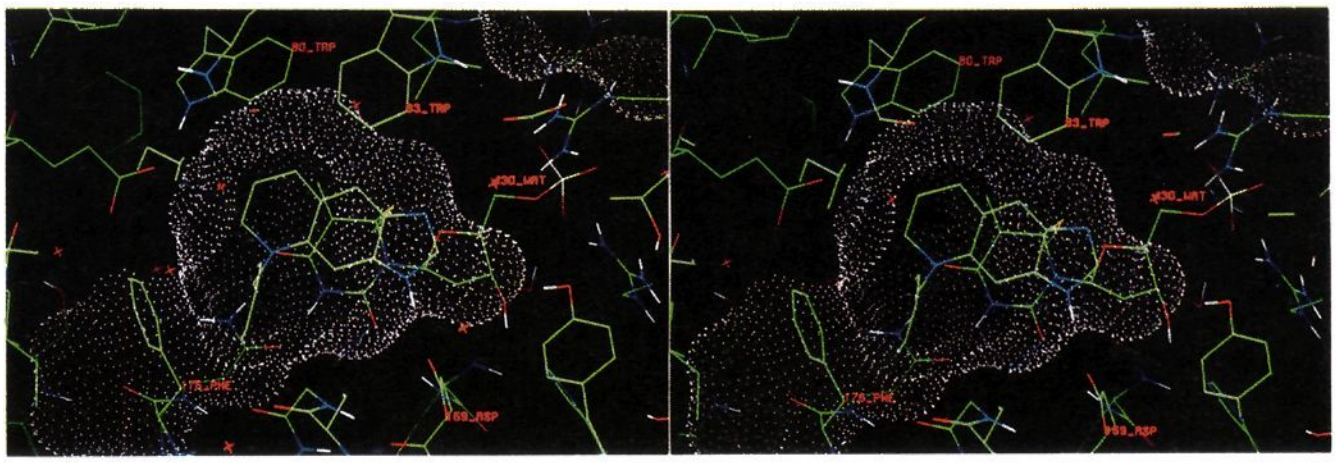


Figure 2. Stereoview of the tricyclic portion of compound **1h** complexed with *E. coli* TS. Protein and ligand atoms are shown in green (carbon), blue (nitrogen), and red (oxygen). Water molecules are represented as red crosses. The protein's solvent accessible surface is shown in beige, and key residues Asp169, Trp80, Trp83, Phe176, and water molecule 430 are labeled. The covalently bound FdUMP molecule which lies up against the inhibitor and comprises the back of the binding pocket can be seen behind the solvent accessible surface.

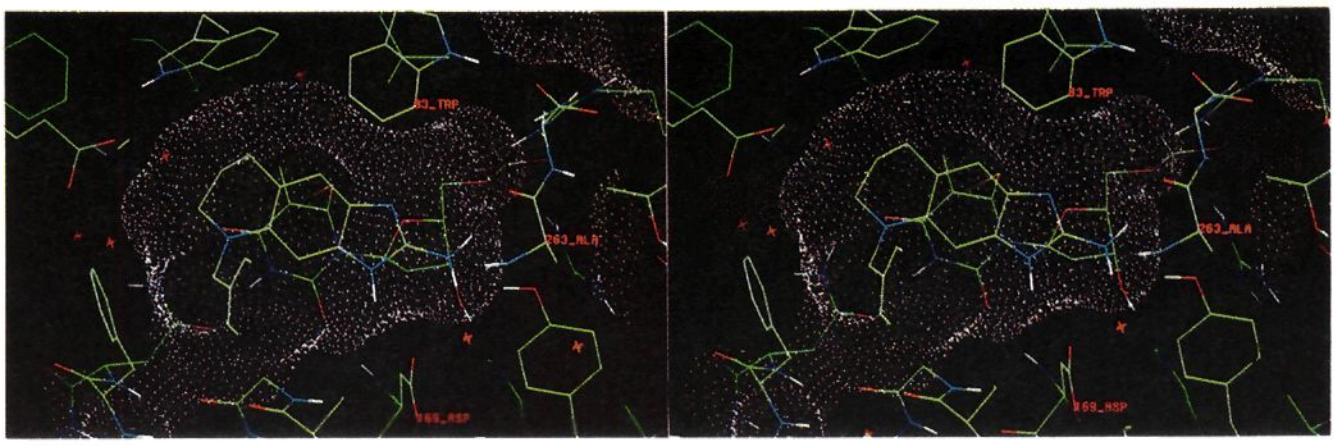


Figure 3. Stereoview of 2-amino compound **2h** complexed with *E. coli* TS. The carbonyl of residue Ala263 involved in a hydrogen bond with the 2-amino group is visible. The unoccupied space where water molecule 430 is normally found is clearly visible in the solvent accessible surface.

same as the 2-amino group in the quinazoline series (CB3717) where an 8-fold enhancement in binding over the 2-hydrogen is observed.[12]

The 2-amino compound **2h** was synthesized and tested, showing an improvement in binding by a factor of 35 (*E. coli* TS) and 120 (human TS) relative to the parent compound **1h** (Table I). The crystal structure of **2h** complexed with TS is shown in Figure 3. The binding mode is essentially the same as observed with **1h** except there is no electron density observed for water molecule 430. In fact, density for this water molecule was absent in cocrystal structures of a number of 2-amino-substituted compounds in this series. The 2-amino group in **2h** does make a hydrogen bond to the Ala263 carbonyl as anticipated although the carbonyl oxygen is slightly out of the plane of the imidazole ring. The 2,5-diaminobenzimidazole system is most likely protonated when bound in the active site of TS. We determined the pK_a of compound **21** to be 8.2 in DMF–H_2O.[13] The pK_a of the benzimidazole ring system in unsubstituted compound **1h** is expected to be below 6.5 on the basis of the pK_a of the 2-methyl compound **6k** (vide infra). In the 2-amino compound **2h** protonation places a hydrogen on N1 and N3 of the imidazole ring so that the imidazole now donates a hydrogen bond from N1 instead of accepting a hydrogen bond as observed in **1h**. This interaction is apparently unfavorable to the extent that the occupancy of water molecule 430 is considerably reduced. The enhanced binding observed in the 2-amino-substituted compounds may, in part, be due to a favorable electrostatic interaction of the protonated aminoimidazole with Asp169.

We next turned our attention to the groups appended to the tetrahydroquinoline nitrogen in search of further improvements in binding. Replacement of the terminal piperazinyl group with a phenyl group resulted in a 2-fold enhancement in binding. Further substitution of this terminal phenyl ring with a hydroxyl group led to an additional 2-fold enhancement in binding observed in phenolic compound **2j** with a K_i of 14 nM[14] against human TS.

In an attempt to diverge from the substituted benzyl group of previous compounds a 2-substituted naphthalene ring was found to fit well when modeled into the pocket normally occupied by the phenylsulfonyl group with the B ring of the naphthalene lying up against Val262. A hydroxymethyl group was added to the 6-position of the naphthalene ring to extend into solvent and increase solubility. The naphthalene compound **2l** was found to

(12) Jones, T. R.; Thornton, T. J.; Flinn, A.; Jackman, A. L.; Newell, D. R.; Calvert, A. H. Quinazoline Antifolates Inhibiting Thymidylate Synthase: 2-Desamino Derivatives with Enhanced Solubility and Potency. *J. Med. Chem.* **1989**, *32*, 847–852.

(13) Potentiometric titrations were performed at 25 °C as described by Albert and Serjeant (Albert, A.; Serjeant, E. P. *The Determination of Ionization Constants. A Laboratory Manual*, 3rd ed.; Chapman and Hall: New York, 1984) in DMF–water (67:33).

(14) The K_{is} of the most active compounds **2j**, **2m**, and **8** have been determined using tight binding kinetics as described by Henderson (Henderson, P. J. F. A Linear Equation That Describes the Steady-State Kinetics of Enzymes and Subcellular Particles Interacting with Tightly Bound Inhibitors. *Biochem. J.* **1972**, *127*, 321–333) and the values are substantially lower, e.g., **2j**:K_{is} = K_{ii} = 2.6 nM; **2m**:K_{is} = 4.5 nM, K_{ii} = 13.5 nM; **8**:K_{is} = 2.2 nM, K_{ii} = 6.0 nM against the human enzyme. Welsh, K. M., manuscript to be published elsewhere.

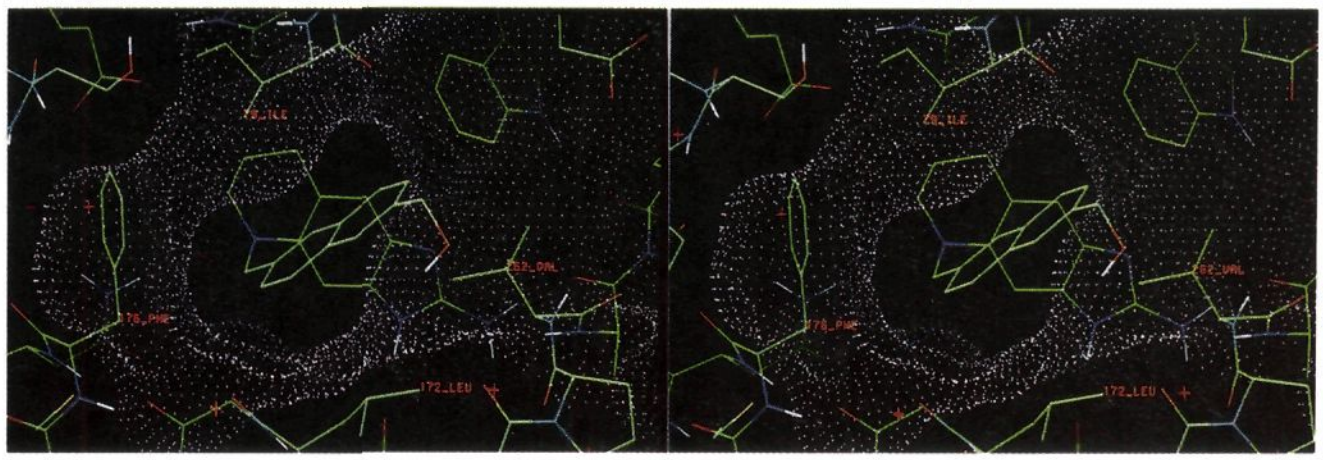
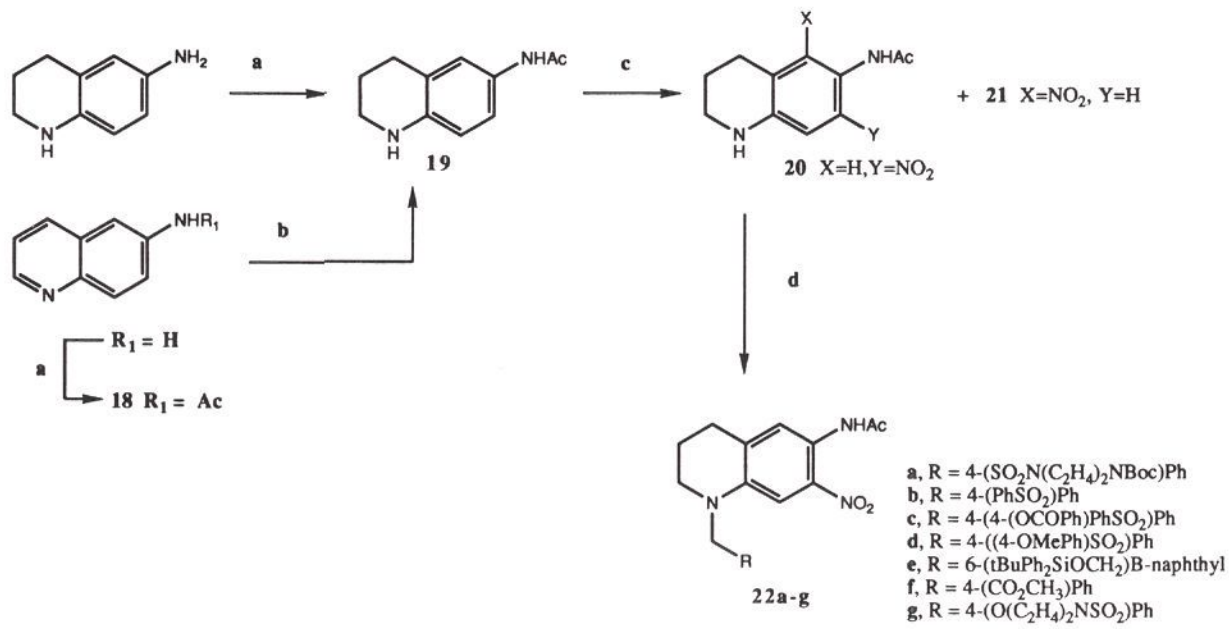


Figure 4. Stereoview of naphthalene-containing compound **2l** complexed with *E. coli* TS highlighting the region where the naphthalene portion binds. Lipophilic residues interacting with the naphthalene ring, Ile79, Leu172, and Val262 are labeled.

Scheme I[a]



[a] (a) Ac_2O; (b) PtO_2, AcOH, H_2; (c) HNO_3; (d) RCH_2Br, iPR_2NEt or $CaCO_3$, DMF or DMA, 85 °C.

be essentially equipotent with compound **2i**, establishing the naphthalene system as an effective replacement for the typical para-disubstituted phenyl ring found in most TS inhibitors. A cocrystal structure of this new naphthalene-containing inhibitor is shown in Figure 4. The naphthalene ring bound largely as modeled making good van der Waals contact with lipophilic residues Ile79, Leu172, and Val262.

Glutamate-containing analogues of the folate cofactor can be subject to active transport across cell membranes and/or polyglutamylation by folylpolyglutamyltransferase (FPGS) as are a number of classical quinazoline-based TS inhibitors.[15] A glutamate-containing compound **8** was prepared and was found to be a potent inhibitor of TS in vitro with K_is of 5 and 11 nM[14] against the *E. coli* and human enzymes, respectively, but had poor cell culture activity (vide infra). This may correlate with a failure of compound **8** to be actively transported and/or to function as a substrate for FPGS.

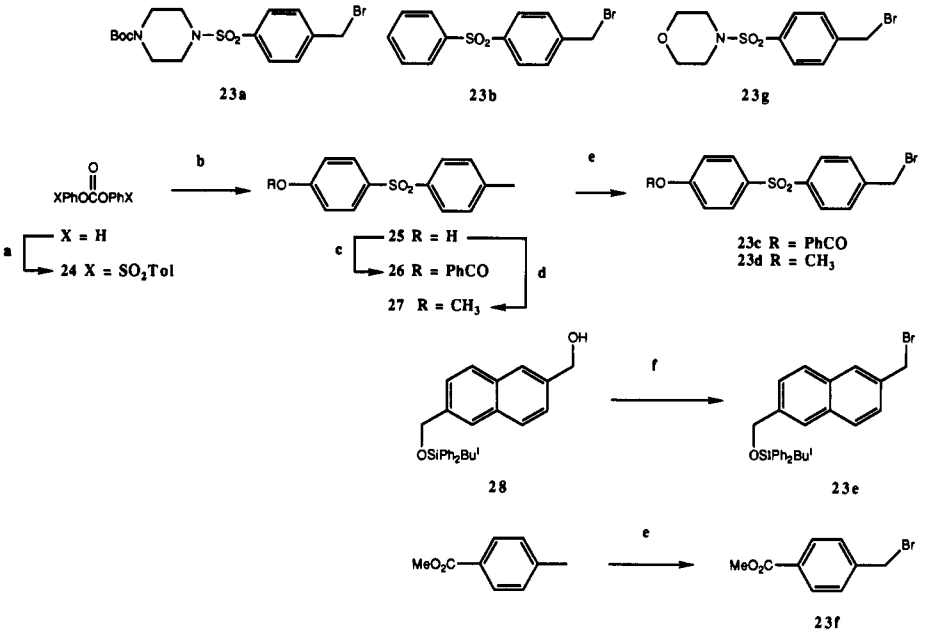
Returning to the benzimidazole portion of the molecule, it was envisioned that the space normally occupied by water molecule 430 might be filled with an appropriate substituent to enhance binding. A series of 1- and 2-substituted derivatives were modeled and synthesized in an attempt to fill the space left by the displaced water molecule. In addition, we were interested in modulating the pK_a of the benzimidazole system to better understand the relationship between pK_a and activity. Compound **6k**, which has a 2-methyl group in place of the 2-amino group, was less active by a factor of 20 relative to **2k**. The pK_a of 2-methylimidazole compound **6k** was found to be 6.4 in DMF–H_2O, suggesting that protonation of the benzimidazole system is in fact a requirement for potency in this series. The 1-methyl-2-amino compound **5k**, which is also expected to be protonated in solution, was equipotent with the parent compound **2k**. The remaining 2-substituted derivatives, 2-methylthio compound **3i** and 2-methylamino compound **4i**, were considerably less active than the 2-amino derivative **2h**.

Another means of modulating the pK_a of the benzimidazole nucleus, albeit in a more subtle way, was by replacing the tetrahydroquinoline nitrogen with a carbon.[16] The tetrahydronaphthalene sulfide **9i** was designed to place the polarizable and lipophilic sulfur atom up against the phenyl ring of Phe176 while lowering the pK_a slightly. This system had the additional appeal of replacing the tetrahydroquinoline nitrogen, which has a variable hybridization state, with the less flexible sp^3 carbon. Sulfide **9i**, synthesized as a mixture of enantiomers, was disappointingly found to be less active than the parent tetrahydroquinoline system by a factor of 7.

(15) Jackman, A. L.; Taylor, G. A.; Bishop, J. A.; O'Connor, B. M.; Bisset, G.; Hughes, L. R.; Moran, R. G. Calvert Biochemical Aspects of a New Thymidylate Synthase Inhibitor, D1649. *Proc. Am. Assoc. Cancer Res.* **1990**, *31*, 342. 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.

(16) The pK_a values of 5-methyl- and 5-aminobenzimidazole in water are 5.81 and 6.11, respectively. Hofmann, K. *Imidazole and Its Derivatives*; Wiley-Interscience: New York, 1953; Part I, p 251.

Scheme II[a]

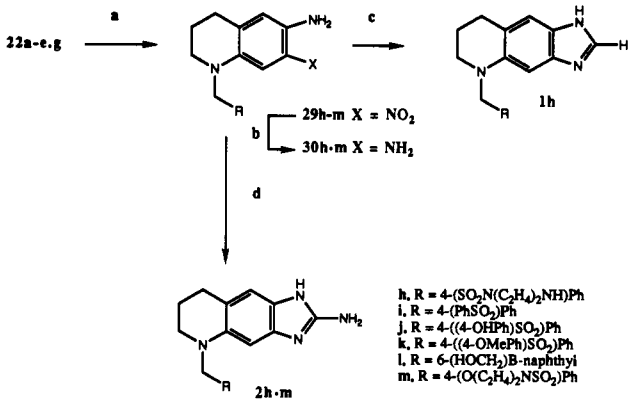


[a] (a) ClO_2SPhCH_3, $PhNO_2$, $FeCl_3$, 120 °C; (b) (i) KOH–EtOH, 80 °C; (ii) HCl; (c) PhCOCl, pyridine; (d) CH_3I, K_2CO_3, acetone; (e) NBS, CCl_4, $h\nu$; (f) CBr_4, Ph_3P.

Chemistry

The syntheses of compounds **1h–9i** are summarized in Schemes I–VI. We initially planned on preparing the 6,7-imidazotetrahydroquinoline nucleus from the 6,7-dinitrotetrahydroquinoline, reportedly obtained in 70% yield from 6-nitrotetrahydroquinoline.[17] Attempts at reproducing this result gave only intractable mixtures of isomeric nitro compounds. The key intermediate **19**, used in our synthesis, could however be prepared from 6-aminotetrahydroquinoline or 6-aminoquinoline, the latter being the method of choice (Scheme I). Selective acylation of 6-aminotetrahydroquinoline, followed by nitration, afforded the 6-(acetylamino)-7-nitro- and 6-(acetylamino)-5-nitrotetrahydroquinoline isomers **20** and **21** in a 2.5:1 ratio, respectively.[18] Alternatively, intermediate **19** could be obtained by acylation of commercially available 6-aminoquinoline, followed by catalytic hydrogenation.[19] Alkylation of **20** with the benzylic bromides **23a–g** proceeded smoothly with base in DMF or DMA to provide the desired coupled products **22a–g**. Bromides **23a**, **23b**, and **23g** were generously provided by Varney et al. and their preparation has been described elsewhere.[10] Bromides **23c** and **23d** were prepared beginning with Friedel–Crafts sulfonylation of diphenyl carbonate[20] to afford the sulfonylphenyl carbonate **24** (Scheme II). Hydrolysis of **24** to the phenol **25** and acylation or methylation yielded the phenyl sulfones **26** and **27**, respectively. Finally, bromination of the phenyl sulfones **26** and **27** with NBS and light afforded the benzylic bromides **23c** and **23d**. Bromide **23e** was obtained by bromination of alcohol **28**[10] using CBr_4–Ph_3P.[21]

Scheme III[a]

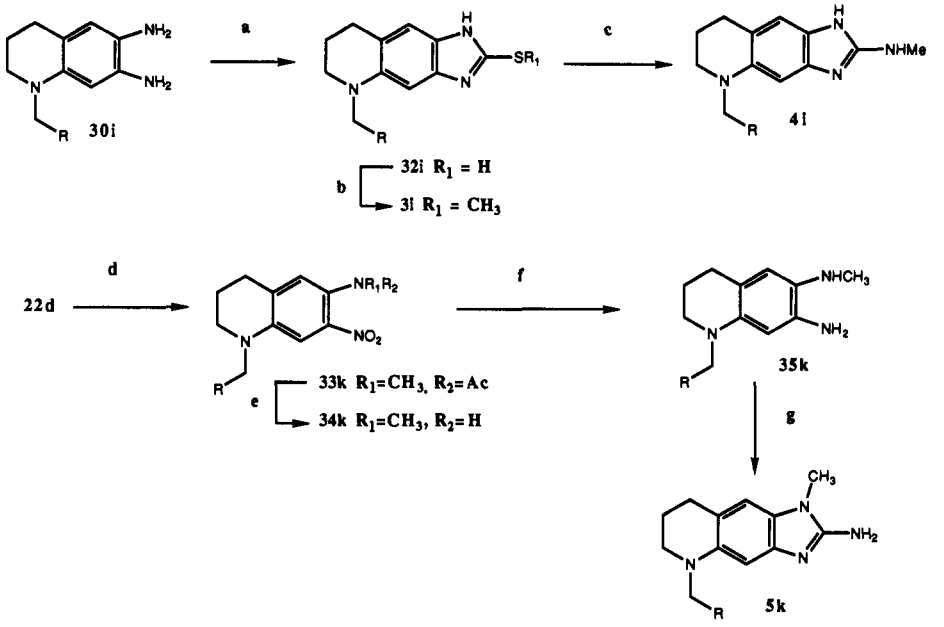


[a] (a) Aqueous HCl, reflux; (b) RaNi, NH_2NH_2 or Zn, AcOH; (c) $HC(OMe)_3$, HCl; (d) CNBr.

Removal of the acetyl group to give intermediates **29h–m** followed by reduction of the nitro group (RaNi/hydrazine[22] or Zn/AcOH[23]) yielded the ortho diamines **30h–m** as shown in Scheme III. Cyclization of the diamines with cyanogen bromide afforded the desired 2-aminoimidazotetrahydroquinoline targets **2h–m** in moderate yields.[24] Treatment of the diamino compound **29h**

(17) Il'ina, I. G.; Kazennova, N. B.; Terent'ev, A. P. *Khim. Geterotsikl. Soedin.* **1973**, *1*, 80–83.
(18) Amit, B.; Ben-Efraim, D. A.; Patchornick, A. Light Sensitive Amides. Photocleavage of N-Acyl-1,2,3,4-tetrahydro-8-nitroquinolines to give Free Carboxylic Acids. *J. Chem. Soc., Perkins Trans. 2* **1976**, 57–63.
(19) Ishikawa, H.; Tabusa, F.; Miyamoto, H.; Kano, M.; Ueda, H.; Tamaoka, H.; Nakagawa, K. Studies on Antibacterial Agents. I. Synthesis of Substituted 6,7-Dihydro-1-oxo-1*H*,5*H*-benzo-[i,j]-quinolizine-2-carboxylic Acids. *Chem. Pharm. Bull.* **1989**, *37*, 2103–2108.
(20) Attwood, T. E.; Barr, D. A.; Feasey, V. J.; Leslie, V. J.; Newton, A. B.; Rose, J. B. Poly(arylene ether sulfones) by Polyetherification: 1. Synthesis of Halogenophenols. *POLYMER* **1977**, *18*, 354–58.
(21) Lee, J. B.; Nolan, T. J. Sugar Esters III. New Reagent for Deoxyhalo Sugar Preparation. *Can. J. Chem.* **1966**, *44*, 1331–1334.
(22) Abuzar, S.; Dubey, R.; Sharma, S. Syntheses of 2,5-(6)-Disubstituted Benzimidazoles and 1,4-Disubstituted Piperazines as Potential Antiparasitic Agents. *Indian J. Chem.* **1985**, *24B*, 848–52.
(23) Fieser and Fieser, *Reagents for Organic Synthesis*; John Wiley and Sons, Inc.: New York, 1967; Vol. I, p 1276.
(24) Wright, J. B. Synthesis, Reactions, and Spectroscopic Properties of Benzimidazoles. *Chem. Rev.* **1974**, *74*, 279–314. Ohemeng, K. A.; Roth, B. Receptor-Based Design of Novel Dihydrofolate Reductase Inhibitors: Benzimidazole and Indole Derivatives. *J. Med. Chem.* **1991**, *34*, 1383–1394.

Scheme IV[a]



[a] (a) $(Imid)_2C{=}S$; (b) MeI; (c) $MeNH_2$; (d) MeI, K_2CO_3, DMF, 60 °C; (e) aqueous HCl, reflux; (f) RaNi, NH_2NH_2; (g) CNBr.

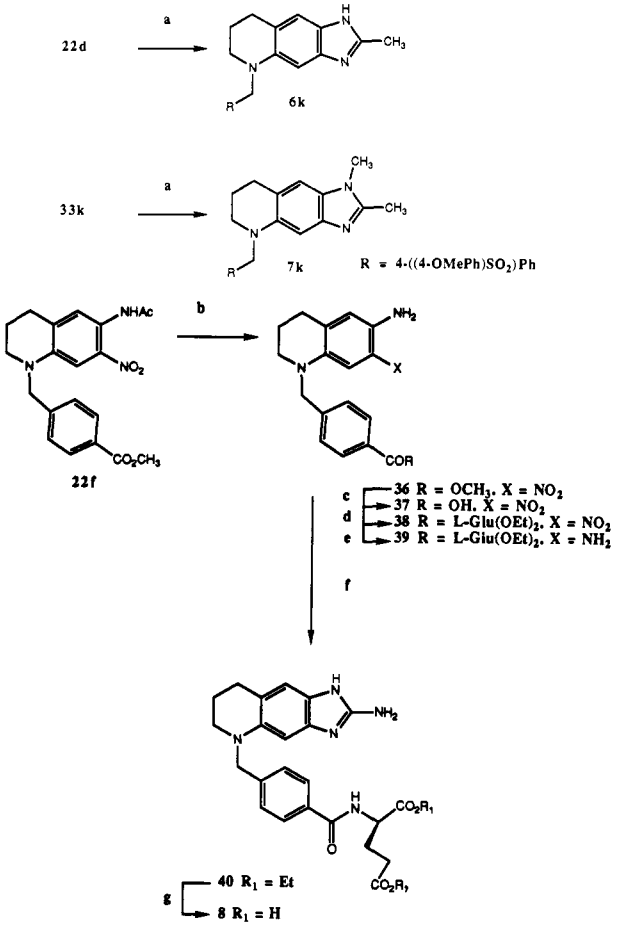
with trimethyl orthoformate–HCl[25] gave the unsubstituted benzimidazole **1h**.

The 2-methylthio derivative **3i** and 2-methylamino compound **4i** were prepared from the diamino intermediate **30i** through formation of the imidazoline-2-thione[26] **32i** followed by methylation[27] (Scheme IV). Displacement of the 2-methylthio group with methylamine required forcing conditions, resulting in a poor yield of the 2-methylamino compound **4i**. The 1-methyl-2-amino compound **5k** was obtained by methylation of the *N*-acetyl derivative **22d** with MeI–K_2CO_3–DMF to give **33k** followed by deacylation to give **34k**. Reduction as before to give diamine **35k** and cyclization with cyanogen bromide yielded **5k**.

The 2-methyl derivatives **6k** and **7k** were prepared by a one-step reduction–cyclization of the *N*-acetyl nitro compounds **22d** and **33k**, respectively, with Sn in acetic acid[28] (Scheme V). Synthesis of the glutamylated compound **8** began with the methyl ester **22f**. Attempted base hydrolysis of both the *N*-acetyl and the methyl ester functionality led to decomposition of the tetrahydroquinoline nucleus. The acetyl group was therefore first removed with acid. This yielded a mixture of mostly the deacylated ester with a small amount of the deacylated acid. This mixture was saponified directly with 1 N NaOH to afford the desired amino acid compound **37** in good yield. Coupling of this material with L-glutamate diethyl ester using EDC in DMF proceeded smoothly to yield the desired amide **38** in good yield. Reduction and cyclization as before to give the 2-aminobenzimidazole **40** and hydrolysis of the diester gave the final compound **8**.

The (phenylthio)tetrahydronaphthimidazole compound **9i** required a new synthesis starting with the known 6-acetylamino tetralone **41** (Scheme VI).[29] Nitration[30] of

Scheme V[a]



[a] (a) Sn^0, AcOH; (b) aqueous HCl, reflux; (c) NaOH; (d) L-Glut-$(OEt)_2$, EDC, DMF; (e) Zn, AcOH; (f) CNBr; (g) NaOH.

41 yielded a mixture of 5- and 7-nitro isomers **42a** and **42b** in a 2:1 ratio, respectively. Sodium borohydride reduction

(25) Martin, P. K.; Matthews, H. R.; Rapoport, H.; Thyagarajan, G. The Synthesis of 1,4-Substituted Imidazoles. *J. Org. Chem.* **1968**, *33*, 3758–3761.

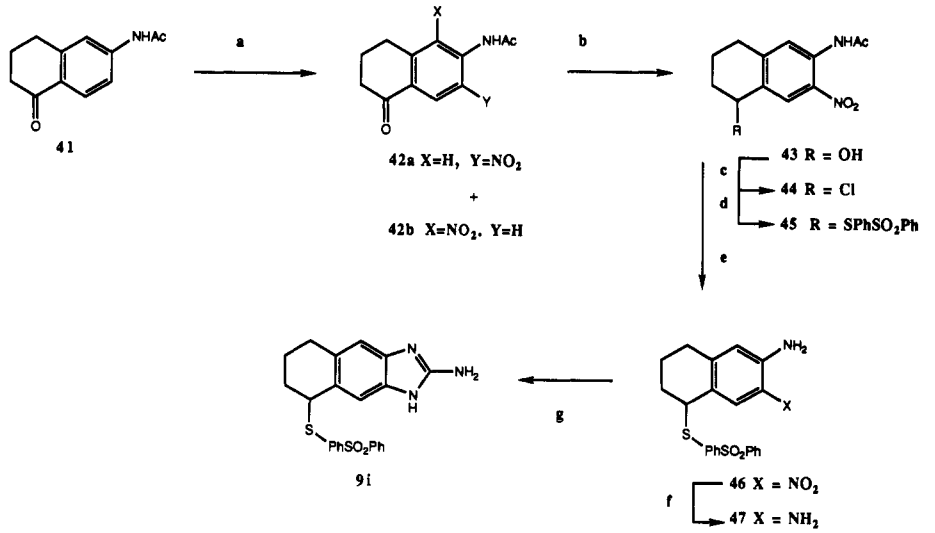
(26) Pozharskii, A. F.; Garnovskii, A. D.; Simonov, A. M. Advances in the Chemistry of Imidazole. *Russ. Chem. Rev.* **1966**, *35*, 122–144.

(27) See ref 16, pp 285–291.

(28) Abou-Zeid, Y. M.; Abou-Ouf, A. A.; Ragab, H. *Bull. Fac. Pharm. Cairo Univ.* **1963**, *2*, 27; *Chem. Abstr.* **62**, 5269b.

(29) Allinger, N. L.; Jones, E. S. Synthesis of Some Functionally Substituted Benzocyclanones. *J. Org. Chem.* **1962**, *27*, 70–76.

Scheme VI[a]



[a] (a) HNO_3, H_2SO_4; (b) $NaBH_4$; (c) HCl, benzene; (d) $PhSO_2PhSSPhSO_2Ph$, NaH, DMF; (e) Na_2O_2; (f) Zn, AcOH; (g) CNBr.

provided the desired alcohol **43**. Activation of this alcohol as the mesylate or tosylate was thwarted with elimination problems; however, the stable chloride **44** could be obtained in good yield simply by treatment with anhydrous HCl. Displacement of the chloride with the (phenylsulfonyl)benzenethiolate anion at −78 °C (generated from the disulfide and NaH in DMF) furnished the coupled thioether **45**. Deacetylation of **45** proved difficult under standard acidic conditions but was cleanly effected using sodium peroxide,[31] yielding the nitro amine **46**. Reduction of **46** to give diamine **47** and cyclization with cyanogen bromide afforded the desired sulfide **9i**.

Inhibition of Cellular Growth

The ability of compounds **1h–9i** to inhibit cellular growth was measured using three continuous cell lines: the mouse (L1210) and human (CCRF-CEM) leukemias and a thymidine kinase deficient human adenocarcinoma line (GC_3/MTK$^-$). The results are shown in Table II. The glutamate-containing compound **8** and phenolic compound **2j**, two of the more potent inhibitors of isolated TS (K_i < 15 nM, human), had poor activity in cell culture, possibly a result of poor transport properties. While a number of the inhibitors have IC_{50}s in the 1–10 μM range against L1210, the best activity across all cell lines including the thymidine kinase deficient line GC_3/MTK$^-$ is observed with the 1-methyl-2-amino compound **5k**. It has IC_{50}s under 1 μM against the L1210 and CCRF-CEM lines.

The ability of thymidine to reverse the growth inhibitory effects was examined to determine whether TS was the primary locus of intracellular activity. Results ranged from an absence of salvage effects (eight compounds) to small magnitude changes.[32] The largest effect was observed with compound **2m** where the IC_{50} was increased 2.6-fold by thymidine treatment. In contrast, thymidine produced a 15-fold increase in the IC_{50} for CB3717, a known TS active agent.[6] These data suggest that the effects observed with these novel inhibitors on cellular growth do not result primarily from the inhibition of TS although the possibility that these compounds interfere with cellular nucleoside uptake has yet to be explored.[33] Compounds **2i**, **2m**, and **5k** were evaluated further for activity against DHFR. Their inhibition of cellular growth was monitored in the presence and absence of leucovorin. No effects were observed in these experiments, suggesting that DHFR is not the locus of activity.

As was described in the introduction, the information from a protein crystal structure has the potential of increasing the selectivity for a given target protein. Having a tight binding inhibitor of a given enzyme does not necessarily result in specific targeting of that enzyme in cells.

Table II. TS Inhibition and Inhibition of Cellular Growth Data

	K_i (μM)[a]		IC_{50} (μM)[a]		
compd	*E. coli* TS	human TS	L1210	CCRF-CEM	GC3M(−)
1h	4.9 (34)	7.7 (11)	20	11	43
2h	0.14 (1.2)	0.064 (0.12)	2.0	2.2	>50[b]
2i	0.043 (0.48)	0.031 (0.043)	2.0	3.9	>5[b]
2j	0.008 (0.076)	0.020[c] (0.014)	12	22	>50[b]
2k	0.040 (0.81)	0.024 (0.086)	2.6	5.1	9.0
2l	0.039 (0.60)	0.038 (0.051)	3.1	>5[b]	>5[b]
2m	0.011 (0.11)	0.006[c] (0.024)	4.0	4.0	8.6
3i	>3[b] (>3[b])	4.2 (8.1)	>10[b]	>10[b]	>10[b]
4i	8.5 (41)	0.77 (2.9)	3.0	3.3	>5[b]
5k	0.5 (1.4)	0.051 (0.14)	0.5	0.9	3.0
6k	1.3 (10)	0.44 (1.1)	2.8	8.0	4.1
7k	5.2 (6.8)	0.48 (2.0)	1.05	1.8	>5[b]
8	0.005 (0.055)	0.011[c] (0.013)	20	40	>50[b]
9i	0.35 (1.2)	0.23 (0.30)	7.5	>10[b]	>10[b]

[a] See Experimental Section for details of assay conditions. Inhibition constants (K_{is} is listed and K_{ii} is shown in parentheses) are as described by Cleland (ref 35) and values are averaged over multiple data sets. K_{is} values are cited in the text. Reported K_i values have an average standard deviation of 29%. [b] Concentrations above this level could not be tested because of insolubility. [c] These compounds were also assayed using tight binding kinetics resulting in considerably lower K_i values: **2j**: $K_{is} = K_{ii} = 2.6$ nM; **2m**: $K_{is} = 4.5$ nM, $K_{ii} = 13.5$ nM; **8**: $K_{is} = 2.2$ nM, $K_{ii} = 6.0$ nM; see ref 14 in text.

(30) Cereda, E.; Turconi, M.; Ezhaya, A.; Bellora, E.; Brambilla, A.; Pagani, F.; Donetti, A. Anti-secretory and Anti-ulcer Activities of Some New 2-(2-pyridylmethyl-sulfinyl)-benzimidazoles. *Eur. J. Med. Chem.* **1987**, *22*, 527–537.

(31) Vaughn, H. L.; Robbins, M. D. Rapid Procedure for the Hydrolysis of Amides to Acids. *J. Org. Chem.* **1975**, *40*, 1187–89.

(32) The ratio of the IC_{50} determined in the presence of thymidine to that observed in its absence was 1.0 (no TS targeting) for compounds **1h**, **2h**, **2k**, **2l**, **3i**, **4i**, **5k**, and **7k**. Small thymidine salvage effects were measured for the following compounds where the IC_{50} ratio is indicated in parentheses: **9i** (1.3); **2i**, **2j**, **6k** (1.4); **8** (1.8); **2m** (2.6).

(33) Paterson, A. R. P.; Kim, S. C.; Bernard, O.; Cass, C. E. Transport of Nucleosides. *Ann. N.Y. Acad. Sci.* **1975**, *255*, 402–410.

Factors which are difficult to predict such as binding to other cellular proteins or transport properties can render a potent inhibitor ineffective in cell culture and in vivo. Experiments which monitor TS activity in cells and the uptake of thymidine as well as exploration of structural modifications of these compounds which may impart enhanced targeting of TS in cell culture are currently in progress.

Conclusions

In the present study, using a high-resolution crystal structure of the therapeutically important receptor thymidylate synthase, we have been able to identify and rapidly optimize a lead structure which bears no resemblance to known inhibitors or substrates of this enzyme. The initial imidazotetrahydroquinoline inhibitor had a K_i of ca. 5 μM. A crystal structure of this inhibitor complexed with *E. coli* TS experimentally verified the binding mode of this new series. Additional derivatives were designed using this structural information to optimize the starting inhibitor. Subsequent cycles of iterative design, synthesis, and crystal structure analysis produced compounds having structural variety which inhibit both human and *E. coli* TS in the low-nanomolar range. It was also found that some of the compounds inhibit the growth of three tumor cell lines at micromolar levels. Further investigation is required to establish the exact locus of action of these novel TS inhibitors in cells.

Experimental Section

Melting points were determined on a Mel-Temp apparatus and are uncorrected. The structure of all compounds were confirmed by proton magnetic resonance spectroscopy, infrared spectroscopy, and either elemental microanalysis or mass spectrometry. Proton magnetic resonance spectra were determined using a General Electric QE-300 spectrometer operating at a field strength of 300 MHz. Chemical shifts are reported in parts per million (ppm) and by setting the references such that, in $CDCl_3$, the $CHCl_3$ peak is at 7.26 ppm and, in DMSO-d_6, the DMSO peak is at 2.49 ppm, and in acetone-d_6 the acetone peak is at 2.04 ppm. Standard and peak multiplicities are designated as follows: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; br s, broad singlet; br d, broad doublet; br, broad signal; and m, multiplet. Mass spectra were determined using a VG 7070E-HF high-resolution mass spectrometer. Infrared absorption spectra were taken on a Perkin-Elmer 457 spectrometer or a MIDAC high-resolution FT IR, and values are reported in cm^{-1}. Elemental microanalysis gave results for the elements stated within ±0.4% of the theoretical values. *N,N*-Dimethylformamide and *N,N*-dimethylacetamide was used as received from Aldrich. Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl under nitrogen. Flash chromatography was performed using silica gel 60 (Merck Art 9385) unless stated otherwise. Thin-layer chromatographs (TLC) were performed on precoated sheets of silica 60 F254 (Merck Art 5719).

6-(Acetylamino)-1,2,3,4-tetrahydroquinoline (19). A solution of 6-aminoquinoline (5.00 g, 34.68 mmol) in 50 mL of CH_2Cl_2 at 25 °C was treated with acetic anhydride (4.00 mL, 42.39 mmol, 1.2 equiv). After 10 min, the reaction mixture was diluted in 100 mL of CH_2Cl_2 and washed with water. The aqueous layer was basified with $NaHCO_3$ and extracted three times with EtOAc. The combined organic layers were dried over $MgSO_4$ and concentrated to give 6-(acetylamino)quinoline (18) as a beige solid, 4.99 g (26.80 mmol, 77%), which was used directly in the next step: ^1H NMR ($CDCl_3$) δ 8.83 (1 H, d, J = 3.4 Hz), 8.36 (1 H, s), 8.12 (1 H, d, J = 8.3 Hz), 8.03 (1 H, d, J = 9.0 Hz), 7.60 (1 H, br s), 7.53 (1 H, dd, J = 9.0, 2.4 Hz), 7.37 (1 H, dd, J = 8.3, 4.1 Hz), 2.25 (3 H, s).

A solution of 18 (10.00 g, 53.70 mmol) in 100 mL of AcOH over PtO_2 (0.49 g, 2.14 mmol) was shaken on a Parr apparatus under 30 psi of hydrogen pressure for 1.5 h. The mixture was filtered through Celite, neutralized with 6 N NaOH, and filtered to give 19 as a light purple solid, 8.71 g (45.78 mmol, 85%). Amine 19 could also be obtained by selective acylation (1 equiv of Ac_2O, pyridine, −10 °C) of 6-amino-1,2,3,4-tetrahydroquinoline. 19: ^1H NMR ($CDCl_3$) δ 7.08 (1 H, d, J = 2.0 Hz), 6.98 (1 H, dd, J = 8.5, 2.0 Hz), 6.40 (1 H, d, J = 8.5 Hz), 3.79 (1 H, br s), 3.26 (2 H, t, J = 5.5 Hz), 2.73 (2 H, t, J = 6.4 Hz), 2.10 (3 H, s), 1.95 (2 H, m).

6-(Acetylamino)-7-nitro-1,2,3,4-tetrahydroquinoline (20). A solution of 19 (3.72 g, 19.55 mmol) in 30 mL of 98% H_2SO_4 at −10 °C was treated with 70% HNO_3 (1.3 mL, 20.22 mmol), during which the temperature increased to 5 °C. The mixture was poured into 400 mL of water, neutralized with 6 N NaOH, and extracted with EtOAc (3 × 500 mL). ^1H NMR of the crude material showed a 2.5:1 mixture of 20 and its 5-nitro isomer 21. The extract was dried over Na_2SO_4, concentrated, and purified by flash chromatography (65% EtOAc–hexanes) to give 19 as a dark red solid (2.58 g, 56%): mp 150–155 °C; ^1H NMR ($CDCl_3$) δ 9.80 (1 H, br s), 8.22 (1 H, s), 7.22 (1 H, s), 4.10 (1 H, br s), 3.32 (2 H, t, J = 5.5 Hz), 2.82 (2 H, t, J = 6.4 Hz), 2.22 (3 H, s), 1.55–1.95 (2 H, m); IR (KBr) 3410, 3320, 2930, 1650, 1580, 850; HRMS exact mass calculated for $C_{11}H_{13}N_3O_3$ 235.0957 (M^+), found 235.0950.

Representative Procedure for the Alkylation of 20. 6-(Acetylamino)-7-nitro-*N*-[4-(phenylsulfonyl)benzyl]-1,2,3,4-tetrahydroquinoline (22b). A solution of 20 (0.40 g, 1.17 mmol), 4-(phenylsulfonyl)benzyl bromide (23b) (0.76 g, 2.46 mmol), and diisopropylethylamine (0.44 mL, 2.53 mmol) in 4 mL of DMF was heated at 70 °C for 5 h. The mixture was diluted in EtOAc, washed twice with water, dried over Na_2SO_4, and concentrated. Purification by flash chromatography (50% EtOAc–hexanes) afforded 22b as a red-orange solid (0.58 g, 73%): mp 202–205 °C; ^1H NMR ($CDCl_3$) δ 9.90 (1 H, br s), 8.28 (1 H, s), 7.89–7.95 (4 H, m), 7.50–7.52 (3 H, m), 7.35 (2 H, d, J = 8.2 Hz), 7.07 (1 H, s), 4.52 (2 H, s), 3.36 (2 H, m), 2.87 (2 H, m), 2.22 (3 H, s), 2.02 (2 H, m); IR (KBr) 3376, 3071, 2932, 2865, 1692, 1574, 1512, 1447, 1296, 1154, 1105, 727; HRMS exact mass calculated for $C_{24}H_{23}N_3O_5S$ 465.1358 (M^+), found 465.1373.

6-(Acetylamino)-7-nitro-*N*-[4-[[*N*-(*tert*-butyloxycarbonyl)piperazinyl]sulfonyl]benzyl]-1,2,3,4-tetrahydroquinoline (22a). Reaction of 20 and bromide 23a as described above for 22b followed by flash chromatography (50% EtOAc–hexanes) afforded 22a as a purple solid (60%): mp 190–195 °C; ^1H NMR ($CDCl_3$) δ 9.95 (1 H, br s), 8.29 (1 H, s), 7.71 (2 H, d, J = 8.3 Hz), 7.39 (2 H, d, J = 8.2 Hz), 7.12 (1 H, s), 4.57 (2 H, s), 3.50 (4 H, m), 3.40 (2 H, m), 2.98 (4 H, m), 2.90 (2 H, m), 2.22 (3 H, s), 2.04 (2 H, m), 1.40 (9 H, s); IR (KBr) 1680, 1510, 1330; HRMS exact mass calculated for $C_{27}H_{35}N_5O_7S$ 573.2257 (M^+), found 573.2257.

6-(Acetylamino)-7-nitro-*N*-[4-[[4-(benzoyloxy)phenyl]-sulfonyl]benzyl]-1,2,3,4-tetrahydroquinoline (22c). Compound 22c was prepared from 20 and 23c in 57% yield as described for 22b: mp 174–178 °C; ^1H NMR ($CDCl_3$) δ 9.95 (1 H, s), 8.29 (1 H, s), 8.16 (2 H, d, J = 8.3 Hz), 8.01 (2 H, d, J = 6.8 Hz), 7.92 (2 H, d, J = 8.3 Hz), 7.65 (1 H, m), 7.51 (2 H, m), 7.37 (4 H, dd, J = 8.6, 2.1 Hz), 7.21 (1 H, s), 4.54 (2 H, s), 3.36 (2 H, m), 2.88 (2 H, m), 2.22 (3 H, s), 2.04 (2 H, m); IR (KBr) 3360, 1730, 1670, 1570, 1150, 880, 830, 810, 700; HRMS exact mass calculated for $C_{31}H_{27}N_3O_7S$ 585.1570 (M^+), found 585.1567.

6-(Acetylamino)-7-nitro-*N*-[4-[(4-methoxyphenyl)-sulfonyl]benzyl]-1,2,3,4-tetrahydroquinoline (22d). Compound 22d was prepared from 20 and bromide 23d in 82% yield as described above: mp 168–171 °C; ^1H NMR ($CDCl_3$) δ 8.30 (1 H, s), 7.86 (4 H, d, J = 8.9 Hz), 7.33 (2 H, d, J = 8.4 Hz), 7.08 (1 H, s), 6.96 (2 H, d, J = 8.9 Hz), 4.52 (2 H, s), 3.83 (3 H, s), 3.37 (2 H, m), 2.87 (2 H, m), 2.22 (3 H, s), 2.01 (2 H, m); IR (KBr) 2920, 2851, 1688, 1593, 1510, 1460, 1337, 1296, 1260, 1150, 1105, 1022. Anal. ($C_{25}H_{25}N_3O_6S$) C, H, N.

6-(Acetylamino)-7-nitro-*N*-[6-[(*tert*-butyldiphenylsilyloxy)methyl]-2-naphthobenzyl]-1,2,3,4-tetrahydroquinoline (22e). Compound 22e was prepared from 20 and bromide 23e in 91% yield as described above: mp 70–76 °C; NMR ($CDCl_3$) δ 8.28 (1 H, s), 7.70–7.82 (8 H, m), 7.63 (1 H, s), 7.35–7.43 (8 H, m), 7.30 (1 H, s), 4.91 (2 H, s), 4.65 (2 H, s), 3.45 (2 H, m), 2.90 (2 H, m), 2.22 (3 H, s), 2.05 (2 H, m), 1.11 (9 H, s); IR (neat) 3360, 1680, 1080 (broad), 880, 820, 750, 700; HRMS exact mass calculated for $C_{39}H_{41}N_3O_4Si$ 643.2866 (M^+), found 643.2822.

6-(Acetylamino)-7-nitro-*N*-(4-carbomethoxybenzyl)-1,2,3,4-tetrahydroquinoline (22f). Reaction of bromide 23f and amine 20 afforded 22f as described above in 88% yield: mp

183–185 °C; ^{1}H NMR ($CDCl_3$) δ 9.93 (1 H, br s), 8.28 (1 H, s), 8.00 (2 H, d, J = 8.2 Hz), 7.30 (2 H, d, J = 8.2 Hz), 7.13 (1 H, s), 4.55 (2 H, s), 3.90 (3 H, s), 3.41 (2 H, t, J = 5.8 Hz), 2.89 (2 H, t, J = 6.4 Hz), 2.22 (3 H, s), 2.04 (2 H, m); IR (KBr) 3630, 3345, 2947, 1727, 1690, 1505; HRMS exact mass calculated for $C_{20}H_{21}N_3O_5$ 383.1481 (M^+), found 383.1493.

6-(Acetylamino)-7-nitro-*N*-[4-(morpholinosulfonyl)benzyl]-1,2,3,4-tetrahydroquinoline (22g). Reaction of bromide **23a** and amine **20** afforded **22g** as described above in 84% yield: mp 157–160 °C; ^{1}H NMR ($CDCl_3$) δ 9.92 (1 H, br s), 8.29 (1 H, s), 7.72 (2 H, dd, J = 1.7 Hz, J = 6.7 Hz), 7.40 (2 H, d, J = 8.30 Hz), 7.12 (1 H, s), 4.58 (2 H, s), 3.74 (4 H, m), 3.41 (2 H, t, J = 5.6 Hz), 3.01 (4 H, m), 2.90 (2 H, t, J = 6.3 Hz), 2.23 (3 H, s), 2.05 (2 H, m); IR (KBr) 3420, 2920, 2800, 2340, 1740, 1690, 1570, 1500, 1450, 1335, 1285, 1260, 1160, 1115, 940, 850; HRMS exact mass calculated for $C_{22}H_{27}SO_6N_4$ 475.1651 (MH^+), found 475.1668.

6-(Methylacetylamino)-7-nitro-*N*-[4-[(4-methoxyphenyl)sulfonyl]benzyl]-1,2,3,4-tetrahydroquinoline (33k). A solution of **22d** (3.25 g, 6.5 mmol) and K_2CO_3 (6.2 g, 45.5 mmol) in 70 mL of DMF was treated with MeI (1.38 g, 0.6 mL, 9.75 mmol) at 25 °C. The mixture was heated to 80 °C for 48 h during which additional MeI was added (2 × 0.6 mL). The reaction mixture was then diluted with 200 mL of EtOAc and washed with 400 mL of H_2O. The layers were separated, and the organic portion was washed with H_2O (50 mL) and then dried with Na_2SO_4. Concentration yielded 3.5 g of crude product which was chromatographed on silica (4:1 EtOAc–hexanes) to afford **33k** as a black solid (1.61 g, 49% yield): mp 105–109 °C; ^{1}H NMR ($CDCl_3$) δ 7.34 (2 H, d, J = 8.26 Hz), 6.97 (2 H, m), 6.92 (1 H, s), 6.89 (1 H, s), 3.84 (3 H, s), 3.42 (2 H, t, J = 5.7 Hz), 3.13 (3 H, s), 2.85 (2 H, t, J = 6.5 Hz), 2.05 (2 H, m); IR (KBr) 3560, 3400, 2900, 2850, 1785, 720, 1640, 1510, 1320, 1255, 1145, 1100, 1015, 830, 800. Anal. ($C_{26}H_{27}N_3S_1O_2$) C, H, N, S.

Bis[4-(*p*-tolylsulfonyl)phenyl] Carbonate (24). A solution of diphenyl carbonate (50.16 g, 0.23 mol), *p*-toluenesulfonyl chloride (90.04 g, 0.47 mol), and $FeCl_3$ (1.12 g, 0.01 mol) in 75 mL of nitrobenzene was heated to 120 °C, while evolving HCl was bubbled through a water trap. After 2 h, the mixture was cooled to 23 °C, forming a light beige precipitate. The solid was filtered and washed with MeOH to give 87.61 g of crude **24** (0.17 mol, 72%), which was used directly in the next step: mp 149–160 °C; ^{1}H NMR ($CDCl_3$) δ 7.98 (2 H, d, J = 8.7 Hz), 7.82 (2 H, d, J = 8.3 Hz), 7.40 (2 H, m), 7.24–7.32 (2 H, m), 2.40 (3 H, s).

4-(*p*-Tolylsulfonyl)phenol (25). A solution of **24** (87.61 g, 0.17 mol) in 150 mL of EtOH and 150 mL of 5N KOH was heated to 80 °C for 1 h. The mixture was poured into 500 mL of MeOH and neutralized with 6 N HCl, causing precipitation of the KCl. The salt was filtered and the liquid dissolved in CH_2Cl_2, washed with water, dried over Na_2SO_4, and concentrated to yield **25** as an off-white solid: yield 69.65 g (0.28 mol, 82%); mp 143–144 °C; ^{1}H NMR ($CDCl_3$) δ 7.76 (4 H, d, J = 8.6 Hz), 7.27 (2 H, d, J = 8.4 Hz), 6.90 (2 H, d, J = 8.8 Hz), 6.47 (1 H, br s), 2.38 (3 H, s); IR (KBr) 3325, 1900, 1200, 830, 800. Anal. ($C_{13}H_{12}O_3S$) C, H, S.

4-(*p*-Tolylsulfonyl)phenyl Benzoate (26). A solution of **25** (7.64 g, 30.77 mmol) and 12 mL of pyridine in 30 mL of $CHCl_3$ was treated with benzoyl chloride (4.2 mL, 36.18 mmol) at 23 °C. After 1 h, the mixture was diluted in $CHCl_3$, washed with water, dried over Na_2SO_4, and concentrated. The pale beige solid was azeotroped with toluene to give 9.22 g (26.16 mmol, 85%) of **26**, which was used without purification: mp 192–198 °C; ^{1}H NMR ($CDCl_3$) δ 8.17 (2 H, m), 8.01 (2 H, d, J = 8.8 Hz), 7.83 (2 H, d, J = 8.3 Hz), 7.66 (1 H, m), 7.52 (2 H, t, J = 7.7 Hz), 7.30–7.37 (4 H, m), 2.41 (3 H, s); IR (KBr) 1745, 1200, 1045, 820, 730, 700; HRMS exact mass calculated for $C_{20}H_{16}O_4S$ 352.0770 (M^+), found 352.0755.

4-(*p*-Tolylsulfonyl)phenyl Methyl Ether (27). A mixture of compound **25** (20.13 g, 81.07 mmol), K_2CO_3 (16.61 g, 120.18 mmol), and CH_3I (6.20 mL, 99.58 mmol) in 500 mL of acetone was refluxed for 4 h (an additional 1.00 mL of CH_3I (16.00 mmol) was added after 2 h). The resulting mixture was filtered through Celite and concentrated. The yellow-white solid was dissolved in $CHCl_3$, washed with H_2O and then with brine, and dried over Na_2SO_4. The volume was reduced, and the compound was triturated with hexanes and filtered to give **27** as a white solid (119.53 g, 92%): ^{1}H NMR ($CDCl_3$) δ 7.85 (2 H, d, J = 8.9 Hz), 7.79 (2 H, d, J = 8.3 Hz), 7.27 (2 H, d, J = 8.4 Hz), 6.95 (2 H, d, J = 8.9 Hz), 3.83 (3 H, s), 2.38 (3 H, s). Anal. ($C_{14}H_{14}O_3S$) C, H, S.

Representative Bromination Procedure Using NBS-hv: 4-[[4-(Benzoyloxy)phenyl]sulfonyl]benzyl Bromide (23c). A suspension of 4-(*p*-tolylsulfonyl)phenyl benzoate (7.43 g, 21.08 mmol) and *N*-bromosuccinimide (3.76 g, 21.13 mmol) in 150 mL of CCl_4 was heated to reflux under a 200-W light. After 1 h, ^{1}H NMR of an aliquot showed approximately 53% desired product, along with 30% dibromide and 17% starting material. The mixture was cooled, diluted in CH_2Cl_2, washed with water, dried (Na_2SO_4), and concentrated to a yellow-white solid, 8.36 g (ca. 4.35 g of **23c**, 48%). The product was used without purification: ^{1}H NMR ($CDCl_3$) δ 8.19 (2 H, m), 7.90–8.05 (4 H, m), 7.70 (1 H, m), 7.50–7.56 (4 H, m), 7.39 (2 H, m), 4.49 (2 H, s).

4-[(4-Methoxyphenyl)sulfonyl]benzyl Bromide (23d). Compound **27** was brominated as described above for **23c** to give **23d** in ca. 64% yield based on ^{1}H NMR. The product was used without purification: ^{1}H NMR ($CDCl_3$) δ 7.86 (4 H, m), 7.48 (2 H, d, J = 8.4 Hz), 6.95 (2 H, d, J = 8.9 Hz), 4.44 (2 H, s), 3.83 (3 H, s).

2-(Bromomethyl)-6-[(*tert*-butyldiphenylsiloxy)methyl]naphthalene (23e). To a solution of triphenylphosphine (6.85 g, 26.17 mmol) in 80 mL of CH_2Cl_2 at 0 °C was added carbon tetrabromide (4.34 g, 13.10 mmol). The resulting orange solution was treated with alcohol **28** (4.66 g, 10.92 mmol) in 10 mL of CH_2Cl_2 and warmed to 25 °C. After 5 min, the mixture was poured into water and extracted twice with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and concentrated. Purification by flash chromatography (CH_2Cl_2) gave a straw-colored oil: 4.08 g (76%); ^{1}H NMR ($CDCl_3$) δ 7.82–7.71 (8 H, m), 7.41–7.38 (8 H, m), 4.92 (2 H, s), 4.68 (2 H, s), 1.12 (9 H, s); IR (neat film) 3071, 2932, 2857, 1472, 1209, 1159, 1113, 1088, 893, 818; HRMS exact mass calcd for $C_{28}H_{29}BrOSi$ 488.1172 (M^+), found 488.1157.

Methyl 4-(Bromomethyl)benzoate (23f). Methyl toluate (10.0 g, 66.6 mmol) was brominated following the general procedure for **23c** above to afford 11 g (72%) of bromide **23f** as white plates after recrystallization from hexanes: mp 39–43 °C; ^{1}H NMR ($CDCl_3$) δ 8.01 (2 H, d, J = 8.3 Hz), 7.45 (2 H, d, J = 8.3 Hz), 4.50 (2 H, s), 3.92 (3 H, s). Anal. ($C_9H_9O_2Br$) C, H, Br.

Representative Deacylation of 6-(Acetylamino)-7-nitro-1,2,3,4-tetrahydroquinolines. 6-Amino-7-nitro-*N*-[4-(piperazinylsulfonyl)benzyl]-1,2,3,4-tetrahydroquinoline (29h). The following procedure, described for **29h**, is representative: A suspension of **22a** (0.40 g, 0.70 mmol) in 3 N HCl (12 mL) was heated to 60 °C for 5.5 h. The mixture was poured into 100 mL of saturated $NaHCO_3$, extracted with EtOAc, dried over Na_2SO_4, and concentrated. Purification by flash chromatography (10% EtOH–$CHCl_3$) yielded **29h** as a purple solid (0.22 g, 71%): mp 194–196 °C; ^{1}H NMR ($CDCl_3$) δ 7.71 (2 H, d, J = 8.2 Hz), 7.42 (2 H, d, J = 8.1 Hz), 7.06 (1 H, s), 6.52 (1 H, s), 5.67 (2 H, br s), 4.50 (2 H, s), 3.31 (2 H, t, J = 5.7 Hz), 2.95–2.99 (4 H, m), 2.90–2.93 (4 H, m), 2.80 (2 H, t, J = 6.0 Hz), 2.01–2.03 (2 H, m); IR (KBr) 3460, 3420, 1240; HRMS exact mass calculated for $C_{20}H_{25}N_5O_4S$ 431.1627 (M^+), found 431.1626.

6-Amino-7-nitro-*N*-[4-(phenylsulfonyl)benzyl]-1,2,3,4-tetrahydroquinoline (29i): 75% yield; mp 163–169 °C; ^{1}H NMR ($CDCl_3$) δ 7.88–7.94 (4 H, m), 7.50–7.54 (3 H, m), 7.38 (2 H, d, J = 8.3 Hz), 7.02 (1 H, s), 6.50 (1 H, s), 5.65 (2 H, br s), 4.46 (2 H, s), 3.25–3.27 (2 H, m), 2.75–2.77 (2 H, m), 1.90–2.04 (2 H, m); IR (KBr) 3480, 3378, 1562, 1503, 1422, 1304, 1246, 1154, 1105, 725; HRMS exact mass calculated for $C_{22}H_{21}N_3O_4S$ 423.1254 (M^+), found 423.1255.

6-Amino-7-nitro-*N*-[4-(4-hydroxyphenyl)sulfonyl]benzyl]-1,2,3,4-tetrahydroquinoline (29j): 39% yield; mp 200–205 °C; ^{1}H NMR ($CDCl_3$) δ 7.80–7.86 (4 H, m), 7.37 (2 H, d, J = 8.2 Hz), 7.01 (1 H, s), 6.90 (2 H, d, J = 8.7 Hz), 6.50 (1 H, s), 5.66 (2 H, br s), 4.46 (2 H, s), 3.28 (2 H, t, J = 5.7 Hz), 2.77 (2 H, t, J = 6.2 Hz), 1.99 (2 H, m); IR (KBr) 3460, 3380, 2940, 1570, 1150, 880, 840; HRMS exact mass calculated for $C_{22}H_{21}N_3O_5S$ 439.1202 (M^+), found 439.1213.

6-Amino-7-nitro-*N*-[4-[(4-methoxyphenyl)sulfonyl]benzyl]-1,2,3,4-tetrahydroquinoline (29k): 54% yield; mp 141–143 °C; ^{1}H NMR ($CDCl_3$) δ 7.86 (4 H, d, J = 8.9 Hz), 7.35 (2 H, d, J = 8.3 Hz), 7.03 (1 H, s), 6.96 (1 H, s), 6.50 (2 H, d, J = 8.8 Hz), 5.65 (2 H, br s), 4.45 (2 H, s), 3.83 (3 H, s), 3.26 (2 H, t, J = 5.8 Hz), 2.77 (2 H, m), 1.98 (2 H, m); IR (KBr) 3400, 1500,

1250, 1150, 1100, 860, 800; HRMS exact mass calculated for $C_{23}H_{23}N_3O_5S$ 453.1360 (M^+), found 453.1368.

6-Amino-7-nitro-*N*-[6-(hydroxymethyl)-2-naphthobenzyl]-1,2,3,4-tetrahydroquinoline (29l): 57% yield; ^1H NMR ($CDCl_3$) δ 7.79 (3 H, m), 7.68 (1 H, s), 7.39–7.48 (2 H, m), 7.25 (1 H, s), 6.51 (1 H, s), 5.63 (2 H, br s), 4.85 (2 H, d, J = 5.9 Hz), 4.58 (2 H, s), 3.33 (2 H, t, J = 5.7 Hz), 2.79 (2 H, t, J = 6.3 Hz), 2.04 (2 H, m), 1.71 (1 H, t, J = 6.0 Hz); IR (KBr) 3480, 3320, 2910, 1560, 880, 810; HRMS exact mass calculated for $C_{21}H_{21}N_3O_3$ 363.1583 (M^+), found 363.1574.

6-Amino-7-nitro-*N*-[4-(morpholinosulfonyl)benzyl]-1,2,3,4-tetrahydroquinoline (29m): 89% yield; mp 162–167 °C; ^1H NMR ($CDCl_3$) δ 7.72 (2 H, d, J = 8.3 Hz), 7.45 (2 H, d, J = 8.3 Hz), 7.09 (1 H, s), 6.53 (1 H, s), 5.68 (2 H, br s), 4.51 (2 H, s), 3.74 (4 H, m), 3.29 (2 H, t, J = 5.7 Hz), 3.01 (4 H, m), 2.80 (2 H, t, J = 6.3 Hz), 2.02 (2 H, m); IR (KBr) 3480, 3376, 2930, 2860, 1720, 1590, 1560, 1490, 1415, 1340, 1300, 1245, 1160, 1110, 1070, 940, 895, 850. Anal. ($C_{20}H_{24}SO_5N_4$) C, H, N, S.

6-(Methylamino)-7-nitro-*N*-[4-[(4-methoxyphenyl)-sulfonyl]benzyl]-1,2,3,4-tetrahydroquinoline (34k): 32% yield; mp 192–194 °C; ^1H NMR ($CDCl_3$) δ 7.85 (4 H, m), 7.37 (2 H, d, J = 8.4 Hz), 7.11 (1 H, s), 6.96 (2 H, m), 6.55 (1 H, s), 4.45 (2 H, s), 3.83 (3 H, s), 3.25 (2 H, t, J = 5.7 Hz), 2.97 (3 H, d, J = 5.2 Hz), 2.84 (2 H, t, J = 6.3 Hz), 1.99 (2 H, m); IR (KBr) 3455, 2900, 1560, 1490, 1470, 1400, 1290, 1280, 1250, 1210, 1140, 1100, 1015, 960, 890, 830. Anal. ($C_{24}H_{25}N_3O_5S$) C, H, N, S.

Representative Procedure for the Reduction of 6-Amino-7-nitro-1,2,3,4-tetrahydroquinolines: With Hydrazine–Raney Nickel. 6,7-Diamino-*N*-[4-(piperazinylsulfonyl)benzyl]-1,2,3,4-tetrahydroquinoline (30h). A solution of **29h** (0.215 g, 0.50 mmol) in 6 mL of MeOH and 2 mL of THF over ca. 10 mg of Raney nickel/H_2O was heated to reflux and treated with anhydrous hydrazine (1.00 mL, 31.54 mmol). After 1 h, during which loss of color occurred, the mixture was filtered through Celite, concentrated, and azeotroped with CH_3CN–benzene to give 0.16 g of **29h** (0.39 mmol, 79%). In all cases, the diamine was used immediately in the next cyclization reaction: ^1H NMR ($CDCl_3$) δ 7.69 (2 H, d, J = 8.2 Hz), 7.44 (2 H, d, J = 8.2 Hz), 6.44 (1 H, s), 5.85 (1 H, s), 4.42 (2 H, s), 3.24 (2 H, m), 2.97 (4 H, m), 2.93 (4 H, m), 2.70 (2 H, m), 1.98 (2 H, m).

With Zn–AcOH. 6,7-Diamino-*N*-[4-(L-glutamocarbonyl)benzyl]-1,2,3,4-tetrahydroquinoline Diethyl Ester (39). 6-Amino-7-nitrotetrahydroquinoline **38** (0.60 g, 1.17 mmol) in 10 mL of AcOH was treated portionwise with Zn^0 (459 mg, 7.02 mmol) at 23 °C. After 2–3 min the solution had turned from deep purple to clear green. The reaction mixture was poured into saturated aqueous $NaHCO_3$ and neutralized further with solid $NaHCO_3$. Extraction of the aqueous layer with EtOAc, drying (Na_2SO_4), and concentration afforded 0.76 g of the crude diamine **39** as a dark green oil which was cyclized immediately in the next step (see procedure for **2j** below).

***N*^{5}-[4-(Piperazinylsulfonyl)benzyl]-5,6,7,8-tetrahydro-1*H*-imidazo[4,5-*g*]quinoline (1h).** A solution of diamine **30h** (0.08 g, 0.21 mmol) in 2 mL of $HC(OMe)_3$ at 23 °C was treated with 1 drop of concentrated HCl, causing formation of a precipitate. The mixture was stirred for 1 h, poured into saturated $NaHCO_3$ solution (20 mL), and extracted with 50 mL of $CHCl_3$. The organic layer was dried with Na_2SO_4, concentrated, and purified by flash chromatography (1.5%–NH_3–13.5%–EtOH/85%–$CHCl_3$) to afford **1h** as a yellow-orange solid (0.03 g, 0.08 mmol, 38%): ^1H NMR (acetone-d_6) δ 8.01 (1 H, s), 7.76 (1 H, s), 7.70 (2 H, d, J = 8.1 Hz), 7.56 (2 H, d, J = 8.1 Hz), 7.20 (1 H, s), 6.52 (1 H, s), 4.64 (2 H, s), 3.45 (2 H, t, J = 5.7 Hz), 2.92 (2 H, t, J = 6.1 Hz), 2.84 (4 H, br s), 2.81 (4 H, br s); IR (KBr) 2951, 2919, 2855, 1638, 1508, 1454, 1406, 1343, 1165, 1094, 949; HRMS exact mass calculated for $C_{21}H_{25}N_5O_2S$ 411.1729 (M^+), found 411.1719.

2-Mercapto-*N*^{5}-[4-(phenylsulfonyl)benzyl]-5,6,7,8-tetrahydro-1*H*-imidazo[4,5-*g*]quinoline (32i). Compound **29i** (1.00 g, 2.36 mmol) was reduced with hydrazine–RaNi as described above for **30h** to afford the crude diamine, which was azeotroped with benzene and treated with thiocarbonyldiimidazole (1.38 g, 7.8 mmol) in 100 mL of THF at 25 °C. After stirring overnight, the reaction mixture was diluted with 400 mL of EtOAc and washed with 1 N HCl (2 × 50 mL). The organic layer was then dried with Na_2SO_4 and concentrated to give **32i** as a solid (0.94 g, 88%): mp 278–283 °C; ^1H NMR ($CDCl_3$) δ 7.94 (2 H, d, J = 7.2 Hz), 7.87 (2 H, d, J = 8.2 Hz), 7.53 (3 H, m), 7.36 (2 H, d, J = 8.1 Hz), 6.83 (1 H, s), 6.12 (1 H, s), 4.46 (2 H, s), 3.35 (2 H, t, J = 5.5 Hz), 2.82 (2 H, m), 2.01 (2 H, m); IR (KBr) 3350, 3150, 2940, 2758, 2100, 1720, 1640, 1610, 1470, 1405, 1330, 1300, 1152, 1008, 1070, 900; HRMS exact mass calculated for $C_{23}H_{21}N_3O_2S_2$ 436.1153 (MH^+), found 436.1170.

2-(Methylthio)-*N*^{5}-[4-(phenylsulfonyl)benzyl]-5,6,7,8-tetrahydro-1*H*-imidazo[4,5-*g*]quinoline (3i). To a flask containing K_2CO_3 (6 g, 43.40 mmol) and mercaptoimidazole **32i** (3.00 g, 6.88 mmol) in 60 mL of THF was added dropwise MeI (2.07 g, 14.60 mmol) at 25 °C. After stirring for 1 h, the reaction mixture was filtered and the solvent removed. The crude product (2.72 g) was then purified via flash chromatography (1% MeOH–$CHCl_3$) which gave a solid (1.8 g, 58% yield): mp 130–135 °C; ^1H NMR ($CDCl_3$) δ 8.8 (1 H, br), 7.73 (2 H, m), 7.85 (2 H, d, J = 8.4 Hz), 7.52 (3 H, m), 7.37 (2 H, d, J = 8.4 Hz), 7.2 (1 H, br), 6.2 (1 H, br), 4.50 (2 H, s), 3.38 (2 H, t, J = 5.7 Hz), 2.91 (2 H, t, J = 6.2 Hz), 2.64 (3 H, s), 2.03 (2 H, m); IR (KBr) 3400, 2930, 2840, 1720, 1630, 1590, 1430, 1405, 1308, 1175, 1150, 1105, 1070, 1015, 982, 965, 984; HRMS exact mass calculated for $C_{24}H_{23}N_3S_2O_2$ 449.1232 (M^+), found 449.1244. Anal. ($C_{24}H_{23}N_3S_2O_2 \cdot 1.2H_2O$) C, H, N, S.

2-(Methylamino)-*N*^{5}-[4-(phenylsulfonyl)benzyl]-5,6,7,8-tetrahydro-1*H*-imidazo[4,5-*g*]quinoline (4i). Compound **3i** (0.41 g, 0.9 mmol) and 7.5 mL of MeOH was added to a sealed tube. Methylamine (ca. 7 mL) was then bubbled into the mixture, which was cooled to 0 °C. The temperature was raised to 170 °C for 30 h. The reaction was then allowed to cool and the mixture was concentrated. The crude product was purified via flash chromatography (8% MeOH–CH_2Cl_2), giving a solid (55 mg, 20% yield): mp 203–208 °C; ^1H NMR ($CDCl_3$) δ 7.90 (2 H, m), 7.82 (2 H, d, J = 8.3 Hz), 7.51 (3 H, m), 7.31 (2 H, d, J = 8.3 Hz), 6.84 (1 H, s), 6.22 (1 H, s), 4.40 (2 H, br), 3.28 (2 H, br), 2.77 (5 H, br), 1.96 (2 H, br); IR (KBr) 3450, 3050, 2930, 2860, 1730, 1680, 1495, 1450, 1385, 1300, 1240, 1180, 1155, 1110, 1020, 990; HRMS exact mass calculated for $C_{24}H_{24}N_4O_2S$ 432.1620 (M^+), found 432.1600.

2-Amino-*N*^{5}-[4-[(4-hydroxyphenyl)sulfonyl]benzyl]-5,6,7,8-tetrahydro-1*H*-imidazo[4,5-*g*]quinoline (2j). The following procedure, described for **2j**, is representative: A solution of diamine **30j** (0.69 g, 1.59 mmol) in 6 mL of CH_3CN–2 mL of MeOH was heated to 70 °C and treated with 5.0 M CNBr–CH_3CN solution (0.5 mL, 2.5 mmol). After 2 h, the mixture was diluted in 0.5 N HCl and extracted twice with EtOAc. The aqueous layer was neutralized with 6 N NaOH, saturated with NaCl, and extracted twice with EtOAc. The organic layer was dried over Na_2SO_4 and concentrated to a brown solid. Purification by flash chromatography (5 → 10% EtOH–CH_2Cl_2) afforded **2j** as a light brown solid (0.17 g, 25%); mp 230–245 °C; ^1H NMR (DMSO-d_6) δ 7.86 (3 H, m), 7.75 (2 H, d, J = 8.7 Hz), 7.43 (2 H, d, J = 8.2 Hz), 6.91 (3 H, m), 6.16 (1 H, s), 4.53 (2 H, s), 3.35 (2 H, m), 2.80 (2 H, m), 1.90 (2 H, m); IR (KBr) 3400 (broad), 1660, 1280; HRMS exact mass calculated for $C_{23}H_{22}N_4O_3S$ 434.1412 (M^+), found 434.1401.

2-Amino-*N*^{5}-[4-(piperazinylsulfonyl)benzyl]-5,6,7,8-tetrahydro-1*H*-imidazo[4,5-*g*]quinoline (2h): 20% yield; ^1H NMR ($CDCl_3$) δ 7.65 (2 H, d, J = 8.3 Hz), 7.48 (2 H, d, J = 8.2 Hz), 6.93 (1 H, s), 6.10 (1 H, s), 4.52 (2 H, s), 3.43 (2 H, t, J = 5.5 Hz), 3.29 (4 H, m), 3.04 (4 H, m), 2.92 (2 H, t, J = 6.5 Hz), 2.17 (2 H, m); IR (KBr) 3420, 1610; HRMS exact mass calculated for $C_{21}H_{26}N_6O_2S$ 427.1911 (MH^+), found 427.1940.

2-Amino-*N*^{5}-[4-(phenylsulfonyl)benzyl]-5,6,7,8-tetrahydro-1*H*-imidazo[4,5-*g*]quinoline (2i): 39% yield; ^1H NMR ($CDCl_3$) δ 7.92 (2 H, d, J = 8.3 Hz), 7.83 (2 H, d, J = 8.3 Hz), 7.47–7.55 (3 H, m), 7.39 (2 H, m), 6.89 (1 H, s), 6.23 (1 H, s), 4.44 (2 H, s), 3.32 (2 H, m), 2.85 (2 H, m), 2.01 (2 H, m); IR (film) 1630, 1290, 1150, 820; HRMS exact mass calculated for $C_{23}H_{22}N_4O_2S$ 418.1463 (M^+), found 418.1467.

2-Amino-*N*^{5}-[6-(hydroxymethyl)-2-naphthobenzyl]-5,6,7,8-tetrahydro-1*H*-imidazo[4,5-*g*]quinoline (2l): 27% yield; ^1H NMR (DMSO-d_6) δ 7.71–7.84 (4 H, m), 7.41 (2 H, m), 6.68 (1 H, s), 6.30 (1 H, s), 5.74 (2 H, br s), 4.62 (2 H, s), 4.54 (2 H, s), 3.32 (2 H, m), 2.77 (2 H, m), 1.93 (2 H, m); IR (KBr) 3440, 1720; HRMS exact mass calculated for $C_{22}H_{22}N_4O$ 358.1793 (M^+), found 358.1814.

2-Amino-N^5-[4-(morpholinosulfonyl)benzyl]-5,6,7,8-tetrahydro-1*H*-imidazo[4,5-*g*]quinoline (2m): 63% yield; mp 167–173 °C; ^{1}H NMR (DMSO-d_6) δ 10.18 (1 H, br s), 7.67 (2 H, d, J = 8.3 Hz), 7.53 (2 H, d, J = 8.3 Hz), 6.68 (1 H, s), 6.16 (1 H, s), 5.71 (2 H, br s), 4.51 (2 H, s), 3.60 (4 H, m), 3.28 (2 H, t, J = 5.6 Hz), 2.81 (4 H, m), 2.76 (2 H, t, J = 2.8 Hz), 1.90 (2 H, m); IR (KBr) 3450, 2930, 2839, 1730, 1631, 1556, 1490, 1366, 1333, 1258, 1126, 1127, 1085, 944, 911, 861, 828; HRMS exact mass calculated for $C_{21}H_{25}N_5S_1O_3$ 427.1678 (M^+), found 427.1678.

2-Amino-N^5-[4-[(4-methoxyphenyl)sulfonyl]benzyl]-5,6,7,8-tetrahydro-1*H*-imidazo[4,5-*g*]quinoline (2k): 29% yield; mp 217–228 °C; ^{1}H NMR (DMSO-d_6) δ 7.84 (4 H, d, J = 8.9 Hz), 7.46 (2 H, d, J = 8.3 Hz), 7.09 (2 H, d, J = 8.9 Hz), 6.66 (1 H, s), 6.10 (1 H, s), 5.74 (1 H, br s), 4.45 (2 H, s), 3.80 (3 H, s), 3.15–3.26 (2 H, m), 2.74 (2 H, m), 2.47–2.49 (2 H, m); IR (KBr) 3400, 2950, 1650, 1600, 1400, 1140, 1100, 830, 800; HRMS exact mass calculated for $C_{24}H_{24}N_4O_3S$ 448.1569 (M^+), found 448.1559.

N^1-Methyl-2-amino-N^5-[4-[(4-methoxyphenyl)sulfonyl]benzyl]-5,6,7,8-tetrahydro-1*H*-imidazo[4,5-*g*]quinoline (5k): 56% yield; mp ca. 230 °C (softening at 170 °C); ^{1}H NMR (CD_3OD) δ 7.81 (2 H, d, J = 8.9 Hz), 7.80 (2 H, d, J = 8.2 Hz), 7.43 (2 H, d, J = 8.2 Hz), 7.01 (2 H, d, J = 8.8 Hz), 6.71 (1 H, s), 6.22 (1 H, s), 5.47 (2 H, s), 4.44 (2 H, s), 3.80 (3 H, s), 3.38 (3 H, s), 2.95 (2 H, t, J = 6.2 Hz), 1.78 (2 H, m); IR (KBr) 3450, 2940, 2860, 1730, 1592, 1540, 1490, 1410, 1300, 1262, 1180, 1150, 1110, 1020, 1035, 840, 805; HRMS exact mass calculated for $C_{25}H_{26}N_4O_3S$ 462.1726 (M^+), found 462.1747.

2-Methyl-N^5-[4-[(4-methoxyphenyl)sulfonyl]benzyl]-5,6,7,8-tetrahydro-1*H*-imidazo[4,5-*g*]quinoline (6k). A solution of compound **22d** (0.11 g, 0.23 mmol) in 3 mL of glacial acetic acid was treated with Sn^0 (0.14 g, 1.21 mmol) and refluxed overnight. The mixture was filtered through Celite, neutralized with saturated $NaHCO_3$ solution, and extracted twice with EtOAc. The combined organic layers were dried over Na_2SO_4, concentrated, and purified by flash chromatography (10% MeOH–CH_2Cl_2) to give **6k** as a light yellow solid (0.72 g, 71%): mp 162–165 °C; ^{1}H NMR ($CDCl_3$) δ 7.84 (4 H, m), 7.38 (2 H, d, J = 8.3 Hz), 7.16 (1 H, s), 6.96 (2 H, d, J = 8.9 Hz), 6.33 (1 H, s), 4.49 (2 H, s), 3.83 (3 H, s), 3.38 (2 H, m), 2.92 (2 H, m), 2.47 (3 H, s), 2.04 (2 H, m); IR (KBr) 3400, 1590, 1250, 1140; HRMS exact mass calculated for $C_{25}H_{25}N_3O_3S$ 447.1616 (M^+), found 447.1586.

N^1,2-Dimethyl-N^5-[4-[(4-methoxyphenyl)sulfonyl]benzyl]-5,6,7,8-tetrahydro-1*H*-imidazo[4,5-*g*]quinoline (7k). Using the same procedure described above for **6k**, nitro compound **33k** (0.21 g, 0.41 mmol) was cyclized to yield **7k** (0.11 g, 63%) as light yellow crystals after chromatography on silica (3% MeOH–CH_2Cl_2): mp 222–225 °C (sealed cap); ^{1}H NMR ($CDCl_3$) δ 7.85 (2 H, d, J = 8.9 Hz), 7.80 (2 H, d, J = 8.3 Hz), 7.37 (2 H, d, J = 8.3 Hz), 6.95 (2 H, d, J = 8.9 Hz), 6.88 (1 H, s), 6.55 (1 H, s), 4.51 (2 H, s), 3.83 (3 H, s), 3.59 (3 H, s), 3.39 (2 H, t, J = 5.7 Hz), 2.95 (2 H, t, J = 5.7 Hz), 2.45 (3 H, s), 2.06 (2 H, m); HRMS exact mass calculated for $C_{26}H_{27}N_3O_3S$ 461.1773 (M^+), found 461.1761.

6-Amino-7-nitro-*N*-(4-carboxybenzyl)-1,2,3,4-tetrahydroquinoline (37). *N*-Acyl nitro compound **22f** (5.00 g, 13.0 mmol) was deacylated as described above for **29h**. The crude acidic reaction mixture was placed in an ice bath and neutralized with 6 N NaOH and solid NaOH was added to make the aqueous layer 1 N in NaOH. After stirring for 12 h, the reaction mixture was diluted with brine and neutralized with 6 N HCl followed by saturation with NaCl and extraction with EtOAc. The combined organic layer was dried ($MgSO_4$) and concentrated to afford 5.5 g of a purple solid. Chromatography on silica (5→10% MeOH–CH_2Cl_2) yielded **37** as a purple solid (3.14 g, 74%): mp 197–200 °C; ^{1}H NMR (DMSO-d_6) δ 7.89 (2 H, d, J = 8.1 Hz), 7.36 (2 H, d, J = 8.1 Hz), 6.96 (1 H, s), 6.95 (1 H, s), 6.73 (1 H, s), 6.69 (1 H, s), 4.48 (2 H, s), 3.35 (2 H, m, obscured by water peak), 2.74 (2 H, t, J = 6.3 Hz), 1.91 (2 H, m); IR (KBr) 3482, 3368, 2948, 1690, 1505, 1246; HRMS exact mass calculated for $C_{17}H_{17}N_3O_4$ 327.1219 (M^+), found 327.1231.

6-Amino-7-nitro-*N*-[4-(L-glutamocarbonyl)benzyl]-1,2,3,4-tetrahydroquinoline Diethyl Ester (38). A solution of amino acid **37** (2.2 g, 6.8 mmol), diethyl L-glutamate hydrochloride (2.13 g, 8.9 mmol), and triethylamine (0.9 g, 8.9 mmol) in 60 mL of DMF was treated with 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC) (1.70 g, 8.9 mmol) at 0 °C. The reaction was allowed to warm to 25 °C and stirred an additional 12 h. The mixture was diluted with water and extracted with EtOAc. The combined organic layers were washed with brine, dried ($MgSO_4$), and concentrated to give 5.1 g of a purple oil. Chromatography on silica (1:1 EtOAc–hexanes) afforded 2.25 g (65%) of **38** as an oil: ^{1}H NMR ($CDCl_3$) δ 7.80 (2 H, d, J = 8.3 Hz), 7.35 (2 H, d, J = 8.3 Hz), 7.12 (1 H, s), 7.0 (1 H, d, J = 7.4 Hz), 6.52 (1 H, s), 5.66 (2 H, s), 4.79 (1 H, m), 4.49 (2 H, s), 4.24 (2 H, q, J = 1.4 Hz), 4.12 (2 H, q, J = 1.8 Hz), 3.31 (2 H, t, J = 5.8 Hz), 2.79 (2 H, t, J = 6.2 Hz), 2.30–2.60 (3 H, m), 2.00–2.20 (3 H, m), 1.31 (3 H, t, J = 7.1 Hz), 1.22 (3 H, t, J = 7.1 Hz); IR (KBr) 3474, 3364, 3337, 2978, 2942, 1734, 1640, 1503; HRMS exact mass calculated for $C_{26}H_{32}N_4O_7$ 512.2270 (M^+), found 512.2262. Anal. ($C_{26}H_{32}N_4O_7$) C, H, N.

2-Amino-N^5-[4-(L-glutamocarbonyl)benzyl]-5,6,7,8-tetrahydro-1*H*-imidazo[4,5-*g*]quinoline Diethyl Ester (40). The diamine **39** (0.76 g crude, 1.17 mmol) was cyclized with CNBr as previously described above for **2j** to afford 224 mg 38% (two steps) of **40** as a brown glass: ^{1}H NMR ($CDCl_3$) δ 7.66 (2 H, d, J = 8.1 Hz), 7.22 (2 H, d, J = 8.1 Hz), 6.84 (1 H, s), 6.19 (1 H, s), 4.74 (4 H, m), 4.39 (2 H, s), 4.20 (2 H, q, J = 7.1 Hz), 4.09 (2 H, q, J = 7.1 Hz), 3.35 (2 H, m), 2.83 (2 H, m), 2.47 (2 H, m), 2.30 (1 H, m), 2.18 (1 H, m), 2.01 (2 H, m), 1.27 (3 H, t, J = 7.1 Hz), 1.20 (3 H, t, J = 7.1 Hz); IR (film) 2900–3500 (broad), 2986, 1732, 1649, 1500. Anal. ($C_{27}H_{33}N_5O_5 \cdot 0.6H_2O$) C, H, N.

2-Amino-N^5-[4-(L-glutamocarbonyl)benzyl]-5,6,7,8-tetrahydro-1*H*-imidazo[4,5-*g*]quinoline (8). To a solution of diester **40** (0.09 g, 0.17 mmol) in 1 mL of ethoxyethanol was added 1 mL of 1 N NaOH at 23 °C. After 1.5 h the reaction was diluted with 1 mL of water and the pH was adjusted to 4–5 with AcOH and the mixture was concentrated to a light brown solid. This solid was taken up in ca. 1 mL of water and centrifuged. The aqueous layer was decanted and the process was repeated twice. The remaining solid was lyophilized to yield 58 mg (73%) of **8** as a tan powder: mp 226–230 °C; ^{1}H NMR (DMSO-d_6) δ 8.29 (1 H, d, J = 7.2 Hz), 7.78 (2 H, 2, J = 8.1 Hz), 7.32 (2 H, 2, J = 8.1 Hz), 7.20 (2 H, br s), 6.78 (1 H, s), 6.22 (1 H, s), 4.48 (2 H, s), 4.27 (1 H, m), 3.40 (2 H, m, obscured by water peak), 2.77 (2 H, m), 2.28 (2 H, m), 1.90 (4 H, m); IR (KBr) 3349, 3173, 2949, 2859, 1705, 1686, 1638, 1499; HRMS-FAB exact mass calculated for $C_{23}H_{26}N_5O_5$ 452.1933 (M^+), found 452.1935.

6-(Acetylamino)-7-nitro-1,2,3,4-tetrahydronaphthalen-1-one (42a/42b). A suspension of 41[29] (9.20 g, 45.25 mmol) in 55 mL of acetic anhydride was cooled to 0 °C and a solution of 5.2 mL of concentrated nitric acid in 8.8 mL of glacial acetic acid was added at the same temperature. The resulting dark green mixture was stirred 2 h at 0 °C and then warmed to 25 °C for 2 h. The mixture was poured into a dilute H_2SO_4–ice water solution and extracted with ethyl acetate. The organic layer was washed with dilute $NaHCO_3$, dried over $MgSO_4$, and concentrated to a brown oil. Crude yield of **42a** and **42b**, 5.85 g, 52%. TLC analysis indicated that **42a** had a higher R_f value in 3% MeOH–CH_2Cl_2. Purification on silica gel (1% MeOH–CH_2Cl_2) gave a ca. 2:1 ratio of the yellow-orange solids **42a**:**42b**. Recrystallization (ethyl acetate–hexanes) yielded pure **42a**: 3.85 g; mp 152–153 °C; ^{1}H NMR ($CDCl_3$) δ 10.55 (1 H, br s), 8.86 (1 H, s), 8.74 (1 H, s), 3.03 (2 H, t, J = 6.0 Hz), 2.67 (2 H, t, J = 6.5 Hz), 2.32 (3 H, s), 2.18 (2 H, m); IR (KBr) 3372, 1703, 1686, 1510. Anal. ($C_{12}H_{14}O_4N_2$) C, H, N. **42b**: 1.90 g, mp 198–200 °C; ^{1}H NMR ($CDCl_3$) δ 8.53 (1 H, s), 8.38 (1 H, d, J = 8.9 Hz), 8.23 (1 H, d, J = 8.9 Hz), 2.98 (2 H, t, J = 6.1 Hz), 2.67 (2 H, t, J = 6.6), 2.24 (3 H, s), 2.15 (2 H, m); IR (KBr) 3372, 1700, 1669, 1515.

6-(Acetylamino)-7-nitro-1,2,3,4-tetrahydronaphthalen-1-ol (43). A solution of **42a** (3.00 g, 12.08 mmol) in 80 mL of MeOH was added dropwise to $NaBH_4$ (0.50 g, 13.19 mmol) in 20 mL of MeOH at 0 °C. The resulting orange solution was warmed to 25 °C and stirred for 1 h. After the solution was concentrated to half the volume, the product was extracted with ethyl acetate (3 × 40 mL), washed with $NaHCO_3$, dried over anhydrous $MgSO_4$, and concentrated to a yellow solid (2.70 g, 89%). The alcohol, **43**, was recrystallized from a mixture of ethyl acetate–hexanes: mp 178–179 °C; ^{1}H NMR ($CDCl_3$) δ 10.32 (1 H, s), 8.51 (1 H, s), 8.34 (1 H, s), 4.78 (1 H, m), 2.85 (2 H, m), 2.28 (3 H, s), 2.05 (2 H, m), 1.86 (2 H, m); IR (KBr) 3350, 1707, 1578, 1507, 1333, 1046. Anal. ($C_{12}H_{14}O_4N_2$) C, H, N.

6-(Acetylamino)-1-chloro-7-nitro-1,2,3,4-tetrahydronaphthalene (44). HCl gas was bubbled through a suspension of alcohol **43** (1.90 g, 7.59 mmol) in 75 mL of benzene. After 5 min the reaction was complete and the solution heterogeneous. Concentration of the reaction gave a yellow-orange solid (2.02 g, 93%). The crude solid can be purified by flash chromatography on silica gel (3:1 hexanes–ethyl acetate) or by recrystallization from the same solvent mixture: mp 143–144 °C; ^{1}H NMR ($CDCl_3$) δ 10.33 (1 H, s), 8.54 (1 H, s), 8.26 (1 H, s), 5.26 (1 H, t, J = 3.5 Hz), 2.98 (1 H, dt, J = 3.9, 4.0 Hz), 2.85 (1 H, m), 2.28 (3 H, s), 2.24 (3 H, m), 1.88 (1 H, m); IR (KBr) 3353, 1707, 1577, 1505, 1331, 1289. Anal. ($C_{12}H_{13}O_3N_2Cl$) C, H, N.

6-(Acetylamino)-7-nitro-1-[[4-(phenylsulfonyl)phenyl]thio]-1,2,3,4-tetrahydronaphthalene (45). A DMF solution of $PhSO_2PhSSPhSO_2Ph$ (1.12 g, 2.25 mmol) was treated with NaH (80% dispersion, 0.14 g, 4.50 mmol) at 25 °C and stirred for 1 h. The resulting anion was transferred via cannula into a 12-mL DMF solution of **44** (0.93 g, 3.46 mmol) at −43 °C and stirred for 2 h. The reaction mixture was poured into a −43 °C THF solution containing glacial acetic acid (3.46 mmol, 0.21 mL). After the mixture was warmed to 25 °C, 40 mL of water was added and the mixture was extracted with ethyl acetate. The organic layer was washed several times with aqueous $NaHCO_3$ and brine, dried with $MgSO_4$, and concentrated to give an orange oil. Purification on silica (2:1 hexanes–ethyl acetate) gave 1.17 g (70%) of **45** as a yellow solid: mp 77–80 °C; ^{1}H NMR ($CDCl_3$) δ 10.29 (1 H, s), 8.53 (1 H, s), 8.22 (1 H, s), 7.94 (2 H, m), 7.87 (2 H, d, J = 8.5 Hz), 7.56 (5 H, m), 4.68 (1 H, m), 2.95 (1 H, m), 2.82 (1 H, m), 2.28 (3 H, s), 2.06 (3 H, m), 1.82 (1 H, m); IR (neat) 3356, 1705, 1578, 1503, 1080; HRMS exact mass calcd for $C_{24}H_{23}O_5N_2S_2$ 483.1048 (MH^+), found 483.1069.

6-Amino-7-nitro-1-[[4-(phenylsulfonyl)phenyl]thio]-1,2,3,4-tetrahydronaphthalene (46). A solution of compound **45** (1.00 g, 2.07 mmol) and Na_2O_2 (0.16 g, 2.07 mmol) in 65 mL of EtOH was heated at 45 °C for 45 min. The red mixture was cooled to 0 °C and quenched with dilute HCl. The product was extracted with ethyl acetate, washed with dilute $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated to a red oil. Purification on silica (3:2 hexanes–ethyl acetate) gave 0.77 g (85%) of **46** as an orange solid: mp 89–92 °C; ^{1}H NMR ($CDCl_3$) δ 8.14 (1 H, s), 7.93 (2 H, m), 7.85 (2 H, d, J = 8.4 Hz), 7.56 (3 H, m), 7.43 (2 H, m), 6.53 (1 H, s), 5.94 (2 H, br s), 4.67 (1 H, t, J = 3.6 Hz), 2.80 (1 H, m), 2.67 (1 H, m), 2.10 (1 H, m), 2.03 (2 H, m), 1.57 (1 H, m); IR (neat) 3507, 3395, 1632, 1265; HRMS exact mass calcd for $C_{22}H_{21}O_4N_2S_2$ 441.0943 (MH^+), found 441.0958.

6,7-Diamino-1-[[4-(phenylsulfonyl)phenyl]thio]-1,2,3,4-tetrahydronaphthalene (47). Compound **47** was prepared in 90% yield according to the general procedure for the Zn–AcOH reduction used above for **39**: ^{1}H NMR ($CDCl_3$) δ 7.94 (2 H, d, J = 7.6 Hz), 7.82 (2 H, d, J = 8.4 Hz), 7.53 (3 H, m), 7.40 (2 H, d, J = 8.3 Hz), 6.69 (1 H, s), 6.42 (1 H, s), 4.64 (1 H, t, J = 3.1 Hz), 3.40 (2 H, br s), 3.25 (2 H, br s), 2.65 (2 H, m), 2.02 (3 H, m), 1.71 (1 H, m); HRMS exact mass calcd for $C_{22}H_{23}O_2N_2S_2$ 411.1201 (MH^+), found 411.1188.

2-Amino-5-[[4-(phenylsulfonyl)phenyl]thio]-5,6,7,8-tetrahydronaphth[2,3-*d*]imidazole (9i). To a solution of compound **47** (0.28 g, 0.68 mmol) in 7 mL of CH_3CN–5 mL of MeOH was added CNBr (as a 5 M solution in CH_3CN, 17.05 mmol) at 0 °C. After 1 h at 0 °C, the reaction mixture was warmed to 25 °C, poured into water, and extracted with ethyl acetate. The solvent was washed with dilute $NaHCO_3$ and concentrated to give a brown oil, which was subjected to flash chromatography on silica (8% MeOH–CH_2Cl_2), and **9i** (0.09 g, 31%) was obtained as a fine brown solid after solvent removal: mp 150–154 °C; ^{1}H NMR ($CDCl_3$) δ 7.88 (2 H, d, J = 7.6 Hz), 7.72 (2 H, d, J = 7.9 Hz), 7.44 (3 H, m), 7.28 (2 H, m), 7.11 (1 H, s), 6.83 (1 H, s), 5.14 (3 H, br s), 4.70 (1 H, s), 2.70 (2 H, m), 2.01 (3 H, m), 1.95 (1 H, m); IR (KBr) 3358, 1647, 1568, 1469, 1155; HRMS exact mass calcd for $C_{23}H_{22}O_2N_3S_2$ 436.1153 (MH^+), found 436.1139.

Biochemical Assays. TS activity was assayed by a modified procedure of the tritium release method of Lomax and Grunberg.[34] Inhibition constants (K_{is} and K_{ii}) were as described by Cleland[35] and were determined by steady-state analysis against the cofactor 5,10-methylenetetrahydrofolate as the variable substrate under conditions of saturating dUMP. Reaction conditions in 0.1 mL were 50 mM Tris pH 7.6, 10 mM dithiothreitol, 1 mM ethylenediaminetetraacetic acid, 25 mM $MgCl_2$, 15 mM formaldehyde, 25 μM dUMP ([5-^{3}H]; specific activity 2×10^8 cpm/μmol), and tetrahydrofolate (eight concentrations ranging from 5 to 150 μM). Bovine serum albumin at up to 100 mg/mL was present when human TS was assayed. These reactions were either in the absence of inhibitor or in the presence of inhibitor at concentrations ranging, at a minimum, between $0.5 \times K_i$ to $2.0 \times K_i$ except when the solubility of the inhibitor was limiting. Reactions were run at room temperature by initiating with the addition of enzyme. After 5 min, the reactions were quenched by the addition of charcoal and centrifuged to remove unreacted dUMP, and the supernatant was counted to determine the release of tritium from the 5-position of dUMP. Experimental results were analyzed by a nonlinear regression analysis program[36] which fit the data to a mixed noncompetitive inhibition scheme.

Measurement of Tissue Culture IC_{50}s. IC_{50} values for the inhibition of cellular growth were measured using a modification[37] of the MTT[38] colorimetric assay of Mosmann[39] using mouse (L1210) and human (CCRF-CEM) leukemia lines (ATCC) and a human adenocarcinoma (GC_3/M TK$^-$) deficient in thymidine kinase. Cells were seeded at 1000 (L1210) or 10 000 (CCRF-CEM, GC_3/M TK$^-$) cells per well in 96-well plates and growth measured over a range of nine 2-fold serial dilutions of each compound. Culture medium (RPM1-1640) contained 5% (L1210, CCRF-CEM) or 10% (GC_3/M TK$^-$) fetal calf serum and 0.5% DMSO. Following a 3-day (L1210) or 5-day (CCRF-CEM, GC_3/M TK$^-$) incubation and a 4-h treatment with MTT, cells were harvested and growth measured spectrophotometrically after dissolution of the deposited formazan in DMSO. IC_{50} values were determined from semilogarithmic plots of compound concentration vs the mean of the four growth assessments made at each serial dilution of the agent relative to the growth of control cultures.

Measurement of IC_{50} Shift Due to Thymidine and Leucovorin. The ability of thymidine or leucovorin to reverse growth inhibition was assessed against L1210 by comparing the IC_{50} measured under standard conditions (RPM1-1640 medium containing 5% fetal calf serum) with that obtained in the presence of 10 μM thymidine (or 0.5 μM leucovorin) which was replenished daily during the three days of growth. The magnitude of the ratio of the IC_{50} measured in the presence of thymidine (or leucovorin) to that measured without additive was used to reflect the extent to which the inhibition of growth could be attributed to intracellular inhibition of thymidylate synthase (or DHFR). A value of 1.0 under these conditions would reflect a probable locus of action other than TS while larger values probably reflect a direct relationship between growth inhibition and TS targeting (or DHFR).

Acknowledgment. We express our appreciation to members of the Organic Chemistry Group at Agouron Pharmaceuticals Inc. that supported this work. We thank Russ Bacquet, Dominic Zichi, and Rami Reddy for their assistance with computer graphics software and with the computational analysis of inhibitors. Finally, we are grateful to Peter Johnson for his continued support and encouragement of this work.

(34) Lomax, M. I. S.; Greenberg, G. R. A New Assay of Thymidylate Synthetase Activity Based on the Release of Tritium from Deoxyuridylate-5-^{3}H. *J. Biol. Chem.* **1967**, *242*, 109–113.

(35) Cleland, W. W. The Kinetics of Enzyme-catalyzed Reactions with Two or More Substrates or Products II. Inhibition: Nomenclature and Theory. *Biochim. Biophys. Acta* **1963**, *67*, 173–187.

(36) Perrella, F. W. EZ-FIT, Perrella Scientific, Springfield, PA, 1989.

(37) Alley, M. C.; et al. Feasibility of Drug Screening with Panels of Human Tumor Cell Lines Using a Microculture Tetrazolium Assay. *Cancer Res.* **1988**, *48*, 589–601.

(38) MTT is 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

(39) Mosmann, T. Rapid Colorimetric Assay for Cellular Growth and Survival: Application to Proliferation and Cytotoxicity Assays. *J. Immunol. Methods* **1983**, *65*, 55–63.