Design of Thymidylate Synthase Inhibitors Using Protein Crystal Structures: The Synthesis and Biological Evaluation of a Novel Class of 5-Substituted Quinazolinones

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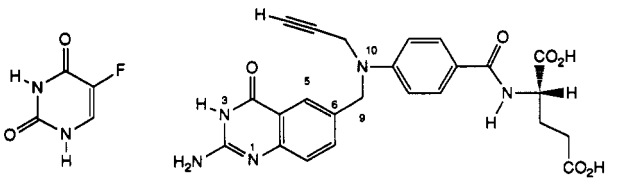
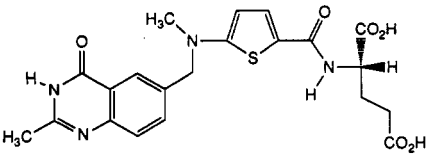
The design, synthesis, and biological evaluation of a new class of inhibitors of thymidylate synthase (TS) is described. The molecular design was carried out by a repetitive crystallographic analysis of protein-ligand structures. At the onset of this project, we focused on the folate cofactor binding site of a high-resolution ternary crystal complex of *Escherichia coli* TS, 5'-fluorodeoxyuridylate (5-FdUMP) and a classical glutamate-containing folic acid analog. A preliminary ternary crystal structure of a novel compound was successfully solved. Upon analysis of this initial complex, further structural elaborations were made, and a series of active 5-(arylthio)quinazolinones was developed. The synthetic strategy was based on the displacement of a halogen at the 5-position of a quinazolinone by various aryl thioanions. The compounds were tested for inhibition of purified *E. coli* and/or human TS, and were assayed for cytotoxicity against three tumor cell lines in vitro. Significant thymidine protection effects were observed with several of the inhibitors, indicating that TS was the intracellular locus of activity.

Introduction

With the goal of developing new cancer chemotherapeutics, several research groups, including our own, have chosen the enzyme thymidylate synthase (TS, EC 2.1.1.45) as a biochemical target for drug design. The enzyme catalyzes the reaction of deoxyuridine monophosphate (dUMP) to deoxythymidylate (dTMP) by a reductive methylation using 5,10-methylenetetrahydrofolate as the cofactor. This rate-limiting step is the exclusive de novo source of dTMP for DNA biosynthesis. Without providing an exogenous supply of thymidine, halting TS activity would lead to a "thymineless cell death". Several inhibitors are known that bind in either the pyrimidine substrate or folate cofactor site.[1] The anticancer agent 5-fluorouracil (5-FU, 1) acts as a TS inhibitor by the binding of its metabolite, 5-fluorodeoxyuridine monophosphate (5-FdUMP), in the substrate site. Although 5-FU is a clinically useful drug, it possesses several detrimental features, including neurotoxicity and overt cardiotoxicity.[2] Not being a pure TS inhibitor, 5-FU can also become an unnatural substrate for RNA biosynthesis. The development of drug resistance is common due to metabolic changes associated with decreases in kinase levels. Amplified pools of dUMP are created, decreasing the efficacy of 5-FU by competition. Keeping these problems in mind, TS inhibitors designed to bind in the folate cofactor site would be devoid of such negative properties. The antifolates *N*-[4-[*N*-[(2-amino-3,4-dihydro-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoyl]-L-glutamic acid (CB3717, 2),[3] and *N*-[5-[*N*-(3,4-dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-methylamino]-2-thenoyl]-L-glutamic acid (D1694, 3)[4] represent examples that display antitumor activity. However, classical folic acid analogs are not free of complications. Renal and hepatic toxicities have been prominent problems.[5] The glutamic acid component, necessary for active transport across cell membranes, can lead to drug resistance.[6] In addition, these molecules are often good substrates for folylpolyglutamate synthase (FPGS).[7a-c] Once polyglutamated, the compounds frequently become extremely tight binding TS inhibitors and are not effluxed from cells.[7d,e] Polyglutamation seems necessary for cytotoxicity of tumor cells but may also be implicated as a possible cause of detrimental side effects to the host.



1 (5-FU) 2 (CB3717)



3 (D1694)

Attempting to overcome the aforementioned complications, our primary objective was to discover lipophilic inhibitors of TS based on the folate site.[8,9] Theoretically, lipophilic compounds would diffuse into cells passively, thus avoiding drug resistance due to active transport. Any effects associated with polyglutamation would be eliminated. The compounds would also be expected to have very different physical properties than those of classical antifolates. However, we were also interested in designing novel molecules retaining the glutamate moiety, with the intent of modulating their negative properties.

By using a high-resolution crystal structure and molecular modeling techniques, initial design work led to the synthesis of four different quinazolinones, each substituted

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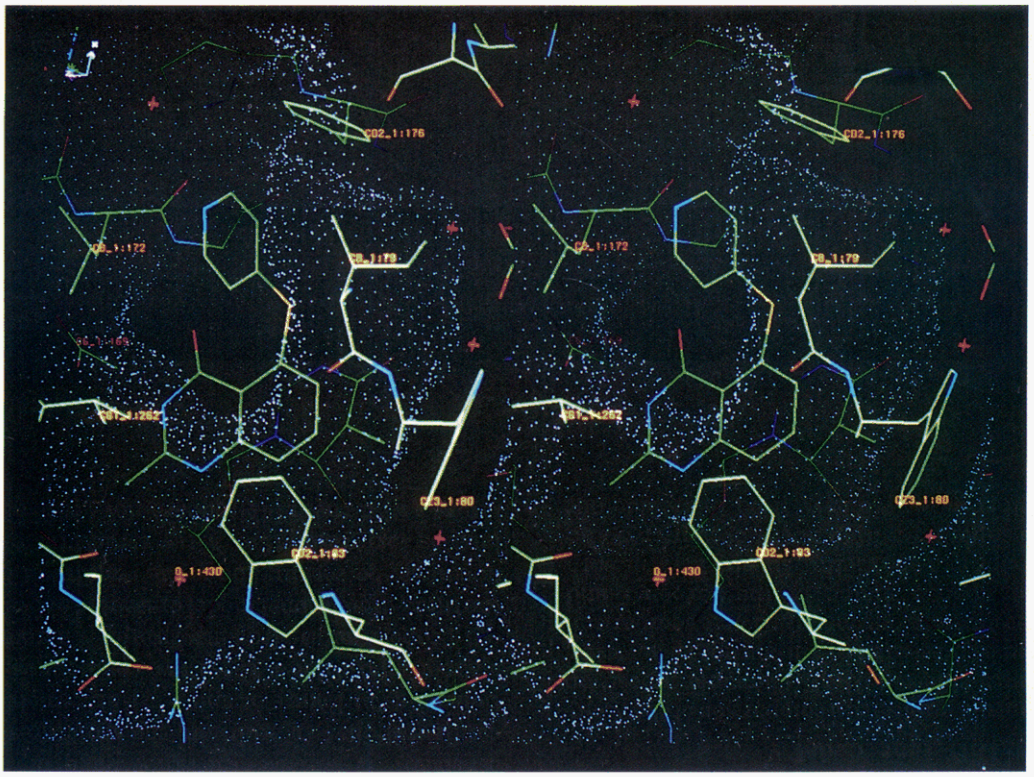


Figure 1. Stereoview of the ternary X-ray structure of compound **6**, *E. coli* TS, and 5-FdUMP. The protein and ligand atoms are displayed in green (carbon), red (oxygen), blue (nitrogen), and yellow (sulfur). The water molecules are displayed as red crosses, and the solvent accessible surface of the protein is highlighted in light blue. Ile79, Trp80, Trp83, Asp169, Leu 172, Phe176, Val262, and H_2O 430 are labeled.

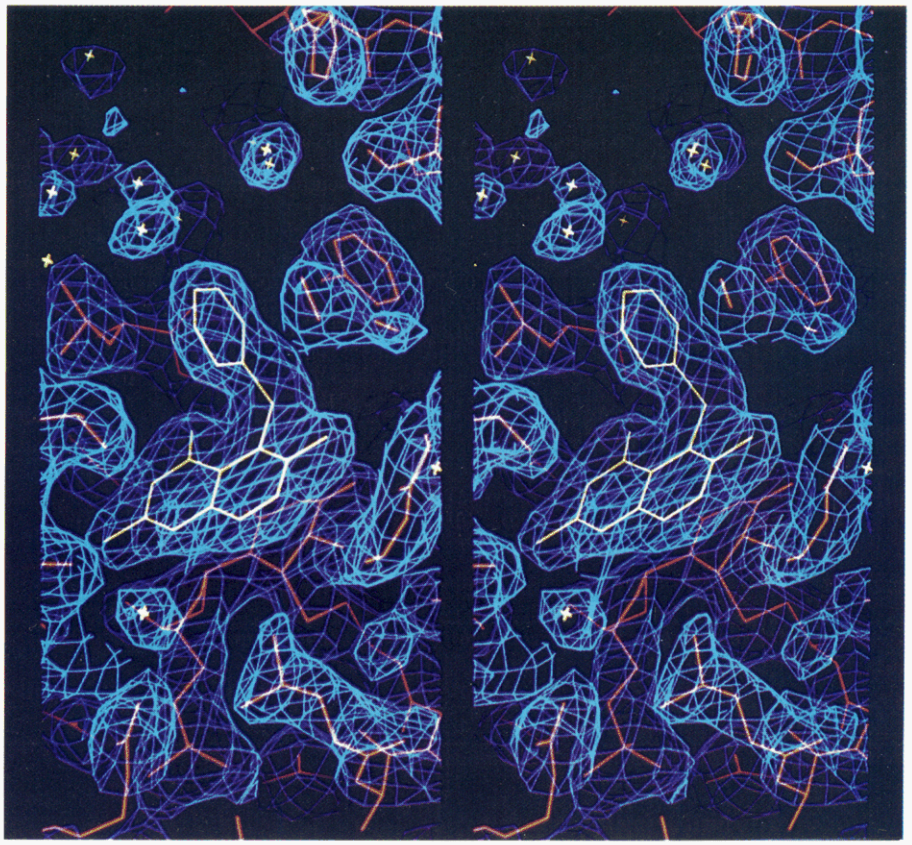


Figure 2. Stereoview of the electron density map of the crystal structure of compound 8 complexed with *E. coli* TS and 5-FdUMP at 2.5-Å resolution computed with coefficients $2F_{obs} - 2F_{calc}$ and phases calculated after 18 cycles of refinement for which the R factor was 17.8%.

at C-5. Of these derivatives, compound **6** exhibited measurable inhibition against human and *Escherichia coli* TS. The protein–ligand crystal structure of **6** was solved and utilized for subsequent design. Exploiting the struc-

tural information enabled us to make several improvements to our original ideas. The development of this new family of compounds, including synthesis and biological evaluation, is discussed.

Design

At the time we started our investigation, human TS was not available, whereas the purified *E. coli* form was accessible in large amounts and crystallization conditions were well established. With the exception of the three residues His51, Trp83, and Val262 (Phe80, Asn112, and Met311 in human), the folate binding sites of *E. coli* and human TS are homologous. Therefore, we surmised that a crystal complex of the *E. coli* form would serve as a good model to do structure-based drug design.[9] Our research began by examination of a 2.3-Å resolution ternary complex of *E. coli* TS, 5-FdUMP, and CB3717.[10] We decided to keep the quinazolin-4-one ring system intact based on several important observations. These factors included H-bonding between the carboxylate of Asp169 and N3-H, and H-bonding between H_2O·430 and the lone pair of N1, as well as ring stacking between the pyrimidine nucleus of FdUMP and the quinazolinone. We elected to start with a quinazolin-4-one substituted with the 2-methyl rather than the 2-amino group in order to simplify the initial synthesis and to increase the solubility of the molecules. Using the quinazolin-4-one system as a molecular scaffold, our design strategy was focused upon the C5–C6–C9 and *p*-aminobenzoyl regions of CB3717. The direction of the binding pocket turns sharply and then begins to expand as you move from C6 to the C9–N10–phenyl area of CB3717. When a solvent-accessible surface is displayed in the absence of the ligand, the cavity created by this space resembles the shape of a cone or funnel. The base of this cone is the opening of the active site where the glutamic acid fragment resides. A large percentage of the surrounding domain is comprised of hydrophobic side chains, including Ile79, Trp80, Trp83, Leu172, Phe176, and Val262.

By examining the binding conformation of CB3717 and models of 5,10-methylenetetrahydrofolate, we observed that the hydrophobic cavity associated with the para-aminobenzoyl portion could be accessed from C-5 or N-5 of these respective structures. A one-atom linkage to a phenyl or aromatic ring via C-5 of the quinazolinone system was envisaged. This general idea led to the design of molecules **4–7**, which were then subjected to calculations to establish their minimum-energy conformations.[11] For each of the three variants, two relatively equal low-energy conformers were found. Of these two orientations, one could be modeled well in the active site by superimposing the structures onto CB3717. The compounds were synthesized, and of the three, only the pyridine derivative **6** had measurable inhibition constants of 10.0 μM against *E. coli* and 0.96 μM against human TS. When this inhibitor was subjected to crystallization experiments with *E. coli* TS and 5-FdUMP, a complex was formed and the ternary structure was solved.[12] Figure 1 illustrates how compound **6** binds to the protein. The small molecule is found complexed within the active site in a similar orientation as originally modeled. Some movement of the side chain of Ile79 toward the inhibitor was observed.

Exploiting the information generated from this new crystal structure, we then concentrated on the C-6-Trp80 region of this complex. A void had been created in this area since C-6 of compound **6** is unsubstituted, as compared to CB3717, where a methylene unit occupies the space and makes hydrophobic interactions with Trp80. The next logical step was the replacement of the hydrogen atom with a methyl group at C-6. Quinazolinone **8** was found to be a very rigid molecule having one low-energy conformation. When docked into the active site, the C-6-methyl group of **8** fills the empty gap efficiently, while the thiopyridine moiety, being locked in a configuration orthogonal to the bicyclic ring system, interacts favorably with the surrounding hydrophobic residues as described previously. Compound **8** was prepared and determined to have a greater affinity for *E. coli* (0.50 μM) and human TS (0.093 μM). A highly refined ternary crystal structure was thus obtained.[13] An electron density map of this complex is shown in Figure 2.

Various substitutions were thus modeled at C-6, and compounds **9–11** and **22** were prepared. Of these, the chloro derivative **22** had comparable properties,[14a] while the larger ethyl and methoxy derivatives **9** and **10** were found to be inferior. We believe that the chlorine atom and methyl group, being of relative size and hydrophobic nature,[14b] sterically interact better with Trp80. The effects of having a free hydroxyl group at this position were extremely detrimental, the rationale being due solely to desolvation factors and not electrostatic ones.[14c] The biological test results for these variations are summarized in Table I.

The next part of our strategy was focused on replacing or modifying the pyridine ring. From previous work,[8] potent TS inhibitors were discovered by replacement of the CO-glutamate function of classical antifolates with different electron-withdrawing groups. Conversely, electron-donating groups on the phenyl ring para to N-10 diminished binding properties. A plausible explanation for this phenomenon may be a dipole–dipole interaction between the protein and the substituted phenyl segment of the inhibitor. Electron-withdrawing groups generate favorably oriented dipoles while electron-donating groups both diminish the magnitude of the dipole and alter the direction. Hence, the 4-thiopyridyl system may interact with the protein similar to other lipophilic inhibitors having phenyl rings substituted with electron-withdrawing groups.[8] When the 4-thiopyridyl group of **6** is replaced with the 2-thiopyridyl (**7**), a dramatic decrease in binding is observed. The change in direction of the dipole is notable, but we must also take entropic or desolvation effects of the pyridine nitrogen into account. In the case of compounds **6** and **8**, the pyridine nitrogen remains in contact with solvent upon binding. This is observed in the ternary crystal complex of **8** as shown in Figure 2. Whereas in the case of analog **7**, if we assume that binding in the active site is analogous to **6**,[15] the pyridyl nitrogen can only be positioned in one of two hydrophobic regions. Compound **7** would therefore lose binding energy upon desolvating this atom as compared with either **6** or **8** where no such free energy would be lost. The 3-thiopyridyl derivative, **12**, was also prepared and shown to be a weaker binder to TS than **8**. By the replacement of the pyridine of **8** with pyridazine, an enhancement of the dipole effect might be expected, although no significant change in potency was observed. The K_is of compound **13** against *E. coli* and human TS were found to be 0.60 and 0.087 μM, respectively. One last test of the dipole theory was accomplished by exchanging the 4-pyridyl ring with either

Table I. Biological Data and Calculated log *P*

	K_i (μM)[a]				IC_{50} (μM)[c]				
	E. coli TS		human TS						
no.	K_{is}	K_{ii}	K_{is}	K_{ii}	L1210	CCRF-CEM	GC_3/M TK⁻	thymidine[e] shift	clog *P*[f]
4	>100[d]		>100[d]		>50[d]	>50[d]			2.05
5	>10[d]		>10[d]		>4.2[d]	>4.2[d]			2.5
6	10	30	0.96	2.1	14	>26.9[d]	>26.9[d]	2	1.94
7	>80[d]	>80[d]	120	190	>50[d]	>50[d]	>50[d]	1	1.92
8	0.50	0.82	0.093	0.058	3.5	5.2	6.0	≫14	2.16 (2.17)
9			0.20	0.59	>10[d]	>10[d]	>10[d]	~10	2.3
10	1.5	3.9	0.26	0.64	21	26	32	10	1.65
11	51	190	8.8	19	>50[d]	>50[d]	>50[d]	1	1.50
12	1.7	9.3	0.31	1.1	>2.5[d]	>2.5[d]	>2.5[d]		2.1
13	0.6	1.1	0.087	0.12	8.0	10.5	>12.5[d]	1.4	2.02
14			0.141	0.065	50	>50[d]	>50[d]	>5	
15	0.49	0.73	0.056	0.078	3.0	2.9	>4.0[d]	3.3	3.29 (3.04)
16	0.096	0.22	0.076	0.12	1.8	2.1	4.5	5.6	4.67 (3.81)
17	0.24	0.53	0.039	0.066	4.2	4.2	5	>20	2.34 (2.45)
18	0.28	0.71	0.037	0.046	3.1	3.8	>5[d]	>7	1.97
19	0.23	0.54	0.07	0.13	8.1	8.6	15	3.1	2.46
20			0.080	0.058	4.05	10.5	18	4.25	2.08
21	0.049	0.14	0.015	0.016	1.0	0.81	1.0	~40	1.83
22			0.020	0.018	1.3	2.1	2.05	~14	1.86
23			0.028	0.024	1.8	>2[d]	>2[d]	>5	2.29
24			0.032	0.050	1.8	4.9	5.2	>20	1.66
25	0.051	0.14	0.013	0.011	1.6	0.88	1.5	17.6	1.80
26			(0.00131)[b]		1.05	0.99	4.1	>22	
27			(0.000124)[b]		2.5	0.52	1.7	~8.0	

[a] TS activity was assayed by the tritium release method of Lomax and Greenfield.[33] See Experimental Section for details. K_{is} values are cited in the text. [b] Tight binding kinetics were performed as described in the Experimental Section. [c] Inhibition of cell growth was measured using a modification[35] of the MTT[36] colorimetric assay of Mosmann.[37] [d] Concentrations above this level could not be tested due to insolubility of the inhibitor. [e] The ratio of the IC_{50} of the inhibitor against L1210 cells cultured in the presence of 10 μM exogenous thymidine divided by the IC_{50} observed in the absence of thymidine. See Experimental Section for details. [f] Calculated log *P* using the method of Bodor et al.[39] Experimental log *P* values[40] are shown in parentheses.

the 4-phenyl carboxylic acid, 4-nitrophenyl, or 4-diphenyl sulfone functions. Each of these replacements could be accommodated by the protein when modeled in the active site. Compounds **14–16** were prepared and found to be active against *E. coli* and/or human TS as listed in Table I.

Further examination of the three-dimensional structure of **8** complexed with TS revealed an unoccupied pocket created by the side chains of Leu172 and Val262 which could be accessed from the 2-position of the pyridine ring. Derivatives **17–20** are examples that have been synthesized and tested. Each one showed some improvement over the parent with respect to binding. This data suggests that the enzyme may be indiscriminate to a variety of functional groups located at this position. Therefore, other physical properties, including solubility and lipophilicity, may be modified by changes made at this site.

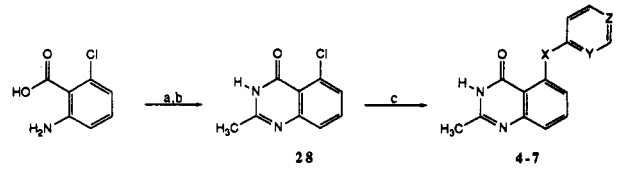
Alterations at position C-2 of classical antifolate TS inhibitors have been studied extensively.[16–18] Upon inspection of the crystal structure of **8**, it became apparent that hydrogen bonding to the carbonyl oxygen of Ala263 may be possible by replacement of the quinazoline C-2 methyl with an amino group. Compound **21** was synthesized and binding was enhanced by a factor of 10 against *E. coli* TS (49.0 nM) and by a factor of 6 against human TS (15.0 nM). The biological data for the 2-aminoquinazoline analogs is listed in Table I.

In the final stage of our research we decided to explore the concept of including a glutamic acid residue into our designs. Physiochemical properties for these unique molecules may be very different from those of other glutamate-containing TS inhibitors, whereby improvements with respect to solubility, bioavailability, and toxicity may result. An initial model was created by replacing the pyridine ring of **8** with the 4-(COGlu)phenyl system. After minimization, the structure was overlaid onto the 3D coordinates of **8** in the active site. When this model is compared with CB3717 (**2**), the phenylglutamic acid amides do not superimpose exactly, but occupy the same general space. Two representatives, **26** and **27**, were synthesized and tested. Both compounds were found to inhibit human TS at extremely low concentrations. Crystallization experiments are currently in progress.

Chemistry

A convergent synthetic plan utilizing the Ullmann reaction[19] was used to prepare the 5-(arylthio)quinazolinones. The 5-haloquinazolin-4-ones were made from commercially available anthranilic acids or via the appropriate isatin employing the modified procedure of Marvel and Hiers.[20] Compounds **4–7** were synthesized from 5-chloro-2-methyl-3*H*-quinazolin-4-one[21] (**28**) as depicted in Scheme I. The 2-methyl- or 2-aminoquinazolin-4-ones **34a–c** and **37a,d** were prepared as outlined in Scheme II. The first attempts to synthesize methyl anthranilate **36a** by acid-catalyzed esterification of anthranilic acid **32a** failed. Surprisingly the sole product isolated was the starting aniline **29a**, formed by decarboxylation. The methyl ester could be prepared cleanly with diazomethane, but this method was impractical on a large scale. The next approach was through ring opening of benzoxazinone **33a** with methanol, followed by acid hydrolysis of the intermediate acetamide group. The yields for this sequence varied, and careful monitoring of the reaction was necessary in order to avoid dimer and polymerization. We found the best route to **36a** to be the opening of isatoic anhydride **35a** with methanol. The isatoic anhydrides **35a** and **35d** were prepared from their corresponding anthranilic acids with bis(trichloromethyl) carbonate. Attempts to synthesize **35a** directly from isatin

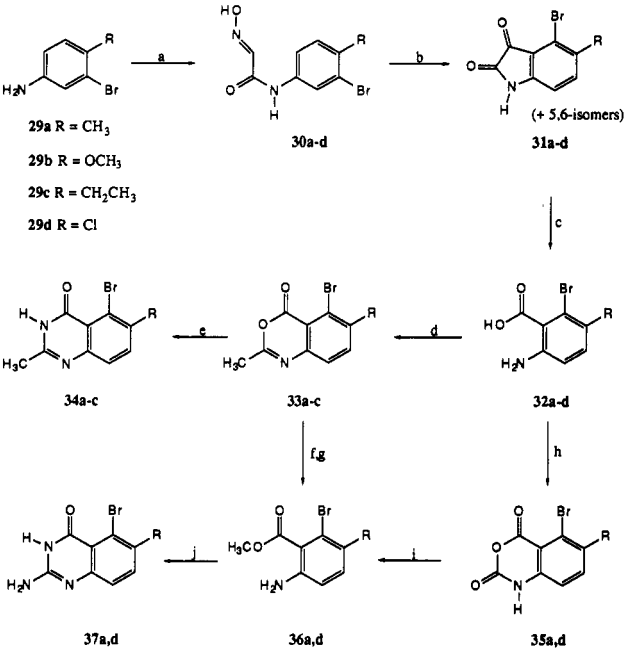
Scheme I[a]



#	X	Y	Z	coupling method
4	O	CH	CH	A + C_6H_5OH
5	S	CH	CH	A + C_6H_5SH
6	S	CH	N	A + 4-(SH)C_5H_4N
7	S	N	CH	A + 2-(SH)C_5H_4N

[a] (a) AcOAc; (b) NH_3; aqueous 1-N NaOH; (c) coupling method A, NaH, DMA, CuBr, Cu_2O.

Scheme II[a]

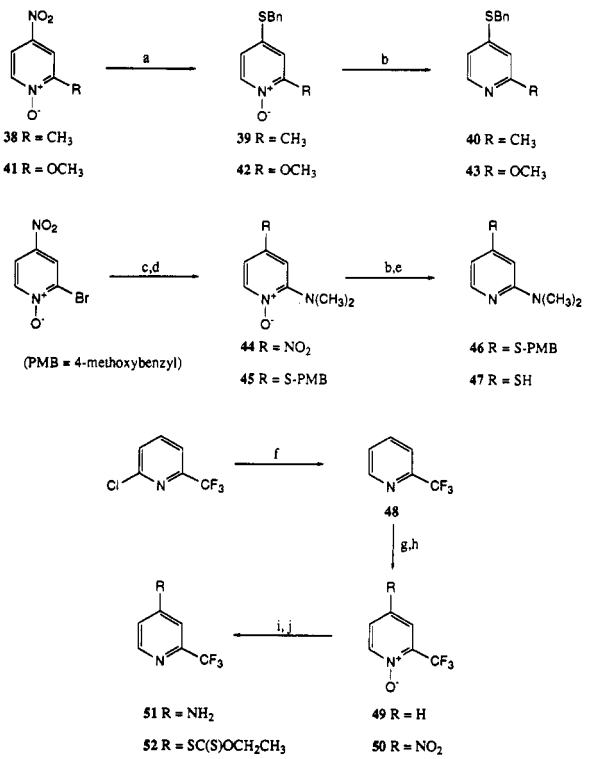


[a] (a) $Cl_3CCH(OH)_2$, Na_2SO_4, NH_2OH, HCl, H_2O; (b) H_2SO_4; (c) NaOH, H_2O_2; (d) AcOAc; (e) NH_3; aqueous 1 N NaOH; (f) CH_3OH; (g) HCl; (h) $(Cl_3CO)_2CO$; (i) CH_3OH; (j) $ClC(NH)NH_2$·HCl, diglyme.

31a by peracid oxidation were unsuccessful. When esters **36a** and **36d** were exposed to chloroformamidine hydrochloride at elevated temperatures, transformation to their respective quinazolinones, **37a** and **37d**, took place in high yield. Key intermediate **37a** could not be produced from the reaction of anhydride **35a** with guanidine carbonate.

Not being commercially available, we found it necessary to prepare many of the mercaptopyridines. 3-Mercaptopyridine[22] and 4-mercaptopyridazine[23] were synthesized using methods reported in the literature. The syntheses of the 2,4-disubstituted pyridines are illustrated in Scheme III. At first, we envisioned that the 4-mercaptopyridines could be generated from their corresponding pyridones. The reaction of 2-methyl- or 2-(trifluoromethyl)-4(1*H*)-pyridone[24] with either P_4S_{10} or Lawesson's reagent[25] failed or gave poor yields. An alternative way around this problem was to introduce the sulfur atom using a direct displacement reaction of 4-nitropyridine *N*-oxides. Nitration of pyridine *N*-oxides were performed as described in the literature.[26-28] The anion of benzyl mercaptan, generated with KH, was initially used in the displacement reactions. Debenzylation was performed with sodium metal in liquid ammonia. In some instances we discovered

Scheme III[a]



[a] (a) Benzyl mercaptan, KH, THF; (b) PCl_3, $CHCl_3$; (c) $HN(CH_3)_2$, THF; (d) 4-methoxy-α-toluenethiol, DMF, NaH; (e) $Hg(OAc)_2$; (f) 10% Pd/C, H_2, Me_2NEt, MeOAc; (g) H_2O_2, AcOH; (h) HNO_3, H_2SO_4; (i) 10% Pd/C, H_2, EtOH; (j) H^+, $NaNO_2$, potassium ethyl xanthate.

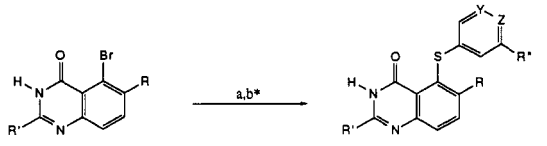
that it was not necessary to isolate the free thiols, whereas the sulfanions used in the Ullmann reaction could be generated in situ upon deprotection.[29] This technique worked well for the transformation of **40** to **17**, but not as well in the case of **43** to **18**. The method completely failed with the 4-*S*-benzyl derivatives of 2-(dimethylamino)- or 2-(trifluoromethyl)pyridine. Thiol **47** was made by a similar plan incorporating the *p*-methoxybenzyl group instead of benzyl, which was then cleaved effectively with mercuric acetate.[30] After testing several ideas, the anion of 4-mercapto-2-(trifluoromethyl)pyridine was finally obtained by hydrolysis of xanthate **52**. The synthesis of **52** is shown in Scheme III. Scheme IV illustrates the Ullmann coupling and the variations used for compounds **8–13** and **17–25**.

The synthetic procedures used for compounds **14–16**, **26**, and **27** are summarized in Scheme V. Methyl 4-mercaptobenzoate was prepared according to the literature,[31] or by the sulfuric acid catalyzed esterification of 4-mercaptobenzoic acid. The preparation of 4-(phenylsulfonyl)-thiophenol[32] was achieved with 4-(phenylsulfonyl)fluorobenzene and NaSH in DMSO at 100 °C. After trying several methods, we found that the peptide coupling reaction of carboxylic acid **14** or **55** with L-glutamic acid diethyl ester worked best with diphenyl phosphorazidate, DMF, and Et_3N.[31]

Inhibition Studies and Biological Evaluation

The TS inhibition constants for compounds **4–27** were measured versus the cofactor 5,10-methylenetetrahydrofolate.[33] The data is reported in Table I. Precise K_i values for compounds **4**, **5**, and **7** could not be obtained due to solubility limitations. In the case of **26** and **27**, tight binding kinetics were also used to analyze these compounds

Scheme IV[a]



34a R = R' = CH_3

34b R = OCH_3 R' = CH_3

34c R = CH_2CH_3 R' = CH_3

37a R = CH_3 R' = NH_2

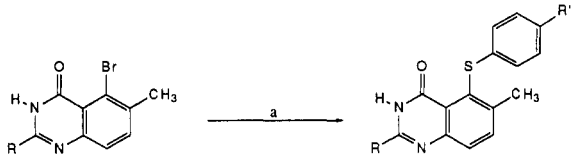
37d R = Cl R' = NH_2

8-13,17-25

#	R	R'	R''	Y	Z	coupling method
8	CH_3	CH_3	H	CH	N	A + 4-(SH)C_5H_4N
9	CH_2CH_3	CH_3	H	CH	N	A + 4-(SH)C_5H_4N
10	OCH_3	CH_3	H	CH	N	D + 4-(SH)C_5H_4N
11	OH	CH_3	H	CH	N	b*
12	CH_3	CH_3	H	N	CH	A + 3-(SH)C_5H_4N
13	CH_3	CH_3	H	N	N	A + 4-(SH)$C_4H_3N_2$
17	CH_3	CH_3	CH_3	CH	N	B + 40
18	CH_3	CH_3	OCH_3	CH	N	B + 43
19	CH_3	CH_3	CF_3	CH	N	C + 52
20	CH_3	CH_3	$N(CH_3)_2$	CH	N	A + 47
21	CH_3	NH_2	H	CH	N	A + 4-(SH)C_5H_4N
22	Cl	NH_2	H	CH	N	A + 4-(SH)C_5H_4N
23	CH_3	NH_2	CF_3	CH	N	C + 52
24	CH_3	NH_2	$N(CH_3)_2$	CH	N	A + 47
25	CH_3	NH_2	H	N	N	A + 4-(SH)$C_4H_3N_2$

[a] (a) Coupling methods: (A) NaH, DMA, CuBr, Cu_2O; (B) Na, NH_3; DMA, CuBr, Cu_2O; (C) KOH, CH_3OH; DMA, CuBr, Cu_2O; (D) NaOH, DMA, CuBr, Cu_2O; (b*) conversion of **10** to **11**; HBr, AcOH.

Scheme V[a]

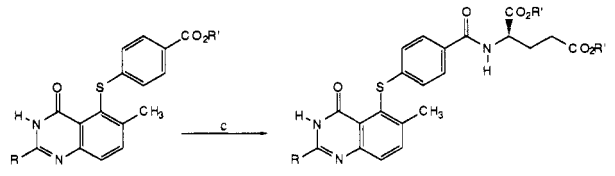


34a R = CH_3

37a R = NH_2

15, 16, 53, 54

#	R	R'	coupling method
15	CH_3	NO_2	A + 4-(SH)$C_6H_4NO_2$
16	CH_3	SO_2Ph	A + 4-(SH)$C_6H_4SO_2Ph$
53	CH_3	CO_2CH_3	A + 4-(SH)$C_6H_4CO_2CH_3$
54	NH_2	CO_2CH_3	A + 4-(SH)$C_6H_4CO_2CH_3$



53 R = R' = CH_3 → b → 14 R = CH_3 R' = H

54 R = NH_2 R' = CH_3 → b → 55 R = NH_2 R' = H

56 R = CH_3 R' = CH_2CH_3 → b → 26 R = CH_3 R' = H

57 R = NH_2 R' = CH_2CH_3 → b → 27 R = NH_2 R' = H

[a] (a) Coupling method A: NaH, DMA, CuBr, Cu_2O; (b) EtOH, aqueous 1 N NaOH; (c) L-glutamic acid diethyl ester, DMF, $(PhO)_2P(O)N_3$, Et_3N.

since they inhibit TS at extremely low concentrations.[34] It has not yet been determined if these compounds are substrates for FPGS. The compounds were also screened for cell growth inhibition against three tumor cell lines: L1210 (murine leukemia), CCRF-CEM (human lymphoblastic leukemia), and the human thymidine kinase deficient adenocarcinoma GC_3/M TK$^-$.[35-38] The results are listed as IC_{50}s in Table I.

To establish whether TS was the principal locus of action responsible for inhibiting cell growth, a "thymidine protection" assay was used. This procedure tested whether exogenous thymidine, which can be salvaged to circumvent TS inhibition, could reverse an inhibitor's cytotoxic effects. The experiments were carried out in L1210 cells by comparing the IC_{50} observed under normal culture conditions with that observed when cells were cultured in media supplemented with 10 μM thymidine. An increase in IC_{50} in the presence of thymidine under these conditions is suggestive of TS targeting. Larger shifts are judged to be the most significant. Additional salvage experiments were conducted with hypoxanthine and leucovorin for compounds **8**, **16**, **21**, **26**, and **27**. No significant reversal of cell growth inhibition was observed in these experiments indicating that GART (glycinamide ribonucleotide transformylase) and DHFR (dihydrofolate reductase) are not targets for these compounds.

The logarithm of the partition coefficients (log *P*s) of the molecules may provide useful information about various physiochemical properties which in turn could possibly aid in the discovery of lipophilic drugs. The log *P*s were estimated[39] for each compound (excluding **14**, **26**, and **27**), and in specific examples (**8**, **15–17**) compared to experimental values.[40] These calculated values, with respect to in vitro cell growth inhibition, solubility, and transport properties are currently being evaluated.

Conclusions

In an iterative fashion, we have successfully utilized ternary *E. coli* TS crystal structures for drug design. The wealth of information abstracted from an initial crystal complex enabled us to rapidly generate a novel lead compound that otherwise might have been overlooked. Using this information, in conjunction with other reported data on TS, we developed a series of 5-(arylthio)-quinazolinones. The initial design of a 0.96 μM inhibitor (**6**) without a glutamate residue was transformed into a 15.0 nM inhibitor (**21**) by making two minor changes to the quinazolinone nucleus. Changes at the 2-position of the 4-thiopyridyl component were made in order to explore the possibility of modifying solubility and lipophilicity properties of the molecules without affecting enzyme binding. More work in this area is necessary in order to construct a useful SAR with respect to in vitro antitumor effects. Interestingly, the in vitro inhibitory effects of many of the 4-pyridyl derivatives were abated by the addition of exogenous thymidine, an observation which strongly implies selectivity for TS. Replacement of the pyridine ring with the "classical" benzoyl glutamate system also produced potent inhibitors of human TS. When compounds **26** and **27** were tested against tumor cell lines in vitro, the cytotoxicity of the 2-amino derivative **27** was comparable to that of CB3717, **2**,[3,9a] whereas the 2-methyl version **26** was significantly less active than D1694, **3**.[4] The differences may be due to changes in active transport and/or polyglutamation. The physicochemical properties of these compounds are currently being analyzed.

From this work, compound **21** was chosen as a candidate for pharmaceutical formulation and clinical evaluation as an anticancer agent. The results of these studies will be presented at a future time elsewhere.

Experimental Section

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 (δ) and setting the references such that in $CDCl_3$ the $CHCl_3$ is at 7.26 ppm and in d_6-DMSO the DMSO is at 2.49 ppm. Standard and peak multiplicities are designated as follows: s, singlet; d, doublet; dd, doublet of doublets; ddd, doublet of doublet of doublets; t, triplet; q, quartet; bs, broad singlet; m, multiplet. Mass spectra were determined at either the University of California Riverside or the University of California Berkeley Mass Spectrometry Centers. Infrared absorption spectra were taken on either a Perkin-Elmer 457 spectrometer or a MIDAC Corporation FTIR. Elemental microanalysis were performed by Atlantic Microlab Inc., Norcross, GA, or MHW Laboratories, Phoenix, AZ, and gave results for the elements stated with ±0.4% of the theoretical values. Analytical HPLC was performed using a Gilson Model 805 module, equipped with a Gilson Model 811c mixer, two Gilson Model 306 pumps, and a Gilson Model 117 UV detector set at 254 nm; sensitivity 0.1 AUFS. A Zorbax CN column (250 mm × 4.6 mm; 5 μm) was used. A gradient mobile phase consisting of 20% CH_3CN, 80% 0.1 M aqueous AcOH 50 60% CH_3CN, 40% 0.1 M aqueous AcOH and then isocratic at this final ratio was used. Flow rate = 1.5 mL/min; injection volume = 10 μL. Anhydrous *N,N*-dimethylformamide (DMF) and *N,N*-dimethylacetamide (DMA) (Aldrich Gold Label grade) were used as is. Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl under nitrogen. Ether refers to diethyl ether. Petroleum ether refers to petroleum ether of bp 36–53 °C. Flash chromatography was performed using silica gel 60 (Merck Art 9385). Thin-layer chromatographs (TLC) were performed on precoated sheets of silica 60 F_{254} (Merck Art 5719). Melting points were determined on a Mel-Temp apparatus and are uncorrected. log *P*s were measured at SRI International, Menlo Park, CA.

Biochemical Assays. TS activity was assayed by a modified procedure of the tritium release method of Lomax and Greenberg.[33] Inhibition constants were determined by steady-state analysis against the cofactor 5,10-methylenetetrahydrofolate (generated in situ by the reaction of formaldehyde with tetrahydrofolate) as the variable substrate under conditions of saturating dUMP. Reaction conditions in 0.1 mL were 50 mM Tris at 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 μg/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 0.5K_i to 2.0K_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[41] which fit the data to a mixed noncompetitive inhibition scheme. Tight binding kinetic[34] assays were performed as described above but with the following changes: human TS was diluted to 8 nM; the tetrahydrofolate concentrations were 10, 20, 50, and 80 μM; 12 different concentrations of the inhibitor ranging between 0 and 100 nM were used; apparent K_i values were obtained by fitting the data to the tight binding equation of Morrison[34] with the nonlinear curve-fitting program of Jackson.[42] The K_is were then calculated by the relationship:

$$K_{i\ app} = K_i\left(1 + \frac{[s]}{K_m}\right)$$

Measurement of Tissue Culture IC_{50}'s. IC_{50} values for the inhibition of cellular growth were measured using a modification[35] of the MTT[36] colorimetric assay of Mosmann[37] using mouse (L1210) and human (CCRF-CEM) leukemia lines (ATCC) and a human adenocarcinoma cell line (GC_3/M TK$^-$) deficient in thymidine kinase.[38] Cells were seeded at 1000 (L1210) or 10 000 (CCRF-CEM, GC_3/M TK$^-$) cells per well in 96-well plates, and growth was 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 was 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. The ability of thymidine to reverse growth inhibition in L1210 cells was assessed 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 which was replenished daily during the 3 days of growth. The magnitude of the ratio of the IC_{50} measured in the presence of thymidine to that measured without added nucleoside was used to reflect the extent to which the inhibition of growth could be attributed to intracellular inhibition of TS. A value of >1.0 under these conditions would be consistent with a direct relationship between growth inhibition and TS targeting.

2-Methyl-5-phenoxy-3*H*-quinazolin-4-one (4). (Method A) A solution of phenol (0.94 g, 10 mmol) in 20 mL of anhydrous DMA was deprotonated at 0 °C with NaH (0.4 g, 10 mmol, 60% oil dispersion). After being stirred for 1 h at room temperature, 5-chloro-2-methyl-3*H*-quinazolin-4-one (**28**)[21] (0.82 g, 4.2 mmol) was added followed by CuBr (0.3 g) and Cu_2O (0.3 g). The heterogeneous mixture was heated at 130 °C for 12 h. At this time the DMA was removed under vacuum, and the residual solid was treated with 10 mL of H_2S/MeOH (20 g/L). The precipitated Cu_2S was removed by filtration, and the methanolic solution was concentrated. The residue was purified by flash column chromatography on silica with MeOH/CH_2Cl_2 (5:95) yielding 52% of **4** as an off-white solid: mp 257–258 °C; IR (KBr) 3470, 3418, 3051, 2897, 1674, 1631, 1485, 1250, 1042, 767 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.27 (s, 3 H), 6.80 (d, 1 H, *J* = 8.0 Hz), 6.85 (d, 2 H, *J* = 8.1 Hz), 7.04 (d, 1 H, *J* = 7.4 Hz), 7.30 (m, 3 H), 7.65 (t, 1 H, *J* = 8.1 Hz). Anal. ($C_{15}H_{12}N_2O_2 \cdot H_2O$) C, H, N.

2-Methyl-5-(phenylsulfanyl)-3*H*-quinazolin-4-one (5). (Method A) This compound was prepared in 43% yield from thiophenol and **28** using the procedure described to prepare **4**. Beige solid: mp 280 °C dec; IR (KBr) 3412, 2886, 1676, 1628, 1584, 1458, 1300, 930, 814 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.30 (s, 3 H), 6.38 (d, 1 H, *J* = 7.9 Hz), 7.16 (d, 1 H, *J* = 8.0 Hz), 7.40 (t, 1 H, *J* = 8.0 Hz), 7.52 (m, 5 H), 12.15 (bs, 1 H). Anal. ($C_{15}H_{12}N_2OS \cdot H_2O$) C, H, N, S.

2-Methyl-5-(pyridin-4-ylsulfanyl)-3*H*-quinazolin-4-one (6). (Method A) Compound **6** was prepared as described above in 30% yield from 4-mercaptopyridine and **28**. Tan solid: mp 260–262 °C; IR (KBr) 3408, 2893, 2361, 1676, 1626, 1581, 1460, 1302, 812 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.28 (s, 3 H), 6.75 (d, 1 H, *J* = 7.8 Hz), 7.32 (d, 1 H, *J* = 8.0 Hz), 7.50 (m, 3 H), 8.60 (d, 2 H, *J* = 4.5 Hz), 12.30 (bs, 1 H). Anal. ($C_{14}H_{11}N_3OS$) C, H, N, S.

2-Methyl-5-(pyridin-2-ylsulfanyl)-3*H*-quinazolin-4-one (7). (Method A) Compound **6** was prepared from 2-mercaptopyridine and **28** as outlined above in 23% yield. Tan solid: mp 275–276 °C; IR (KBr) 3420, 2890, 1680, 1634, 1420, 610 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.30 (s, 3 H), 6.74 (d, 1 H, *J* = 7.8 Hz), 7.27 (d, 1 H, *J* = 7.9 Hz), 7.38 (m, 1 H), 7.46 (t, 1 H, *J* = 8.0 Hz), 7.58 (d, 1 H, *J* = 7.9 Hz), 7.83 (m, 1 H), 8.60 (d, 1 H, *J* = 4.5 Hz). Anal. ($C_{14}H_{11}N_3OS \cdot 0.5H_2O$) C, H, N, S.

3-Bromo-4-methyl-α-isonitrosoacetanilide (30a). A mixture of chloral hydrate (45.0 g, 0.27 mol), sodium sulfate (65.0 g, 0.46 mol), 3-bromo-4-methylaniline (**29a**)[43] (40.0 g, 0.21 mol), 20 mL of concentrated HCl, hydroxylamine hydrochloride (55.0 g, 0.79 mol), and 1.5 L of H_2O were heated at 100 °C for 1 h. The reaction mixture was cooled to 0 °C, and the precipitate was collected by filtration. The solid was washed with H_2O and dried to yield 41.0 g (76%) as a tan solid: mp 195–197 °C; IR (KBr) 3439, 3310, 3110, 2998, 2876, 2749, 1636, 1591, 1466, 1256, 905, 691 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.28 (s, 3 H), 3.50 (bs, 1 H), 7.28 (d, 1 H, *J* = 8.3 Hz), 7.53 (dd, 1 H, *J* = 8.2, 2.1 Hz), 8.02 (d, 1 H, *J* = 2.0 Hz), 10.26 (s, 1 H), 12.21 (s, 1 H). Anal. ($C_9H_9BrN_2O_2$) C, H, Br, N.

4-Bromo-5-methylisatin (31a). To 160 mL of concentrated sulfuric acid at 80 °C was added 40 g (0.156 mol) of **30a**, and the mixture was stirred for 1 h. The reaction mixture was cooled to room temperature and then poured onto 2 L of crushed ice. The precipitate was filtered, washed with water, and then washed with benzene. The red solid was added to 800 mL of boiling ethanol. The solution was allowed to cool to room temperature. The precipitate was collected and then washed with cold ethanol. 6-Bromo-5-methylisatin, as well as some of the desired product remains in the mother liquor, and can be separated by silica gel flash column chromatography. The filter cake was dried to yield 19 g (50.7%) of a red solid: mp 245–248 °C; IR (KBr) 3302, 1750, 1609, 1466, 1273, 675 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.26 (s, 3 H), 6.8 (d, 1 H, J = 7.9 Hz), 7.5 (d, 1 H, J = 8.3 Hz), 11.06 (s, 1 H). Anal. ($C_9H_6BrNO_2$) C, H, Br, N.

6-Bromo-5-methylanthranilic Acid (32a). A mixture of 80 mL of 3 N aqueous NaOH and 19 g of isatin **31a** (0.08 mol) was heated at 80 °C. To the solution was added 18 mL of 30% H_2O_2, and the mixture was stirred for 1 h. The mixture was cooled to 5 °C and acidified to pH 5 with concentrated HCl. The solution was evaporated to dryness and then added to 300 mL of methanol. The mixture was filtered, and the filtrate was evaporated to yield 18 g of a tan solid (98%): mp (HCl salt) 290–294 °C; IR (KBr) 3619, 3229, 1578, 1478, 1412, 1381, 1084, 1010, 820, 706 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.13 (s, 3 H), 4.9 (s, 2 H), 6.4 (d, 1 H, J = 7.9 Hz), 6.74 (d, 1 H, J = 7.8 Hz).

5-Bromo-2,6-dimethylbenzo[*d*][1,3]oxazin-4-one (33a). A mixture of 18 g of anthranilic acid **32a** (0.078 mol) in 300 mL of acetic anhydride was heated at reflux for 3 h. The solution was cooled to 0 °C and filtered. The filter cake was washed with acetone to yield 16 g (81%) of a white solid (mp 190–194 °C) which was used without further purification: IR (KBr) 3460, 1750, 1660, 1574, 1416, 1260, 1070, 841 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.45 (s, 3 H), 2.55 (s, 3 H), 7.40 (d, 1 H, J = 8.2 Hz), 7.64 (d, 1 H, J = 8.0 Hz); HRMS calcd for $C_{10}H_8BrNO_2$ 252.9738 (M^+), found 252.9743.

5-Bromo-2,6-dimethyl-3*H*-quinazolin-4-one (34a). Anhydrous ammonia (50 mL) was condensed into a flask containing anthranil **33a** (8.5 g, 34.0 mmol), and the reaction was stirred for 3 h. The solvent was evaporated to give a residue, and 75 mL of 1 N NaOH was then added. The reaction mixture was heated at reflux temperature for 1 h. The resulting solution was cooled to 0 °C and acidified to pH 4 with concentrated hydrochloric acid. The mixture was filtered, and the filter cake was washed with water and then dried to yield 7.1 g (82.5% theory) of **34a** as a tan solid: mp 288–291 °C dec. The product was used without further purification: IR (KBr) 2910, 2620, 1680, 1630, 1460, 1377, 1298, 1128, 872 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.33 (s, 3 H), 2.43 (s, 3 H), 7.49 (d, 1 H, J = 8.3 Hz), 7.70 (d, 1 H, J = 8.3 Hz), 12.20 (bs, 1 H); HRMS calcd for $C_{10}H_9BrN_2O$ 251.9898 (M^+), found 251.9908.

Methyl 2-Amino-6-bromo-5-methylbenzoate (36a). A mixture of 10 g (0.039 mol) of anthranil **33a** in 75 mL of methanol was heated at reflux for 2 h. To the solution was added 10 mL of concentrated hydrochloric acid, and the mixture was heated for an additional 2 h. The solution was then evaporated to dryness. The residue was dissolved in 20 mL of H_2O and neutralized to pH 7 with triethylamine. The aqueous solution was extracted with methylene chloride. The layers were separated, and the organic layer was dried over magnesium sulfate, and evaporated to dryness to yield 6.0 g of **36a** as an orange oil (63% theory): IR (neat) 3483, 3410, 3220, 3000, 2950, 2851, 1720, 1620, 1560, 1430, 1288, 1120, 1015, 816 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.31 (s, 3 H), 3.95 (s, 3 H), 4.10 (bs, 2 H), 6.60 (d, 1 H, J = 8.2 Hz), 7.05 (d, 1 H, J = 8.1 Hz); HRMS calcd for $C_9H_{10}BrNO_2$ 242.9890 (M^+), found 242.9895. An improved route to methyl ester **36a** via isatoic anhydride **35a** was developed and used.

5-Bromo-6-methyl-1*H*-benzo[*d*][1,3]oxazine-2,4-dione (35a). Under anhydrous conditions, 6-bromo-5-methylanthranilic acid (**32a**) (2.3 g, 10 mmol) was stirred with bis(trichloromethyl) carbonate (1.0 g, 3.4 mmol) in THF (25 mL) for 12 h. The resultant solid was filtered, washed with cold acetone, and dried under vacuum. Isatoic anhydride **35a** was isolated in quantitative yield and used without further purification: mp 271–275 °C; IR (KBr) 3140, 2933, 2329, 1774, 1741, 1617, 1510, 1402, 1311, 1030, 740 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.37 (s, 3 H), 7.06 (d, 1 H, J = 8.4 Hz), 7.66 (d, 1 H, J = 8.4 Hz), 11.76 (s, 1 H). Anal. ($C_9H_6BrNO_3$) C, H, N.

The conversion of **35a** to methyl ester **36a** was accomplished quantitatively with refluxing dry methanol. This reaction could be accelerated by the addition of catalytic DMAP.[44]

2-Amino-5-bromo-6-methyl-3*H*-quinazolin-4-one (37a). To a solution of methyl ester **36a** (6 g, 24 mmol) in 50 mL of dry diglyme was added 3 g (24 mmol) of chloroformamidine hydrochloride.[45] The mixture was heated at reflux for 1 h. The mixture was cooled to 0 °C and filtered. The solid was washed with ether and then dried to yield 6.25 g (88% theory) of a tan solid (hydrochloride): mp >390 °C. The product was used without further purification: IR (KBr) 3140, 2950, 1670, 1620, 1471, 1402, 816, 600 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.28 (s, 3 H), 6.75 (bs, 2 H), 7.0 (d, 1 H, J = 8.3 Hz), 7.40 (d, 1 H, J = 8.0 Hz), 11.8 (bs, 1 H). Anal. ($C_9H_8BrN_3O$·HCl) C, H, N. HRMS calcd for $C_9H_8BrN_3O$ 253.9927 (M^+), found 253.9929.

3-Bromo-4-methoxy-α-isonitrosoacetanilide (30b). In a 250-mL 3-neck round-bottom flask was added 84 mL of water to 6.3 g (37.8 mmol) of chloralhydrate. The flask was fitted with a mechanical stirrer and reflux condenser, and 90 g of anhydrous sodium sulfate powder was added over a period of 1 min with constant stirring. A solution of 6.3 g (31.2 mmol) of 3-bromo-4-methoxyaniline (**29b**)[46] in 3.0 mL of concentrated HCl and 21 mL of water was added, followed by a solution of 7.7 g (112 mmol) of H_2NOH·HCl in 35 mL of water. The mixture was slowly heated to reflux with constant sitrring and continued for 2 min, at which time brown crystals formed. The mixture was cooled, and the solid was filtered off, washed well with water, and dried to constant weight by vacuum. The resulting solid weighed 5.65 g (66%) and was pure enough for the next step. An analytical sample was prepared by recrystallization: mp 202–203 °C (hexane, EtOAc); IR (KBr) 3409, 2875, 2056, 2023, 1643, 1634, 1543, 1502, 1295, 1270, 1047, 799 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.88 (s, 3 H), 6.87 (d, 1 H, J = 8.9 Hz), 7.53 (m, 2 H), 7.83 (d, 1 H, J = 2.5 Hz), 8.49 (s, 1 H), 11.60 (s, 1 H, NH). Anal. ($C_9H_9BrN_2O_3$·0.11EtOAc) C, H, Br, N.

4-Bromo-5-methoxyisatin (31b). Vacuum-dried α-isonitrosoacetanilide **30b** (3.0 g, 11 mmol) was slowly added to 8 mL of concentrated H_2SO_4 at 50 °C while being stirred. The reaction mixture first became yellow and then turned dark. The temperature was raised to 65 °C for 10 min, and the reaction was followed by TLC (EtOAc/hexane, 40:60). Heating at 65–70 °C was resumed until all the starting material was consumed as judged by TLC. Upon completion, the reaction mixture was cooled and added to 80 g of crushed ice with stirring. A dark red solid formed and was filtered off, washed free of acid by water, and dried under vacuum. The resulting substance was purified by chromatography on a flash silica column using a gradient system of EtOAc/hexane, 40:60; 50:50; 60:40; 70:30; 80:20. The undesired isomer, 6-bromo-5-methoxyisatin eluted first, followed by the desired isomer **31b**, which was isolated as a red solid (0.71 g; 25% yield): mp 250–251 °C; IR (KBr) 2064, 1758, 1750, 1634, 1278 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.91 (s, 3 H), 6.84 (d, 1 H, J = 8.8 Hz), 7.09 (d, 1 H, J = 8.8 Hz), 10.88 (s, 1 H). Anal. ($C_9H_6BrNO_3$) C, H, Br, N.

5-Bromo-6-methoxy-2-methylbenzo[*d*][1,3]oxazin-4-one (33b). A stirred solution of isatin **31b** (2.28 g, 8.9 mmol) in 2 N aqueous NaOH (13.4 mL, 26.7 mmol) was cooled to 0 °C. To this cold solution was added gradually 30% H_2O_2 (0.90 mL, 8.9 mmol), keeping the temperature below 20 °C. The progress of the reaction was followed by TLC (EtOAc/hexane, 40:60). An additional 0.20 mL of 30% H_2O_2 was added, and the reaction mixture was stirred for 20 min at room temperature. At this time TLC indicated consumption of the starting material. The mixture was acidified with glacial acetic acid to pH 4 and concentrated via a cryogenic trap at −78 °C, leaving crude 6-bromo-5-methoxyanthranilic acid (**32b**) as a gray semisolid and was used as is. This slurry was treated with 28 mL of acetic anhydride and refluxed for 40 min. The dark mixture was then concentrated as before. To the residue was added an excess of ethyl acetate/hexane (2:1). The mixture was heated and filtered hot through silica gel to remove insoluble and colored particulants. The solution was partially concentrated and allowed to cool, and the product crystallized yielding 1.71 g (71% based on starting isatin **31b**): mp 228–229 °C dec; IR (KBr) 3397, 2039, 1717, 1651,

1625, 1543, 1295, 1055, 881, 617 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.42 (s, 3 H), 3.98 (s, 3 H), 7.34 (d, 1 H, J = 8.9 Hz), 7.51 (d, 1 H, J = 8.9 Hz). Anal. ($C_{10}H_8BrNO_3$) C, H, Br, N.

5-Bromo-6-methoxy-2-methyl-3*H*-quinazolin-4-one (34b). To 1.25 g (4.6 mmol) of the anthranil **33b**, in a dried round-bottom flask, equipped with a dry ice condenser, was condensed approximately 50 mL of anhydrous NH_3. The mixture was magnetically stirred for 40 min. At this time, the dry ice condenser was removed and the NH_3 was allowed to evaporate. Upon evaporation, 15 mL of water and 1.5 mL of 2 N NaOH were added, and the solution was refluxed for 1 h. The solution was then cooled to room temperature, and 1 N HCl was added, adjusting the pH to approximately 7 and thus precipitating the quinazoline. The white substance was filtered off, washed with water, and dried, yielding 0.71 g (57%): mp 273–274 °C; IR (KBr) 3189, 3074, 2990, 2974, 2899, 2362, 1676, 1643, 1552, 1461, 1303, 1286, 1063, 872, 832 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.39 (s, 3 H), 3.98 (s, 3 H), 7.39 (d, 1 H, J = 9.0 Hz), 7.59 (d, 1 H, J = 9.0 Hz), 11.60 (s, 1 H). Anal. ($C_{10}H_9BrN_2O_2$) C, H, Br, N.

3-Bromo-4-ethyl-α-isonitrosoacetanilide (30c). This compound was prepared from 3-bromo-4-ethylaniline[47] following the procedure used for **30a**: mp 192–193 °C; IR (KBr) 3414, 3310, 2965, 2878, 1631, 1620, 1591, 1531, 1464, 1258, 817, 699 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.13 (t, 3 H, J = 7.5 Hz), 2.64 (q, 2 H, J = 7.5 Hz), 7.28 (d, 1 H, J = 8.4 Hz), 7.56 (dd, 1 H, J = 8.3, 2.0 Hz), 7.61 (s, 1 H), 8.01 (d, 1 H, J = 2.0 Hz), 10.26 (s, 1 H), 12.21 (s, 1 H).

4-Bromo-5-ethylisatin (31c). Using the method to synthesize **31a**, this isatin was prepared in 41% yield from **30c** as an orange solid: mp 253–254 °C (EtOAc/hexane); IR (KBr) 3476, 3256, 2965, 2913, 1759, 1730, 1612, 1462, 1254, 1138, 849, 667 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.19 (t, 3 H, J = 7.5 Hz), 2.75 (q, 2 H, J = 7.5 Hz), 6.82 (d, 1 H, J = 7.9 Hz), 7.35 (d, 1 H, J = 7.9 Hz), 10.41 (bs, 1 H). Anal. ($C_{10}H_8BrNO_2$·0.1hexane) C, H, N, Br.

6-Bromo-5-ethylanthranilic Acid (32c). Isatin **31c** was converted to **32c** as described for **32a**: tan solid; mp 156–157 °C dec; IR (KBr) 3466, 3430, 3192, 2965, 2920, 1726, 1613, 1472, 1373, 804, 699 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.18 (t, 3 H, J = 7.5 Hz), 2.68 (q, 2 H, J = 7.5 Hz), 3.25 (bs, 2 H), 6.62 (d, 1 H, J = 7.8 Hz), 7.02 (d, 1 H, J = 7.8 Hz).

5-Bromo-6-ethyl-2-methylbenzo[*d*][1,3]oxazin-4-one (33c). As done in the case of **33a**, **33c** was prepared from **32c** and acetic anhydride: beige solid: mp 198–200 °C; IR (KBr) 3484, 3270, 3100, 2932, 1738, 1651, 1584, 1532, 1406, 1360, 822, 681 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.26 (t, 3 H, J = 7.6 Hz), 2.43 (s, 3 H), 2.93 (q, 2 H, J = 7.6 Hz), 7.44 (d, 1 H, J = 7.9 Hz), 7.63 (d, 1 H, J = 8.3 Hz). Anal. ($C_{11}H_{10}BrNO_2$·0.05hexane) C, H, N, Br.

5-Bromo-6-ethyl-2-methyl-3*H*-quinazolin-4-one (34c). Benzoxazinone **33c** was converted to quinazolinone **34c** quantitatively with ammonia as described for **34a**: tan solid; mp 230–233 °C dec; IR (KBr) 3474, 3418, 2963, 2930, 1678, 1634, 1460, 1379, 1298, 849 cm^{-1}; ^{1}H NMR (DMSO-d_6) 1.16 (t, 3 H, J = 7.4 Hz), 2.29 (s, 3 H), 2.84 (q, 2 H, J = 7.3 Hz), 7.48 (d, 1 H, J = 8.4 Hz), 7.67 (d, 1 H, J = 8.3 Hz), 12.18 (bs, 1 H); HRMS calcd for $C_{11}H_{11}BrN_2O$ 267.0133 (M^+), found 267.0141.

3-Bromo-4-chloro-α-isonitrosoacetanalide (30d). This compound was prepared from 3-bromo-4-chloroaniline (**29d**)[48] in 77% yield using the procedure to synthesize **30a**: tan solid; mp 175–178 °C dec; IR (KBr) 1661, 1620, 1588, 1537, 1470 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.57 (d, 1 H, J = 8.8 Hz), 7.68 (dd, 1 H, J = 8.8, 2.3 Hz), 8.19 (d, 1 H, J = 2.3 Hz), 10.44 (bs, 1 H), 12.29 (bs, 1 H). Anal. ($C_8H_6BrClN_2O_2$) C, H, Br, Cl, N.

4-Bromo-5-chloroisatin (31d). As described for **31a**, **31d** was prepared in 58% yield from **30d**: orange solid; mp 255–265 °C dec; IR (KBr) 1738, 1607, 1437, 1236 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 6.89 (d, 1 H, J = 8.1 Hz), 7.74 (d, 1 H, J = 8.1 Hz), 11.22 (s, 1 H). Anal. ($C_8H_3BrClNO_2$) C, H, Br, Cl, N.

6-Bromo-5-chloroanthranilic Acid (32d). The compound was prepared from **31d** in 78% yield using the procedure to synthesize **32a**: tan solid; mp 169–170 °C dec; IR (KBr) 1609, 1565, 1537, 1451, 1362 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 6.73 (d, 1 H, J = 8.8 Hz), 7.27 (d, 1 H, J = 9.1 Hz), 8.25 (bs, 2 H), 10.2 (bs, 1 H). Anal. ($C_7H_5BrClNO_2$) C, H, Br, Cl, N.

5-Bromo-6-chloro-1*H*-benzo[*d*][1,3]oxazine-2,4-dione (35d). Anthranilic acid **32d** and triphosgene were reacted as described for **35a** to give **35d** in 72% yield: tan solid; mp >270 °C dec; IR (KBr) 1805, 1778, 1597, 1458, 1333, 1252, 1038 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.15 (d, 1 H, J = 8.8 Hz), 7.91 (d, 1 H, J = 8.8 Hz), 11.93 (s, 1 H). Anal. ($C_8H_3BrClNO_3$) C, H, Br, Cl, N.

2-Amino-5-bromo-6-chloro-3*H*-quinazolin-4-one (37d). Isatoic anhydride **35d** was reacted with dry methanol as described before to give methyl ester **36d**. The crude ester was not isolated. It was then reacted with chloroformamidine hydrochloride[45] in refluxing diglyme, yielding **37d** (67% from **35d**) as described for **37a**: light brown solid (hydrochloride); mp >400 °C; IR (KBr) 3459, 3331, 1649, 1591, 1560, 1451 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 6.61 (bs, 2 H), 7.17 (d, 1 H, J = 8.9 Hz), 7.69 (d, 1 H, J = 8.9 Hz), 11.20 (bs, 1 H). Anal. ($C_8H_5BrClN_3O$·0.5HCl·0.5H_2O) C, H, Br, Cl, N.

4-(Benzylsulfanyl)-2-methylpyridine *N*-Oxide (39). Mineral oil was removed from KH (0.11 M; 35 wt % dispersion in mineral oil) by several washings with petroleum ether (5 × 50 mL). The remaining petroleum ether was removed under vacuum. To this dry solid was added 350 mL of anhydrous THF cautiously. The well-stirred suspension was cooled to 0 °C. To this mixture was added dropwise 14.1 mL (0.12 mol) of benzylmercaptan over a period of 30 min. The resultant milky white mixture was warmed to room temperature and allowed to stir for an additional 30 min. The mixture was then cooled to −30 °C, and 15.41 g (0.1 mol) of 4-nitro-2-picoline *N*-oxide was added portionwise. The mixture became dark orange-brown in color. Once warmed to room temperature, the mixture was refluxed for 1 h. At this time, the reaction was cooled to 0 °C and quenched with 50 mL of water. The pH of the mixture was adjusted to approximately 6 with 2 M HCl and extracted with CH_2Cl_2 (3 × 300 mL). The combined organic layers were dried (anhydrous Na_2SO_4), and the solvent was removed under reduced pressure. The crude residue was chromatographed on flash silica gel with MeOH/CH_2Cl_2 (gradient: 3:97, 4:96; 5:95). The pure product was isolated (6.94 g; 30% yield) as a tan solid: mp 98–99 °C; IR (KBr) 3063, 3028, 1612, 1466, 1236, 831, 715, 675 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.45 (s, 3 H), 4.16 (s, 2 H), 6.97 (dd, 1 H, J = 6.8, 2.7 Hz), 7.07 (d, 1 H, J = 2.7 Hz), 7.32 (m, 5 H), 8.09 (d, 1 H, J = 6.8 Hz). Anal. ($C_{13}H_{13}NOS$) C, H, N, S.

4-(Benzylsulfanyl)-2-methylpyridine (40). Compound **39** (1.97 g, 8.5 mmol) was dissolved into 50 mL of $CHCl_3$. The solution was stirred and cooled to 0 °C, and 1.75 mL (17.4 mmol) of PCl_3 was added dropwise. Once the addition was complete, the reaction mixture was brought to room temperature and then heated slightly below reflux (approximately 55 °C) until no starting *N*-oxide was present by TLC (MeOH/CH_2Cl_2, 5:95). The solution was then recooled to 0 °C, and 10 g of ice was added with vigorous stirring. The mixture was made basic (pH 8) by careful addition of 1 M NaOH, and the organic phase was separated. The aqueous layer was extracted with CH_2Cl_2 (3 × 50 mL), and the organic layers were combined and dried over Na_2SO_4. Removal of the solvent under reduced pressure gave an oil which was chromatographed on a short flash silica column using MeOH/CH_2Cl_2, 3:97. The product was isolated as a white solid (1.54 g; 84% yield): mp 69–70 °C; IR (KBr) 3028, 3003, 2920, 1583, 1454, 864, 815, 719, 702 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.55 (s, 3 H), 4.22 (s, 2 H), 7.03 (m, 2 H), 7.35 (m, 5 H), 8.28 (d, 1 H, J = 5.5 Hz). Anal. ($C_{13}H_{13}NS$) C, H, N, S.

2-Methoxy-4-nitropyridine *N*-Oxide (41). The nitration was carried out using the method of Den Hertog and Van Ammers.[27] The results obtained from this experiment differ from those reported. Concentrated H_2SO_4 (35 mL) was cooled to 0 °C, and *N*-oxide **41** (15.3 g, 0.12 mL) was cautiously added portionwise. To this stirred solution, kept at 0 °C, was added the nitrating mixture (35 mL of concentrated H_2SO_4:60 mL of fuming HNO_3) dropwise. The ice bath was removed, and mixture was heated to 75 °C for 90 min. The mixture was recooled to 0 °C and cautiously poured onto 150 g of ice. With vigorous stirring, portions of solid K_2CO_3 were added until the pH was 7. The liquid was then extracted several times with CH_2Cl_2 (3 × 200 mL). The aqueous layer was continuously extracted with $CHCl_3$. The organic layers were combined, dried over anhydrous Na_2SO_4, and concentrated to give a yellow solid. The solid was chromatographed on a flash silica column using a gradient system of MeOH/CH_2Cl_2, 2:98, 3:97; 4:96; 5:95. A mixture of 2-methoxy-4-nitropyridine and 2-methoxy-5-nitropyridine (2.9 g) eluted first, followed by 2-methoxy-4-nitropyridine *N*-oxide (6.4 g), and then

2-methoxy-5-nitropyridine *N*-oxide (2.9 g). Compound **42** was obtained as a yellow solid (30%): mp 176–178 °C dec (lit. mp 154.5–158.5 °C dec); IR (KBr) 3106, 3082, 1601, 1528, 1346, 1296, 1231, 1088, 1011, 660 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.18 (s, 3 H), 7.73 (d, 1 H, J = 2.9 Hz), 7.78 (dd, 1 H, J = 7.1, 2.9 Hz), 8.35 (d, 1 H, J = 7.1 Hz). Anal. ($C_6H_6N_2O_4$) C, H, N.

4-(Benzylsulfanyl)-2-methoxypyridine *N*-Oxide (42). This compound was prepared in similar fashion as outlined for compound **39**, with the following changes: Once the 4-nitro-2-methoxypyridine *N*-oxide was added, the reaction mixture was allowed to warm to room temperature. Stirring was continued for 12 h. The precipitated solid that formed was filtered and washed with ice-cold THF. The solid was dried under vacuum and shown to be one spot by TLC ($MeOH/CH_2Cl_2$, 10:90). The filtrate was concentrated and flash chromatographed on silica with $MeOH/CH_2Cl_2$ (gradient: 4:96, 5:95, 6:94). An analytically pure tan solid was isolated. The total combined yield was 70%: mp 131–133 °C; IR (KBr) 3105, 3038, 3005, 1670, 1610, 1543, 1483, 1290, 1211, 1132, 1016, 802 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.95 (s, 3 H), 4.19 (s, 2 H), 6.64 (d, 1 H, J = 2.4 Hz), 6.78 (dd, 1 H, J = 6.9, 2.4 Hz), 7.33 (m, 5 H), 8.09 (d, 1 H, J = 6.9 Hz). Anal. ($C_{13}H_{13}NO_2S$) C, H, N, S.

4-(Benzylsulfanyl)-2-methoxypyridine (43). The starting pyridine *N*-oxide **42** (1.85 g) was reduced using the method to prepare compound **40**, except heating of the mixture was not necessary. The reaction was complete in approximately 90 min. Flash silica chromatography using ether/petroleum ether, 5:95, yielded 1.57 g (90%) of compound **43** as a tan solid: mp 35–36 °C; IR (KBr) 3028, 2943, 1589, 1543, 1385, 1307, 1037, 715 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.98 (s, 3 H), 4.23 (s, 2 H), 6.64 (d, 1 H, J = 1.6 Hz), 6.84 (dd, 1 H, J = 5.9, 1.6 Hz), 7.35 (m, 5 H), 7.98 (d, 1 H, J = 5.9 Hz). Anal. ($C_{13}H_{13}NOS$) C, H, N, S.

2-(Dimethylamino)-4-nitropyridine *N*-Oxide (44). To a solution of 2-bromo-4-nitropyridine *N*-oxide[28] (5.0 g, 23.0 mmol) dissolved in 75 mL of anhydrous THF, was added dimethylamine (1.1 g, 24.0 mmol). The mixture was stirred for 3 h followed by filtration to remove the dimethylamine hydrobromide salt. The filtrate was evaporated to dryness, and the crude solid was purified by flash column chromatography on silica using $MeOH/CH_2Cl_2$, 4:96. The product was isolated as an orange solid (mp 128–130 °C) in 83% yield: IR (KBr) 3422, 3109, 3040, 2499, 1610, 1529, 1348, 1275, 1169, 979, 887, 744, 659 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.14 (s, 6 H), 7.67 (m, 2 H), 8.24 (d, 1 H, J = 7.1 Hz). Anal. ($C_7H_9N_3O_3$) C, H, N.

2-(Dimethylamino)-4-((4-methoxybenzyl)sulfanyl)pyridine *N*-Oxide (45). To a solution of 4-methoxy-α-toluenethiol (1.1 g, 7.1 mmol) dissolved in 75 mL of anhydrous DMF was added NaH (0.28 g, 7.0 mmol; 60 wt % dispersion in mineral oil). After stirring for 1 h, a solution of pyridine *N*-oxide **44** (1.2 g, 6.55 mmol) in 25 mL of anhydrous DMF was added dropwise. The reaction mixture was stirred for 2 h and was then poured into 200 mL of H_2O. The aqueous solution was extracted with 500 mL of diethyl ether, separated, and dried over anhydrous $MgSO_4$. The ether was evaporated to give compound **45** as a tan solid (mp 97–100 °C) in 63% yield: IR (KBr) 3475, 3416, 1616, 1548, 1512, 1246, 1203, 615 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.06 (s, 6 H), 3.83 (s, 3 H), 4.16 (s, 2 H), 6.60 (d, 1 H, J = 2.5 Hz), 6.70 (d, 1 H, J = 7.0 Hz), 6.90 (d, 2 H, J = 8.7 Hz), 7.30 (d, 2 H, J = 8.7 Hz), 8.0 (d, 1 H, J = 7.0 Hz); HRMS calcd for $C_{15}H_{18}N_2O_2S$ 291.1177 (M + 1), found 291.1167.

2-(Dimethylamino)-4-((4-methoxybenzyl)sulfanyl)pyridine (46). The starting pyridine *N*-oxide **45** (0.60 g, 2.1 mmol) was reduced using the method to prepare compound **40**. Upon completion of the reaction, the mixture was poured into 200 mL of H_2O, and the pH was adjusted to 7. The aqueous solution was extracted with ethyl acetate (500 mL), and the organic phase was dried over anhydrous Na_2SO_4, filtered, and concentrated. No chromatography was necessary, and the product, **46**, was isolated as a yellow solid in 88% yield: mp 85–86 °C; IR (KBr) 3420, 1585, 1510, 1412, 1309, 1253, 1174, 1105, 985, 823, 781 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.08 (s, 3 H), 3.82 (s, 3 H), 4.17 (s, 2 H), 6.34 (d, 2 H, J = 1.3 Hz), 6.48 (d, 1 H, J = 5.5 Hz), 6.87 (d, 2 H, J = 8.7 Hz), 7.33 (d, 2 H, J = 8.7 Hz), 8.0 (d, 1 H, J = 5.5 Hz); HRMS calcd for $C_{15}H_{18}N_2OS$ 274.1152 (M^+), found 274.1139.

6-(Dimethylamino)-4-mercaptopyridine (47). A formic acid (10 mL) solution of pyridine **46** (0.40 g, 1.46 mmol) was cooled to 0 °C. To this solution was added 1.2 g of $Hg(OAc)_2$ dissolved in 3 mL of H_2O. The ice bath was removed, and the reaction mixture was allowed to stir for 12 h. At this time the pH was adjusted to 8 by the addition of aqueous ammonia. A gray precipitate forms which was filtered and washed with an excess of H_2O and air-dried. The solid was then taken up in a saturated H_2S/methanol solution. A black solid (HgS) formed and was filtered off. The filtrate was evaporated to dryness to yield a yellow solid (89%) which was used without further purification: mp 116 °C dec; IR (KBr) 2833, 2364, 1585, 1535, 1402, 1248, 1086, 984, 812 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.12 (s, 3 H), 3.5 (bs, 1 H), 6.5 (d, 1 H, J = 5.3 Hz), 6.57 (d, 1 H, J = 3.6 Hz), 7.69 (d, 1 H, J = 5.5 Hz); HRMS calcd for $C_7H_{10}N_2S$ 154.0554 (M^+), found 154.0564.

2-(Trifluoromethyl)pyridine (48).[49] Under an argon atmosphere, 2-chloro-6-(trifluoromethyl)pyridine (29.0 g, 0.15 mol) was dissolved in 250 mL of methyl acetate. With stirring, *N*,*N*-dimethylethylamine (41 mL, 0.375 mol) was added followed by 10% Pd on carbon (2.0 g). Using a balloon of H_2, the mixture was dehalohydrogenated. The reaction was complete in approximately 90 min. The vessel was flushed with argon and pentane was added. The Pd, carbon, and precipitated *N*,*N*-dimethylethylamine hydrochloride were removed by filtration through Celite. The filtrate was concentrated with care since 2-(trifluoromethyl)pyridine is fairly volatile. The product could be purified by fractional distillation, but for our purposes we simply used the concentrate which was contaminated (^{1}H NMR) with traces of methyl acetate and pentane (yellow liquid): ^{1}H NMR ($CDCl_3$) δ 7.50 (ddd, 1 H, J = 7.6, 4.9, 0.3 Hz), 7.69 (d, 1 H, J = 7.9 Hz), 7.88 (dt, 1 H, J = 7.8, 0.9 Hz), 8.74 (d, 1 H, J = 4.7 Hz).

2-(Trifluoromethyl)pyridine *N*-Oxide (49). Using the procedure described above to prepare 2-methoxypyridine *N*-oxide, 2-(trifluoromethyl)pyridine *N*-oxide **49** was synthesized from 2-(trifluoromethyl)pyridine (**48**) (74%; based on two steps from 2-chloro-6-(trifluoromethyl)pyridine): yellow oil; IR (neat) 3125, 3085, 1721, 1615, 1439, 1329, 1269, 1115, 1071, 1044, 852, 771, 662 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.38 (t, 1 H, J = 7.9 Hz), 7.48 (dt, 1 H, J = 7.0, 2.1 Hz), 7.71 (dd, 1 H, J = 7.9, 2.1 Hz), 8.35 (d, 1 H, J = 6.5 Hz). Anal. ($C_6H_4F_3NO{\cdot}0.5H_2O$) C, H, F, N.

4-Nitro-2-(trifluoromethyl)pyridine *N*-Oxide (50). The nitration of pyridine *N*-oxide **49** was carried out using the method to prepare compound **41**, with the following changes: The reaction mixture was heated at 125–130 °C for 3.5 h. During workup, no continuous extraction of the aqueous layer was necessary; the crude solid was purified, employing flash column chromatography on silica using ethyl acetate/hexane, 20:80. The product was isolated as a yellow solid (mp 112–114 °C) in 38% yield: IR (KBr) 3416, 3125, 1620, 1591, 1537, 1449, 1354, 1306, 1281, 1165, 1130, 916, 693 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.28 (dd, 1 H, J = 7.2, 3.1 Hz), 8.36 (d, 1 H, J = 7.2 Hz), 8.52 (d, 1 H, J = 3.1 Hz). Anal. ($C_6H_3F_3N_2O_3$) C, H, F, N.

4-Amino-2-(trifluoromethyl)pyridine (51). In a hydrogenation bottle, *N*-oxide **50** (8.32 g, 0.04 mol) was dissolved in 275 mL of 95% ethanol. The bottle was flushed with argon, and 0.83 g of 10% Pd on activated carbon was added. The bottle was shaken under 35 psi of H_2 for 45 min on a Parr hydrogenator. At this time, the catalyst was filtered off through a Celite pad. The ethanolic filtrate was concentrated in vacuo, and the oil was dissolved in 50 mL of CH_2Cl_2. This solution was filtered through a small pad of silica gel to remove traces of catalyst and carbon. The filtrate was concentrated, and traces of solvent were removed under vacuo. The oil slowly crystallized to give 5.77 g (89% yield) of an analytically pure light orange solid: mp 56–58 °C; IR (KBr) 3501, 3335, 3175, 1657, 1611, 1472, 1373, 1300, 1169, 1117, 993, 850 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.40 (bs, 2 H), 6.64 (dd, 1 H, J = 5.6, 2.3 Hz), 6.89 (d, 1 H, J = 2.3 Hz), 8.30 (d, 1 H, J = 5.6 Hz); HRMS calcd for $C_6H_5F_3N_2$ 162.0405 (M^+), found 162.0402. Anal. ($C_6H_5F_3N_2$) C, H, F, N.

Dithiocarbonic Acid *O*-Ethyl *S*-(2-(Trifluoromethyl)-pyridin-4-yl) Diester (52). A solution of amine **51** (4.86 g, 0.03 mol) in 30 mL of concentrated H_2SO_4 was cooled to 0 °C. An aqueous solution (30 mL of H_2O) of $NaNO_2$ (2.69 g, 39.0 mmol) was cooled to 0 °C and added dropwise over a period of 15 min. Stirring of the brown mixture was continued at 0 °C for 5 additional min. At this time, an ice-cold solution of potassium

ethyl xanthate (8.17 g, 51.0 mmol) in 30 mL of H_2O was added dropwise, maintaining the reaction temperature between 0 and 5 °C. The mixture was warmed to room temperature, and CH_2Cl_2 (125 mL) was added. The aqueous layer was neutralized to pH 7 with solid Na_2CO_3. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate (3 × 50 mL). The organic layers were combined, dried over anhydrous Na_2SO_4, and concentrated. The residue was subjected to flash column chromatography using silica gel and a gradient solvent system of ethyl acetate/hexane (2:98, 2.5:97.5, 3:97). Compound **52** was isolated as a yellow oil in 36% yield and was used without further purification: IR (neat) 3061, 2988, 2901, 1738, 1584, 1555, 1406, 1323, 1252, 1184, 1146, 1038, 845, 720 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.38 (t, 3 H, J = 7.1 Hz), 4.66 (q, 2 H, J = 7.1 Hz), 7.60 (dd, 1 H, J = 5.0, 1.3 Hz), 7.83 (d, 1 H, J = 1.0 Hz), 8.77 (d, 1 H, J = 5.0 Hz); HRMS calcd for $C_9H_8F_3NOS_2$ 268.0077 (M + 1), found 268.0065.

2,6-Dimethyl-5-(pyridin-4-ylsulfanyl)-3*H*-quinazolin-4-one (8). (Method A) To a solution of 4-mercaptopyridine (3.2 g, 28.8 mmol) in 50 mL of anhydrous DMA at 0 °C was added NaH (1.24 g, 28.8 mmol; 60% dispersion in mineral oil). The heterogeneous mixture was stirred for 1 h at room temperature. To the reaction mixture was added bromoquinazoline **34a** (3.1 g, 0.012 mol), copper(I) bromide (1.4 g), and copper(I) oxide (0.7 g). The vessel was heated at 90 °C for 4 h. The reaction mixture was evaporated to dryness, 50 mL of a H_2S/methanol solution (20 g/L) was added to the residue, and the mixture was stirred for 1 h. The mixture was filtered, and the filtrate was evaporated to dryness. The solid was purified via flash chromatography on silica gel using MeOH/CH_2Cl_2 (5:95) to yield 1.7 g (48% theory) of a tan solid: mp 235–238 °C; IR (KBr) 3430, 1670, 1633, 1575, 1460, 1408, 1300, 841, 820, 714 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.28 (s, 3 H), 2.40 (s, 3 H), 6.80 (d, 2 H, J = 5.9 Hz), 7.60 (d, 1 H, J = 8.3 Hz), 7.80 (d, 1 H, J = 8.5 Hz), 8.24 (d, 2 H, J = 6.5 Hz), 12.10 (bs, 1 H); HRMS calcd for $C_{15}H_{13}N_3OS$ 283.0773 (M^+), found 283.0779. Anal. ($C_{15}H_{13}N_3OS \cdot H_2O$) C, H, N, S.

6-Ethyl-2-methyl-5-(pyridin-4-ylsulfanyl)-3*H*-quinazolin-4-one (9). (Method A) Final product **9** was prepared in 84% yield from **34c** and 4-mercaptopyridine as described for **8**. The compound was purified by flash column chromatography on silica using 0.5% NH_3, 4.5% MeOH, 95% $CHCl_3$ and isolated as an off-white solid: mp 205–206 °C; IR (KBr) 3474, 3414, 2963, 2926, 2870, 1676, 1630, 1582, 1462, 1300, 1107, 847, 810, 711 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.09 (t, 3 H, J = 7.5 Hz), 2.29 (s, 3 H), 2.87 (q, 2 H, J = 7.5 Hz), 6.82 (d, 2 H, J = 6.2 Hz), 7.68 (d, 1 H, J = 8.5 Hz), 7.84 (d, 1 H, J = 8.5 Hz), 8.23 (d, 2 H, J = 6.0 Hz), 12.02 (bs, 1 H); HRMS calcd for $C_{16}H_{15}N_3OS$ 298.1014 (M^+), found 298.1009. Anal. ($C_{16}H_{15}N_3OS \cdot 0.75H_2O$) C, H, N, S.

6-Methoxy-2-methyl-5-(pyridin-4-ylsulfanyl)-3*H*-quinazolin-4-one (10). (Method D) To 78 mg (0.7 mmol) of 4-mercaptopyridine was added 34 mg (0.5 mmol) of solid NaOH in 1 mL of dry DMA. To the resulting solution was added 134 mg (0.5 mmol) of quinazolinone **34b**, dissolved in 2 mL of dry DMA. The mixture was kept under N_2, and a finely ground catalyst mixture containing 44 mg of CuBr and 22 mg of Cu_2O was added. The mixture was stirred magnetically and heated to 135 °C until the reaction was complete as judged by TLC (anhydrous NH_3/MeOH/$CHCl_3$, 0.5:4.5:9.5). The solvent was removed under high vacuum through a cryogenic trap cooled to −78 °C. The desired product was isolated by flash chromatography (anhydrous NH_3/MeOH/$CHCl_3$, 0.5:4.5:9.5) on silica, yielding 0.13 g (89%) of **10** as a white powder: mp 248–249 °C dec; IR (KBr) 3358, 3073, 2933, 1682, 1634, 1574, 1475, 1462, 1318, 1277, 1059, 835, 710 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.36 (s, 3 H), 3.84 (s, 3 H), 6.90 (d, 2 H, J = 5.1 Hz), 7.48 (d, 1 H, J = 9.1 Hz), 7.79 (d, 1 H, J = 9.1 Hz), 8.28 (d, 2 H, J = 5.1 Hz), 10.86 (s, 1 H); HRMS calcd for $C_{15}H_{13}N_3O_2S$ 299.0730 (M^+), found 299.0718. Anal. ($C_{15}H_{13}N_3O_2S$) C, H, N, S.

6-Hydroxy-2-methyl-5-(pyridin-4-ylsulfanyl)-3*H*-quinazolin-4-one (11). To cleave the methyl ether, quinazoline **10** (100 mg, 0.30 mmol) was gently refluxed with 2 mL of a 1:1 mixture of 48% aqueous HBr and glacial AcOH for 8 h. At this time the solvent was removed via high vacuum through a cryogenic trap at −78 °C. The residue obtained was dissolved in 10% anhydrous NH_3 in MeOH and subjected to flash column chromatography on silica (anhydrous NH_3/MeOH/$CHCl_3$, 0.5:4.5:95) yielding 62 mg of **10** as a white powder (65%): mp 246–247 °C dec; IR (KBr) 3450, 3240, 3073, 1667, 1634, 1580, 1464, 629 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.40 (s, 3 H), 6.83 (d, 2 H, J = 6.0 Hz), 7.40 (d, 1 H, J = 9.0 Hz), 7.59 (d, 1 H, J = 9.0 Hz), 8.20 (d, 2 H, J = 6.0 Hz), 8.51 (s, 1 H), 11.51 (s, 1 H); HRMS calcd for $C_{14}H_{11}N_3O_2S$ 285.05733 (M^+), found 285.05720. Anal. ($C_{14}H_{11}N_3O_2S$) C, H, N, S.

2,6-Dimethyl-5-(pyridin-3-ylsulfanyl)-3*H*-quinazolin-4-one (12). (Method A) This compound was prepared from **34a** and 3-mercaptopyridine[22] in 28% as described for **8**: tan solid; mp 212–214 °C; IR (KBr) 3472, 3414, 3060, 2878, 1668, 1639, 1462, 1302, 1103, 831 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.25 (s, 3 H), 2.40 (s, 3 H), 7.22 (m, 2 H), 7.55 (d, 1 H, J = 8.4 Hz), 7.71 (d, 1 H, J = 8.5 Hz), 8.20 (m, 2 H), 12.05 (bs, 1 H); HRMS calcd for $C_{15}H_{13}N_3OS$ 283.0778 (M^+), found 283.0779; HPLC t_R = 11.7 min; 96.2%.

2,6-Dimethyl-5-(pyridazin-4-ylsulfanyl)-3*H*-quinazolin-4-one (13). (Method A) This compound was prepared from **34a** and 4-mercaptopyridazine[23] in 30% as described for **8**: tan solid: mp >200 °C dec; IR (KBr) 1669, 1632, 1595, 1537 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.30 (s, 3 H), 2.45 (s, 3 H), 6.94 (dd, 1 H, J = 5.6, 2.6 Hz), 7.68 (d, 1 H, J = 8.4 Hz), 7.85 (d, 1 H, J = 8.5 Hz), 8.77 (dd, 1 H, J = 5.6, 1.1 Hz), 8.88 (dd, 1 H, J = 2.7, 1.1 Hz), 12.12 (bs, 1 H); HRMS calcd for $C_{14}H_{12}N_4OS$ 284.0732 (M^+), found 284.0730; HPLC t_R = 5.2 min; 94.1%.

2,6-Dimethyl-5-((4-nitrophenyl)sulfanyl)-3*H*-quinazolin-4-one (15). (Method A) This compound was synthesized from **34a** and 4-nitrothiophenol in 41% yield as described for **8**: light brown solid: mp 310 °C dec; IR (KBr) 3487, 3418, 3067, 1666, 1518, 1338, 1084, 854 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.26 (s, 3 H), 2.39 (s, 3 H), 7.05 (d, 2 H, J = 8.7 Hz), 7.63 (d, 1 H, J = 9.0 Hz), 7.79 (d, 1 H, J = 9.0 Hz), 8.00 (d, 2 H, J = 8.7 Hz), 12.05 (bs, 1 H); HRMS calcd for $C_{16}H_{13}N_3O_3S$ 327.0679 (M^+), found 327.0677; HPLC t_R = 14.75 min; 96%.

5-[[(Phenylsulfonyl)phenyl]sulfanyl]-2,6-dimethyl-3*H*-quinazolin-4-one (16). (Method A) This compound was prepared from **34a** and 4-(phenylsulfonyl)thiophenol[32] in 32% yield as described for **8**: beige solid: mp 292–294 °C; IR (KBr) 3464, 3049, 2903, 1666, 1631, 1593, 1518, 1339, 1084, 855, 740 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.24 (s, 3 H), 2.35 (s, 3 H), 7.01 (d, 2 H, J = 8.5 Hz), 7.57 (m, 3 H), 7.69 (d, 2 H, J = 8.5 Hz), 7.74 (d, 2 H, J = 7.2 Hz), 7.85 (d, 2 H, J = 7.2 Hz), 12.00 (bs, 1 H); HRMS calcd for $C_{22}H_{18}N_2O_3S_2$ 422.0741 (M^+), found 422.0758. Anal. ($C_{22}H_{18}N_2O_3S_2$) C, H, N, S.

2,6-Dimethyl-5-((2-methylpyridin-4-yl)sulfanyl)-3*H*-quinazolin-4-one (17). (Method B) To a solution of 5 mL of anhydrous NH_3 condensed into 5 mL of THF kept at −78 °C was added sodium metal (0.115 g, 5.0 mmol). The deep blue solution was stirred for 15 min. To the mixture was added 4-(benzylsulfanyl)-2-picoline (**40**) (1.0 g, 4.65 mmol), and the reaction was stirred for 90 min at 0 °C. The solvent was removed under vacuum, and to the resulting solid was added 10 mL of anhydrous DMA, 0.5 g of quinazolinone **34a** (2.0 mmol), 0.25 g of CuBr, and 0.25 g of Cu_2O. The heterogeneous mixture was heated at 90 °C for 4 h. The solvent was removed under vacuum, and the solid was treated with 10 mL of H_2S/MeOH solution (20 g/L). The insoluble Cu_2S was filtered off, and the filtrate was evaporated to dryness. The solid was purified using flash chromatography on silica with MeOH/CH_2Cl (5:95) to yield 400 mg (84% theory) of a tan solid: mp 225–227 °C; IR (KBr) 3480, 3160, 3053, 2960, 1670, 1630, 1590, 1460, 1298, 831 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.28 (s, 6 H), 2.36 (s, 3 H), 6.60 (bs, 1 H), 6.80 (bs, 1 H), 7.60 (d, 1 H, J = 8.4 Hz), 7.80 (d, 1 H, J = 8.4 Hz); HRMS calcd for $C_{16}H_{15}N_3OS$ 297.0936 (M^+), found 297.0936. Anal. ($C_{16}H_{15}N_3OS \cdot 0.5H_2O$) C, H, N, S.

2,6-Dimethyl-5-((2-methoxypyridin-4-yl)sulfanyl)-3*H*-quinazolin-4-one (18). (Method B) This compound was prepared in 10% yield as described above as a tan solid: mp 223–226 °C; IR (KBr) 3445, 1684, 1675, 1669, 1452, 1394, 1320, 1038 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.28 (s, 3 H), 2.35 (s, 3 H), 3.70 (s, 3 H), 6.05 (s, 1 H), 6.49 (dd, 1 H, J = 4.1, 2.9 Hz), 7.60 (d, 1 H, J = 8.5 Hz), 7.78 (d, 1 H, J = 8.4 Hz), 7.85 (d, 1 H, J = 5.4 Hz), 12.10 (s, 1 H); HRMS calcd for $C_{16}H_{15}N_3O_2S$ 313.0885 (M^+), found 313.0882; HPLC t_R = 13.0 min; 98.2%.

2,6-Dimethyl-5-((2-(trifluoromethyl)pyridin-4-yl)sulfanyl)-3*H*-quinazolin-4-one (19). (Method C) To a solution of xanthate **52** (0.67 g, 2.5 mmol) in 2 mL of MeOH was added 2.5

mL of 1 N KOH in MeOH, and the mixture was stirred for 90 min. The mixture was evaporated to dryness, and to the residue was added 10 mL of anhydrous DMA, quinazolinone **34a** (0.25 g, 10.0 mmol), 0.1 g of CuBr, and 0.1 g of Cu_2O. The mixture was heated at 90 °C for 6 h, and then the solvent was evaporated. The solid was treated with 50 mL of H_2S/MeOH solution (20 g/L) for 1 h. The mixture was filtered, and the filtrate was evaporated to dryness. The solid was purified via flash chromatography on silica using MeOH/CH_2Cl_2 (5:95) to yield 65 mg (18.5% theory) of a yellow solid: mp 240–245 °C. IR (KBr) 3440, 3190, 3057, 2950, 1675, 1630, 1595, 1321, 1140, 720 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.28 (s, 3 H), 2.42 (s, 3 H), 6.97 (d, 1 H, J = 5.2 Hz), 7.46 (d, 1 H, J = 1.1 Hz), 7.67 (d, 1 H, J = 8.4 Hz), 7.84 (d, 1 H, J = 8.4 Hz), 8.37 (d, 1 H, J = 5.2 Hz), 12.05 (bs, 1 H); HRMS calcd for $C_{16}H_{12}F_3N_3OS$ 351.0656 (M^+), found 351.0653; HPLC t_R = 14.2 min; 92%.

5-((2-(Dimethylamino)pyridin-4-yl)sulfanyl)-2,6-dimethyl-3*H*-quinazolin-4-one (20). (Method A) This compound was prepared from intermediates **34a** and **47** using the exact procedure to generate compound **8**. The crude product was purified by flash column chromatography on silica gel using MeOH/CH_2Cl_2 (8:92) to give a tan solid in 21% yield: mp 242–245 °C; IR (KBr) 3489, 3427, 3049, 2885, 1666, 1583, 1517, 1400, 1302, 987, 833 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.97 (s, 3 H), 2.08 (s, 3 H), 2.56 (s, 3 H), 5.55 (d, 1 H, J = 5.4 Hz), 5.82 (d, 1 H, J = 1.2 Hz), 7.27 (d, 1 H, J = 8.4 Hz), 7.44 (d, 1 H, J = 5.2 Hz), 7.45 (d, 2 H, J = 8.4 Hz), 12.75 (bs, 1 H). Anal. ($C_{17}H_{18}N_4OS$·0.5H_2O) C, H, N, S.

2-Amino-6-methyl-5-(pyridin-4-ylsulfanyl)-3*H*-quinazolin-4-one (21). (Method A) To a solution of 4-mercaptopyridine (1.72 g, 15.5 mmol) in 25 mL of anhydrous DMA at 0 °C was added NaH (0.62 g, 15.5 mmol; 60% dispersion in mineral oil), and the reaction was stirred for 1 h. To the solution was added aminoquinazoline **37a** (1.6 g, 6.3 mmol), CuBr (0.45 g), and Cu_2O (0.45 g). The mixture was heated at 90 °C for 4 h and then concentrated under vacuum. To the resulting solid was added 15 mL of H_2S/MeOH solution (20 g/L). The dark mixture was stirred for 1 h, the precipitated Cu_2S was removed by filtration, and the methanolic filtrate was evaporated. The solid was washed with methylene chloride, followed by ethyl ether and finally boiling 2-propanol to yield 0.75 g (50%) of **21** as a tan solid: mp 301–302 °C; IR (KBr) 3320, 3150, 2750, 1670, 1575, 1466, 1305, 1220, 804, 710, 482 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.30 (s, 3 H), 6.35 (bs, 2 H), 6.80 (d, 2 H, J = 5.9 Hz), 7.26 (d, 1 H, J = 8.4 Hz), 7.58 (d, 1 H, J = 8.5 Hz), 8.25 (bs, 2 H), 10.85 (bs, 1 H); HRMS calcd for $C_{14}H_{12}N_4OS$ 284.0734 (M^+), found 284.0732. Anal. ($C_{14}H_{12}N_4$-OS·1.5H_2O) C, H, N, S.

2-Amino-6-chloro-5-(pyridin-4-ylsulfanyl)-3*H*-quinazolin-4-one (22). (Method A) This compound was prepared from **37d** and 4-mercaptopyridine in 12% and was isolated as the dihydrochloride salt: tan solid; mp >210 °C dec; IR (KBr) 1695, 1622, 1478, 1454 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.45 (bs, 2 H), 7.49 (d, 2 H, J = 6.2 Hz), 7.56 (d, 1 H, J = 9.0 Hz), 7.97 (d, 1 H, J = 9.0 Hz), 8.50 (d, 2 H, J = 6.5 Hz); HRMS ($C_{13}H_9ClN_4OS$) 304.0186 (M^+), found 304.0201. Anal. ($C_{13}H_9ClN_4OS$·2HCl·1H_2O·0.1ether) C, H, Cl, N, S.

2-Amino-6-methyl-5-((2-(trifluoromethyl)pyridin-4-yl)-sulfanyl)-3*H*-quinazolin-4-one (23). (Method C) This compound was prepared in 22% yield from **37a** and **52** as described for **19**: tan solid; mp 247–249 °C; IR (KBr) 3421, 2056, 1650, 1625, 1485, 1419, 1328, 1146, 815, 724 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.30 (s, 3 H), 6.50 (bs, 2 H), 6.97 (dd, 1 H, J = 4.1, 1.2 Hz), 7.30 (d, 1 H, J = 8.4 Hz), 7.39 (d, 1 H, J = 1.0 Hz), 7.62 (d, 1 H, J = 8.6 Hz), 8.36 (d, 1 H, J = 5.2 Hz), 12.10 (bs, 1 H); HRMS calcd for $C_{15}H_{11}F_3N_4OS$ 353.0677 (M + 1), found 353.0684; HPLC t_R = 13.1 min; 99.3%.

2-Amino-5-((2-(dimethylamino)pyridin-4-yl)sulfanyl)-6-methyl-3*H*-quinazolin-4-one (24). (Method A) This compound was prepared in 15% yield from **37a** and **47** as described for **21**: tan solid; mp 120 °C dec; IR (KBr) 3431, 2924, 1703, 1633, 1469, 1361, 1290, 1089, 995 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.38 (s, 3 H), 3.08 (s, 6 H), 6.24 (d, 1 H, J = 6.5 Hz), 6.61 (s, 1 H), 7.54 (d, 1 H, J = 8.4 Hz), 7.66 (d, 1 H, J = 6.6 Hz), 7.80 (d, 1 H, J = 8.5 Hz), 8.10 (bs, 2 H), 12.10 (bs, 1 H); HRMS calcd for $C_{16}H_{17}N_5OS$ 327.1156 (M^+), found 327.1153; HPLC t_R = 2.2 min; 96%.

2-Amino-6-methyl-5-(pyridazin-4-ylsulfanyl)-3*H*-quinazolin-4-one (25). (Method A) This compound was prepared in 47% yield from **37a** and 4-mercaptopyridazine:[23] tan solid; mp 265–266 °C dec; IR (KBr) 3435, 3168, 1659, 1620, 1549, 1470 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.36 (s, 3 H), 6.39 (bs, 2 H), 6.93 (dd, 1 H, J = 5.6, 2.6 Hz), 7.32 (d, 1 H, J = 8.5 Hz), 7.63 (d, 1 H, J = 8.6 Hz), 8.77 (d, 1 H, J = 5.1 Hz), 8.86 (bs, 1 H), 10.84 (bs, 1 H); HRMS calcd for $C_{13}H_{11}N_5OS$ 286.0763 (M + 1), found 286.0760. Anal. ($C_{13}H_{11}N_5OS$·0.78H_2O) C, H, N, S.

4-((2,6-Dimethyl-4-oxo-3,4-dihydroquinazolin-5-yl)sulfanyl)benzoic Acid Methyl Ester (53). (Method A) This compound was synthesized from intermediate **34a** and methyl 4-mercaptobenzoate[31] in the same manner as **8**. The compound was purified by flash column chromatography on silica with MeOH/CH_2Cl_2, 5:95, to give a tan solid in 85% yield: mp 275–278 °C; IR (KBr) 3481, 3340, 2850, 1725, 1595, 1301, 1090, 783 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.26 (s, 3 H), 2.43 (s, 3 H), 3.75 (s, 3 H), 6.94 (d, 2 H, J = 8.4 Hz), 7.22 (d, 1 H, J = 8.5 Hz), 7.52 (d, 1 H, J = 8.5 Hz), 7.71 (d, 2 H, J = 8.4 Hz), 11.7 (bs, 1 H); HRMS calcd for $C_{18}H_{16}N_2O_3S$ 340.0898 (M^+), found 340.0882.

4-((2,6-Dimethyl-4-oxo-3,4-dihydroquinazolin-5-yl)sulfanyl)benzoic Acid (14). An ethanolic solution (5 mL) consisting of methyl ester **53** (0.19 g, 0.55 mmol) and 0.5 mL of aqueous 1 N NaOH was heated at 50 °C for 4 h. At this time the solution was evaporated to dryness, and the sodium salt was dissolved in 3 mL of H_2O. The solution was carefully acidified to pH 4 with concentrated HCl. The free acid precipitated which was filtered and washed with 5 mL of cold H_2O. The solid was dried in a desicator over $CaSO_4$ to yield 0.15 g (84%) of acid **14** as a beige solid: mp 298 °C dec; IR (KBr) 3412, 2866, 2610, 1730, 1700, 1584, 1310, 1113, 740 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.29 (s, 3 H), 2.45 (s, 3 H), 7.0 (d, 2 H, J = 8.5 Hz), 7.44 (d, 1 H, J = 8.6 Hz), 7.71 (d, 2 H, J = 8.4 Hz), 7.74 (d, 1 H, J = 8.3 Hz); HRMS calcd for $C_{17}H_{14}N_2O_3S$ 326.0742 (M^+), found 326.0725; HPLC t_R = 8.7 min; 97.9%.

Diethyl *N*-[4-[(2,6-Dimethyl-4-oxo-3,4-dihydroquinazolin-5-yl)sulfanyl]benzoyl]-L-glutamate (56). Benzoic acid **14** (60.0 mg; 18.4 mmol), and L-glutamic acid diethyl ester hydrochloride (0.144 g, 0.6 mmol) were dissolved in 5 mL of anhydrous DMF and cooled to 0 °C. To the stirred solution was added diphenyl phosphorazidate (0.15 mL, 0.7 mmol). After 15 min, 0.2 mL (1.4 mmol) of triethylamine was added, and the reaction mixture was allowed to stir for 12 h at room temperature. The solvent was then removed under vacuum, and the remaining solid was taken up in 5 mL of H_2O. The pH was carefully adjusted to 6 with concentrated HCl, and the aqueous solution was extracted with $CHCl_3$ (3 × 10 mL). The organic layers were combined, dried over $MgSO_4$, filtered, and evaporated to dryness. The product was purified by flash chromatography on silica using MeOH/CH_2Cl_2, 10:90. A tan solid (78.0 mg, 82%) was isolated: mp 152 °C dec; IR (KBr) 3400, 2925, 1711, 1618, 1550, 1410, 1220, 836 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.11 (m, 6 H), 1.61 (m, 2 H), 1.79 (m, 2 H), 2.26 (s, 3 H), 2.37 (s, 3 H), 3.26 (m, 1 H), 4.05 (m, 4 H), 6.96 (d, 2 H, J = 8.4 Hz), 7.55 (d, 1 H, J = 8.5 Hz), 7.64 (d, 2 H, J = 8.4 Hz), 7.71 (d, 1 H, J = 8.5 Hz), 8.60 (d, 1 H, J = 5.3 Hz), 12.10 (bs, 1 H); HRMS calcd for $C_{28}H_{29}N_3O_6S$ 512.1843 (M + 1), found 512.1855.

***N*-[4-[(2,6-Dimethyl-4-oxo-3,4-dihydroquinazolin-5-yl)-sulfanyl]benzoyl]-L-glutamic Acid (26).** Diethyl ester **56** (78.0 mg, 0.15 mmol) was dissolved in 5 mL of ethanol, and to this solution was added 0.5 mL of an aqueous 1 N NaOH solution. The reaction mixture was stirred at 50 °C for 3 h, whereupon disappearance of starting material was indicated by TLC (MeOH/CH_2Cl_2, 10:90). The solution was then evaporated to dryness, and the disodium salt was taken up in 2 mL of H_2O and acidified to pH 4 with concentrated HCl. The solid was filtered upon precipitation and washed with 5 mL of cold H_2O. The final product was dried under vacuum over $CaSO_4$ yielding 50 mg (72%) of an off-white solid: mp 173 °C dec; IR (KBr) 3557, 3418, 2366, 1637, 1298, 1207, 1148, 1084, 760, 621 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.95 (m, 2 H), 2.05 (m, 2 H), 2.26 (s, 3 H), 2.46 (s, 3 H), 4.40 (m, 1 H), 6.93 (d, 2 H, J = 8.4 Hz), 7.55 (d, 1 H, J = 8.4 Hz), 7.65 (d, 2 H, J = 8.5 Hz), 7.71 (d, 1 H, J = 8.4 Hz), 8.40 (bd, 1 H, J = 5.4 Hz), 12.00 (bs, 1 H). Anal. ($C_{22}H_{21}N_3O_6$-S·2.0H_2O·1.0HCl) C, H, N, S.

4-[(2-Amino-6-methyl-4-oxo-3,4-dihydroquinazolin-5-yl)sulfanyl]benzoic Acid Methyl Ester (54). This compound was prepared from **37a** and methyl 4-mercaptobenzoate[31] as described for **53**. After treatment with H_2S/MeOH the suspension was filtered through a medium porosity glass frit to remove the copper salts, and the filtrate was concentrated. The residue was washed with minimal CH_2Cl_2, triturated with ether, and then dried under vaccum over $CaSO_4$ giving a off-white solid: mp 273–275 °C; IR (KBr) 3481, 3414, 2928, 1722, 1659, 1593, 1537, 1466, 1275, 1111, 756 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.28 (s, 3 H), 3.78 (s, 3H), 6.39 (bs, 2 H), 6.96 (d, 2 H, J = 8.5 Hz), 7.25 (d, 1 H, J = 8.4 Hz), 7.56 (d, 1 H, J = 8.5 Hz), 7.74 (d, 2 H, J = 8.5 Hz), 12.10 (bs, 1 H); HRMS calcd for $C_{17}H_{15}N_3O_3S$ 341.0839 (M^+), found 341.0834.

4-[(2-Amino-6-methyl-4-oxo-3,4-dihydroquinazolin-5-yl)sulfanyl]benzoic Acid (55). As outlined in the hydrolysis of **53** to **14**, carboxylic acid **55** was prepared in 96% yield: off-white solid; mp >300 °C; IR (KBr) 3404, 3106, 2820, 1692, 1609, 1490, 1394, 1270, 1146, 1022 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.31 (s, 3 H), 6.74 (bs, 2 H), 6.98 (d, 2 H, J = 8.4 Hz), 7.32 (d, 1 H, J = 8.4 Hz), 7.63 (d, 1 H, J = 8.5 Hz), 7.75 (d, 2 H, J = 8.4 Hz); HRMS calcd for $C_{16}H_{13}N_3O_3S$ 327.0685 (M^+), found 327.0677.

Diethyl *N*-[4-[(2-Amino-6-methyl-4-oxo-3,4-dihydroquinazolin-5-yl)sulfanyl]benzoyl]-L-glutamate (57). The amino acid coupling reaction of **55** and L-glutamic acid diethyl ester hydrochloride was performed as described in the procedure for **56**. Upon evaporation of the reaction solvents, the residue was taken up in CH_2Cl_2 and washed with 10% aqueous $NaHCO_3$, followed by H_2O. The organic layers were dried over anhydrous $MgSO_4$, filtered, concentrated, and triturated with ether and then with acetone. The beige solid was dried under vacuum over $CaSO_4$ (78%): mp 125 °C dec; IR (KBr) 3380, 2950, 1708, 1625, 1543, 1485, 1386, 1260, 848 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.13 (m, 6 H), 2.00 (m, 2 H), 2.24 (s, 3 H), 2.40 (t, 2 H, J = 5.2 Hz), 4.05 (m, 4 H), 4.39 (m, 1 H), 6.35 (bs, 2 H), 6.94 (d, 2 H, J = 8.5 Hz), 7.23 (d, 1 H, J = 8.5 Hz), 7.54 (d, 1 H, J = 8.5 Hz), 7.66 (d, 2 H, J = 8.5 Hz), 8.58 (d, 1 H, J = 7.0 Hz), 10.8 (bs, 1 H). HRMS calcd for $C_{25}H_{28}N_4O_6S$ 512.1713 (M^+), found 512.1729. Anal. ($C_{25}H_{28}N_4O_6S \cdot 1.5H_2O$) C, H, N.

***N*-[4-[(2-Amino-6-methyl-4-oxo-3,4-dihydroquinazolin-5-yl)sulfanyl]benzoyl]-L-glutamic Acid (27).** Diethyl ester **57** was hydrolyzed in 95% yield using the procedure to prepare **26**: beige solid; mp 210 °C dec; IR (KBr) 3363, 2908, 1750, 1650, 1576, 1468, 1212, 1096, 980 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.90 (m, 1 H), 2.10 (m, 1 H), 2.27 (s, 3 H), 2.32 (t + s, 2 H, J = 5.2 Hz), 4.35 (m, 1 H), 6.80 (bs, 2 H), 6.94 (d, 2 H, J = 8.4 Hz), 7.27 (d, 1 H, J = 8.4 Hz), 7.58 (d, 1 H, J = 8.5 Hz), 7.66 (d, 2 H, J = 8.3 Hz), 8.47 (d, 1 H, J = 7.7 Hz), 12.30 (bs, 1 H). HRMS calcd for $C_{21}H_{20}N_4O_6S$ 457.1165 (M^+), found 457.1181. Anal. ($C_{21}H_{20}N_4O_6S \cdot 1.25H_2O \cdot 1.0HCl$) C, H, N, S.

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(13) X-ray diffraction quality crystals of *E. coli* TS complexed with 5-FdUMP and **8** were grown in space group $P6_1$ as described in ref 10. The structure was solved at 2.5-Å resolution with the *R* factor being 17.8% upon refinement.

(14) (a) The 2-methyl derivative of **8**, 6-chloro-2-methyl-5-(pyridin-4-ylsulfanyl)-3*H*-quinazolin-4-one was not prepared; therefore, a direct comparison of the K_is between the 2-amino derivatives, **21** and **22**, was made. (b) The π values for the Cl and CH_3 fragments derived from chlorobenzene and toluene are 0.71 and 0.56, respectively. See: Hansch, C.; Leo, A. J. *Substituents Constants for Correlation Analysis in Chemistry and Biology*; John Wiley and Son Inc.: New York, 1979. (c) Bacquet, R. J. Electrostatic Contributions to Binding Affinity for the Enzyme Thymidylate Synthase and a Series of Inhibitors. Submitted to *Trends in QSAR and Molecular Modeling '92*; Wermuth, C. G., Ed.; ESCOM: Leiden, 1992.

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