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Synthesis and In Vitro Antitumor Activity of Thiophene Analogues of 5-Chloro-5,8-dideazafolic Acid and 2-Methyl-2-desamino-5-chloro-5,8-dideazafolic Acid

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Abstract—*N*-[5-[*N*-(2-Amino-5-chloro-3,4-dihydro-4-oxoquinazolin-6-yl)methylamino]-2-thenoyl]-L-glutamic acid (**6**) and *N*-[5-[*N*-(5-chloro-3,4-dihydro-2-methyl-4-oxoquinazolin-6-yl)methylamino]-2-thenoyl]-L-glutamic acid (**7**), the first reported thiophene analogues of 5-chloro-5,8-dideazafolic acid, were synthesized and tested as inhibitors of tumor cell growth in culture. 4-Chloro-5-methylisatin (**10**) was converted stepwise to methyl 2-amino-5-methyl-6-chlorobenzoate (**22**) and 2-amino-5-chloro-3,4-dihydro-6-methyl-4-oxoquinazoline (**19**). Pivaloylation of the 2-amino group, followed by NBS bromination, condensation with di-*tert*-butyl *N*-(5-amino-2-thenoyl)-L-glutamate (**28**), and stepwise cleavage of the protecting groups with ammonia and TFA yielded **6**. Treatment of **9** with acetic anhydride afforded 2,6-dimethyl-5-chlorobenz[1,3-*d*]oxazin-4-one (**31**), which on reaction with ammonia, NaOH was converted to 2,6-dimethyl-5-chloro-3,4-dihydroquinazolin-4-one (**33**). Bromination of **33**, followed by condensation with **28** and ester cleavage with TFA, yielded **7**. The IC_{50} of **6** and **7** against CCRF-CEM human leukemic lymphoblasts was 1.8 ± 0.1 and 2.1 ± 0.8 μM, respectively.

Introduction

5-Chloro-5,8-dideazafolic acid (**1**) was first synthesized by Davoll and Johnson[1] and later reported by Jones and co-workers[2a,b] to bind tightly to dihydrofolate reductase (DHFR) and thymidylate synthase (TS), two key enzymes used by cells to synthesize the pyrimidine and purine nucleotide precursors of DNA that are essential for normal growth. The potency of **1** as an inhibitor of the growth of leukemia cells in culture was essentially the same as that of 5,8-dideazafolic acid (**2**). A major synthetic effort was subsequently undertaken to optimize the activity of 5-unsusbtituted-5,8-dideazafolates, led to the development of *N*-[4-[*N*-(2-amino-3,4-dihydro-4-oxoquinazolin-6-yl)methyl]-*N*-propargylamino]benzoyl-L-glutamic acid (**3**)[3a–c] and its more soluble 2-desamino-2-methyl analogue (**4**, ICI 198583).[4a,b] An even better third-generation analogue, *N*-[5-[*N*-(3,4-dihydro-2-methyl-4-oxoquinazolin-6-yl)methyl]-*N*-methylamino]-2-thenoyl-L-glutamic acid (**5**) was then developed and subjected to intensive clinical evaluation.[5a–e] Interestingly, with the exception of a series of papers by Hynes and co-workers[6a–h] on 5,8-dideazaisofolic acid derivatives (N9–C10 bridge), among which was 5-chloro-5,8-dideazaisofolic acid, the effect of 5-chloro substitution on the in vitro antitumor activity of 2-amino-3,4-dihydro-4-oxoquinazoline antifolates with a *para*-aminobenzoyl-L-glutamate side chain has remained unexplored. Moreover, 5-chloro-3,4-dihydro-4-oxoquinazoline antifolates with a conventional C9–N10 bridge and a heterocyclic ring in place of the phenyl ring have not been described. Thus it was of interest to synthesize compounds **6** and **7**, the first reported examples of 5-chloro-5,8-dideazafolic acid analogues in which the phenyl ring is replaced by thiophene. The structures of **1**–**7** are shown in Figure 1.

Chemistry

The general plan we adopted for the synthesis of **6** and **7** was similar to one that had been used earlier to prepare the 5,8-dideazafolic acid analogues **1**[1,2a] and **8**[5a] (Fig. 1), the sole difference being that the starting material we chose to use was the commercially unavailable compound 2-amino-5-methyl-6-chlorobenzoic acid (**9**)

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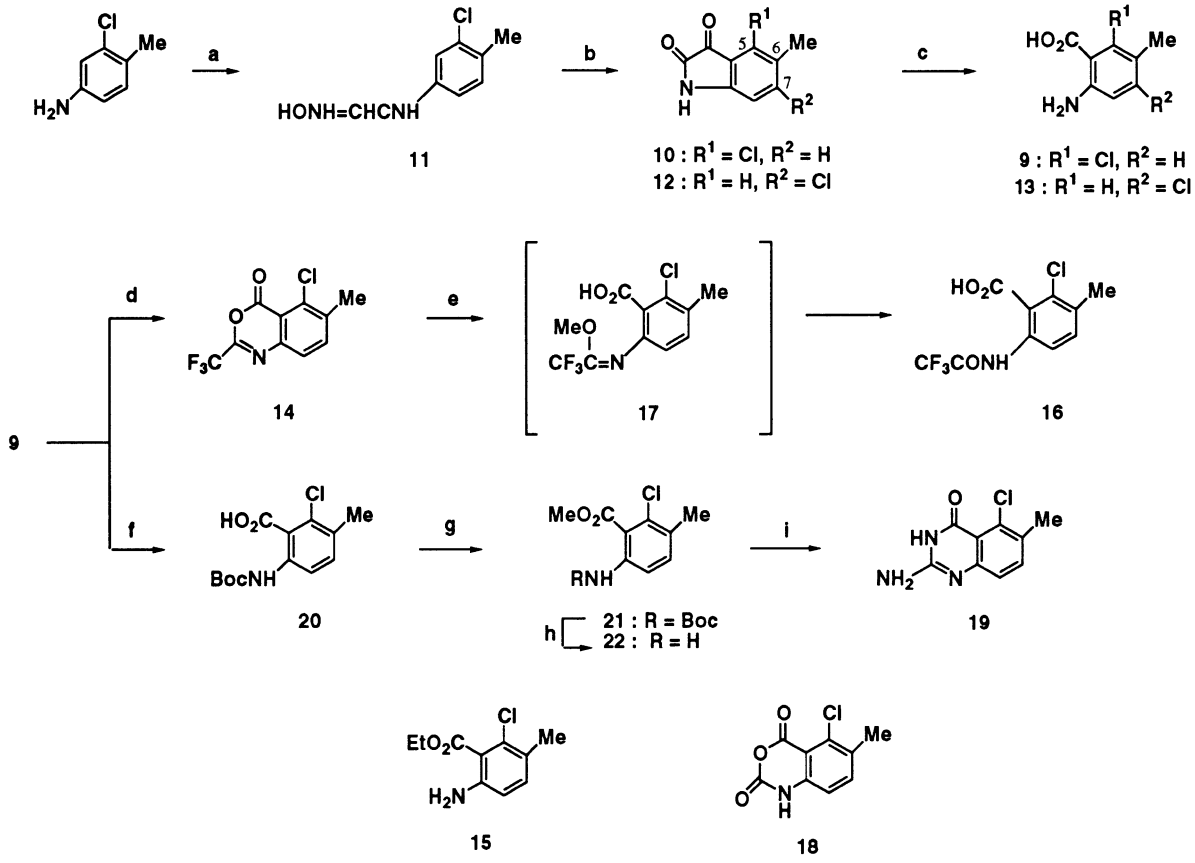
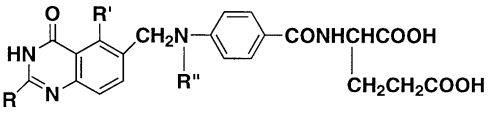
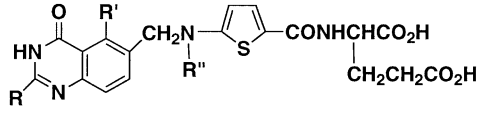


Scheme 1. Synthesis of compound **6**. Reagents: (a) $CCl_3CH(OH)_2$, H_2NOH, Na_2SO_4; (b) (i) H_2S0_4; (ii) separate isomers; (c) H_2O_2, NaOH; (d) $(CF_3CO)_2O$; (e) (i) NaOEt, (ii) aq HCI; (iii) $(t\text{-BuO})_2O$, NaOH, dioxane; (g) Mel, Cs_2CO_3, DMF; (h) TFA; (i) C1C(=NH)NH_2·HC1, 200 °C.

(Scheme 1). We envisaged that it would be possible to obtain **9** from the isatin **10**, which Baker and co-workers[7] described many years ago as one of two isomers produced when the α-isonitrosoanilide **11** was heated at 65 °C under strongly acidic conditions. The two isomers were said to precipitate at different rates upon gradual acidification of an alkaline solution of the mixture with HCl, and were distinguishable by their color, which was red in the case of **10** and orange in the case of the more soluble isomer **12**. In addition, recrystallized samples of the two compounds had different melting points, which were 242–244 °C in the case of **10** and 256–258 °C in the case of **12**. The structures of **10** and **12** were assigned by oxidizing them to the corresponding anthranilic acids **9** and **13** with alkaline H_2O_2, followed by reductive deamination with $NaNO_2$ and H_3PO_2, which yielded the previously known compound 3-methyl-4-chlorobenzoic acid from **12** and the positional isomer 2-chloro-3-methylbenzoic acid from **10**. Although a sample of the latter acid was not synthesized by an alternate route for comparison, the structure of the isatin oxidation product was eventually confirmed unequivocally by ^{1}H NMR,[8] proving that the original formulation of **10** had been correct. This was obviously of critical importance, since it meant that the ultimate product of our synthesis had to be a 5-chloro and not a 7-chloro derivative, the latter of which would be expected to have lower antifolate activity.



1 : R = NH_2, R' = Cl, R" = H
2 : R = NH_2, R' = R" = H
3 : R = NH_2, R' = H, R" = $CH_2C{\equiv}CH$
4 : R = Me, R' = H, R"= $CH_2C{\equiv}CH$



5 : R = R" = Me, R' = H
6 : R = NH_2, R' = Cl, R" = H
7 : R = Me, R' = Cl, R" = H
8 : R = Me, R' = R" = H

Figure 1. Structures of compounds **1**–**7**.

When the original synthesis of **10** and **12** was repeated, separation of the isomers was unexpectedly found to be less straightforward than reported.[7] In our hands, gradual addition of HCl to an alkaline solution of the crude product from the cyclization of **11** never yielded any crops consisting only of the desired isomer **10**. Instead, mixtures of **10** and **12** invariably co-precipitated during gradual acidification and cooling, and it was only when the crude mixture was recrystallized directly from glacial AcOH or acetone that crops of

pure individual isomers could be isolated. Moreover the same isomer did not always crystallize first, and the order of crystallization seemed to be independent of the relative amount of each isomer in the mixture. Thus it was necessary to carefully analyze each crop from every crystallization by TLC in order to make sure that it consisted of the desired isomer. Confirmation that the slower-moving isomer was indeed **10** came from the ^{1}H NMR spectrum which featured a pair of doublets at δ 6.77 (C7-H) and δ 7.53 (C6-H). By contrast, the ^{1}H NMR spectrum of the faster-moving isomer showed only singlets at δ 6.95 (C7-H) and δ 7.55 (C4-H), proving that it was **12**. The larger chemical shift of the C6 proton relative to the C7 proton in **10** and of the C4 proton relative to the C7 proton in **12** can be explained on the basis of an electron-withdrawing effect by the 3-keto group.

In the course of trying to optimize the yield from the ring closure reaction, we made the interesting observation, not noted earlier,[7] that the temperature at which ring closure was carried out could markedly influence regioselectivity. Thus, when the reaction was performed at 60 °C the ratio of **10** to **12** was ca. 2:1, whereas at 70 °C the two isatins formed in nearly equal amount. The fact that the sterically more hindered isomer **10** was favored at the lower temperature suggested that the formation of **10** may be kinetically controlled whereas the less hindered product **12** is thermodynamically preferred.

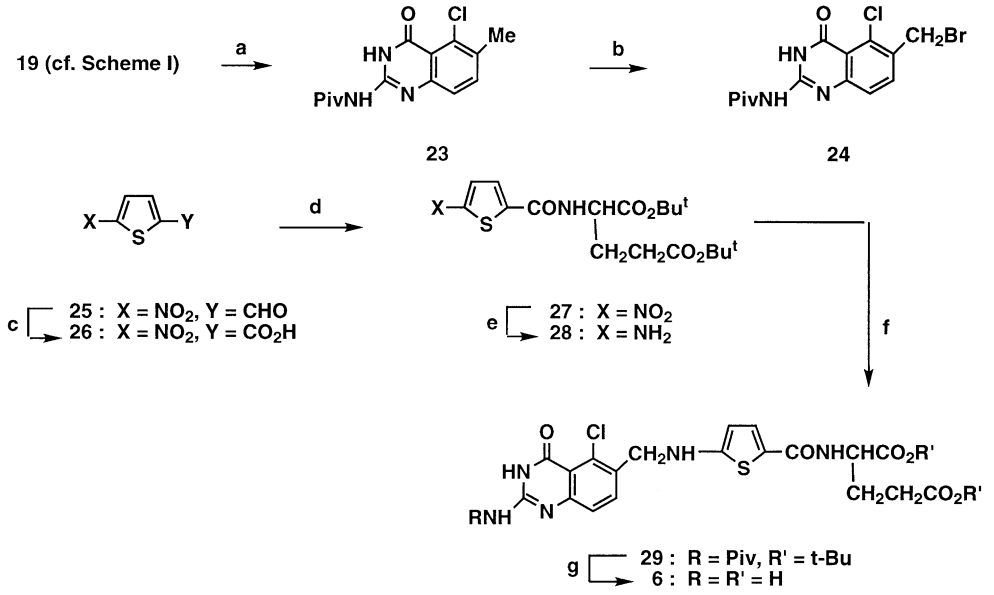
Oxidation of **10** with alkaline H_2O_2 afforded anthranilic acid **9** as reported,[7,8] but there was again a small discrepancy between our results and those of the earlier investigators. Whereas our observed melting point for this compound after recrystallization was 167–168 °C, the value given in the literature is approximately eleven degrees lower,[7,8] suggesting that a completely pure sample may not have been used in the earlier papers. In order to ensure that we had the right compound, isatin **12** was oxidized to **13**, and the melting point of the latter was found to be more or less in agreement with the published value of 210–211 °C, although our sample actually had a double melting point, first at 210–211 °C and then, after resolidifying briefly, at 213–214 °C. The yield of both **9** and **13** was 80%. The most interesting difference in the behavior of the two anthranilic acids, not mentioned by the earlier workers, was that **9** underwent vigorous gas evolution at its melting temperature, whereas **13** did not. The facile decarboxylation of **9** was consistent with the crowded 1,2,3,4-tetrasubstituted structure of this molecule. The effect of steric hindrance on the behavior of the carboxyl group in **9** also became evident from some unexpected chemistry which is discussed below.

Because we had first intended to cyclize **9** to a 2-aminoquinazolin-4(3*H*)-one by reaction of its methyl ester with guanidine, we attempted to esterify the acid via a standard reaction with MeOH in the presence of $SOCl_2$ at 10 °C. To our surprise, when the reaction product was partitioned between aqueous $NaHCO_3$ and an organic solvent, approximately two-thirds of the starting material was recovered from the aqueous layer and the only neutral product in the organic layer was obviously not the desired methyl ester, since its ^{1}H NMR spectrum lacked an OMe signal and the two well-defined doublets in the aromatic region were replaced by a complex multiplet. We therefore concluded that acid-catalyzed decarboxylation had probably occurred even at low temperature. Although Webber and coworkers[9] had made the same observation when they tried to esterify 2-amino-5-methyl-6-bromobenzoic acid by this method, it was striking to see that such a facile loss of CO_2 occurs even with a smaller Cl substituent at C6.

In another approach, we prepared the oxazinone **14** from **9** and trifluoroacetic anhydride in pyridine in the hope that the oxazinone could be cleaved to **15** with NaOEt in dry EtOH. In the event, the only product isolated after these reactions was not **15**, but rather the *N*-trifluoroacetylated acid **16**, probably arising via attack at the imine carbon of **14**, followed by hydrolysis of the putative imino ether intermediate **17** upon neutralization with aqueous acid. The structure of **16** was evident from its ^{1}H NMR spectrum, in which the aromatic doublets appeared at δ 7.73 and δ 7.93, whereas the corresponding doublets in the anthranilic acid **9** were much further upfield at δ 6.63 and δ 7.08. Although this unfavorable outcome was disappointing, it could be explained by increased chemical reactivity of the imine carbon next to the electron-withdrawing CF_3 group.

4-Trifluoromethylisatin has been converted to 5-trifluoromethylisatoic anhydride upon oxidation with monoperphthalic acid, and the anhydride has been converted to 2-amino-5-trifluoromethyl-3,4-dihydro-4-oxoquinazoline by further reaction with guanidine carbonate.[10] We reasoned that if we could isolate 5-chloro-6-methylisatoic anhydride (**18**), it might be possible to convert it directly to 2-amino-5-chloro-3,4-dihydro-6-methyl-4-oxoquinazoline (**19**) by reaction with guanidine. As expected, oxidation of **10** with monoperphthalic acid or *m*-chloroperbenzoic acid was rapid as judged by the disappearance of the characteristic red color of the isatin. However subsequent heating with guanidine did not yield **19**, but instead a product that was highly water-soluble at pH 8 and whose identity was not determined.

The nettlesome problems discussed above were finally resolved when **9** was found to be readily converted to the Boc derivative **20** with di-*tert*-butyl dicarbonate and then to the *N*-protected ester **21** with Cs_2CO_3 and MeI in DMF with a combined two-step yield of 70% (Scheme 1). Deprotection of **21** with TFA was quantitative, affording the amino ester **22**. Although our expectations that heating **22** with guanidine or guanidine carbonate would yield quinazoline **19** failed to materialize, when **22** was heated with chloroformamidine hydrochloride in diglyme at 200 °C,[9] the desired product was obtained in 79% yield. Acylation of the amino group with pivaloyl chloride afforded the more soluble and easily recrystallized derivative **23**

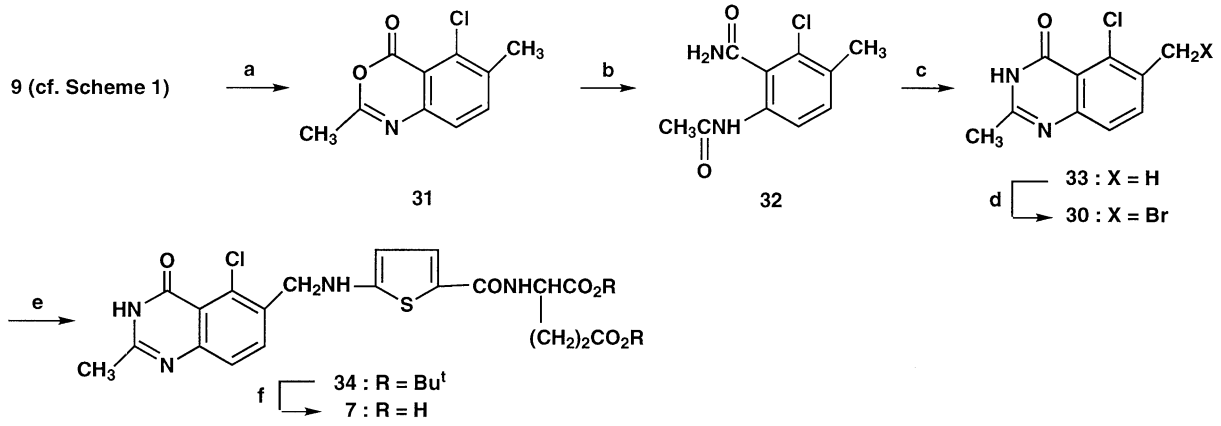


Scheme 2. Synthesis of compound **7**. Reagents: (a) PivCl, Et_3N, THF; (b) NBS, Bz_2O_2, $CHCl_3$ (c) $KMnO_4$, NaH_2PO_4, aq Me_2CO; (d) (i) $SOCl_2$ (ii) di-*tert*-butyl L-glutamate, Et_3N, CH_2Cl_2 (e) Fe powder, $FeSO_4$, aq MeOH; (f) **24**, $NaHCO_3$, DMF; (g) 1:3 TFA–CH_2Cl_2, 5 °C.

(86% yield), which was then brominated with NBS/Bz_2O_2 in $CHCl_3$ to obtain bromide **24** (Scheme 2). Although it was possible to obtain an analytical sample of **24** by recrystallization from MeOH, there was substantial loss of material, and we therefore took this compound to the next step without purification. That bromination of **23** had occurred on the 6-Me group was evident from the ^{1}H NMR spectrum of **24**, in which the Me singlet at δ 2.47 was replaced by a singlet at δ 4.72. Moreover, the doublet we had observed at δ 7.57 for the C7 aromatic proton in the spectrum of **23** was now at δ 7.72. Interestingly, the other aromatic proton in compounds **23** and **24** had almost the same chemical shift (ca. δ 7.3).

With the synthesis of **24** completed, the next task was to synthesize the right-hand fragment of the target compound **6**. Although dimethyl or diethyl esters had generally been used by other workers in the past to protect the glutamate moiety during the synthesis of quinazoline antifolates, pilot experiments showed that, perhaps because of the absence alkyl substitution on nitrogen, the aminothiophene ring would be extensively damaged in aqueous base. Moreover, while the thiophene moiety appeared to be stable in methanolic ammonia at room temperature, we remained concerned that even these mild conditions might convert methyl esters to amides, and thus felt that protection of the glutamate side chain would be more appropriate with *tert*-butyl esters. Accordingly, we synthesized the previously unknown di-*tert*-butyl ester **28** (Scheme 2). Oxidation of commercially available 5-nitro-2-thiophenecarboxaldehyde (**25**) with $KMnO_4$ in the presence of NaH_2PO_4 afforded acid **26**, which on reaction with $SOCl_2$ followed by addition of di-*tert*-butyl L-glutamate was converted to the nitro ester **27** (93% crude yield). The successful synthesis of **26** from **25** with $KMnO_4$ was in surprising contrast to an earlier report[11] that this oxidation does not work, but instead requires alkaline $AgNO_3$.[11,12] Diester **27** was a gum that could not be induced to crystallize even though its purity was of microanalytical quality and its identity was fully supported by its ^{1}H NMR spectrum, in which the protons of the thiophene ring were readily discerned as a pair of doublets at δ 7.50 and δ 7.88, respectively. Reduction of the nitro group was performed with a mixture of activated Fe powder and $FeSO_4$ in refluxing MeOH to obtain the amino ester **28** (60% crude yield). Complete reduction of the NO_2 group was verified by the ^{1}H NMR spectrum, in which the doublets for protons on the thiophene ring were shifted upfield to δ 6.08 and δ 7.22. The doublet with the high chemical shift (δ 7.88) in the spectrum **27** was assigned to C3-H because this proton is next to the electron-withdrawing NO_2 group. Using analogous reasoning, the doublet with the low chemical shift (δ 6.08) in the spectrum of **28** was assigned to C3-H on the basis that this proton would now be shielded rather than deshielded. By a process of exclusion the doublets at δ 7.50 in **27** and δ 7.22 in **28** were therefore assigned to C4-H.

Coupling of the pivaloylated bromide **24** with amine **28** to form **29** was performed by stirring equimolar amounts of the reactants with two mols of $NaHCO_3$ in DMF at room temperature for 2 days. The yield of **29** after chromatography was 69%. Attempted acidolysis of the pivaloyl and ester groups in a single reaction using TFA were unsuccessful, as only the latter were cleaved. Thus, **29** was deprotected by treatment with anhydrous methanolic ammonia at room temperature for 20 h to remove the pivaloyl group, followed by TFA in CH_2Cl_2 at 5 °C for 20 h to cleave the esters. The resulting diacid (**5**) was purified by a two-stage process involving preparative HPLC on a C18 column, followed by ion-exchange chromatography on a DEAE-cellulose (HCO_3- form). Because a number of side products formed during the two-stage deprotection scheme, the final yield of highly pure material suitable for biological



Scheme 3. Reagents: (a) Ac_2O, reflux ; (b) NH_3, −33 °C; (c) 1 N NaOH, reflux; (d) NBS/Bz_2O_2, $CHCl_3$ (e) **28**, $NaHCO_3$, DMF; (1) 1:3 TFA–CH_2Cl_2, 5 °C.

testing was still only 15%. Although protection of the side chain with acid-cleavable *tert*-butyl groups apparently did not provide the advantage we had hoped for, it should be noted that in the only example published thus far of the use of *tert*-butyl groups during the synthesis of a 5,8-dideazafolate containing a 4-aminothiophene ring,[5a] the bridge nitrogen was substituted with a cyanomethyl group which may have had a protective effect. All the other thiophene analogues in the series, including one in which the nitrogen on the ring was unsubstituted, were made from diethyl L-glutamate.

An alternative approach to the synthesis of **5** in which closure of the quinazolinone ring would be left to the end was also briefly explored. Thus, bromination of **21** with NBS/Bz_2O_2 and condensation of the resulting bromide with aminothiophene **28** afforded a carbamate triester which we proposed to subject to acidolysis of the Boc and *tert*-butyl ester groups, followed by condensation with guanidine. In the event, even though pilot experiments showed that the synthesis of the carbamate triester quite feasible, this approach was abandoned after a model ring closure reaction with guanidine and the non-brominated amino ester **22** was found to be completely unsuccessful (see above).

We next turned our attention to the synthesis of the 2-methyl-2-desamino analogue **6** via the previously unknown bromide **30**, which was prepared according to Scheme 3. Treatment of **9** with Ac_2O under reflux afforded the benz[1,3-*d*]oxazine **31** in 87% yield, and further reaction with liquid ammonia presumably yielded the anthranilamide **32**, which was directly cyclized to **33** with hot aqueous NaOH. The structure of **31** was easily identified by a pair of ^1H NMR singlets at δ 2.43 and δ 2.50 for the Me groups. Two singlets were likewise observed in the spectrum of **33**, this time at δ 2.30 and δ 2.40, and it was assumed, as has been done with the corresponding 5-unsubstituted compounds,[13] that the upfield signal in each case corresponded to the Me group at C6. Bromination of **33** with NBS/Bz_2O_2 in $CHCl_3$ under reflux afforded bromide **30**, which precipitated directly from the reaction mixture (crude yield 77%) and was used directly for the next reaction with the expectation that any non-brominated starting material would be easy to remove by chromatography at the next stage. The spectrum of **30** showed a singlet at δ 2.33, attributable to the 2-Me group, and a two-proton singlet at δ 4.87 which we assigned to the CH_2Br group at C6 because it was approximately in the same region as that of the CH_2Br group in **24** (δ 4.72). That bromination had occurred at the 6-position rather than the 2-position was also consistent with an earlier study which unequivocally established that bromination of 3,4-dihydro-2,6-dimethyl-4-oxoquinazoline yields the 6-bromomethyl derivative.[13] Moreover, as in the bromination of **23**, the doublet for the C7 aromatic proton was displaced downfield from δ 7.73 to δ 7.97, and the C8 proton likewise showed a downfield shift, albeit a smaller one, from δ 7.42 to δ 7.52. The magnitude of this shift was greater than would have been expected if the 2-Me group had undergone bromination. In agreement with earlier data for 2-amino-3,4-dihydro-4-oxoquinazolines[14a,b] versus 3,4-dihydro-2-methyl-4-oxoquinazolines[4a] lacking a C5 substituent, when the chemical shifts of the C7 and C8 protons in **33** were compared with those of the C7 and C8 protons in **19**, the latter both had higher δ values consistent with the ability of the 2-amino group to increase electron density at C8 via a resonance effect. Moreover, the absence in either **19** or **33** of a doublet below δ 8.0 confirmed that the values assigned originally to the C5 proton in 5-unsubstituted 6-bromomethyl-3,4-dihydro-4-oxoquinazolines[14a,14b,15] was correct.

The synthesis of **7** was completed by condensing bromide **30** with aminothiophene **28** in DMF in the presence of $NaHCO_3$ to obtain diester **34** (56%) and deprotecting the latter with TFA in CH_2Cl_2 at 5 °C. Whereas the yield of **6** from **29** had been only 15%, that of **7** from **34** after purification by HPLC and ion-exchange chromatography was more than 2-fold higher (39%). In agreement with the method of isolation of these compounds by freeze-drying of NH_4HCO_3 eluates from ion-exchange columns, microchemical analysis indicated that **6** and **7** were both solvated with 3.5 mol of H_2O, and also contained 0.5 and 1.0 mol of ammonia, respectively.

Biological Activity

Compounds **6** and **7** were tested as inhibitors of the growth of CCRF-CEM human leukemic lymphoblasts as described by McGuire and co-workers.[16] The concentrations at which **6** and **7** inhibited cell growth by 50% (IC_{50}) after 120 h of drug exposure in medium containing 10% horse serum were 1.8 ± 0.1 and 2.1 ± 0.8 μM, respectively. The IC_{50} values reported for **5** against human WI-L2 lymphoblastic leukemia cells, which are likewise T-cells, was 0.0035 μM.[16] In assays against L1210 murine leukemic cells the N10-methyl group in **5** is known to increase growth-inhibitory potency versus the corresponding N10-unsubstituted compound at least 100-fold.[5d,e] While it may be speculated that similar modification of **6** and **7** would similarly have a favorable effect on biological activity, we did not address this question in the present work.

Experimental

IR spectra were obtained on a Perkin Elmer model 281 spectrophotometer and UV spectra on a Varian model 210 instrument. For the sake of brevity, only IR peaks with wave numbers greater than 1200 cm^{-1} are reported, and very weak peaks and shoulders are omitted. ^1H NMR spectra were recorded at 60 MHz with a Varian model EM360L instrument using Me_4Si as the reference, or in the case of **6** and **7** at 200 MHz with a Varian model Mercury 200 instrument. The very broad amide NH signal in several compounds was barely discernible at 60 MHz is therefore recorded only for **6** and **7**. Analytical TLC was performed on fluorescent Whatman MK6F silica gel-coated glass slides, with spots viewed under 254 nm illumination. Column chromatography was on Baker silica gel (regular grade, 60–200 mesh; flash grade 40-μm particle size) or on Whatman DE-52 preswollen DEAE-cellulose (HCO_3-form). HPLC separations were on C18 silica gel radial compression cartridges (Millipore, Milford, MA, USA; analytical, 5-μm particle size, 5 × 100 mm; preparative, 15-μm particle size, 24 × 100 mm). In those instances where preparative HPLC was followed by ion-exchange chromatography, the latter step was in order to ensure that the sample was not contaminated with traces of silica gel from the C18 column. MOISTURE-sensitive reactions were carried out in solvents that were of Sure-Seal grade (Aldrich, Milwaukee, WI, USA) or had been stored over Linde 4A molecular sieves. Solids were generally dried over P_2O_5 at 50–80 °C in a vacuum oven or Abderhalden apparatus. Melting points (not corrected) were obtained on a Fisher–Johns hot-stage microscope or in open Pyrex capillary tubes in a Mel-Temp apparatus (Cambridge Laboratory Devices, Cambridge, MA, USA). Starting materials and other reagents and chemicals were purchased from Aldrich (Milwaukee, WI, USA), Lancaster (Windham, NH, USA), or Fisher (Boston, MA, USA). Microanalyses were performed by Quantitative Technologies, Whitehouse, NJ, USA and were within ±0.4% of calculated values unless otherwise noted.

Synthesis and separation of 4-chloro-5-methylisatin (10) and 6-chloro-5-methylisatin (12). Step 1. Solid chloral hydrate (45 g, 0.27 mol) was added to a suspension of Na_2SO_4 (286 g, 2.01 mol) in H_2O (965 mL). In another flask, a solution of 3-chloro-4-methylaniline hydrochloride was prepared by adding the free amine (35.4 g, 0.25 mol) to 12 N HCl (21.5 mL) and H_2O (150 mL), and warming the mixture gently until all the solid dissolved. This warm solution was then added, all in one portion, to the solution of chloral while stirring manually to obtain a fine dispersion. A solution of $H_2NOH{\cdot}HCl$ (55 g, 0.79 mol) in H_2O (250 mL) was then added in one portion, and manual stirring continued until all the Na_2SO_4 dissolved and the organic solids were finely dispersed. The mixture was heated and stirred for 40 min until it came to a vigorous boil, kept at this temperature for 2 min, cooled by immersion in cold water, and left to stand overnight to facilitate filtration. The solid was collected, washed with H_2O, and dried in a lyophilization apparatus to obtain the isonitrosoanilide **11** as a beige solid (median crude yield from several runs: 50.3 g, 95%), mp 168–169 °C. ^1H NMR (DMSO-d_6) δ 2.28 (s, 3H, Me), 7.33 (m, 2H, C5-H and C6-H), 7.65 (s, 1H, CH=N), 7.90 (d, $J = 2$ Hz, 1H, C2-H), 10.28 (br s, 1H, OH, exchangeable with D_2O). Recrystallization of a small sample from aqueous MeOH raised the melting point to 170–172 °C (lit.[8] 177–179 °C), but because there was considerable loss of material during purification the freeze-dried crude product was used in the next reaction directly.

Step 2. Crude **11** (50.3 g, 0.237 mol) was added in small portions to concentrated H_2SO_4 (223 mL) at 60–65 °C at such rate that the internal temperature did not exceed 65 °C. When addition was complete, the solution was warmed to 80 °C and kept at this temperature for 10 min, then cooled to room temperature by immersion in cold water, and poured into ten volumes of ice. After being kept at room temperature overnight to facilitate filtration, the solid was collected, washed with H_2O to remove the acid, and taken up in 0.4 N NaOH (1 L). The insoluble portion was separated by filtration, and the filtrate was acidified, left to stand overnight, and filtered to obtain an orange-red solid (41.1 g, 89% crude yield) consisting of a mixture of **10** and **12**. In some experiments in which the acidified mixture was filtered after only 1 h, the filter cake was washed with H_2O, and the filtrate was left to stand overnight, a small amount of solid precipitated which turned out to be pure **12** (2% yield).

A 10 g portion of the foregoing mixture of **10** and **12** was dissolved in warm acetone (250 mL) and the solution was left to cool in a beaker covered with aluminum foil. The first crop was a red powder consisting of pure **10** (3.73 g) mp 245–247 °C (lit.[7] 242–244 °C); TLC: R_f 0.4 (silica gel, 1:1 isooctane–Me_2CO). ^1H NMR (DMSO-d_6) δ 2.23 (s, 3H, Me), 6.77 (d, $J = 8$ Hz, 1H, C7-H), 7.53 (d, $J = 8$ Hz, 1H, C6-H). Reduction of the volume to 175 mL yielded another 0.66 g of red powder consisting mostly of **10** but also containing some **12**. TLC of the mother liquor showed that it now contained

mostly **12**. Although it can be estimated that the amount of pure **10** theoretically recoverable from the original 41.1 g mixture if the entire sample were recrystallized in one batch would be 15.5 g (33%), actual recoveries of pure **10** from different recrystallizations were not always the same.

A 4.7 g portion of the isomer mixture was recrystallized from glacial AcOH (275 mL). The first crop (2.1 g) consisted of pure **12** as an orange powder: mp 255–256 °C (lit.[7] mp 256–258 °C). TLC R_f 0.5 (silica gel, 1:1 isooctane–Me_2CO). ^{1}H NMR (DMSO-d_6) δ 2.26 (s, 3H, Me), 6.95 (s, 1H, C7-H), 7.55 (s, 1H, C4-H). The second crop (1.8 g) was less pure by TLC, and the supernatant contained mostly **10**. The estimated amount of recoverable pure **12** from the first crop of the 41.1 g mixture if the entire sample had been recrystallized as a single batch was 18.4 g (45%).

2-(*N*-*tert*-Butyloxycarbonyl)amino-5-methyl-6-chlorobenzoic acid (20). Step 1. A stirred solution of **10** (3.91 g, 0.02 mol) in 5% NaOH (100 mL) was treated dropwise over 10 min with 30% H_2O_2 (5.7 mL, calculated to contain 1.71 g, 0.05 mol). After another 20 min of being stirred, during which it became warm and effervesced, the solution was cooled in an ice-bath and acidified to pH 4 with 3 N HCl. The precipitate was collected and dried in a lyophilizer to obtain 2-amino-5-methyl-6-chlorobenzoic acid (**9**) as a beige powder (2.96 g, 80%): mp 163–164 °C dec, gas evolution. ^{1}H NMR (DMSO-d_6) δ 2.13 (s, 3H, Me), 6.63 (d, J = 9 Hz, 1H, C3-H), 7.08 (d, J = 9 Hz, 1H, C4-H), 7.3–8.2 (m, 2H, NH_2). Recrystallization of part of this solid from EtOAc-isooctane afforded glistening beige flakes: mp 167–168 °C, vigorous gas evolution (lit.[8] mp 156–157 °C; lit.[7] mp 156–158 °C). Most of the product was used without recrystallization.

The same procedure with isatin **12** (3.91 g, 0.02 mol) afforded 2-amino-5-methyl-4-chlorobenzoic acid (**13**) as a beige solid (2.95 g, 80%) after recrystallization from toluene: mp 210–211 °C (lit.[7] mp 210–211 °C), with resolidification and a second mp at 213–214 °C. In contrast to **9**, there was very little gas evolution at the mp of **13**.

Step 2. A solution of **9** (11.7 g, 0.0633 mol) in 2 N NaOH (32 mL) and dioxane (65 mL) was treated with di-*tert*-butyl dicarbonate (15.2 g, 0.0696 mol) and stirred at room temperature for 2 days. The dioxane was evaporated and the residue partitioned between EtOAc and H_2O. The aqueous layer was washed with EtOAc to remove neutral impurities, then acidified to pH 2 with 3 N HCl and re-extracted with EtOAc. Evaporation to dryness yielded a foam which solidified on prolonged standing. Recrystallization from a mixture of benzene and isooctane yielded a greenish-gray powder (14.2 g, 79%): mp 130–131 °C, vigorous gas evolution. IR (KBr) ν_{max} 3200, 2980, 2920, 2620, 2550, 1710, 1665, 1600, 1575, 1490, 1455, 1415, 1390, 1370, 1290, 1255, 1225 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 1.50 (s, 9H, *t*-Bu), 2.33 (s, 3H, Me), 7.32 (d, J = 8 Hz, 1H, C3- or C4-H), 7.78 (d, J = 8 Hz, 1H, C3- or C4-H), 8.02 (br s, 1H, NH), 8.68 (br s, 1H, OH). Anal. calcd for $C_{13}H_{16}ClNO_4$: C, 54.65; H, 5.64; N, 4.90; Cl. 12.41. Found: C, 54.71; H, 5.52; N, 4.94; C1, 12.71.

Methyl 2-(*N*-*tert*-butyloxycarbonyl)amino-5-methyl-6-chlorobenzoate (21). A stirred suspension of Cs_2CO_3 (8.06 g, 0.0247 mol) in dry DMF (100 mL) was treated first with **20** (14.1 g, 0.0495 mol) and then, after 15 min, with MeI (3.10 mL, 7.05 g, 0.0493 mol). Stirring was continued for 24 h, during which all the solids dissolved. The DMF was evaporated and the residue partitioned between EtOAc and brine. Rotary evaporation of the EtOAc layer afforded a beige solid which was recrystallized from MeOH to obtain **21** as an off-white powder (13.1 g, 88%): mp 135 °C sharp. IR (KBr) ν_{max} 3210, 3170, 3110, 2980, 2930, 1735, 1705, 1600, 1570, 1495, 1460, 1390, 1370, 1360, 1300, 1280, 1265, 1235 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 1.52 (s, 9H, *t*-Bu), 2.35 (s, 3H, aromatic Me), 3.98 (s, 3H, OMe), 7.28 (d, J = 9 Hz, 1H, C3-H), 7.78 (d, J = 9 Hz, 1H, C4-H). Anal. calcd for $C_{14}H_{18}ClNO_4$: C, 56.10; H, 6.05; N, 4.67; Cl, 11.83. Found: C, 55.90; H, 5.92; N, 4.61; Cl, 11.94.

Methyl 2-amino-5-methyl-6-chlorobenzoate (22). Compound **21** (1.5 g, 0.005 mol) was dissolved in TFA (7 mL), the solution was evaporated to dryness under reduced pressure, and the residue was partitioned between EtOAc and 5% $NaHCO_3$. Evaporation of the EtOAc layer yielded an oil (1.1 g), a small portion of which was purified by flash chromatography on silica gel (5 g, 1.5 × 9 cm) using 2:1 isooctane–EtOAc as the eluent. The resulting material was still an oil, but was homogeneous by TLC (R_f 0.2, silica gel, 2:1 isooctane–EtOAc). IR (thin film) ν_{max} 3470, 3380 2950, 2920, 2850, 1710, 1620, 1565, 1485, 1460, 1445, 1400, 1295, 1275, 1225 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 2.23 (s, 3H, aromatic Me), 3.92 (s, 3H, OMe), 6.52 (d, J = 9 Hz, 1H, C3-H), 7.05 (d, J = 9 Hz, 1H, C4-H). MS: m/e 199 (M, ^{35}Cl), 200 (M + 1, ^{35}Cl), 201 (M, ^{37}Cl), 202 (M + 1, ^{37}Cl). Anal. calcd for $C_9H_{10}ClNO_2$: C, 54.15; H, 5.05; N, 7.02; Cl, 17.76. Found: C, 53.70; H, 4.89; N, 6.88; Cl, 17.49.

2-Amino-5-chloro-3,4-dihydro-4-oxo-6-methylquinazoline (19). Chloroformamidine hydrochloride was freshly prepared by bubbling HCl gas through a solution of cyanamide (0.5 g, 0.012 mol) in Et_2O (100 mL), collecting the precipitate, and drying it under vacuum. This solid was then added to a solution of the entire product from another TFA hydrolysis of **21** (1.5 g, 0.005 mmol) in diglyme (8 mL), and the resulting slurry was plunged into an oil bath preheated to 200 °C. The mixture became homogenous after a few min, and then solidified. Heating was continued for another 10 min, and after being allowed to cool to 80 °C the solid was triturated with EtOH (15 mL), collected, and washed with Et_2O. The solid was then dissolved in a mixture of 1 N HCl (17 mL), DMF (20 mL), and H_2O (20 mL) at 80 °C. The solution was left to cool to room temperature and basified to pH 8 with concentrated NH_4OH (5 mL). The resulting precipitate was gelatinous but became granular and easier to filter when the mixture was reheated to 60 °C and left to cool. The solid was

filtered, washed with H_2O (2 × 15 mL) and EtOH (2 mL), and dried over P_2O_5 in vacuo at 75 °C to obtain **19** as a white solid (0.825 g, 79%). When the reaction was scaled up four-fold the yield was 68%: mp >300 °C; TLC: R_f 0.5 (silica gel, 30:4:1 $CHCl_3$–MeOH–AcOH). IR (KBr) ν_{max} 3450, 3320, 3120, 1665, 1600, 1555, 1515, 1470, 1370, 1325, 1300, 1230 cm^{-1}. ^1H NMR (DMSO-d_6) δ 2.33 (s, 3H, Me), 6.53 (br m, 2H, NH_2), 7.12 (d, J = 8 Hz, 1H, C7-H), 7.55 (d, J = 8 Hz, 1H, C8-H). MS: m/e 210 (M + 1, ^{35}Cl), ^{37}Cl peak obscured by the matrix. Anal. calcd for $C_9H_8ClN_3O.0.25H_2O$: C. 50.48; H, 4.00; N, 19.62; Cl, 16.56. Found: C, 50.21; H, 3.98; N, 19.31; Cl, 16.91.

5-Chloro-3,4-dihydro-6-methyl-4-oxo-2-(*N*-pivaloylamino)-quinazoline (23). A suspension of **19** (2.78 g, 0.0133 mol) in dry THF (200 mL) was treated with Et_3N (2.78 mL, 2.02 g, 0.02 mol) and pivaloyl chloride (2.46 mL, 2.41 g, 0.02 mol), and the mixture was refluxed for 20 h, then cooled and filtered. The filtrate was evaporated under reduced pressure, and the residue crystallized from MeOH to obtain **23** as white needles (2.55 g, 65%). The mother liquor was evaporated to dryness, the residue was extracted with hot EtOAc, and the solid which was insoluble in EtOAc was recrystallized from MeOH afford an additional amount of **23** (0.56 g (14%). The filter cake from the original reaction mixture was dried and the pivaloylation reaction repeated, yielding an additional 0.26 g (8%) of **23**; total yield 3.37 g (86%): mp 232–233 °C. IR (KBr) ν_{max} 3240, 3170, 2960, 1655, 1640, 1595, 1550, 1515, 1490, 1455, 1400, 1380, 1370, 1315, 1290, 1245 cm^{-1}. ^1H NMR ($CDCl_3$ + 2 drops DMSO-d_6) δ 1.35 (s, 9H, *t*-Bu), 2.47 (s, 3H, Me), 7.28 (d, J = 8 Hz, 1H, C7-H), 7.57 (d, J = 8 Hz, C8-H). Anal. calcd for $C_{14}H_{16}ClN_3O_2$: C, 57.24; H, 5.49; N, 14.30; Cl, 12.07. Found: C, 57.32; H, 5.54; N, 14.11; Cl, 11.86.

Di-*tert*-butyl *N*-(5-nitro-2-thenoyl)-L-glutamate (27).

Step 1. A stirred suspension of $KMnO_4$ (15.8 g, 0.10 mol) in 5% aq NaH_2PO_4 (150 mL) was added in a single portion to a stirred solution of **25** (15.7 g, 0.10 mol) in acetone (250 mL). The reaction was mildly exothermic. After the mixture was stirred at ambient temperature for 45 min, the brown MnO_2 precipitate was removed by filtration and the filter cake was washed with H_2O and acetone. Acetone was removed from the combined filtrates, the mixture was acidified with 2 N HCl, and the precipitated solid was filtered, dried in vacuo, and used directly in the next step. After being recrystallized from H_2O, the product melted at 153–155 °C (lit.[12a] 157–158 °C, lit.[12b] 157 °C, lit.[12b] 155–157 °C).

Step 2. Compound **26** (6.92 g, 0.04 mol) was added slowly (foaming!) to $SOCl_2$ (30 mL), and the reaction mixture refluxed for 30 min and evaporated under reduced pressure to obtain the acid chloride as a gum. The crude acid chloride was taken up directly in CH_2Cl_2 (50 mL) and added to a solution of di-*tert*-butyl L-glutamate hydrochloride (8.88 g, 0.03 mol) in CH_2Cl_2 (100 mL). The resulting solution was treated immediately with Et_3N (6.9 mL, 5.06 g, 0.05 mol), producing a moderate exotherm. After several min, the solvent was evaporated under reduced pressure and the residue partitioned between EtOAc and H_2O. The organic layer was washed with 0.5 N HCl, H_2O, 5% $NaHCO_3$, and brine, then evaporated to dryness to obtain diester **27** (11.4 g, 93%) as a brown gum suitable for use in the next reaction. The analytical sample was obtained by flash chromatography (30:1 w/w silica gel, 2:1 isooctane–EtOAc) and drying in vacuo at 65 °C over P_2O_5; TLC: R_f 0.3 (silica gel, 2:1 isooctane–EtOAc). IR (thin film) ν_{max} 3100, 2970, 2930, 2820, 1735, 1660, 1545, 1505, 1475, 1450, 1430, 1390, 1365, 1335, 1285, 1250 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.45 (s, 9H, *t*-Bu), 1.50 (s, 9H, *t*-Bu), 1.90–2.67 (m, 4H, β- and γ-CH_2), 4.63 (m, 1H, α-CH), 7.50 (d, J = 4 Hz, 1H, thiophene C4-H), 7.88 (d, J = 4H, 1H, thiophene C3-H). Anal. calcd for $C_{18}H_{26}N_2O_7S$: C, 52.16; H, 6.32; N, 6.76; S, 5.74. Found: C, 52.00; H, 5.98; N, 7.17; S, 5.45.

Di-*tert*-butyl *N*-(5-amino-2-thenoyl)-L-glutamate (28). Iron powder (14 g, 0.25 mol)[5a] was kept under 2 N HCl for 10 min with occasional swirling, then filtered, washed with H_2O, rinsed with acetone, and dried in vacuo. The activated iron and a catalytic amount of $FeSO_4 \cdot 7H_2O$ (4.63 g, 0.016 mol) were added to a solution of the nitro diester **27** (5.9 g, 0.014 mol) in a mixture of MeOH (75 mL) and H_2O (25 mL). After being refluxed for 24 h, the mixture was filtered through Celite on a bed of glass fiber paper, and the filtrate was concentrated to a small volume to remove the MeOH. The product was then partitioned between EtOAc and H_2O, and the organic layer was evaporated to a gum (5.27 g), which was purified by flash chromatography (silica gel, 60 g, 5 × 8 cm, 1:1 isooctane–EtOAc) to obtain **27** as a foam (3.28 g, 60%). The analytical sample was obtained by passing a 0.2 g portion of this material through a second column of flash-grade silica gel (10 g, 20:1 w/w), and drying the resulting gum in vacuo over P_2O_5 at 70 °C until it formed a glass that could be ground to a powder: mp 116–117 °C; TLC: R_f 0.3, turning orange on standing overnight (silica gel, 1:1 isooctane–EtOAc). IR (KBr) ν_{max} 3440, 3380, 3310, 3200, 2970, 2930, 1715, 1625, 1545, 1515, 1470, 1365, 1295, 1235 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.43 (s, 9H, *t*-Bu), 1.48 (s, 9H, *t*-Bu), 2.23 (m, 4H, β- and γ-CH_2), 4.22 (m, 1H, α-CH), 6.08 (d, J = 4 Hz, 1H, thiophene C3-H), 7.22 (d, J = 4 Hz, 1H, thiophene C4-H). Anal. calcd for $C_{18}H_{28}N_2O_5S$: C, 56.23; H, 7.34; N, 7.29; S, 7.74. Found: C, 56.32; H, 7.16; N, 7.17; S, 7.64.

***N*-[5-[*N*-(2-Amino-5-chloro-3,4-dihydro-4-oxoquinazolin-6-yl)methylamino]-2-thenoyl]-L-glutamic acid (6).**

Step 1. A suspension of **23** (669 mg, 2.23 mmol) in $CHCl_3$ (10 mL, previously passed through silica gel to remove EtOH) was treated with NBS (460 mg, 2.58 mmol) and 20 mg of Bz_2O_2, and the mixture was refluxed for 1 h. A second 20 mg portion of Bz_2O_2 was added, reflux was resumed for 20 h, and the solvent was evaporated to dryness under reduced pressure. The residue was partitioned between EtOAc and H_2O, a small amount of MeOH was added to dissolve any remaining solid, and the organic layer was separated, washed with brine, and evaporated to dryness to

obtain **24** as a white powder (837 mg, 99%), which was used directly in the next step; mp 179–180 °C (MeOH). IR (KBr) ν_{max} 3350, 3410, 3120, 2950, 2850, 1665, 1630, 1585, 1535, 1475, 1440, 1385, 1375, 1355, 1300, 1255, 1230 cm^{-1}. ^1H NMR ($CDCl_3$ + 2 drops DMSO-d_6) δ 1.35 (s, 9H, *t*-Bu), 4.72 (s, 2H, CH_2Br), 7.30 (d, $J=8$ Hz, 1H, C7-H, partly obscured by $CHCl_3$), 7.72 (d, $J=8$ Hz, 1H, C8-H).

Step 2. A solution of bromide **24** (372 mg, 1.0 mmol) in DMF (10 mL) was treated with amino diester **28** (386 mg, 1.0 mmol) and solid $NaHCO_3$ (168 mg, 2.0 mmol), and the mixture was stirred at room temperature for 2 days. The solvent was evaporated, and the residue partitioned between EtOAc and H_2O. The organic layer was evaporated under reduced pressure, and the residue purified by flash chromatography on silica gel (25 g, 2 × 20 cm, 3:2 isooctane–acetone) to obtain **29** as a glass (479 mg, 69%), which was used directly in the next step; TLC: R_f 0.3 (3:2 isooctane–acetone).

Step 3. A solution of diester **29** (439 mg, 0.916 mmol) in MeOH (20 mL) was cooled in an ice-bath and saturated with gaseous NH_3. The cooling bath was removed and the solution was kept at room temperature for 20 h in a stoppered flask. The solvent was evaporated, and the residue dissolved in CH_2Cl_2 (30 mL). The solution was cooled in an ice-bath and stirred while TFA (10 mL) was added dropwise over 5 min. The solution was kept at 5° for 20 h, then concentrated to dryness by rotary evaporation. The residue was taken up into a small volume water to which enough 1 N NaOH and 10% AcOH were added to bring the solution to pH 8. After removing a small amount of insoluble material, the filtrate was passed through a preparative HPLC column (7% MeCN in 0.1 M NH_4OAc, pH 6.9; flow rate 10 mL/min; 335 nm). The largest peak, whose elution time on an analytical column (flow rate 1.0 mL/min) was 21 min, was collected and freeze-dried. The resulting product was taken up dilute NH_4OH and passed through a DEAE-cellulose column (1.5 × 17 cm, HCO_3^{-1} form), which was eluted first with H_2O, then with 0.2 M NH_4HCO_3, and finally with 0.4 M NH_4HCO_3 adjusted to pH >10 with ammonia. Pooled fractions containing the desired product were concentrated by rotary evaporation and lyophilization to obtain **6** as a white solid (76 mg, 15%): mp >300 °C. IR (KBr) ν_{max} 3340, 2970, 1705, 1655, 1600, 1555, 1515, 1470, 1400, 1340, 1290, 1260 cm^{-1}. ^1H NMR (DMSO-d_6, 200 MHz) δ 1.77 (m, 2H, β-CH_2), 2.16 (m, 2H, γ-CH_2), 4.12 (m, 1H, α-CH), 4.25 (m, bridge CH_2), 5.72 (d, $J=4$ Hz, thiophene C4-H), 6.46 (br s, 2H, NH_2), 7.04 (d, $J=8$ Hz, 1H, C8-H), 7.29 (d, $J=4$ Hz, 1H, thiophene C3-H), 7.33 (m, 1H, CONH), 7.44 (d, $J=8$ Hz, 1H, C7-H), 7.64 (m, 1H, CONH). UV (pH 7.4) λ_{max} 233 nm (ε 36,900), 273sh (8890), 335 (19,000); (0.1 N NaOH) 234 (37,200), 279 (11,100), 337 (20,100); (0.1 N HCl) 228 (32,200), 235 (30,300), 335 (15,300); MS: *m/e* 478 (M−1, ^{35}Cl), 479 (M, ^{35}Cl), 480 (M−1, ^{37}Cl), 481 (M, ^{37}Cl). Anal. calcd for $C_{19}H_{18}ClN_5O_6S.0.5NH_3{\cdot}3.5H_2O$: C, 41.38; H, 3.84; N, 13.97; S, 5.81. Found: C, 41.51; H, 4.41; N, 14.23; S, 5.72.

5-Chloro-2,6-dimethylbenzo[*d*][1,3]oxaxine-4-one (31). A mixture of the anthranilic acid **9** (5.57 g, 0.03 mol) and Ac_2O (50 mL) was refluxed for 1 h and evaporated to dryness under reduced pressure. Recrystallization from isooctane and EtOAc yielded straw-colored crystals (5.13 g, plus a second crop of 0.29 g; total 87%); mp 180–181 °C. IR (KBr) ν_{max} 1925, 1750, 1660, 1590, 1555, 1465, 1335, 1395, 1370, 1295, 1275, 1255, 1215 cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.43 (s, 3H, 2- or 6-Me), 2.50 (s, 3H, 2- or 6-Me), 7.38 (d, $J=8$ Hz, C8-H), 7.68 (d, $J=8$ Hz, C7-H). Anal. calcd for $C_{10}H_8ClNO_2$; C, 57.30; H, 3.85; N, 6.68; Cl, 16.91. Found: C, 57.41; H, 4.01; N, 6.51; Cl, 17.04.

5-Chloro-2,6-dimethyl-3,4-dihydro-4-oxoquinazoline (33). A suspension of the oxazinone **31** (2.10 g, 0.01 mol) in anhydrous NH_3 (35 mL) in a dry ice-acetone bath was stirred for 1.5 h and allowed to warm to room temperature overnight. The residue was then refluxed with 1 N NaOH (25 mL) for 1 h, and the solution cooled in an ice-bath and acidified with glacial AcOH. The precipitate was collected, washed thoroughly with H_2O, and freeze-dried. Recrystallization from MeOH (250 mL) gave **33** as white needles (1.18 g). Concentration of the mother liquor afforded a second crop (0.87 g); total yield 2.05 g (99%): mp 296–299 °C. IR (KBr) ν_{max} 3170, 3060, 3030, 2920, 2890, 2675, 1635, 1600, 1545, 1500, 1465, 1445, 1375, 1305, 1270, 1230 cm^{-1}. ^1H NMR (DMSO-d_6) δ 2.30 (s, 3H, 6-Me), 2.40 (s, 3H, 2-Me), 7.42 (d, $J=8$ Hz, C8-H), 7.73 (d, $J=8$ Hz, 1H, C7-H). Anal. calcd for $C_{10}H_9ClN_2O$: C, 57.57; H, 4.35; N, 13.43; Cl, 16.99. Found: C, 57.58; H, 4.32; N, 13.41; Cl, 16.77.

***N*-[5-[*N*-(2-Methyl-5-chloro-3,4-dihydro–2-methyl-4-oxoquinazolin-6-yl)methylamino]-2-thenoyl]-L-glutamic acid (7).**

Step 1. A mixture of **33** (209 mg, 1.0 mmol), NBS (178 mg, 1.0 mmol), and 20 mg of Bz_2O_2 (20 mg) in $CHCl_3$ (20 mL) that had been passed through 7 g of silica gel to remove EtOH was refluxed for 1 h, then treated with another 10 mg of Bz_2O_2, and refluxed again for 8 h. The heavy precipitate was collected and washed with $CHCl_3$ to obtain bromide **30** (221 mg, 77%) as a white solid that was used in the next step without purification: mp >300 °C. IR (KBr) ν_{max} 3170, 3070, 3020, 2970, 2950, 2920, 2880, 1675, 1630, 1595, 1545, 1495, 1435, 1425, 1375, 1300, 1270, 1225, 1210 cm^{-1}. ^1H NMR (DMSO-d_6) δ 2.33 (s, 3H, 2-Me), 4.87 (s, 2H, CH_2Br), 7.52 (d, $J=8$ Hz, 1H, C8-H), 7.97 (d, $J=8$H, C7-H).

Step 2. A mixture of the impure bromide **30** (144 mg, 0.5 mmol), amine **28** (192 mg, 0.5 mmol), and $NaHCO_3$ (84 mg, 1.0 mmol) in DMF (5 mL) was stirred at room temperature for 4 days, during which a clear solution was formed. After removal of the solvent under reduced pressure, the residue was partitioned between EtOAc and H_2O. TLC (silica gel, 2:3 isooctane–Me_2CO) revealed a dark spot with R_f 0.25 (turning yellow on standing overnight), corresponding to the desired diester **34**, and additional spots with R_f 0.6 (turning orange on standing overnight), 0.4, and 0.05. The EtOAc layer was evaporated and the residue chromatographed (flash

grade silica gel, 11 g, 1.5 × 16 cm, 2:3 isooctane–Me_2CO) to obtain an off-white solid (165 mg, 56%). IR (KBr) ν_{max} 3300, 3070, 2970, 2930, 1730, 1675, 1630, 1595, 1550, 1510, 1460, 1395, 1365, 1335, 1295 1255 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.40 (s, 9H, *t*-Bu), 1.47 (s, 9H, *t*-Bu), 2.10–2.40 (m, 4H, β- and γ-CH_2), 2.52 (s, 3H, 2-Me), 4.57 (m, 3H, bridge CH_2 and α-CH), 5.88 (d, $J=4$ Hz, 1H, thiophene C3-H), 6.75 (d, $J=8$ Hz, 1H, C8-H), 7.28 (d, $J=4$ Hz, 1H, thiophene C4-H), 7.60 (d, $J=8$ Hz, 1H, C7-H). A high-field ^1H NMR signal at δ 0.8 showed the sample to contain occluded isooctane. This material was used directly in the next step without elemental analysis.

Step 3. Diester **34** (311 mg, 0.526 mmol) was dissolved in CH_2Cl_2 (15 mL), cooled in an ice-bath, and treated dropwise with TFA (5 mL) over 5 min. After being kept at 5 °C for 20 h, the solution was evaporated to dryness under reduced pressure and the residue was taken up in dilute NaOH. The solution adjusted to pH <9 with 10% AcOH and the product isolated by preparative HPLC (C18 silica gel, 7% MeCN in 0.1 M NH_4OAc, pH 6.9, 10 mL/min, 335 nm). The largest peak, whose elution time on an analytical column (flow rate 1.0 mL/min) was 33 min, was collected and concentrated to dryness by rotary evaporation followed by freeze-drying. Further purification by ion-exchange chromatography, as described above for compound **6**, afforded **7** (116 mg, 39%) as a pale-yellow solid; mp >300 °C. IR (KBr) ν_{max} 3200, 3070, 2930, 1675, 1625, 1595, 1550, 1510, 1460, 1395, 1335, 1205 cm^{-1}. ^1H NMR (200 MHz, DMSO-d_6) δ 1.77 (m, 2H, β-CH_2), 2.17 (m, 2H, γ-CH_2), 2.24 (s, 3H, 2-Me), 4.16 (m, 1H, α-CH), 4.36 (m, 2H, bridge CH_2), 5.75 (d, $J=4$ Hz, 1H, thiophene C4-H), 7.31 (d, $J=4$ Hz, 1H, thiophene C3-H), 7.43 (d, $J=8$ Hz, 1H, C8-H), 7.50 (m, 1H, CONH), 7.66 (d, $J=8$ Hz, 1H, C7-H). UV: λ_{max} (pH 7.4) 232 nm (ε 32,800), 238sh (28,800), 270 (9200), 277 (9100), 331 (22,100); (0.1 N NaOH) 232 (28,200), 283sh (10,600), 291 (12,100), 334 (23,000); (0.1 N HCl) 240 (22,200), 280 (6200), 336 (15,700); MS: *m/e* 477 (M−1, ^{35}Cl), 478 (M, ^{35}Cl), 479 (M−1, ^{37}Cl), 480 (M, ^{37}Cl). Anal. calcd for $C_{20}H_{19}ClN_4O6S{\cdot}NH_3{\cdot}3.5H_2O$: C, 42.97; H, 5.23; N, 12.53; S, 5.74. Found: C, 42.87; H, 4.74; N, 12.43; S, 5.45.

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