Synthesis and Antitumor Activity of Thieno-Separated Tricyclic Purines

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Received August 2, 2000

The purine ring system is undoubtedly one of the most ubiquitous heterocyclic ring systems in nature as it has the distinction of being the parent ring in countless derivatives of biological relevance. It is not surprising then that modified purines possess the potential to impact several areas, including a better understanding of the biological effects of DNA damaging agents, enzyme/substrate interactions, and in the development of more potent medicinal agents. One focus for our research at Georgia Tech has centered around the design and synthesis of a series of extended purine analogues containing a heterocyclic spacer ring, with sites set on investigations into their use as (i) potential anticancer and antiviral agents, (ii) dimensional probes for enzyme and coenzyme binding sites, and (iii) structural probes of the minor groove of DNA. The synthesis and preliminary antitumor activity of two thieno-separated purine analogues are described herein. Tricyclic **1** was synthesized in 12 steps from tribromoimidazole and with an overall yield of 7%. Tricyclic **2** was synthesized in 9 steps with an overall yield of 13%. Both **1** and **2** exhibited growth inhibitory effects on HCT116 colorectal cancer cells in vitro.

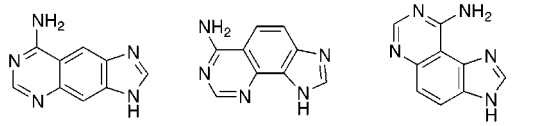
Background and Significance

DNA, the primary genetic material, is a key participant in a wide range of biological processes, and disruption of its function has a detrimental effect in many areas, in particular, on cell growth.[1−3] Most anticancer agents interact directly with DNA. Others block the synthesis of DNA or are incorporated into DNA, interfering with function. Furthermore, drugs targeted toward the nucleic acids act at the earliest possible stage of gene expression and should, therefore, be biologically significant. Understanding the structural interactions of DNA damaging agents and the minor groove of DNA will give direction toward the synthesis of more effective anticancer agents.

In that regard, understanding the structural interactions between enzymes and their substrates or cofactors is also vital to the design of more effective antiviral agents. Leonard and co-workers[4,5] designed a series of extended ring systems (such as depicted in Chart 1) for the purpose of testing the dimensional restrictions of enzyme binding sites.[6−9] These extended purine analogues, such as *lin*-benzoadenine, *dist*-benzoadenine, and *prox*-benzoadenine, have several interesting properties: (i) they exhibit strong enzyme binding, activation, and inhibition; (ii) they act as cofactors in enzymatic reactions; (iii) many of them exhibit fluorescent[10,11] properties, which can help define their environment.

Although extended purine analogues differ electronically, which results in changes in basicity, nucleophilicity, and π-stacking, the similarities in the peripheral rings allow these analogues to retain the necessary hydrogen-bonding elements required for molecular recognition. This affords them the ability to function as dimensional probes, thereby making it possible to investigate the parameters available for the purine moiety in enzyme−coenzyme binding sites, as well as to help define the structural role of the purine ring with respect to the minor groove of DNA. In addition, their inherent structural resemblance to biologically significant purines should endow them with similar medicinal traits. Interest in extended purines has continued,[12−14] but there have been few reports[15−17] of tricyclic analogues containing a heterocyclic spacer ring.

Chart 1



In that regard, the thiophene ring is considered to be bioisosteric with the benzene ring; however, when used as an internal spacer, it imparts an arc to the heterocyclic moiety, not unlike Leonard's *dist*-benzoadenine shown in Chart 1. As noted by Leonard, the insertion of a benzene ring expands the purine ring system by 2.4 Å at both points of attachment; the thiophene spacer, however, causes an expansion of 2.9 and 1.5 Å between the imidazole and pyrimidine rings.

Few examples of the use of the thiophene as a spacer have been published,[15,17−20] and several possess interesting medicinal properties. To date, however, none of these examples have contained the desired peripheral purine ring components of adenine and guanine. On the basis of the strong interest in structural analogues of adenine and guanine for use in medicinal chemistry,

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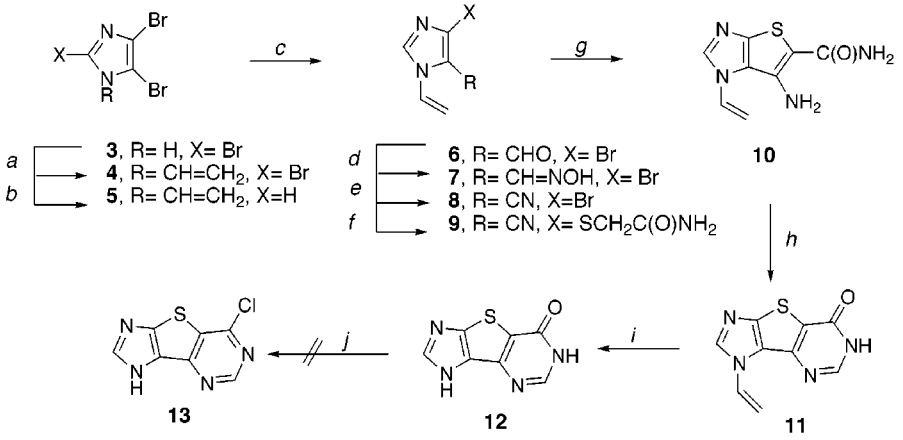
10.1021/jm000326i CCC: $19.00
Published on Web 11/11/2000

Scheme 1



Reaction conditions: *a* , 50% NaOH, Bu_4NBr, 1,2-dibromoethane; *b* , (i) EtMgBr; (ii) H_2O; *c*, (i) EtMgBr; (ii) DMF; *d*, NH_2OH-HCl; *e*, Ac_2O; *f*, $NH_2C(O)CH_2SH$, K_2CO_3; *g*, NaOEt, EtOH; *h*, $(EtO)_2CHOAc$; *i*, $KMnO_4$, acetone; *j*, $POCl_3$, pyridine.

Chart 2

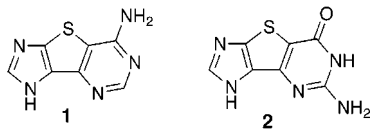
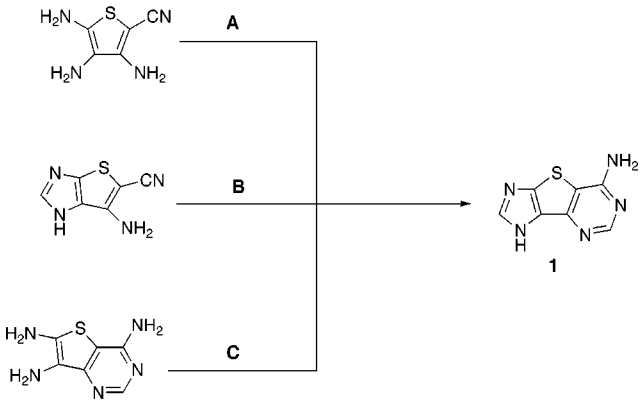


Chart 3



combined with the interesting properties discovered by Leonard for his extended purines, we proceeded with this modification.

Chemistry

The targets chosen for our study are shown in Chart 2, with initial focus on the adenine analogue **1**. In analyzing the possible approaches to **1**, we contemplated the three intermediates shown in Chart 3. The "thiophene pathway" (A) and the "pyrimidine pathway" (C) were both unattractive to us.[19−21] Pathway B was chosen, however, due to a recent report[22] of the synthesis of an intermediate similar to the one shown for pathway B, and we felt this could be readily adapted to meet our needs.

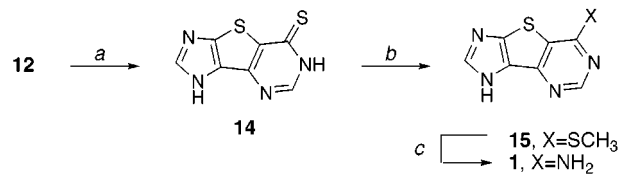
Starting with tribromoimidazole,[23] protection of the imidazole nitrogen was first undertaken. Careful consideration as to the choice of protecting group for this step was critical, since the literature indicated that deprotection of this imidazole nitrogen was difficult at best. Originally we explored two reported procedures[24,25] that showed deprotection of the vinyl group to be more facile than other groups previously tried. This method was initially used on the synthesis of tricyclic target **1**, but unfortunately it proved to be less than satisfactory as well. Subsequently, during the synthesis of **2**, it became apparent that the vinyl group could not be used due to the incompatibility with the conditions of the deprotection method. We then altered our approach to utilize the allyl group instead. The removal of the allyl group has proven to be much more facile.[26,27]

As shown in Scheme 1, treatment of the tribromoimidazole **3**[23] with 50% NaOH, tetrabutylammonium bromide, and 1,2-dibromoethane under reflux conditions produced **4** in 90% yield.[24] Removal of the C-2 bromine of **4** was accomplished with ethylmagnesium bromide and H_2O to form **5** in 90% yield.

Initially it was thought that the nitrile intermediate **8** could be formed directly[28] from displacement of the C-5 bromine with potassium cyanide or lithium cyanide, but all attempts failed. Dibromoimidazole **5** was once again treated with ethylmagnesium bromide in ether followed by addition of *N*-formylpiperidine to provide the aldehyde **6** in 40% yield.[24] Despite a reported[24] yield of 65%, all attempts at optimization in our hands failed to give any better than 40%. The major product (50%) of this reaction was 4-bromo-1-vinylimidazole. In an attempt to improve the yield, *N*,*N*-dimethylformamide (DMF) was then tried in place of *N*-formylpiperidine, and this provided **6** in 60% yield. (Note: Although no NMR data have been published on **6**, Iddon et al.[24] compared it to analogous compounds they had previously synthesized,[29,30] in particular, a benzyl-protected analogue, and found the formylation to have occurred only at the C-5 position. This is not surprising given the reactivity trend of imidazoles to undergo reaction at C-5 prior to C-4.)

Treatment of **6** with hydroxylamine hydrogen chloride and sodium bicarbonate in ethanol produced oxime **7** in 93% yield.[29,30] Subsequent dehydration of **7** with acetic anhydride under reflux gave nitrile **8** in 95% yield. Next, sequential addition (2 equiv, followed by an additional 2 equiv after 1 h) of excess potassium carbonate and thioglycolamide[31] (obtainable in one step from commercially available methyl thioglycolate with

Scheme 2



Reaction conditions: *a*, P_2S_5, anhydrous pyridine, reflux; *b*, K_2CO_3, CH_3I, MeOH; *c*, anhydrous butanol, NH_3, 160 °C, 90 h.

96% yield) afforded **9** (90%). Cyclization of **9** with sodium ethoxide in ethanol gave the appropriately substituted bicyclic intermediate **10** (90%). Ring closure of the third ring was accomplished with diethoxymethyl acetate[32] to provide **11** in 80% yield.

Several methods[21,24] for removal of the vinyl protecting group were then tried, but all proved poor at best. Finally, a modification of the reported[24] procedure employing potassium permanganate and acetone under reflux conditions was utilized. Initially the literature workup and purification for this method proved problematic, resulting in very low yields. However, following the reaction with potassium permanganate and acetone, the mixture was evaporated and the residue treated with acetic acid under reflux conditions to provide the tricyclic hypoxanthine intermediate **12** in 60% yield. Despite numerous efforts to improve the conversion, 60% was the optimal yield that could be achieved. However, since the other 40% was unreacted **11** it could be recovered. Conversion of the oxo moiety to the chloro derivative **13** was then tried using standard conditions with phosphorus oxychloride, but all attempts resulted in decomposition, so an alternative route[4,33,34] was then considered. As shown in Scheme 2, conversion of **12** to the thione derivative **14** was achieved by treatment with phosphorus pentasulfide in dry pyridine, which was immediately converted to its *S*-methyl derivative **15**. Finally, **15** was heated for 90 h with a saturated butanolic ammonia solution to provide the adenine analogue **1** in 12 steps and 7% overall yield.

The second target **2** was envisioned in an analogous fashion, but it was immediately apparent that the oxidative deprotection method used to remove the vinyl group would oxidize the ring present in the guanine analogue. Therefore, an alternative protecting group was sought. After considering several options, the allyl group was selected due to the discovery of a mild deprotection procedure[26] utilizing palladium and phenylsilane, and the synthesis of **2** was then begun.

In an analogous fashion as was carried out for the synthesis of **1**, treatment of **3** with allyl bromide[35] produced **16** in 99% yield (Scheme 3). Removal of the C-2 bromine was accomplished as before to give **17** (90%). Formation of the Grignard, followed by addition of DMF, produced aldehyde **18** (93%). Treatment of **18** gave oxime **19** (63%), which was then converted to the nitrile **20** in 92% yield. Addition of the thiene moiety was accomplished as previously carried out with thioglycolamide[31] to give **21** (74%), which underwent ring closure to afford bicyclic intermediate **22** in 92% yield. Formation of the tricyclic **23** was accomplished with freshly prepared chloroformamidine hydrochloride and methyl sulfone in 58% yield.[14] Removal of the allyl group was achieved employing tetrakis(triphenylphosphine)-palladium(0) and phenylsilane[26] to give the desired guanine analogue **2** (69%).

Tricyclic **1** was synthesized in 12 steps from tribromoimidazole and with an overall yield of 7%. Tricyclic **2** was synthesized in 9 steps with an overall yield of 13%.

Table 1. Tricyclic Compound-Induced Inhibition of HCT116 Growth[a]

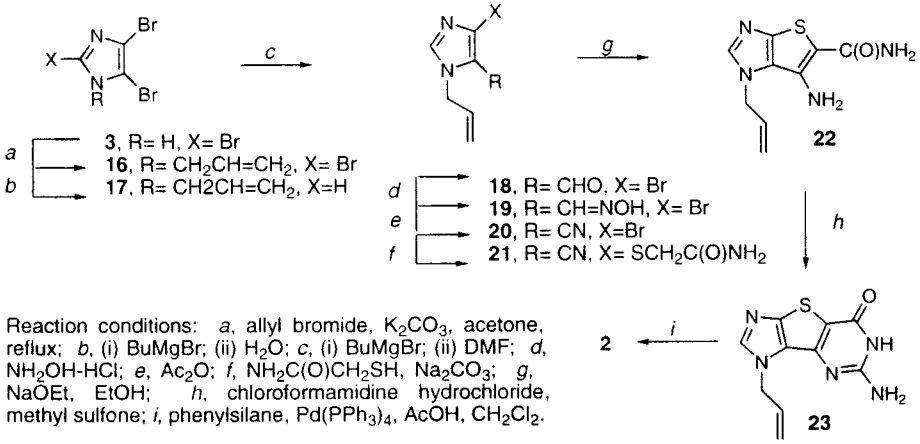
time (h)	compd	0.1 μM	1 μM	10 μM	100 μM
24	**1**	96.1 ± 3.8	106.6 ± 4.8	104.8 ± 3.8	82.0 ± 7.8
	2	98.7 ± 5.7	96.7 ± 3.4	93.2 ± 7.1	75.8 ± 8.9
48	**1**	105.1 ± 3.3	98.3 ± 4.3	105.0 ± 2.1	71.5 ± 3.8[c]
	2	96.8 ± 2.7	95.9 ± 3.3	85.0 ± 2.8[b]	77.0 ± 4.4[b]
72	**1**	101.3 ± 7.3	96.1 ± 6.6	93.8 ± 12.1	51.5 ± 10.7[b]
	2	106.6 ± 4.4	116.7 ± 1.9[b]	99.5 ± 1.3	76.1 ± 3.5[b]

[a] HCT116 cells were treated and growth was assessed as described in the text. Data represent the average ± SEM as a % of control-treated (DMSO) cells ($n = 3-5$). [b] $p < 0.05$. [c] $p < 0.005$ when compared to control-treated cells.

Results

We initially sought to assess the effect of these tricyclic compounds on the growth of HCT116 colorectal cancer cells in vitro. Both **1** and **2** inhibited growth in a dose- and time-dependent manner with **1** exhibiting greater potency after 72 h of treatment (Table 1). Interestingly, **2** also inhibited growth in these cells, with significant inhibitions being observed at lower doses (10 μM) after 48 h of treatment. However, this affect was not sustained for 72 h. In fact, lower doses (1 μM) of **2** produced a slight, but statistically significant, stimulatory effect on growth of HCT116 cells.

Scheme 3



Reaction conditions: *a*, allyl bromide, K_2CO_3, acetone, reflux; *b*, (i) BuMgBr; (ii) H_2O; *c*, (i) BuMgBr; (ii) DMF; *d*, NH_2OH-HCl; *e*, Ac_2O; *f*, $NH_2C(O)CH_2SH$, Na_2CO_3; *g*, NaOEt, EtOH; *h*, chloroformamidine hydrochloride, methyl sulfone; *i*, phenylsilane, $Pd(PPh_3)_4$, AcOH, CH_2Cl_2.

Discussion

Tricyclic **1** and **2** both exhibited growth inhibitory effects on HCT116 colorectal cancer cells in vitro. Our data suggest that **1** is slightly more potent at inhibiting growth in these cells than **2**, with approximately 50% inhibition at 72 h. The reason behind these differences in the dose responses following 72 h of treatment is unknown at this point but may be due to the fact that **1** is a modified adenine analogue. Along these lines, we have demonstrated that an adenine-containing nucleoside, 8-chloroadenosine, potently inhibits growth in these cells.[36] Coupling of **1** or **2** to a *ribo*-sugar to create the thieno-separated nucleosides may increase the growth inhibitory properties of these analogues reported herein. We are actively pursuing this theory as well as other possibilities at this time.

Experimental Section

General. Melting points were recorded on a Meltemp II melting point apparatus and are uncorrected. Combustion analyses were performed by Atlantic Microlabs, Inc., Atlanta, GA. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 300 spectrometer (operated at 300 and 75 MHz, respectively) all referenced to internal tetramethylsilane (TMS) at 0.0 ppm. The spin multiplicities are indicated by the symbols s (singlet), d (doublet), t (triplet), m (multiplet) and b (broad). UV−vis activity was measured on a Shimadzu 1601 UV/Vis spectrophotometer. Reactions were monitored by thin-layer chromatography (TLC) using 0.25-mm Whatman Diamond silica gel 60-F_{254} precoated plates with visualization by irradiation with a Mineralight UVGL-25 lamp. Column chromatography was performed on Whatman silica, 200−400 mesh, 60 Å, and elution with the indicated solvent system. HPLC purification was carried out on a Hewlett-Packard 1090 liquid chromatograph. Yields refer to chromatographically and spectroscopically (^{1}H and ^{13}C NMR) homogeneous materials. All cell culture reagents were obtained from Media-Tech/Cellgro (Herndon, VA), and Fisher Scientific (Pittsburgh, PA). HCT116 colorectal cancer cells were purchased from ATCC (Bethesda, MD).

2,4,5-Tribromo-1-vinylimidazole (4). To a stirred, 0 °C solution of 2,4,5-tribromoimidazole[23] (**3**) (10.0 g, 32.8 mmol) and tetrabutylammonium bromide (10.5 g, 32.8 mmol, 20.1 mL 50% aqueous solution) in 1,2-dibromoethane (30 mL) was added dropwise 50% aqueous sodium hydroxide (30 mL).[24] The reaction was refluxed with vigorous stirring for 3 h. The mixture was then cooled, diluted with H_2O (50 mL) and extracted with methylene chloride (50 mL × 3). The organic layers were combined, washed with 10% HCl (50 mL), then H_2O (50 mL), dried ($MgSO_4$) and evaporated under reduced pressure. The residue was purified via column chromatography eluting with hexane:ethyl acetate (100:3), to give the protected imidazole **4** (9.8 g, 90%) as a white crystalline product following recrystallization with hexane: mp 36 °C; ^{1}H NMR ($CDCl_3$) δ 5.56 (dd, 2.1 Hz, 9.0 Hz, 1H), 5.74 (dd, 2.1 Hz, 16.0 Hz, 1H), 6.68 (dd, 9.0 Hz, 16.0 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 105.6, 116.8, 118.6, 118.7, 129.4. Anal. ($C_5H_3Br_3N_2$) C, H, N.

4,5-Dibromo-1-vinylimidazole (5). To a stirring, room temperature solution of **4** (16.55 g, 50 mmol) in dry ether (500 mL) was added dropwise ethylmagnesium bromide (16.7 mL, 3.0 M in ether, 50 mmol) over a period of 5 min.[24] The solution was stirred for 3 h at which point an additional portion of ethylmagnesium bromide (8.3 mL, 25 mmol, 3.0 M in ether) was added and stirred for 25 min. The solvent was then removed by rotary evaporation, cold water added (400 mL), and extracted with methylene chloride (3 × 150 mL). The organic layers were combined, dried (Na_2SO_4) and evaporated to afford, after recrystallization with hexane, 11.3 g (90%) of **5** as a white solid: mp 55 °C; ^{1}H NMR ($CDCl_3$) δ 5.14 (dd, 2.1 Hz, 9.0 Hz, 1H), 5.48 (dd, 2.1 Hz, 16.0 Hz, 1H), 6.82 (dd, 9.0 Hz, 16.0 Hz, 1H), 7.79 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 103.1, 107.2, 117.17, 128.4, 136.3. Anal. ($C_5H_4Br_2N_2$) C, H, N.

4-Bromo-1-vinylimidazole-5-carbaldehyde (6). To a stirring, room temperature solution of **5** (6.14 g, 24.3 mmol) in dry ether (200 mL) under nitrogen was added dropwise ethylmagnesium bromide (8.5 mL, 25.5 mmol, 3.0 M in ether) and the mixture was stirred for 4 h, at which point DMF (2.0 mL, 25.6 mmol) was added dropwise and the solution stirred for an additional 3 h. Following addition of H_2O (400 mL), the ether layer was separated and the aqueous layer extracted with methylene chloride (3 × 100 mL). The organic layers were combined, washed with brine, dried ($MgSO_4$) and evaporated to give a dark syrup. The residue was purified via column chromatography eluting with hexane:ethyl acetate (2:1) to give **6** as a white crystalline solid (2.94 g, 60%): mp 92−93 °C; ^{1}H NMR ($CDCl_3$) δ 5.19 (dd, 1.8 Hz, 8.5 Hz, 1H), 5.47 (dd, 1.8 Hz, 15.9 Hz, 1H), 7.56 (dd, 8.5 Hz, 15.9 Hz, 1H), 7.79 (s, 1H), 9.80 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 107.9, 125.2, 128.6, 129.6, 140.1, 179.5. Anal. ($C_6H_5BrN_2O$) C, H, N.

4-Bromo-1-vinylimidazole-5-aldoxime (7). A solution of hydroxylamine hydrochloride (14.48 g, 208 mmol) and sodium hydrogen carbonate (17.73 g, 211 mmol) in H_2O (50 mL) was added to 1-vinyl-4-bromo-5-carbaldehyde (**6**; 6.10 g, 30.17 mmol) in ethanol (280 mL) and the solution stirred overnight.[22] The solvent was removed by rotary evaporation and H_2O (50 mL) added to the residue. The aqueous layer was extracted with ethyl acetate (50 mL × 3) and the organic layers were combined, washed with brine (50 mL), dried ($MgSO_4$) and evaporated to give a colorless syrup. The residue was purified via column chromatography eluting with methylene chloride: ethyl acetate (95:5) to give the oxime **7** as a white crystalline solid (6.2 g, 93%): mp 184 °C; ^{1}H NMR (DMSO-d_6) δ 5.10 (dd, 1.5 Hz, 8.7 Hz, 1H), 5.66 (dd, 1.5 Hz, 15.6 Hz, 1H), 7.39 (dd, 8.7 Hz, 15.6 Hz, 1H), 7.99 (s, 1H), 8.30 (s, 1H), 11.68 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 106.0, 119.02, 121.3, 129.7, 137.1, 137.9. Anal. ($C_6H_6BrN_3O$) C, H, N.

4-Bromo-1-vinylimidazole-5-carbonitrile (8). A solution of oxime **7** (5.8 g, 26.85 mmol) in acetic anhydride[22] (150 mL) was heated under reflux for 2 h, at which point the excess acetic anhydride was removed by rotary evaporation to give a reddish brown oil. The residue was coevaporated with toluene (2 × 50 mL) and then purified by column chromatography eluting with hexane:ethyl acetate (3:1) to give nitrile **8** (5.0 g, 94%) as a white crystalline solid following recrystallization with hexane: mp 83−85 °C; ^{1}H NMR ($CDCl_3$) δ 5.31 (dd, 2.4 Hz, 8.7 Hz, 1H), 5.74 (ddd, 0.9, 2.4 Hz, 15.6 Hz, 1H), 6.90 (dd, 8.7 Hz, 15.6 Hz, 1H), 7.73 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 103.4, 108.4, 110.4, 127.3, 128.1, 140.4. Anal. ($C_6H_4BrN_3$) C, H, N.

5-Cyano-*S*-(1-vinylimidazol-4-yl)thioglycolamide (9). A solution of nitrile **8** (123 mg, 0.62 mmol) in anhydrous DMF (25 mL) was added with stirring to a solution of freshly prepared thioglycolamide[31] (212 mg, 2.3 mmol) that had been treated with potassium carbonate (200 mg, 1.5 mmol) and the mixture heated for 12 h maintaining the temperature between 50 and 55 °C. The reaction mixture was cooled, evaporated to dryness, and the residue purified via column chromatography eluting with chloroform:ethanol (9:1) and the resulting solid purified further by recrystallization in ethanol to give **9** as a white crystalline solid (116 mg, 90%): mp 139 °C; ^{1}H NMR (DMSO-d_6) δ 3.75 (s, 2H), 5.22 (dd, 1.8 Hz, 9.0 Hz, 1H), 5.71 (dd, 1.8 Hz, 15.6 Hz, 1H), 7.05 (dd, 9.0 Hz, 15.6 Hz, 1H), 7.13 (br s, 1H), 7.53 (br s, 1H), 8.39 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 35.5, 100.6, 106.9, 111.1, 128.0, 140.4, 147.9, 169.0. Anal. ($C_8H_8N_4OS$) C, H, N, S.

6-Amino-1-vinylthieno[2,3-*d*]imidazole-5-carboxamide (10). A mixture of **9** (2.33 g, 11.2 mmol) and sodium ethoxide (220 mg, 1.0 mmol, 21% in EtOH) in absolute ethanol (150 mL) was heated under reflux for 1.5 h. The solvent was removed under vacuum to one-third of the original volume and the solution was then cooled in an ice bath. The precipitate that formed was filtered and washed with cold ethanol to afford **10** as a white crystalline solid (1.89 g, 81%): mp 179−181 °C; ^{1}H NMR (DMSO-d_6) δ 5.26 (dd, 1.8 Hz, 9.0 Hz, 1H), 5.65 (dd, 1.8 Hz, 15.6 Hz, 1H), 6.78 (br s, 1H), 6.92 (br s, 2H), 7.50 (dd, 9.0 Hz, 15.6 Hz, 1H), 8.48 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 100.0,

104.1, 125.7, 128.8, 138.6, 141.6, 143.7, 167.7. Anal. ($C_8H_8N_4OS$) C, H, N, S.

1-Vinylimidazo[4′,5′:4,5]thieno[3,2-*d*]pyrimidin-5(6*H*)-one (11). A mixture of **10** (180 mg, 0.86 mmol) and diethoxymethyl acetate[32] (0.5 mL, 3 mmol) was refluxed for 3 h, the excess diethoxymethyl acetate evaporated and the residue purified by flash chromatography eluting with ethyl acetate to afford, following recrystallization with ethanol, **11** as a white crystalline solid (153 mg, 81%): mp >250 °C dec; ^{1}H NMR (DMSO-d_6) δ 5.26 (dd, 1.5 Hz, 9.0 Hz, 1H), 6.62 (dd, 1.5 Hz, 15.6 Hz, 1H), 7.45 (dd, 9.0 Hz, 15.6 Hz, 1H), 8.31 (s, 1H), 8.60 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 104.8, 121.3, 127.2, 128.8, 142.8, 145.0, 146.9, 149.8, 157.7. Anal. ($C_9H_6N_4OS\cdot{}^1/_8$EtOH) C, H, N, S.

Imidazo[4′,5′:4,5]thieno[3,2-*d*]pyrimidin-5(6*H*)-one (12). A solution of **11** (109 mg, 0.5 mmol), anhydrous acetone (50 mL) and potassium permanganate[24] (158 mg, 1.0 mmol) was heated for 6 h under reflux. To this was then added an additional portion of potassium permanganate (79 mg, 0.5 mmol) and the mixture allowed to reflux for an additional 12 h. The solvent was removed by rotary evaporation, and the brown residue was dissolved in glacial acetic acid (50 mL) and refluxed for 2 h. The mixture was cooled and evaporated to dryness and the residue purified by column chromatography eluting with methylene chloride:ethanol (4:1) to give **12** as an off-white solid (57 mg, 60%) following recrystallization with ethanol: mp >250 °C dec; ^{1}H NMR (DMSO-d_6) δ 8.23 (s, 1H), 8.25 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 120.2, 129.1, 143.6, 144.1, 147.1, 150.0, 158.0. Anal. ($C_7H_4N_4OS\cdot{}^3/_8H_2O$) C, H, N, S.

Imidazo[4′,5′:4,5]thieno[3,2-*d*]pyrimidine-5(6*H*)-thione (14). A solution of phosphorus pentasulfide[4] (400 mg), dry pyridine (30 mL) and **12** (192 mg, 1.0 mmol) was refluxed for 24 h, cooled, evaporated to dryness under vacuum, and the residue purified by column chromatography eluting with methylene chloride:methanol (4:1) to afford **14** as a pale yellow solid (180 mg, 87%: mp >250 °C dec; ^{1}H NMR (DMSO-d_6) δ 8.41 (s, 1H), 8.24 (s, 1H); which was used directly without further purification.

6-(Methylsulfonyl)imidazo[4′,5′:4,5]thieno[3,2-*d*]pyrimidine (15). To a stirring solution of **14** (104 mg, 0.5 mmol) and potassium carbonate (100 mg, 0.72 mmol) in methanol (20 mL) was added methyl iodide (140 mg, 1.0 mmol).[4] After 10 min the solvent was removed by rotary evaporation and the residue purified via column chromatography eluting with methylene chloride:methanol (7:1) to give **15** (135 mg, 90%) as a white crystalline solid following recrystallization from anhydrous ethanol: mp 308 °C; ^{1}H NMR (DMSO-d_6) δ 2.73 (s, 3H), 3.14 (s, 1H), 8.33 (s, 1H), 8.97 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 48.6, 127.7, 144.6, 144.9, 145.8, 153.6, 153.7, 162.7. Anal. ($C_8H_6N_4S_2$) C, H, N, S.

6-Aminoimidazo[4′,5′:4,5]thieno[3,2-*d*]pyrimidine (1). A solution of 6-(methylsulfonyl)imidazo[4′,5′:4,5]thieno[3,2-*d*]-pyrimidine (**15**; 84 mg, 0.37 mmol) in saturated butanolic ammonia (20 mL) was sealed in a steel bomb and heated at 160 °C for 90 h.[4] The solvent was removed by rotary evaporation and the residue purified on preparative TLC eluting with methylene chloride:methanol (4:1) to give **1** (41 mg, 56%) as an off-white solid: mp >330 °C dec; ^{1}H NMR (DMSO-d_6) δ 3.31 (s, 1H), 7.28 (s, 2H), 8.18 (s, 1H), 8.38 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 112.3, 128.2, 143.0, 145.5, 146.8, 154.3, 158.7; λ_{max} observed at 303 nm, $\epsilon = 1.05 \times 10^4$ M^{-1} cm^{-1}. Anal. ($C_7H_5N_5S\cdot{}^1/_8$EtOH) C, H, N, S.

2,4,5-Tribromo-1-allylimidazole (16). A mixture of **3** (40.0 g, 131 mmol), allyl bromide (12.6 mL, 144 mmol), and potassium carbonate (19.95 g, 144 mmol) in acetone (175 mL) was refluxed for 18 h.[35] After cooling, the mixture was filtered and the filtrate concentrated under reduced pressure. The residual oil was dissolved in methylene chloride (100 mL), filtered through a pad of silica gel and the silica gel washed with additional methylene chloride (200 mL). The filtrates were combined and evaporated to give **16** as a colorless crystalline solid (45.0 g, 99%), which was used directly without further purification: mp 30−32 °C; ^{1}H NMR ($CDCl_3$) δ 4.61 (dt, 1.8 Hz, 5.4 Hz, 2H), 5.06 (d, 17.0 Hz, 1H), 5.29 (d, 10.5 Hz, 1H), 5.82 (ddd, 5.4 Hz, 10.5 Hz, 20.4 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 50.0, 105.2, 116.6, 118.2, 118.9, 129.9.

4,5-Dibromo-1-allylimidazole (17). To a stirring solution of the protected imidazole **16** (50.0 g, 145 mmol) in dry ether (500 mL) was added dropwise butylmagnesium bromide (72.5 mL, 145 mmol, 2.0 M in ether) under N_2. The solution was stirred for 4 h. The ether was then removed by rotary evaporation, saturated NH_4Cl solution added (400 mL) to the residue, and extracted with methylene chloride (3 × 200 mL). The organic layers were combined, dried ($MgSO_4$), filtered over a bed of silica gel, and then evaporated to afford **17** (35.0 g, 90%) as a white crystalline solid: mp 58−59 °C; ^{1}H NMR ($CDCl_3$) δ 4.52 (dt, 1.5 Hz, 5.4 Hz, 2H), 5.06 (d, 19.0 Hz, 1H), 5.30 (d, 10.5 Hz, 1H), 5.88 (ddd, 5.4 Hz, 10.5 Hz, 18.3 Hz, 1H), 7.48 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 49.9, 103.8, 116.6, 119.3, 130.9, 137.0. Anal. ($C_6H_6Br_2N_2$) C, H, N.

4-Bromo-1-allylimidazole-5-carbaldehyde (18). To a stirring solution of **17** (26.6 g, 100 mmol) in dry ether (400 mL) under nitrogen was added dropwise at −78 °C butylmagnesium bromide (50.0 mL, 100 mmol, 2.0 M in ether). The mixture was allowed to warm to room temperature and then stirred for 4 h, at which point DMF (20 mL) was added and the solution stirred for an additional 18 h. The solvents were then removed under reduced pressure, saturated NH_4Cl solution added to the residue, and extracted with methylene chloride. The organic layers were combined, washed with brine, dried ($MgSO_4$), filtered over a bed of silica gel, and evaporated to give **18** as a pale yellow oil (20.1 g, 93%): mp 58−59 °C; ^{1}H NMR ($CDCl_3$) δ 4.91 (dt, 1.5 Hz, 5.4 Hz, 2H), 5.17 (d, 19.0 Hz, 1H), 5.29 (d, 10.5 Hz, 1H), 5.96 (ddd, 5.4 Hz, 10.5 Hz, 21.0 Hz, 1H), 7.58 (s, 1H), 9.76 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 49.9, 119.8, 126.6, 130.6, 131.8, 142.1, 179.6. Anal. ($C_7H_7BrN_2O$) C, H, N.

4-Bromo-1-allylimidazole-5-oxime (19). In an analogous fashion as was employed with **7**, 20.1 g (93.5 mmol) gave oxime **19** as a white crystalline solid (13.6 g, 63%): mp 172 °C; ^{1}H NMR (DMSO-d_6) δ 4.85 (dt, 1.5 Hz, 5.4 Hz, 2 H), 4.93 (dd, 1.5 Hz, 17.1 Hz, 1H), 5.16 (dd, 1.5 Hz, 10.5 Hz, 1H), 5.96 (ddd, 5.4 Hz, 10.5 Hz, 21.0 Hz, 1H), 7.81 (s, 1H), 7.94 (s, 1 H), 11.47 (s, 1 H). Anal. ($C_7H_8BrN_3O$) C, H, N.

4-Bromo-1-allylimidazole-5-carbonitrile (20). In an analogous fashion as was employed with conversion of **7** to **8**, oxime **19** (13.0 g, 56.5 mmol) was converted to the nitrile **20** (11.5 g, 92%) as a white crystalline solid: mp 36−38 °C; ^{1}H NMR ($CDCl_3$) δ 4.68 (d, 5.4 Hz, 2H), 5.32 (d, 16.8 Hz, 1H), 5.45 (d, 10.2 Hz, 1H), 5.95 (ddd, 5.4 Hz, 10.5 Hz, 23.0 Hz, 1H), 7.52 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 50.1, 106.3, 110.0, 121.6, 126.8, 130.1, 139.9. Anal. ($C_7H_6BrN_3$) C, H, N.

5-Cyano-1-allylimidazol-4-ylthioacetamide (21). Conversion of nitrile **20** (9.5 g, 45 mmol) was accomplished in the same fashion as was used for **8** to **9** to give **21** as a white crystalline solid (6.9 g, 74%): mp 141 °C; ^{1}H NMR (DMSO-d_6) δ 3.72 (s, 2 H), 4.73 (dt, 1.5 Hz, 5.4 Hz, 2H), 5.11 (dd, 1.5 Hz, 17.4 Hz, 1H), 5.29 (dd, 1.5 Hz, 10.5 Hz, 1H), 5.95 (ddd, 5.4 Hz, 10.5 Hz, 21.0 Hz, 1H), 7.13 (br s, 1H), 7.53 (br s, 1H), 8.06 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 49.3, 79.8, 103.9, 110.8, 119.3, 133.1, 142.7, 147.9, 169.2. Anal. ($C_9H_{10}N_4OS$) C, H, N, S.

6-Amino-1-allylthieno[2,3-*d*]imidazole-5-carboxamide (22). Compound **21** (5.1 g, 22.0 mmol) was converted to **22** in the same manner as was used for **9** to **10** to give a white crystalline solid (4.7 g, 92%): mp 179 °C; ^{1}H NMR (DMSO-d_6) δ 4.95 (d, 5.4 Hz, 2H), 5.01 (dd, 1.5 Hz, 17.1 Hz, 1H), 5.18 (dd, 1.5 Hz, 10.5 Hz, 1H), 6.04 (ddd, 5.4 Hz, 10.5 Hz, 18.9 Hz, 1H), 6.66 (br s, 2H), 6.82 (br s, 2H), 7.95 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 48.2, 100.1, 117.8, 127.4, 135.3, 139.3, 143.7, 145.6, 168.5. Anal. ($C_9H_{10}N_4OS$) C, H, N, S.

1-Allyl-2-aminoimidazo[4′,5′:4,5]thieno[3,2-*d*]pyrimidin-5(6*H*)-one (23). To a mixture of 6-amino-1-allylthieno[2,3-*d*]-imidazole-5-carboxamide (**22**) and methyl sulfone (5.6 g) was added freshly prepared chloroformamidine hydrochloride[14] (2.5 g, 11.3 mmol) and the mixture heated for 1.5 h at 100 °C with stirring. The mixture was allowed to cool and H_2O (5 mL) was added. The solution was then neutralized with NH_4OH and the solvents were removed under reduced pressure. The

residue was purified via column chromatography eluting with methylene chloride:methanol (15:1) to give **23** (1.6 g, 58%) as a white solid: mp >280 °C dec; ^{1}H NMR (DMSO-d_6) δ 4.99 (d, 5.4 Hz, 2H), 5.15 (dd, 1.5 Hz, 20.4 Hz, 1H), 5.20 (dd, 1.5 Hz, 10.5 Hz, 1H), 6.16 (ddd, 5.4 Hz, 10.5 Hz, 21.0 Hz, 1H), 6.53 (br s, 2 H), 8.09 (s, 1H), 11.0 (br s, 1H); ^{13}C NMR (DMSO-d_6) δ 48.5, 109.7, 118.8, 128.7, 134.5, 145.7, 146.4, 148.4, 155.5, 159.1. Anal. ($C_{10}H_9N_5OS\cdot{}^1/_4$EtOH) C, H, N, S.

2-Aminoimidazo[4′,5′:4,5]thieno[3,2-*d*]pyrimidin-5(6*H*)-one (2). Phenylsilane (1.0 mL, 8.2 mmol) was added dropwise to a mixture of the allyl protected guanine analogue **23** (200 mg, 0.81 mmol), palladium tetrakis (50 mg, 0.043 mmol) and glacial acetic acid (5 mL) in methylene chloride (10 mL) under Ar. The mixture was stirred overnight at 40 °C. The solvent was removed under reduced pressure and purified via column chromatography eluting with methylene chloride:methanol (9:1). The fractions containing product were evaporated and purified further via reverse-phase HPLC eluting with acetonitrile/H_2O to afford **2** (115 mg, 69%) as a white solid: mp 330 °C dec; ^{1}H NMR (DMSO-d_6) δ 6.74 (s, 2H), 8.10 (s, 1H), 11.0 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 109.6, 128.7, 144.21, 146.3, 148.4, 155.3, 159.3; λ_{max} observed at 297 nm, $\epsilon = 3.33 \times 10^4$ M^{-1} cm^{-1}. Anal. ($C_7H_5N_5OS\cdot{}^2/_3H_2O$) C, H, N, S. HPLC analysis on a C-18 reverse-phase column, 5 μm, 250 × 4.60 mm (Phenomenex); detector wavelength 280 nm; flow rate = 1 mL/min; linear gradient 0 to 20 min, 5−20% CH_3CN/H_2O; retention time 8.5 min.

Cell Culture. HCT116 cells were cultured in DMEM supplemented with 2 mM L-glutamine, 50 IU penicillin, 50 μg/mL streptomycin, nonessential amino acids, and 10% heat-inactivated fetal bovine serum. All cell lines were cultured at 37 °C in 5% CO_2 and media changed every 48 h.

Cell Proliferation Assays. Cell proliferation was assessed using the CellTiter96 system from Promega (Madison, WI) according to the manufacturer's instructions. HCT116 cells were seeded in 96-well plates at a density of 500 cells/well. After 72 h the medium was changed and the cells were treated with fresh serum containing medium with either control vehicle (DMSO) or test agent (at various concentrations). Growth was assessed after 24, 48, and 72 h of treatment and reported as a percentage (%) of the control-treated cells.

Statistical Analysis. Statistical differences were determined using a Student's *t*-test (Insat 2.3, GraphPad Software, San Diego, CA), with a p value < 0.05 considered to be significant.

Acknowledgment. We thank Ms. Nadia Boguslavsky and Ms. Julie Ha for their help in the HPLC purification of **2**. The initial work on this project was funded by the Emory/Georgia Tech Biomedical Technology Research Center, and this assistance is appreciated.

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JM000326I