N-(Cycloalkylamino)acyl-2-aminothiazole Inhibitors of Cyclin-Dependent Kinase 2. *N*-[5-[[[5-(1,1-Dimethylethyl)-2-oxazolyl]methyl]thio]-2-thiazolyl]-4-piperidinecarboxamide (BMS-387032), a Highly Efficacious and Selective Antitumor Agent

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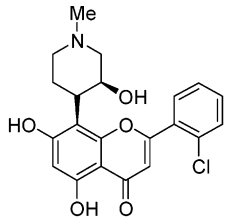
N-Acyl-2-aminothiazoles with nonaromatic acyl side chains containing a basic amine were found to be potent, selective inhibitors of CDK2/cycE which exhibit antitumor activity in mice. In particular, compound **21** {*N*-[5-[[[5-(1,1-dimethylethyl)-2-oxazolyl]methyl]thio]-2-thiazolyl]-4-piperidinecarboxamide, BMS-387032}, has been identified as an ATP-competitive and CDK2-selective inhibitor which has been selected to enter Phase 1 human clinical trials as an antitumor agent. In a cell-free enzyme assay, **21** showed a CDK2/cycE IC_{50} = 48 nM and was 10- and 20-fold selective over CDK1/cycB and CDK4/cycD, respectively. It was also highly selective over a panel of 12 unrelated kinases. Antiproliferative activity was established in an A2780 cellular cytotoxicity assay in which **21** showed an IC_{50} = 95 nM. Metabolism and pharmacokinetic studies showed that **21** exhibited a plasma half-life of 5−7 h in three species and moderately low protein binding in both mouse (69%) and human (63%) serum. Dosed orally to mouse, rat, and dog, **21** showed 100%, 31%, and 28% bioavailability, respectively. As an antitumor agent in mice, **21** administered at its maximum-tolerated dose exhibited a clearly superior efficacy profile when compared to flavopiridol in both an ip/ip P388 murine tumor model and in a sc/ip A2780 human ovarian carcinoma xenograft model.

Introduction

Cyclin-dependent kinases (CDKs) are a family of serine\threonine protein kinases which play key roles in the normal growth and life cycle of eukaryotic cells.[1] Specifically, CDKs, along with their activating subunit cyclins or inhibitory subunit CDKi (e.g., p16, p21, p27), are responsible for cellular response to external stimuli or insults. Together they provide for the orderly coordination of cellular events through the cell cycle and ensure compliance of the genetic integrity of new cells. Because of their key role as drivers of cell growth and division, their misregulation in a number of cancers,[2] and the strong positive correlation between CDK2 activity and poor clinical prognosis in cancer patients,[3] oncology drug discovery programs have directed major efforts toward the identification of small molecule inhibitors of CDKs as potential antitumor therapeutic agents.[4]

Flavopiridol (**1**), a synthetic flavone, was described as a benchmark broad-spectrum ATP-competitive CDK inhibitor a number of years ago and has progressed into advanced clinical trials as an antitumor agent.[5] We recently described *N*-phenylacetyl-2-aminothiazole **2** as a potent CDK2-selective inhibitor with in vivo antitumor activity superior to **1**.[6] In this disclosure we describe the SAR, metabolism, pharmacokinetics, and solid-state structural studies of a series related to **2**, the *N*-(cycloalkylamino)acyl-2-aminothiazoles.[7] It is from this series that our clinical development candidate has emerged. In addition, a preliminary report on the in vivo



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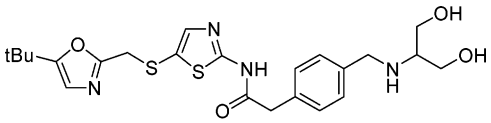

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Figure 1. Structures of CDK inhibitors flavopiridol (**1**) and aminothiazole **2**.

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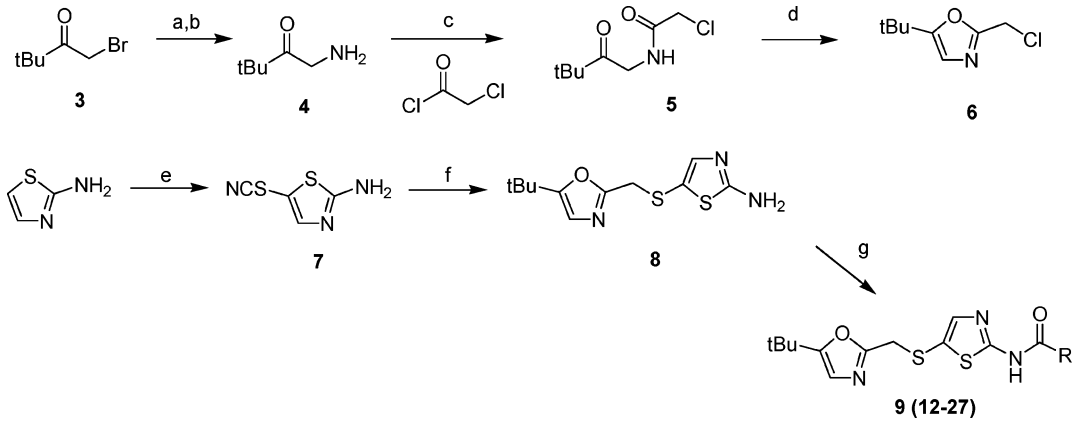
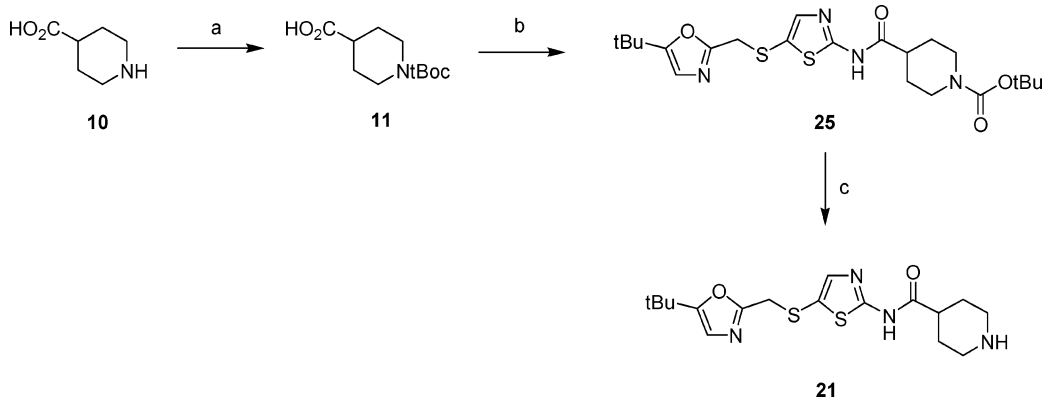
Scheme 1[a]



[a] Reaction conditions. (a) NaN_3, acetone, 25 °C, 100%; (b) Pd/C, H_2, aq HCl, MeOH, 25 °C, 91%; (c) Et_3N/CH_2Cl_2, −10 °C, 98%; (d) $POCl_3$, 105 °C, 80%; (e) Br_2, KSCN, MeOH, 26%; (f) $NaBH_4$ (2 equiv), EtOH, 25 °C followed by chloride **6** (1.1 equiv), reflux, 94%; (g) RCOOH, EDAC (2 equiv), DMAP (1 equiv), DMF, CH_2Cl_2, 25 °C.

Scheme 2[a]



[a] Reaction conditions. (a) $(tBoc)_2O$, aq NaOH, dioxane, CH_3CN, 25 °C, 81%; (b) amine **8**, EDAC (2 equiv), DMAP (0.5 equiv), DMF, CH_2Cl_2, 25 °C, 97%; (c) 4 N HCl in dioxane, $CHCl_3$, 45−55 °C, 83%.

antitumor activity of selected analogues including our clinical development candidate is disclosed.

Synthetic Chemistry Methods. A convergent synthetic route which involved the coupling of chloromethyloxazole **6** with thiazole **7** to access key intermediate amine **8** was utilized to rapidly prepare acylated analogues of general structure **9** (specific examples **12**−**27**). Thus, as shown in Scheme 1, commercially available α-bromopinacolone **3** was converted smoothly to α-amino derivative **4** by treatment with sodium azide followed by catalytic hydrogenation. Acylation of **4** with α-chloroacetyl chloride afforded keto-amide **5** which was cyclized to key chloromethyloxazole **6** in refluxing phosphorus oxychloride. The thiazole core was elaborated by treatment of commercially available 2-aminothiazole with bromine and potassium thiocyanate to give **7** in a low yield but moderately scalable process.[8] Reduction of **7** by exposure to sodium borohydride in methanol followed by alkylation of the resulting thiolate with chloromethyloxazole **6** gave key 2-aminothiazole intermediate **8**.

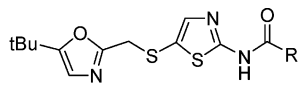
In general, amine **8** was coupled either directly to the appropriate carboxylic acid or when necessary the *N*-t-Boc protected carboxylic acid using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDAC), optionally in the presence of 4-(dimethylamino)pyridine (DMAP) and/or *N*-hydroxybenzotriazole, followed by removal of the protecting group to afford the desired acylated amino analogues **9**. A specific example for the preparation of **21** is shown in Scheme 2. Compounds **22**−**24** were prepared from **21** by elaboration of the amino functionality using standard known synthetic methods.

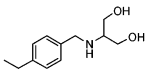
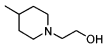
Results and Discussion

In Vitro Biological Evaluation. Compounds were initially evaluated in a cell-free enzyme assay for inhibition of CDK2/cyc E induced phosphorylation of RB protein and subsequently for antiproliferative effects in a whole cell 72 h cytotoxicity assay. An ovarian cancer cell line, A2780, was utilized in the cellular cytotoxity assay. Detailed protocols for both assays were described previously and are included in the Experimental Section.[6] The results of these assays are included in Table 1 as part of the SAR study for this series.

Structure−**Activity Studies.** Summarized in Table 1 is the SAR of a series of *N*-acyl-2-aminothiazoles in which a nonaromatic side chain containing a basic amine has been introduced on the acyl substituent. Previous SAR and structural studies in this series had established that the acyl side chain extends toward the exterior of the protein and was amenable to significant structural modification.[6] This finding was exploited to manipulate the physiochemical properties of the inhibitor and resulted in the identification of inhibitor **2** which contains an *aromatic* acyl side chain with a basic amino group. The basic amine was introduced into the inhibitor

Table 1. Structure−Activity Relationship Studies of Aminothiazoles **12**−**27**[a]



compd	R	CDK2/cycE IC_{50} (nM)[b]	A2780 cytotoxicity IC_{50} (μM)	% serum protein binding
1	flavopiridol	330	0.071	63
2		3	0.029	79
12	-CH_3	5	0.050	83
13		21	0.52	
14		3	0.10	
15		26	0.84	
16		50	1.80	
17		300		
18		50	1.60	
19		83	0.18	82
20		11	0.11	
21		48	0.095	69
22		43	0.078	75
23		74	0.12	75
24		18	0.18	84
25		160	2.9	99
26		22	0.029	68
27		150	0.049	86

[a] See ref 6 and or Experimental Section for description of assay conditions. [b] Assay was performed employing 25 μM [ATP].

to increase solubility and reduce both protein binding and metabolic transformation. Importantly, this modification was a key to improving in vivo antitumor efficacy. This report is the conclusion of this work in which we have focused on examining lower molecular weight *nonaromatic* acyl side chains with basic amines and has led to the identification of our clinical development candidate. Initial comparison of analogues **12** and **13** indicated that introduction of a primary amine into the side chain affords a potent enzyme inhibitor with only a 4-fold decrease in CDK2 inhibitory activity but a larger 10-fold loss of potency in the cellular antiproliferation assay. Subsequently we found that by increasing the lipophilicity of amino analogue **13** and constraining the amine group within or onto a ring we were able to restore cellular antiproliferative activity (see analogues **19**−**23**, vide infra). Analogues in which a methylene group is present between the carbonyl group and the cyclic substituent (**14** vs **15**, **16** vs **17**, **20** vs **21**) exhibited a 4−7-fold increase in enzyme potency versus analogues in which a methylene spacer was absent. This, however, was not necessarily translated into an increase in cellular antiproliferative activity (e.g., **20** vs **21**). We speculate that the allowed orientations of a cyclic ring attached directly to the carbonyl group are sufficiently restricted that it does not allow for simultaneous optimal binding of the side chain and the aminothiazole ring. Introduction of a methylene spacer provides the necessary flexibility to optimize binding of both the side chain and aminothiazole ring. Comparison of phenyl analogues **14** and **15** with their cyclohexyl analogues **16** and **17** indicated that the saturated carbocycles were ∼15-fold less potent than their phenyl counterparts. The reduced enzyme activity of **16** and **17** relative to the phenyl analogues may be due to the increased steric bulk of the cyclohexyl group and/or its increased lipohilicity. Comparison of the cyclohexyl analogues **16** and **17** with their piperidinyl counterparts **20** and **21**, however, revealed a 5 to 6-fold increase in enzyme activity for the piperidinyl analogues. Amines **20** and **21** were only 2 to 3-fold less potent than phenyl analogues **14** and **15**. This comparison supports the hypothesis that there is not a steric issue with the cyclohexyl ring since the piperidinyl ring is essentially the same size. The basis for the diminished activity of **16** and **17** seems to be the high lipophilicity (i.e., low aqueous solubility) of the cyclohexyl side chain. The SAR is consistent with the solid-state structural data which indicates that the acyl side chain extends into the hydrophilic environment at the interface of the protein and aqueous external environment. Highly lipophilic groups would be expected to be poorly accommodated in this region. Because of their acceptable enzyme and cellular antiproliferative activities, increased solubility, and reduced protein binding, we focused more closely on the exploration of analogues containing an aminocycle in the side chain and employed **21** as our benchmark inhibitor. Ring size and substitution were explored through analogues **18** and **19**. Thus, the racemic proline analogue **18** showed similar enzyme inhibitory potency to **21** although it was 16-fold less potent in cells. The racemic 3-piperidinyl analogue **19,** however, was only 2-fold less potent than **21** in both the enzyme and cellular antiproliferation assays but carried the synthetic liability of a chiral center which could not be readily accessed through a commercially purchasable intermediate. The effect of substitution on the piperidinyl nitrogen was explored through analogues **22**−**25**. *N*-Methyl, *N*-hydroxyethyl, and sulfonamide analogues (**22**−**24**) showed similar activity to **21** in both enzyme and cells assays while the *N*-*tert*-butoxycarbonyl analogue **25** was significantly less potent in both of these assays. As noted previously with cyclohexyl analogues

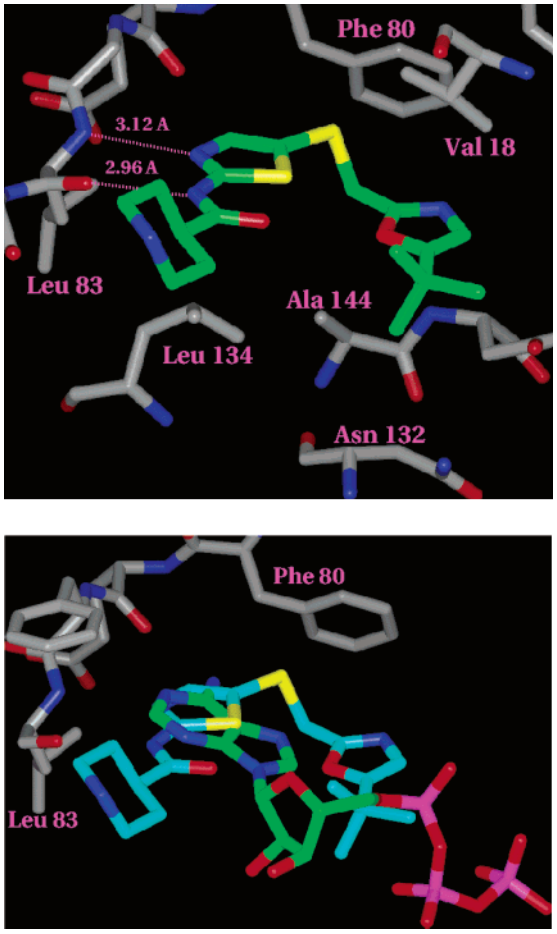


Figure 2. Upper panel: Solid-state structure of piperidinyl aminothiazole **21** bound in the ATP-pocket of CDK2 (no cyclin). The inhibitor carbon atoms are shown in green, the nitrogen atoms are shown in blue, oxygen atoms are shown in red, and the sulfur atoms are shown in yellow. The protein carbons are in gray. Hydrogen bonds are shown by the magenta dotted lines. Lower panel**:** Relative binding mode of inhibitor **21** (carbon atoms in light blue, nitrogen atoms in dark blue, oxygen atoms in red and sulfur atoms in yellow) bound to CDK2 with that of ATP (carbon atoms in green, nitrogen atoms in blue, oxygen atoms in red, and phosphorus atoms in magenta) bound to CDK2.

16 and **17**, this may be the result of introducing a highly lipophilic group into a hydrophilic space. Finally, the isomeric cis and trans exocyclic amino analogues **26** and **27** were prepared and examined. The cis isomer, **26**, was 7-fold more potent than trans isomer **27** in the enzyme assay but only 2-fold more potent in the cellular antiproliferation assay. The origin for the differences in enzyme activity between the two isomers were not apparent. In both of the assays, **26** compared favorably to our benchmark inhibitor **21**.

Solid-State Structure Study. The three-dimensional solid-state structure of **21** in complex with CDK2 was determined by X-ray crystallography (see Supporting Information for summary of crystallographic data). Crystals were obtained by incubating inhibitor **21** (72 h) with crystalline protein in the absence of cyclin. The crystal structure, as shown in the upper panel of Figure 2, confirmed that **21** binds in the ATP-binding site and the inhibitor adopts the same orientation and "folded" conformation previously described with this series.[6] In this conformation the *tert*-butyl oxazole ring wraps back toward the thiazole core and into the ribose pocket rather than extending toward Phe-80. No clear hydrogen bonding interactions are seen between the oxazole ring and the protein. Important hydrogen bonds between the amide backbone atoms of Leu-83 and both the thiazole nitrogen and exocyclic amide proton are clearly discernible. Finally, the piperidinyl ring extends toward the exterior of the protein consistent with the SAR which established that variable substitution at this position of the inhibitor is tolerated. The origins of the CDK2 selectivity of this series are not readily apparent from the structural data. For comparison, the lower panel of Figure 2 is an overlay which shows the relative orientation of inhibitor **21** bound to CDK2 with that of ATP bound to CDK2.[9]

Table 2. Metabolism and Pharmacokinetic Evaluation of Aminothiazole Analogues in Mice[a]

compd	microsomal stability (nmol/min/mg protein)	dose (μmol/kg)	C_{max} (μM)	T_{max} (h)	AUC (μM·h)	$T_{1/2}$ (h)	MRT (h)
2	0.22	18	0.8	1.0	2.7	1.8	2.9
19		26	3.5	0.5	12.1	2.2	3.5
21	0.05	23	1.3	0.5	4.0	2.5	3.5
22		25	1.1	0.5	1.2	0.7	1.1
23		24	1.1	0.5	1.8	1.9	2.4
26[b]	0.07	23	13.6	0.1	11.8	2.2	2.2

[a] See ref 6 for description of assay conditions. [b] Compound was dosed iv.

Metabolism and Pharmacokinetics. Selected analogues were assayed for mouse serum protein binding using an equilibrium dialysis protocol (Table 1) and for metabolic stability toward mouse liver microsomes (Table 2). Inhibitors with suitable in vitro CDK2 inhibitory potency in both enzyme and cell assays (IC_{50} < 100 nM) and low to moderate protein binding (65−85%) were selected, and pharmacokinetic (PK) parameters were determined. Each compound was dosed intraperitoneally (ip) at a dose of 10 mg/kg in mice, with the data shown in Table 2. Although the compounds were all similar in structure, cumulative drug exposure over 6 h as measured by AUC varied 6- to 7-fold. In particular, *N*-methyl analogue **22** showed significantly poorer PK parameters within the group as indicated by lower AUC and, both shorter half-life ($T_{1/2}$) and mean-residence time (MRT) while **19**, **21**, and **26** exhibited relatively high exposures and longer half-lives. We speculate that the poor PK of **22** could be due to either oxidation or demethylation of the tertiary amine.

In Vivo Antitumor Activity. On the basis of their in vitro potency and favorable in vivo exposure, compounds **19** (homochiral isomer, see Experimental Section), **21**, **23**, and **26** were evaluated for in vivo antitumor efficacy in mice using P388 murine leukemia tumors and A2780 human ovarian carcinoma xenografts.[10] In the P388 model, drugs were dosed intraperitonially (ip) once a day for 7 days (qdx7) in immunocompetent mice immediately after the tumors were implanted ip. The antitumor efficacy was measured as an increase in lifespan, and the data are expressed as a ratio of lifespan of drug-treated group (T) versus control group (C). In the A2780 xenograft model, tumors were implanted subcutaneously (sc) in nude mice, and compounds were dosed ip once a day for 8 days (qdx8) starting when tumors had grown to a median weight of

Table 3. In Vivo Antitumor Activity of Aminothiazoles against P388 and A2780 Tumors in Mice[a]

	P388[b]		A2780[c]	
compd	MTD[d] dose (mg/kg)	%*T*/*C*	MTD[d] dose (mg/kg)	LCK[e]
1	7.5	110	7.5	0.6−1.5
2	32	140	26	3.3
19[f]	20	152	22	1.6
21	22.5	140	48	>3.6−5.0
23	22.5	140		
26	8.0	179	8.0	1.7

[a] See refs 6 and 10 for experimental protocols. [b] P388 dosing schedule, ip/qdx7. [c] A2780 dosing schedule, ip/qdx8. [d] MTD = maximum tolerated dose. [e] See ref 10 for definition of LCK. [f] Homochiral isomer, see Experimental Section.

100 mg. The antitumor efficacy was measured as a growth delay and expressed in log cell kill units (LCK).[10] The data are summarized in Table 3. All compounds were active in the P388 model showing %*T*/*C* values between 140 and 179, where %*T*/*C* > 125 was defined as active. In the A2780 xenograft model, 3-piperidinyl analogue **19** and 4-aminocyclohexyl analogue **26** produced a growth delay of 1.6 and 1.7 LCK, respectively. Both compounds were considered comparable in activity to flavopiridol but significantly less efficacious than aminothiazole **2** despite their superior exposure numbers. 4-Piperidinyl analogue **21,** which had shown intermediate exposure, however, produced a growth delay between 3.6 and 5.0 LCK and was significantly more efficacious than either flavopiridol (**1**) or aminothiazole **2**. The enhanced efficacy of **21** will be the subject of future reports.

Biology Summary for 21 (BMS-387032). On the basis of its superior antitumor efficacy, we focused our studies on 4-piperidinyl analogue **21**. Closer examination of the CDK selectivity (Table 4) confirmed that **21** exhibited a similar CDK2-selective profile as reported for earlier analogues in the aminothiazole series. Specifically, **21** was 10-fold selective for CDK2/cycE relative to CDK1/cycB and 20-fold selective relative to CDK4/cycD. In addition, **21** showed remarkable selectivity over a panel of unrelated kinases. It exhibited IC_{50} values >40 μM for PKC$_\alpha$, PKC$_\beta$, PKC$_\gamma$, C*hk*1, IKK, EMT, Lck, FAK, ZAP70, and IC_{50} values >25 μM for HER1, HER2, and IGF-1R. In both mouse and human serum, **21** showed low to moderate protein binding indicating high exposure to free drug.

The pharmacokinetic parameters for **21** administered in mouse, rat, and dog are shown in Table 5. The compound exhibits a moderate half-life ($T_{1/2}$) of between 5 and 7 h across the three species. The $T_{1/2}$ is consistent with in vitro studies which indicated a low rate of metabolism (oxidation and glucuronidation) in liver microsomes. In addition, **21** was well distributed into tissues as indicated by a high volume of distribution (V_{ss}). Examination in three species indicated **21** is orally bioavailable in all three species, although less so in rat and dog than mouse. Metabolism studies in rats indicated that dosed iv (8.9 mg/kg) 28% of parent drug is recovered unchanged in urine and 11% in bile 9 h postadministration. Minor metabolites resulting from cleavage of the amide bond, oxidation of the thioether linker, and hydroxylation of the *tert*-butyl group were identified. Finally, **21** showed IC_{50} values >40 μM across a cytochrome P450 panel which included CyP1A2, CyP2C9, CyP2C19, CyP2D6, and CyP3A4.

The antitumor efficacy of **21** in the A2780 human ovarian carcinoma xenograft model was evaluated in greater detail along with flavopiridol for comparative purposes

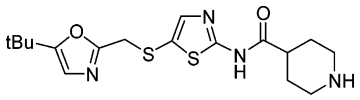
(Figure 3). Tumors were implanted sc in nude mice and grown to 100 mg, and then animals were dosed with **21** once a day ip for 8 days at 18, 36, and 48 mg/kg (MTD). Groups of eight mice were utilized for each dose. Aminothiazole **21** was found to have antitumor activity at all three doses (i.e., LCK > 1.0). At the low 18 mg/kg dose, **21** showed minor tumor regression with a growth delay of 2.1 LCK. At the higher 36 and 48 mg/kg doses, **21** showed rapid, significant tumor regression and growth delays of >5.6 LCK and >6.5, respectively. In addition, after extended times at the two high doses, cures were seen in 9 of 16 animals as determined by no measurable tumor at day 68. At autopsy the cured animals were found to be free of tumor cells. Significantly, **21** was found to be active not only at its MTD but also at both sub-MTD doses (18 and 36 mg/kg), indicating the potential for an enhanced safety window. For comparison, flavopiridol showed a growth delay of 0.4 LCK (inactive in this model, LCK<1.0) with no regression or cures at its MTD (7.5 mg/kg).

Selection of Final Form. Because of the low aqueous solubility of free base **21** (0.28 mg/mL) and an anticipated clinical plan which included an iv dosing protocol, a number of mineral and organic acid salts of **21** were examined with the goal of increasing aqueous solubility and identifying a final form with acceptable physical and stability properties. The 1:2 L-tartrate salt (acid:**21**) was selected as the final form due to its overall favorable physical properties which included examination of polymorph, hydrate, solvate issues, ease, and reproducibility of crystallization, hygroscopicity, solution stability, and aqueous solubility (5.5 mg/mL). On the basis of its physical properties, selectivity, pharmacokinetics, and antitumor activity, **21** (BMS-387032) as the $^1/_2$-L-tartrate salt, was selected to progress into clinical development as an antitumor agent.

Summary

Aminothiazole **21** (BMS-387032, *N*-[5-[[[5-(1,1-dimethylethyl)-2-oxazolyl]methyl]thio]-2-thiazolyl]-4-piperidinecarboxamide) has been identified as a CDK2-selective inhibitor which has been selected to enter clinical development as an antitumor agent. Compound **21** has shown superior antitumor efficacy to both flavopiridol and previously reported analogues in this series. The key modification from our previously reported aminothiazole analogues was replacement of a substituted *aromatic* acyl side chain with a *nonaromatic* amino acyl side chain. This substitution reduced molecular weight, protein binding, and in vitro measured metabolism in liver microsomes while at the same time it increased aqueous solubility. SAR studies were consistent with the solid-state structure of the inhibitor bound to CDK2 protein, which indicated that the acyl side chain of the molecule extended into the hydrophilic extraprotein space and was amenable to modification. Optimization of potency, pharmacokinetics, and evaluation in both an *ip*/*ip* P388 murine tumor model and

Table 4. Summary of in Vitro Evaluation of Aminothiazole **21**



21 (BMS-387032)

CDK1/cycB IC_{50} (nM)	CDK2/cycE IC_{50} (nM)	CDK4/cycD IC_{50} (nM)	A2780 cellular cytotoxicity IC_{50} (nM)	% protein binding mouse	% protein binding human
480	48	925	95	69	63

Table 5. Pharmacokinetic Parameters for Aminothiazole **21** in Mouse, Rat, and Dog

	mouse[a]	rat[a]	dog[b]
dose (μmol/kg)	24	24	1.2
$T_{1/2}$ (h), iv	4.8	5.3	7.0
MRT (h), iv	2.8	3.7	6.3
AUC_{tot} (μM·h), iv	4.8	6.3	2.1
clearance (mL/min/kg), iv	81	64	9.4
V_{ss} (L/kg), iv	14	15	3.6
C_{max} (μM), po	1.4	0.52	0.33
T_{max} (h), po	2.0	4.7	0.4
% oral bioavailability	100	31	28

[a] Dosed in 1:9 EtOH/water vehicle. [b] Dosed in 1:19 dextrose/water, po dose 2.4 μmol/kg.

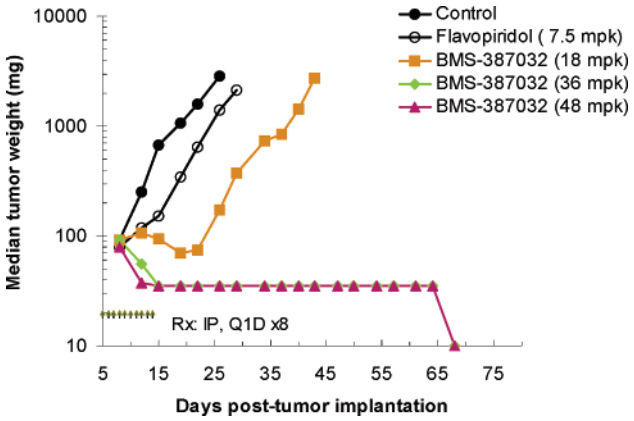


Figure 3. Comparative antitumor efficacy of aminothiazole **21** (BMS-387032) and flavopiridol (**1**) in a A2780 human ovarian carcinoma xenograft nude mouse model.

an A2870 human ovarian cancer xenograft tumor mouse model led to the identification of the 4-piperidinyl analogue, **21**, as our clinical development candidate. Detailed biochemical, antitumor efficacy, and clinical studies will be the subject of future disclosures.

Experimental Section

All air- and moisture-sensitive reactions were conducted under an argon atmosphere. All reagents and starting materials were obtained from commercial sources and used without further purification unless indicated or referenced. DMF refers to *N*,*N*-dimethylforamide, TFA refers to trifluoroacetic acid, DMAP refers to 4-(dimethylamino)pyridine, and EDAC refers to 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride. ^{1}H NMR (400 or 500 MHz) and ^{13}C (100 or 125 MHz) spectra were recorded on JEOL or Bruker spectrometers and chemical shifts are reported in parts per million downfield from internal trimethylsilane. Mass spectra (LCMS) were measured using a Shimadzu HPLC 10A system linked to a Micromass MS detector in positive chemical ionization mode. Analytical high performance liquid chromatography (HPLC) was performed on Shimadzu 10A systems fitted with YMC S5 ODS 4.6 × 50 mm columns, using a UV detector at 220 or 254 nM. All compounds exhibited spectra consistent with their assigned structures. All new compounds were homogeneous (>98%) by HPLC analysis. All previously unreported final analogues showed acceptable ($^+$0.4%) elemental analysis and/or high-resolution mass spectrum (HRMS). Melting points (mp) are uncorrected. Flash chromatography was performed using E. Merck silica gel 60 under medium nitrogen pressure eluting with the solvent systems indicated. Concentration in vacuo refers to the use of a rotorary evaporator then oil pump vacuum for removal of volatile components. Compounds **2**, **6**, **8**, **12** and **15** were prepared previously.[6] Biological assays, metabolism and pharmacokinetic studies, and solid state structural studies were conducted utilizing previously published protocols.[6,10]

Preparation of 2-Amino-5-[[[5-(1,1-dimethylethyl)-2-oxazolyl]methyl]thio]thiazole (8). To a 2L three-necked round-bottom flask fitted with a mechanical stirrer was added α-bromopinacolone (134 g, 747 mmol, 1.0 equiv), acetone (1.2 L), and sodium azide (63.2 g, 971 mmol, 1.3 equiv). The reaction mixture was stirred at room-temperature overnight and then filtered, and the solids were washed with acetone (2 × 100 mL). The filtrate was concentrated in vacuo to provide α-azidopinacolone (105.0 g, 100%) as an oil. ^{1}H NMR ($CDCl_3$) δ 1.17 (s, 9H), 4.07 (s, 2H). The crude material was used in the next step without further purification.

To a 2 L three-necked round-bottom flask fitted with a mechanical stirrer were added α-azidopinacolone (28.6 g, 203 mmol, 1.0 equiv), methanol (1145 mL), concentrated HCl (18 mL), and 10% Pd/C (3.5 g, 50% water wet). The reaction mixture was stirred under hydrogen at 20 psi for 2 h, the mixture was filtered through a pad of Celite, and the residue rinsed with methanol (2 × 50 mL). The filtrate was concentrated under reduced pressure at a temperature below 40 °C. The resulting wet solid was azeotroped with 2-propanol (2 × 100 mL), anhydrous ether (100 mL) was added, and the slurry which formed was stirred for 5 min. The solid product was collected by filtration, and the cake was washed with diethyl ether (2 × 30 mL) and then dried in vacuo to give α-aminopinacolone hydrochloride **4** (28.0 g, 91%), mp 205 °C (lit. 199−200 °C).[11] ^{1}H NMR (DMSO-d_6) δ 1.13 (s, 9H), 4.06 (s, 2H), 8.34 (s, 3H).

To a 1 L three-necked round-bottom flask fitted with a mechanical stirrer were added α-aminopinacolone hydrochloride **4** (15.2 g, 100 mmol, 1.0 equiv) and CH_2Cl_2 (350 mL). The slurry was cooled to −5 °C, and triethylamine (35 mL, 250 mmol, 2.5 equiv) was added. The resulting mixture was stirred and cooled to −10 °C. A solution of α-chloroacetyl chloride (8.8 mL, 110 mmol, 1.1 equiv) in CH_2Cl_2 (20 mL) was added dropwise over 15 min while keeping the reaction temperature below −5 °C. The reaction was stirred for 1 h and then quenched with 1 N aq HCl (200 mL). The phases were separated, and the organic phase was washed with 1 N aq HCl (200 mL), and water (50 mL), dried (Na_2SO_4), and concentrated in vacuo to afford α-*N*-2-(chloroacetylamino)pinacolone **5** (18.9 g, 98%) as a white solid, mp 50−52 °C. ^{1}H NMR ($CDCl_3$) δ 1.21 (s, 9H), 4.09 (s, 2H), 4.30 (s, 2H), 7.35 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 210.13, 166.62, 45.23, 43.51, 42.79, 27.45. Anal. ($C_8H_{14}NO_2Cl$): C, H, N, Cl.

To a 100 mL rounded bottom flask fitted with a magnetic stirrer were added **5** (18.9 g, 98.6 mmol, 1 equiv) and $POCl_3$ (38 mL, 407 mmol, 4.1equiv). The reaction mixture was heated

to 105 °C and stirred for 1 h. After being cooled to room temperature, the reaction mixture was poured carefully into ice (180 g). The mixture was extracted with ether (6 × 150 mL). The organic extracts were combined and neutralized to pH 7−8 with saturated sodium bicarbonate (∼700 mL). The organic phase was separated and washed successively with saturated sodium bicarbonate (100 mL), water (100 mL), and brine (50 mL), dried ($MgSO_4$), and concentrated in vacuo. The crude material was distilled under reduced pressure to give 5-*tert*-butyl-2-chloromethyloxazole, **6**[6] (13.6 g, 80%), as a colorless oil, bp 38−40 °C (0.25 mm). ^{1}H NMR ($CDCl_3$) δ 1.32 (s, 9H), 4.60 (s, 2H), 6.70 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 28.92, 31.91, 36.57, 120.87, 158.03, 162.90.

To a solution of thiocyanate **7**[8] (10.0 g, 63.3 mmol) in absolute EtOH (600 mL) was added $NaBH_4$ (4.8 g, 120 mmol) portionwise at room temperature. The mixture was stirred for 1 h, and then acetone (300 mL) was slowly introduced. After 1 h, a solution of oxazole chloride **6** (12.0 g, 69 mmol) in EtOH (100 mL) was added, and the resulting dark reaction mixture heated to reflux for 1 h. The resulting mixture was cooled, concentrated in vacuo, and then partitioned between EtOAc and brine. The organic phase was separated, dried ($MgSO_4$), and concentrated in vacuo to give a crude solid. The crude material was triturated with diethyl ether/hexane to provide amine **8**[6] (16.0 g, 94%) as a pale red-brown solid.

Preparation of *N*-[5-[[[5-(1,1-Dimethylethyl)-2-oxazolyl]-methyl]thio]-2-thiazolyl]-2-aminoethanecarboxamide Hydrochloride (13). To a stirred solution of amine **8** (1.20 g, 4.45 mmol) and *N*-tBoc-*β*-alanine (2.10 g, 11.1 mmol) in anhydrous DMF (10 mL) was added EDAC in several portions (2.55 g, 13.3 mmol) over 5 min at room temperature. The mixture was stirred for 1.5 h and then partitioned between EtOAc (75 mL) and 1 M aq HCl (75 mL). The organic phase was separated and washed successively with 1 M HCl (75 mL), 5% aq sodium bicarbonate (75 mL), and brine (50 mL), dried (Na_2SO_4) and concentrated in vacuo to give an oil. The crude material was purified by flash chromatography (EtOAc) to afford *N*-[5-[[[5-(1,1-dimethylethyl)-2-oxazolyl]methyl]thio]-2-thiazolyl]-2-(*tert*-butyloxycarbonyl)aminoethanecarboxamide (1.93 g, 98%) as a solid white foam. ^{1}H NMR ($CDCl_3$) δ 11.9 (s, 1H), 7.35 (s, 1H), 6.62 (s, 1H), 5.28 (br s, 1H), 4.09 (s, 2H), 3.54 (m, 2H), 2.72 (m, 2H), 1.43 (s, 9H), 1.27 (s, 9H). LCMS: 441 $[M + H]^+$.

To a solution of the above t-Boc intermediate (1.82 g, 4.13 mmol) in CH_2Cl_2 (10 mL) was added TFA (5 mL) rapidly at room temperature. After 1 h, the solution was concentrated in vacuo. The residue was partitioned between EtOAc (25 mL) and water (35 mL) and then neutralized by addition of solid sodium bicarbonate. The organic layer was separated, washed successively with 5% aq sodium bicarbonate (25 mL) and brine (25 mL), dried (Na_2SO_4), and concentrated in vacuo to give the free amine (1.26 g) as a solid foam. The free amine was solubilized with 1 M aq HCl (3.7 mL, 1 equiv) and water (5 mL) and then lyophilized to afford the hydrochloride salt of **13** (1.37 g, 88%) as an off-white amorphous solid foam. ^{1}H NMR (DMSO-d_6) δ 12.5 (br s, 1H), 8.02 (br s, 2H), 7.39 (s, 1H), 6.71 (s, 1H), 4.06 (s, 2H), 3.08 (m, 2H), 2.84 (t, J = 7, 2H), 1.20 (s, 9H); LCMS: 341 $[M + H]^+$. HRMS FAB $[M + H]^+$ calcd for $C_{14}H_{20}N_4O_2S_2$ 340.1028; found 341.1095.

Preparation of *N*-[5-[[[5-(1,1-Dimethylethyl)-2-oxazolyl]-methyl]thio]-2-thiazolyl]phenylmethanecarboxamide Trifluoroacetic Acid Salt (14). To a stirred mixture of phenylacetic acid (50 mg, 0.37 mmol), amine **8** (100 mg, 0.37 mmol), *N*-hydroxybenzotriazole hydrate (60 mg, 0.44 mmol), and DMAP (45 mg, 0.37 mmol) in DMF (1.4 mL) were added diisopropylethylamine (0.16 mL, 0.89 mmol) and EDAC (71 mg, 0.37 mmol) at room temperature. The reaction mixture was cooled to room temperature. This was purified by preparative HPLC (YMC ODS S5 20 × 100 mm column, 30% to 100% gradient over 10 min with 0.1%TFA in MeOH-water solvent system, 20 mL/min) to give the TFA salt of **14** (40 mg, 28%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 7.49−7.25 (m, 6 H), 6.54 (s, 1 H), 3.93 (s, 2 H), 3.79 (s, 2 H), 1.21 (s, 9 H). LCMS: 388 $[M + H]^+$. HRMS FAB $[M + H]^+$ calcd for $C_{19}H_{21}N_3O_2S_2$ 387.1075; found 388.1167.

Preparation of *N*-[5-[[[5-(1,1-Dimethylethyl)-2-oxazolyl]-methyl]thio]-2-thiazolyl]cyclohexylmethanecarboxamide (16). To a stirred mixture of cyclohexylacetic acid (53 mg, 0.37 mmol), amine **8** (100 mg, 0.37 mmol), *N*-hydroxybenzotriazole hydrate (60 mg, 0.44 mmol), and DMAP (45 mg, 0.37 mmol) in DMF (1.4 mL) were added diisopropylethylamine (0.16 mL, 0.89 mmol) and EDAC (71 mg, 0.37 mmol) at room temperature. This mixture was stirred at room temperature for 6 h and then heated to 80 °C for 16 h. The reaction mixture was cooled to room temperature. This was purified by preparative HPLC (YMC ODS S5 20 × 100 mm column, 30% to 100% gradient over 10 min with 0.1%TFA in MeOH-water solvent system, 20 mL/min) to give the TFA salt of **16** (73 mg, 50%) as a white solid. ^{1}H NMR($CDCl_3$) δ 7.32 (s, 1H), 6.60 (s, 1H), 3.97 (s, 2H), 2.36 (d, 7.2 Hz, 2H), 1.94−1.88 (m, 1H), 1.78−1.70 (m, 5H), 1.28−1.25 (m, 12H), 1.04−0.97 (m, 2H). LCMS: 394 $[M + H]^+$. HRMS FAB $[M + H]^+$ calcd for $C_{19}H_{27}N_3O_2S_2$ 393.1545; found 394.1621.

Preparation of *N*-[5-[[[5-(1,1-Dimethylethyl)-2-oxazolyl]-methyl]thio]-2-thiazolyl]cyclohexanecarboxamide (17). To a solution of amine **8** (1.00 g, 3.70 mmol), cyclohexylcarboxylic acid (711 mg, 5.55 mmol), and DMAP (138 mg, 1.11 mmol) in DMF (4.5 mL) and CH_2Cl_2 (13.5 mL) was added EDAC (2.12 g, 11.1 mmol) at room temperature. The reaction mixture was stirred overnight then poured into EtOAc and water to which were added aq sodium bicarbonate and brine. The precipitate that formed was collected by filtration, washed, and dried to give **17** (844 mg, 60%) as a white solid, mp 189−192 °C. ^{1}H NMR ($CDCl_3$) δ 7.29 (s, 1H), 6.57 (s, 1H), 3.92 (s, 2H), 2.50 (m, 1H), 2.1−1.2 (br m, 10H), 1.23 (s, 9H). LCMS: 380 $[M + H]^+$. HRMS FAB $[M + H]^+$ calcd for $C_{18}H_{27}N_3O_2S_2$ 379.1388; found 380.1463.

Preparation of (±)-*N*-[5-[[[5-(1,1-Dimethylethyl)-2-oxazolyl]methyl]thio]-2-thiazolyl]-2-pyrrolidinecarboxamide (18). To a stirred solution of amine **8** (0.50 g, 1.86 mmol) and *N*-tBoc-DL-proline (0.80 g, 3.71 mmol) in CH_2Cl_2 (20 mL) at room temperature was added EDAC (0.71 g, 3.71 mmol). The reaction mixture was stirred for 1 h and then diluted with CH_2Cl_2 (50 mL). The organic phase was washed with aq $NaHCO_3$ solution twice and brine once, dried ($MgSO_4$), and concentrated in vacuo. The crude material was purified by flash chromatography (EtOAc/hexane 1:1) to give *N*-tBoc-**18** as a light color solid (0.71 g, 82%). ^{1}H NMR ($CDCl_3$) δ 7.33 (s, 1H), 6.58 (s, 1H), 4.12 (m, 1H), 3.93 (s 2H,), 1.94 (m, 2H), 1.44 (m, 4H), 1.26 (s, 9H). LCMS: 467 $[M + H]^+$.

To a stirred solution of *N*-tBoc-**18** (0.70 g, 1.50 mmol) in CH_2Cl_2 (2 mL) at room temperature was added TFA (2.5 mL). The reaction mixture was stirred for 1.5 h and then concentrated in vacuo. The resulting residue was made basic by addition of aq $NaHCO_3$ solution and extracted with EtOAc (3 × 50 mL). The organic extracts were dried ($MgSO_4$) and concentrated in vacuo to afford a light foam. The foam was treated with 1 N aq HCl (1.45 mL) and lyophilized to give the hydrochloride of **18** as a white solid (0.54 g, 89%), mp 118−120 °C. ^{1}H NMR ($CDCl_3$) δ 10.50 (s, 1H), 9.2 (s, 1H), 7.19 (s, 1H), 6.62 (s, 1H), 4.8 (m, 1H), 4.01 (s, 2H), 3.56 (m, 2H), 2.63 (m, 1H), 2.14 (m, 3H) 1.30 (s, 9H). LCMS: 367 $[M + H]^+$. HRMS FAB $[M + H]^+$ calcd for $C_{16}H_{22}N_4O_2S_2$ 366.1184; found 367.1266.

Preparation of (±)-*N*-[5-[[[5-(1,1-Dimethylethyl)-2-oxazolyl]methyl]thio]-2-thiazolyl]-3-piperidinecarboxamide (19) and Separation of Enantiomers. Nipecotic acid (1.3 g, 10 mmol, 1 equiv) was combined with dioxane (10 mL), acetonitrile (2 mL), water (10 mL), and 1 N aqueous NaOH (10 mL, 1 equiv). Di-*tert*-butyl dicarbonate (3.3 g, 15 mmol, 1.5 equiv) was added, the reaction was stirred at room-temperature overnight and then concentrated in vacuo, and 10% aqueous citric acid was added. The mixture was extracted with EtOAc (3 × 100 mL). The organic extracts were dried (Na_2SO_4), filtered through silica gel, and concentrated in vacuo to give a solid. The resulting crude material was recrystallized

(EtOAc/hexanes) to provide *N*-*tert*-butoxycarbonylnipecotic acid[12] (2.2 g, 96%) as a white solid.

To a mixture of amine **8** (2.7 g, 10 mmol, 1 equiv), *N*-*tert*-butoxycarbonylnipecotic acid (3.4 g, 1.5 mmol, 1.5 equiv) in DMF (10 mL), and CH_2Cl_2 (30 mL) was added EDAC (3.8 g, 20 mmol, 2 equiv) at room temperature. The reaction mixture was stirred for 4 h. The resulting black solution was concentrated in vacuo, diluted with water (90 mL), and extracted with EtOAc (100 mL, and then 2 × 75 mL). The organic extracts were dried (Na_2SO_4), concentrated in vacuo, and purified by flash chromatography (50−100% EtOAc in hexanes gradient) to provide (±)-*N*-[5-[[[5-(1,1-dimethylethyl)-2-oxazolyl]methyl]-thio]-2-thiazolyl]-3-(*N*-*tert*-butoxycarbonyl)piperidinecarboxamide (3.80 g, 79%) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ 7.31 (s, 1H), 6.57 (s, 1H), 3.98−3.92 (m, 1H), 3.92 (s, 2H), 3.72 (br m), 3.35 (br m) 3.09 (br), 2.54 (br m), 1.95 (br, 2H), 1.7−1.3 (br), 1.45 (s, 9H), 1.23 (s, 9H). LCMS: 481 $[M + H]^+$.

To a solution of the above t-Boc intermediate (355 mg, 0.74 mmol, 1 equiv) in CH_2Cl_2 (3 mL) was added TFA (3 mL), and the mixture was stirred at room temperature for 20 min. The solution was concentrated in vacuo and neutralized with saturated aqueous $NaHCO_3$. The resulting mixture was extracted with EtOAc. The organic extracts were dried (Na_2SO_4), concentrated in vacuo, and recrystallized (EtOAc) to provide racemic **19** (142 mg, 50%) as a white solid, mp 175−177 °C. ^{1}H NMR (DMSO-d_6) δ 7.23 (s, 1H), 6.69 (s, 1H), 3.96 (s, 2H), 3.2−2.8 (b, 2H), 2.7−2.6 (m, 1H), 2.5−2.3 (m, 2H), 1.87−1.78 (m, 1H), 1.60−1.50 (m, 2H), 1.38−1.26 (m, 1H), 1.19 (s, 9H). LCMS: 381 $[M + H]^+$. HRMS FAB $[M + H]^+$ calcd for $C_{17}H_{24}N_4O_2S_2$ 380.1341; found 381.1429.

For in vivo evaluation, homochiral **19** was obtained as follows. The racemic t-Boc-protected intermediate from above was separated into the individual enantiomers by chiral preparative HPLC (Chiral Pak AD column, 5 × 50 cm, 20 μm, 10% 0.1% triethylamine/2-propanol in hexanes; 45 mL/min, detection at 254 nm, loading 300 mg of racemic **19** in 5 mL of 2-propanol). The two individual tBoc-protected enantiomers were separately deprotected using TFA and isolated as the free bases as described above. They were then converted to their hydrochloride salts for in vivo evaluation. The homochiral isomer of **19** obtained from the slower eluting t-Boc intermediate was evaluated in vivo. The absolute configuration was not determined.

Preparation of *N*-[5-[[[5-(1,1-Dimethylethyl)-2-oxazolyl]-methyl]thio]-2-thiazolyl]-4-methylpiperidinecarboxamide (20). To a stirred solution of amine **8** (0.20 g, 0.74 mmol) and *N*-tBoc-piperidine-4-acetic acid (0.34 g, 1.46 mmol) in CH_2Cl_2 (10 mL) at room temperature was added EDAC (0.28 g, 1.46 mmol). The reaction mixture was stirred overnight and then concentrated in vacuo. The crude material was purified by flash chromatography (EtOAc/hexane 1:1 to 3:2) to give *N*-tBoc-**20** as a white solid (0.32 g, 89%). ^{1}H NMR ($CDCl_3$) δ 11.1 (s, 1H), 7.28 (s, 1H), 6.57 (s,1H), 4.09 (m, 1H), 3.94 (s, 2H), 2.71 (m, 2H), 2.37 (d, J = 7 Hz, 2H), 2.05 (m, 1H), 1.71 (m, 2H), 1.43 (s, 9H), 1.23 (s, 9H), 1.17 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 169.5, 161.8, 158.8, 154.7, 143.9, 121.2, 120.1, 79.5, 42.9, 34.9, 33.2, 31.9, 31.6, 28.6, 28.4. LCMS: 495 $[M + H]^+$.

To a stirred solution of *N*-tBoc-**20** (0.30 g, 0.62 mmol) in dioxane (11 mL) at room temperature was added HCl solution (4 N in dioxane, 3.0 mL). The reaction mixture was stirred overnight, and then MeOH (4 mL) was added to facilitate completion of the reaction by solublizing the solids which had formed. The mixture was concentrated in vacuo, and the residue was dissolved in water and lyophilized to give the hydrochloride of **20** as a light yellow hygroscopic solid (0.234 g, 90%), mp 143−145 °C. ^{1}H NMR (DMSO-d_6) δ 9.14 (br s, 1H), 8.97 (m,1H), 7.36 (s, 1H), 6.71 (s, 1H), 4.04 (s, 2H), 3.17 (d, J = 12, 2H), 2.82 (q, J = 12, 2H), 2.39 (d, J = 7, 2H), 2.03 (m, 1H), 1.73 (d, J = 13, 2H), 1.42 (q, J = 11, 2H), 1.17 (s, 9H). ^{13}C NMR ($CDCl_3$) δ 170.3, 161.2, 161.1, 159.0, 145.3, 120.2, 118.98, 42.9, 41.1, 34.2, 31.1, 30.8, 28.5, 28.1. LCMS: 395 $[M + H]^+$. HRMS FAB $[M + H]^+$ calcd for $C_{18}H_{26}N_4O_2S_2$ 394.1497; found 395.1566.

Preparation of *N*-[5-[[[5-(1,1-Dimethylethyl)-2-oxazolyl]-methyl]thio]-2-thiazolyl]-4-piperidinecarboxamide (21), Scheme 2. To a mixture of amine **8** (9.60 g, 35.6 mmol, 1 equiv), *N*-*tert*-butoxycarbonylisonipecotic acid **11** (12.6 g, 55 mmol, 1.5 equiv), DMAP (2.0 g, 16 mmol, 0.45 equiv) in DMF (36 mL), and CH_2Cl_2 (100 mL) was added EDAC (13.8 g, 72 mmol, 2 equiv) at room temperature. The reaction mixture was stirred for 3.5 h. Water (300 mL) and EtOAc (200 mL) were added, and the resulting precipitate was collected by filtration. The filtrate was extracted with EtOAc. The organic extracts were dried ($MgSO_4$) and concentrated in vacuo to provide a yellow solid which was combined with the precipitate. The solid was heated to reflux in a mixture of ethanol, acetone, and water for 20 min. The solid was filtered, washed with an ethanol/water mixture, and dried under vacuum to give crude *N*-tBoc-**25** (16.6 g, 97%) as a white solid.

A magnetically stirred suspension of crude *N*-tBoc-**25** (20.2 g, 42.1 mmol) in 200 mL of $CHCl_3$ was warmed until homogeneous, and then a solution of HCl (31 mL, 4 N in dioxane) was added at 55 °C. Gas was evolved, and a precipitate formed within a few minutes. After 7 h, HPLC indicated the reaction was about 2/3 complete. Additional HCl solution (10 mL) was introduced, and the reaction mixture was stirred at 60 °C for 1 h. A third portion of HCl solution (10 mL) was added and the reaction mixture stirred at 45 °C for 6 h. The resultant heavy suspension was cooled in an ice-bath, and saturated aq sodium bicarbonate solution (200 mL) was added slowly. Gas was evolved during the addition. The heavy suspension became homogeneous and then formed a light suspension. The light suspension was treated with solid sodium carbonate (6 g), heated at 60 °C for 20 min, and then diluted with $CHCl_3$ (100 mL). The aqueous phase was separated and extracted with $CHCl_3$ (2 × 100 mL). The organic extracts were combined, washed with brine (100 mL), dried (Na_2CO_3/Na_2SO_4), and concentrated in vacuo to give a crude yellow solid. The yellow solid was recrystallized (50% aq EtOH, 400 mL) to afford **21** (13.3 g, 83%) as a white solid, mp 171−173 °C. ^{1}H NMR (DMSO-d_6) δ 7.38 (s, 1H), 6.72 (s, 1H), 4.05 (s, 2H), 2.97 (d, J = 12 Hz, 2H), 2.6−2.4 (m, 3H, overlapped with solvent peak, but discernible), 1.67 (d, J = 12 Hz, 2H), 1.47 (dq, J = 4 Hz, 12 Hz, 2H), 1.17 (s, 9H). ^{13}C NMR (DMSO-d_6) δ 174.36, 161.90, 161.14, 159.11, 145.48, 120.47, 118.63, 45.62, 42.38, 34.32, 31.26, 29.24, 28.63. LCMS: 381 $[M + H]^+$. HPLC: HI > 99% at 3.12 min (YMC S5 ODS column 4.6 × 50 mm, 10−90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). Anal. ($C_{17}H_{24}N_4O_2S_2$) C, H, N, S.

To a warm solution of **21** (1.75 g, 4.6 mmol) in absolute ethanol (70 mL) was added a solution of L-tartaric acid (345 mg, 2.3 mmol, 0.5 equiv) in absolute ethanol (5 mL). A precipitate started to form after several minutes. The mixture was allowed to stand for 4 h at room temperature, and then the solid precipitate was collected on a Buchner funnel, washed with absolute ethanol, and dried in vacuo at 85 °C to afford the $^1/_2$-L-tartrate salt of **21** (326 mg, 94%) as off-white crystals, mp 234−236 °C. Anal. ($C_{17}H_{24}N_4O_2S_2$·0.5-L-Tartaric acid) C, H, N, S.

Preparation of 1-Methyl-*N*-[5-[[[5-(1,1-dimethylethyl)-2-oxazolyl]methyl]thio]-2-thiazolyl]-4-piperidinecarboxamide (22). To a suspension of amine **21** (700 mg, 1.80 mmol) and sodium triacetoxyborohydride[13] (1.70 g, 8.1 mmol) in 1,2-dichloroethane (45 mL) was added aq formaldehyde solution (37%, 0.61 mL, 8.1 mmol) at room temperature. The reaction mixture was stirred overnight, sat aq sodium bicarbonate solution was added, and the mixture was extracted with CH_2Cl_2. The organic extracts were dried (Na_2SO_4) and concentrated in vacuo. The crude material was purified by flash chromatography (1:2:17 MeOH/Et_3N/EtOAc) to afford **22** (530 mg, 75%) as a white solid, mp 177−180 °C. ^{1}H NMR ($CDCl_3$) δ 9.10 (br s, 1H), 7.29 (s, 1H), 6.57 (s, 1H), 3.91 (s, 2H), 2.91 (d, J = 11.4 Hz, 2H), 2.28 (s, 3H), 2.33−2.26 (b, 1H overlapped with 2.28 peak), 1.99 (t, J = 11.4 Hz, 2H), 1.83−1.93 (m, 4H), 1.23 (s, 9H). LCMS: 395 $[M + H]^+$. Anal. ($C_{18}H_{26}N_4O_2S_2$) C, H, N, S.

Preparation of *N*-[5-[[[5-(1,1-Dimethylethyl)-2-oxazolyl]-methyl]thio]-2-thiazolyl]-1-(2-hydroxyethyl)-4-piperidinecarboxamide (23). To a solution of amine **21** (1.4 g, 3.7 mmol, 1 equiv) in DMF (30 mL) and THF (100 mL) were added 2-bromoethoxy-*tert*-butyldimethylsilane (0.79 mL, 3.7 mmol, 1 equiv) and solid sodium bicarbonate (1.8 g) at room temperature. The reaction was stirred at 50 °C for 23 h. Additional bromide (0.9 mL) was added, and the reaction was stirred at 50 °C for 22 h. The reaction was cooled and concentrated in vacuo, water (25 mL) was added, and the reaction was extracted with EtOAc (50 mL). The organic extract was dried (Na_2SO_4), concentrated in vacuo, and purified by flash chromatography (0−5% triethylamine in EtOAc gradient) to provide TBDS-protected **23** (1.70 g, 84%) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ 7.32 (s, 1H), 6.58 (s, 1H), 3.93 (s, 2H), 3.75 (t, J = 6.3 Hz, 2H), 3.0 (d, J = 14 Hz, 2H), 2.53 (t, J = 6.3 Hz, 2H), 2.36−2.30 (m, 1H), 2.15 (t, J = 14 Hz, 2H), 1.93−1.79 (m, 4H), 1.24 (s, 9H), 0.89 (s, 9H), 0.06 (s, 6H). LCMS: 539 $[M + H]^+$.

To a solution of the TBDS intermediate from above (1.45 g, 2.7 mmol, 1 equiv) in acetonitrile (100 mL) was added aq HF solution (48%, 2.5 mL) at room temperature.[14] The reaction was stirred for 4 h, an additional 2.5 mL of aq HF was added, and the reaction was stirred overnight. EtOAc (100 mL) and saturated aq $NaHCO_3$ (50 mL) were slowly added. Additional solid $NaHCO_3$ was added to make the mixture basic. The mixture was extracted with EtOAc (2 × 50 mL). The organic extracts were dried (Na_2SO_4) and filtered through a pad of silica gel, and the filtrate was concentrated in vacuo. The resulting white solid was recrystallized (aq EtOH) to provide **23** (1.60 g, 59%) as a white solid, mp 153−155 °C. ^{1}H NMR (DMSO-d_6) δ 12.20 (s, 1H), 7.38 (s, 1H), 6.72 (s, 1H), 4.35 (t, J = 5.4 Hz, 1 Hz), 4.05 (s, 2H), 3.48 (q, J = 5.7 Hz, 2H), 2.89 (d, J = 11.3 Hz, 2H), 2.36 (t, J = 6.0 Hz, 2H), 1.94 (t, J = 11.3 Hz, 2H), 1.72 (d, J = 11.3 Hz, 2H), 1.64−1.55 (m, 2H), 1.15 (s, 9H). LCMS: 425 $[M + H]^+$. HRMS FAB $(M + H)^+$ calcd for $C_{19}H_{28}N_4O_3S_2$ 424.1603; found 425.1690.

Preparation of *N*-[5-[[[5-(1,1-Dimethylethyl)-2-oxazolyl]-methyl]thio]-2-thiazolyl]-(*N*-methylsulfonyl)-4-piperidinecarboxamide (24). To a stirred solution of amine **21** (45 mg, 0.12 mmol) in pyridine (0.15 mL) and CH_2Cl_2 (1 mL) was added methanesulfonyl chloride (28 μL, 0.36 mmol) at room temperature. The reaction mixture was stirred for 1 h. Anhydrous potassium carbonate powder (130 mg, mmol) was then added, and the mixture was stirred at room temperature for 1 h. Water (1 mL) was added. The mixture was stirred vigorously for 30 min. The aqueous solution was extracted with CH_2Cl_2 (3 × 1 mL). The combined organic extracts were dried (Na_2SO_4), concentrated in vacuo, and purified by flash chromatography (50% EtOAc/heptane and then EtOAc) to give **24** (47 mg, 85%) as a white solid, mp 212−214 °C. ^{1}H NMR ($CDCl_3$) δ 9.11 (s, 1H), 7.29 (s, 1H), 6.57 (s, 1H), 3.92 (s, 2H), 3.79 (dt, J = 13.3, 4.8 Hz, 2H), 2.89 (dt, J = 2.7, 11.4 Hz, 2H), 2.48 (m, 1H), 2.06−2.01 (m, 2H), 1.98−1.89 (m, 2H), 1.24 (s, 9H). LCMS: 459 $[M + H]^+$. HRMS FAB $(M + H)^+$ calcd for $C_{18}H_{26}N_4O_4S_3$ 458.1116; found 59.1190.

Preparation of *N*-[5-[[[5-(1,1-Dimethylethyl)-2-oxazolyl]-methyl]thio]-2-thiazolyl]-(*N*-*tert*-butoxycarbonyl)-4-piperidinecarboxamide (25). To a mixture of amine **8** (270 mg, 1.10 mmol), *N*-*tert*-butoxycarbonylisonipecotic acid (344 mg, 1.50 mmol), DMAP (61 mg, 0.50 mmol), and triethylamine (0.28 mL, 2.0 mmol) in DMF (1 mL) and CH_2Cl_2 (3 mL) was added EDAC (383 mg, 2.00 mmol) at room temperature. The reaction mixture was stirred for 1.5 h, diluted with EtOAc, washed sequentially with brine, aq HCl, and aq sodium bicarbonate, dried (Na_2SO_4), and concentrated in vacuo. The crude material was purified by flash chromatography (1:1 EtOAc/hexanes and then EtOAc) to give a yellow solid which was recrystallized (EtOAc) to afford **25** (363 mg, 76%) as a white solid, mp 216−218 °C. ^{1}H NMR ($CDCl_3$) δ 8.94 (s, 1H), 7.29 (s, 1H), 6.56 (s, 1H), 4.18 (b, 2H) 3.91 (s, 2H), 2.80 (b, 2H), 2.5−2.41 (m, 1H), 1.88 (d, J = 15 Hz, 2H), 1.75−1.65 (m, 2H), 1.45 (s, 9H), 1.23 (s, 9H). LCMS: 481 $[M + H]^+$. Anal. ($C_{22}H_{32}N_4O_4S_2$) C, H, N, S.

Preparation of *cis*-4-Amino-*N*-[5-[[[5-(1,1-dimethylethyl)-2-oxazolyl]methyl]thio]-2-thiazolyl]cyclohexanecarboxamide Hydrochloride (26) and *trans*-4-Amino-*N*-[5-[[[5-(1,1-dimethylethyl)-2-oxazolyl]methyl]thio]-2-thiazolyl]cyclohexanecarboxamide Hydrochloride (27). To a solution of 4-aminocyclohexane carboxylic acid (2.86 g, 20 mmol) in 0.5 M aqueous NaOH (40 mL), dioxane (20 mL), and acetonitrile (4 mL) was added portionwise di-*tert*-butyl dicarbonate (6.5 g total, 30 mmol) at room temperature. After 20 h, EtOAc (100 mL) and 10% aqueous citric acid solution (100 mL) were introduced. The aqueous layer which formed was separated and extracted with EtOAc (3 × 50 mL). The organic phases were combined, dried (Na_2SO_4), and concentrated in vacuo to give crude 4-(*tert*-butoxycarbonylamino)-cyclohexanecarboxylic acid (6.0 g, 125%) as a colorless oil which solidified upon standing.

To a solution of crude 4-(*tert*-butoxycarbonylamino)cyclohexane carboxylic acid (5.0 g, <21 mmol) and amine **8** (3.50 g, 13 mmol) in DMF (13 mL) and CH_2Cl_2 (36 mL) was added EDAC (5.0 g, 26 mmol) at room temperature. The reaction mixture was stirred overnight and diluted with water (100 mL). The aqueous layer was separated and extracted with EtOAc (2 × 150 mL). The combined organic phases were dried (Na_2SO_4) and then filtered through a pad of silica gel. The filtrate was concentrated in vacuo to afford an orange solid. The crude material was recrystallized (95% EtOH) to give tBoc-**26/27** (2.78 g, 43%) as a yellow solid.

To a suspension of the tBoc-protected product (2.6 g, 5.3 mmol) in CH_2Cl_2 (15 mL) was added TFA (3 mL). The mixture was stirred at room temperature for 2 h, concentrated in vacuo, neutralized with base, and extracted with EtOAc. The organic layer was separated, dried (Na_2SO_4), and concentrated in vacuo to give a crude mixture of **26** and **27** (2.7 g). A portion of the crude mixture (1.2 g) was dissolved in methanol and mixed with sodium bicarbonate powder to ensure neutralization of residual TFA. The mixture was stirred for 1 h and filtered to remove solids, and the filtrate was concentrated in vacuo. The crude material was purified by flash chromatography (10% isopropylamine in EtOAc, and then 10% methanol and 20% isopropylamine in EtOAc) to give cis-product **26** (0.80 g, 16% from amine **8**) as a white solid and trans product **27** (0.08 g, 1.6% from amine **8**) as a yellow solid.

Amine **26** was converted to the HCl salt with 1 N aq HCl (1 equiv) to give a pale yellow solid, mp 224−226 °C. ^{1}H NMR (CD_3OD) δ 7.30 (s, 1H), 6.66 (s, 1H), 3.98 (s, 2H), 3.26 (m, 1H), 2.74 (quintet, J = 4.8 Hz, 1H), 2.06−2.00 (m, 2H), 1.90−1.74 (m, 6H), 1.24 (s, 9H). LCMS: 359 $[M + H]^+$. Anal. ($C_{18}H_{27}N_4O_2S_2Cl$ + 1.4 H_2O) C, H, N, S, Cl.

Amine **27** was converted to the HCl salt with 1 N aq HCl (1 equiv) to give a pale yellow solid. ^{1}H NMR (CD_3OD) δ 7.32 (s, 1H), 6.68 (s, 1H), 3.99 (s, 2H), 3.12 (tt, J = 3, 12 Hz, 1H), 2.50 (d, J = 6 Hz, ∼1H), 2.47 (tt, J = 3, 12 Hz, ∼1H overlapped with doublet at 2.50 ppm), 2.13 (d, J = 12 Hz, 2H), 2.03 (d, J = 12 Hz, 2H), 1.65 (dq, J = 3, 12 Hz, 2H), 1.47 (dq, J = 3, 12 Hz, 2H), 1.24 (s, 9H). LCMS: 359 $[M + H]^+$. Anal. ($C_{18}H_{27}N_4O_2S_2Cl$ + 1.03 H_2O) C, H, N, S, Cl.

CDK2/Cyclin E Kinase Assay. Kinase reactions consisted of 5 ng of baculovirus expressed GST-CDK2/cyclin E complex, 0.5 μg GST-RB fusion protein (amino acids 776−928 of retinoblastoma protein), 0.2 μCi ^{33}P γ-ATP, and 25 μM ATP in 50 μL of kinase buffer (50 mM HEPES, pH 8.0, 10 mM $MgCl_2$, 1 mM EGTA, 2 mM DTT). Reactions were incubated for 45 min at 30 °C and stopped by the addition of cold TCA to a final concentration of 15%. TCA precipitates were collected onto GF/C unifilter plates (Packard Instrument Co.) using a Filtermate universal harvester (Packard Instrument Co.). The filters were quantified using a TopCount 96 well liquid scintillation counter (Packard Instrument Co.). Dose−response curves were generated to determine the concentration required for inhibiting 50% of kinase activity (IC_{50}). Compounds were dissolved at 10 mM in DMSO and evaluated at six concentrations, each in triplicate. The final concentration of DMSO in the assay was 2%. IC_{50} values were derived by nonlinear

regression analysis and have a coefficient of variance (SD/mean, $n = 6$) of 16%.

72 h Antiproliferation Assay. In vitro cytotoxicity was assessed in tissue culture cells by MTS {3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfenyl)-2*H*-tetrazolium, inner salt} assay.[15] Depending on the cell line employed, cells were plated at a density of 3000−6000 cells/well in a 96-well plate. Drugs were added after 24 h and serial diluted. The cells were incubated at 37 °C for 72 h at which time the tetrazolium dye MTS, in combination with phenazine methosulfate, was added. After 3 h, the absorbency was measured at 492 nm, which is proportional to the number of viable cells. The results are expressed as IC_{50} values.

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Supporting Information Available: A summary of X-ray crystallographic data for Figure 2. This material is available via the Internet at http://pubs.acs.org.

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