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ARTICLE

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Structure-Based Design of Potent and Selective 3-Phosphoinositide-Dependent Kinase-1 (PDK1) Inhibitors†

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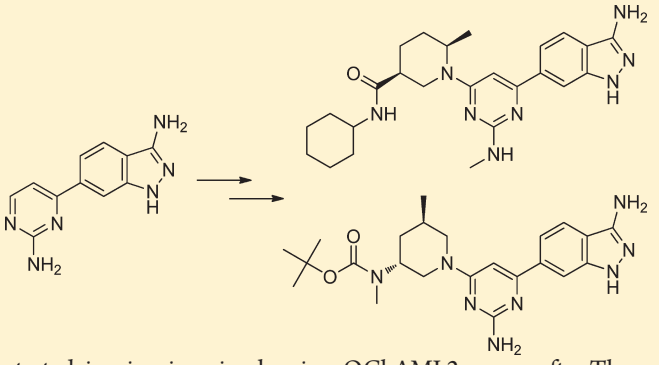
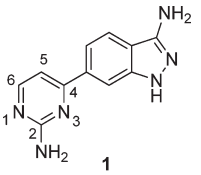
ABSTRACT: Phosphoinositide-dependent protein kinase-1 (PDK1) is a master regulator of the AGC family of kinases and an integral component of the PI3K/AKT/mTOR pathway. As this pathway is among the most commonly deregulated across all cancers, a selective inhibitor of PDK1 might have utility as an anticancer agent. Herein we describe our lead optimization of compound **1** toward highly potent and selective PDK1 inhibitors via a structure-based design strategy. The most potent and selective inhibitors demonstrated submicromolar activity as measured by inhibition of phosphorylation of PDK1 substrates as well as antiproliferative activity against a subset of AML cell lines. In addition, reduction of phosphorylation of PDK1 substrates was demonstrated in vivo in mice bearing OCl-AML2 xenografts. These observations demonstrate the utility of these molecules as tools to further delineate the biology of PDK1 and the potential pharmacological uses of a PDK1 inhibitor.



INTRODUCTION

Phosphoinositide-dependent protein kinase-1 (PDK1), a master regulator of the AGC kinase signal transduction, phosphorylates and activates at least 23 related AGC protein kinases that are often constitutively activated in human cancers and enable tumorigenesis.[1−4] PDK1 is a direct downstream effector of PI3K that positively regulates the AKT pathway,[5−7] resulting in inhibition of apoptosis, promotion of cell division, and stimulation of glucose uptake and storage. Activation of PI3K, mediated by the interaction of insulin and growth factors with their receptors, results in the production of phosphatidylinositol 3,4,-diphosphate (PIP2) and phosphatidylinositol 3,4,5-triphosphate (PIP3), which colocalize AKT and PDK1 to the plasma membrane through interaction with their pleckstrin homology (PH) domains, allowing PDK1 to phosphorylate the activation loop of AKT at Thr 308 and hence initiate the activation of AKT in a PI3K-dependent manner.[1−4] Furthermore, PDK1 is an activator of additional kinases involved in tumor progression such as protein kinase C (PKC), serum-and glucocorticoid-induced protein kinase (SGK), p70 ribosomal S6 kinase (S6K1), and p90 ribosomal S6 kinase (RSK).[2] These kinases lack a PH domain and are therefore not dependent on colocalization with PDK1 at the plasma membrane. For these kinases, phophorylation at the hydrophobic motif by distinct upstream kinases enables PDK1 to recognize and interact with these enzymes through its PIF-pocket, a small phosphate binding groove located in the PDK1 catalytic domain.



1
PDK1 pIC_{50} = 6.4
LE = 0.52

Figure 1. Lead compound **1**.

This interaction facilitates the T-loop phosphorylation of these substrates, activating them in a PI3K-independent manner.[1−4] Because PDK1 is involved in several distinct signaling pathways that are important for tumor progression, inhibitors of PDK1 might be beneficial for the treatment of cancer.[8−11] As such, several classes of small-molecule PDK1 inhibitors have been reported.[12,13] Herein, we report our structure-based optimization of compound **1**, a lead generated from screening an in-house fragment library against PDK1 (Figure 1).[14]

CHEMISTRY

Analogues substituted at the 2-position of the pyrimidine ring were synthesized according to Scheme 1. Suzuki coupling of

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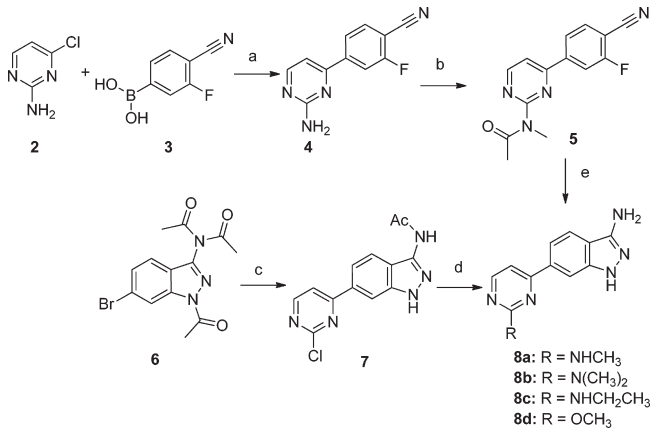
4-chloro-2-aminopyrimidine **2** with boronic acid **3** provided intermediate **4**, which was acetylated and then methylated to give **5**. Reaction with hydrazine monohydrate in ethanol at 95 °C both removed the acetyl group and formed the indazole ring to afford **8a**. Compounds **8b** and **8c** were prepared from intermediate **7**, which was synthesized by the bis-pinacolato diboron coupling of **6**, followed by Suzuki coupling with 2,4-dichloropyrimidine. Treatment of intermediate **7** with dimethylamine or ethylamine followed by removal of the acetyl group with acidic methanol afforded **8b** and **8c**, respectively. Compound **8d** was prepared by direct treatment of intermediate **7** with acidic methanol.

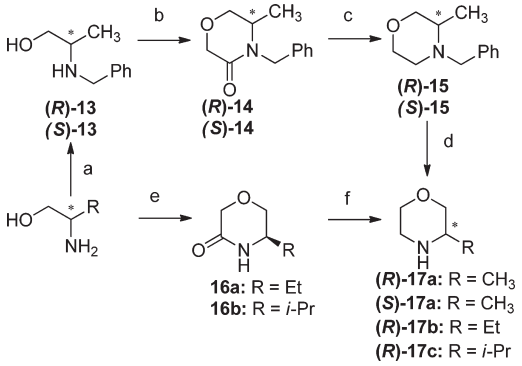
Two general synthetic routes were employed to obtain a series of 6-substituted pyrimidine analogues **12** as described in

Scheme 1. Synthesis of Compounds of General Structure 8[a]



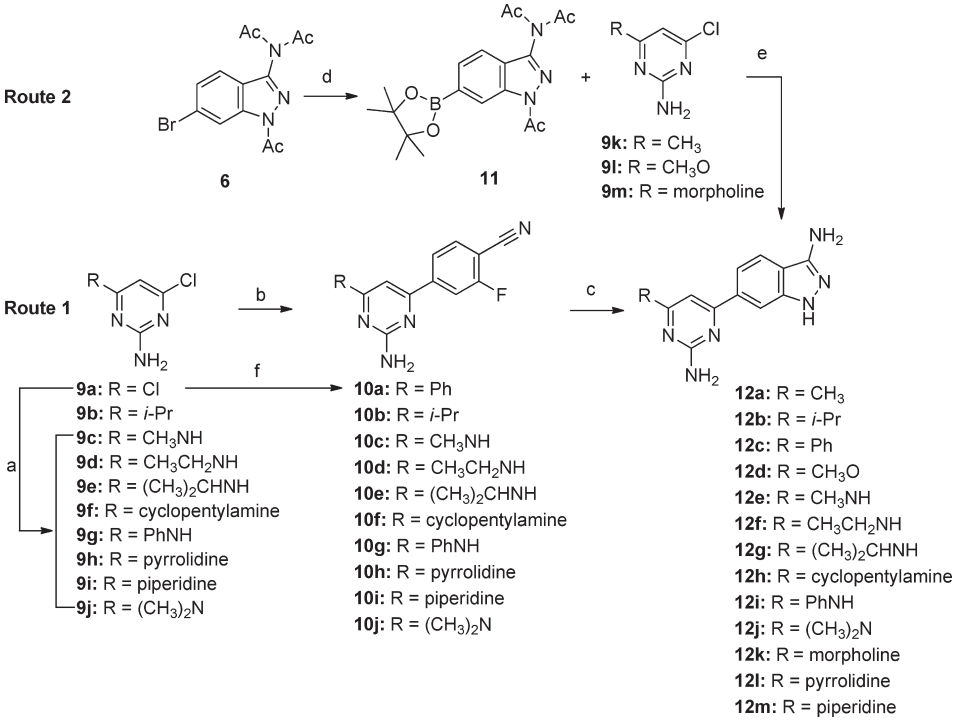
[a] Reagents and conditions: (a) $Pd(PPh_3)_4$, 1,4-dioxane, $NaHCO_3$(aq), 95 °C; (b) (i) Ac_2O, 80 °C, (ii) CH_3I, Cs_2CO_3, DMF, rt; (c) (i) bis(pinacolato)diboron, KOAc, $PdCl_2(dppf)\cdot CH_2Cl_2$, 1,4-dioxane, 100 °C, (ii) 2,4-dichloropyrimidine, $PdCl_2(dppf)\cdot CH_2Cl_2$, 1,4-dioxane, $NaHCO_3$(aq), 100 °C; (d) for **8b** and **8c**, (i) amine, THF, 100 °C, (ii) HCl, CH_3OH, 60 °C; for **8d**, HCl, CH_3OH, 50 °C; (e) $H_2NNH_2\cdot H_2O$, EtOH, 95 °C.

Scheme 3. Synthesis of α-Alkyl Substituted Cyclic Amines 17[a]



[a] Reagents and conditions: (a) (i) benzaldehyde, toluene, reflux, (ii) $NaBH_4$, EtOH, 0 °C; (b) (i) chloroacetyl chloride, K_2CO_3, THF, H_2O, 0 °C, (ii) $NaOH_{(aq)}$ (pH > 13), rt; (c) Red-Al, toluene, 0−60 °C; (d) H_2, Pd/C, HCl, CH_3OH, rt; (e) (i) NaH, toluene, 0 °C, (ii) chloroacetyl chloride, toluene, 90−110 °C; (f) $LiAlH_4$, THF, 70 °C.

Scheme 2. Synthesis of Compounds 12[a]



[a] Reagents and conditions: (a) amine, (EtOH, CH_3OH or THF), 50−100 °C; (b) **3**, $Pd(PPh_3)_4$, 1,4-dioxane, $NaHCO_3$(aq), 90−100 °C; (c) $H_2NNH_2\cdot H_2O$, EtOH, 80−100 °C; (d) bis(pinacolato)diboron, KOAc, $PdCl_2(dppf)\cdot CH_2Cl_2$, 1,4-dioxane, 100 °C; (e) (i) (**9k**, **9l**, or **9m**), $PdCl_2(dppf)\cdot CH_2Cl_2$, 1,4-dioxane, $NaHCO_3$(aq), 100 °C, (ii) HCl, CH_3OH, 60 °C; (f) (i) **3**, $Pd(PPh_3)_4$, 1,4-dioxane, $NaHCO_3$(aq), 95 °C, (ii) $PhB(OH)_2$, $Pd(PPh_3)_4$, 1,4-dioxane, $NaHCO_3$(aq), 95 °C.

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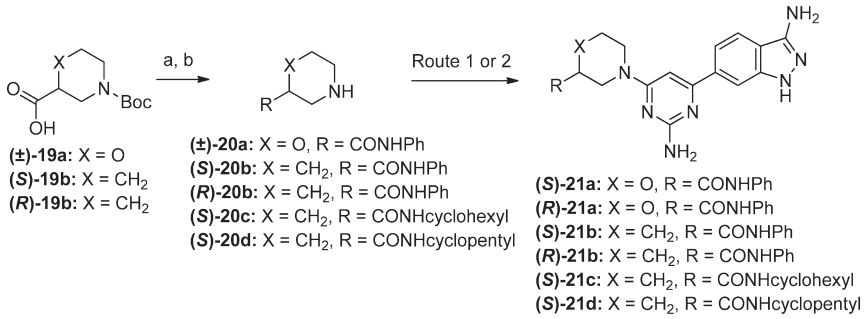
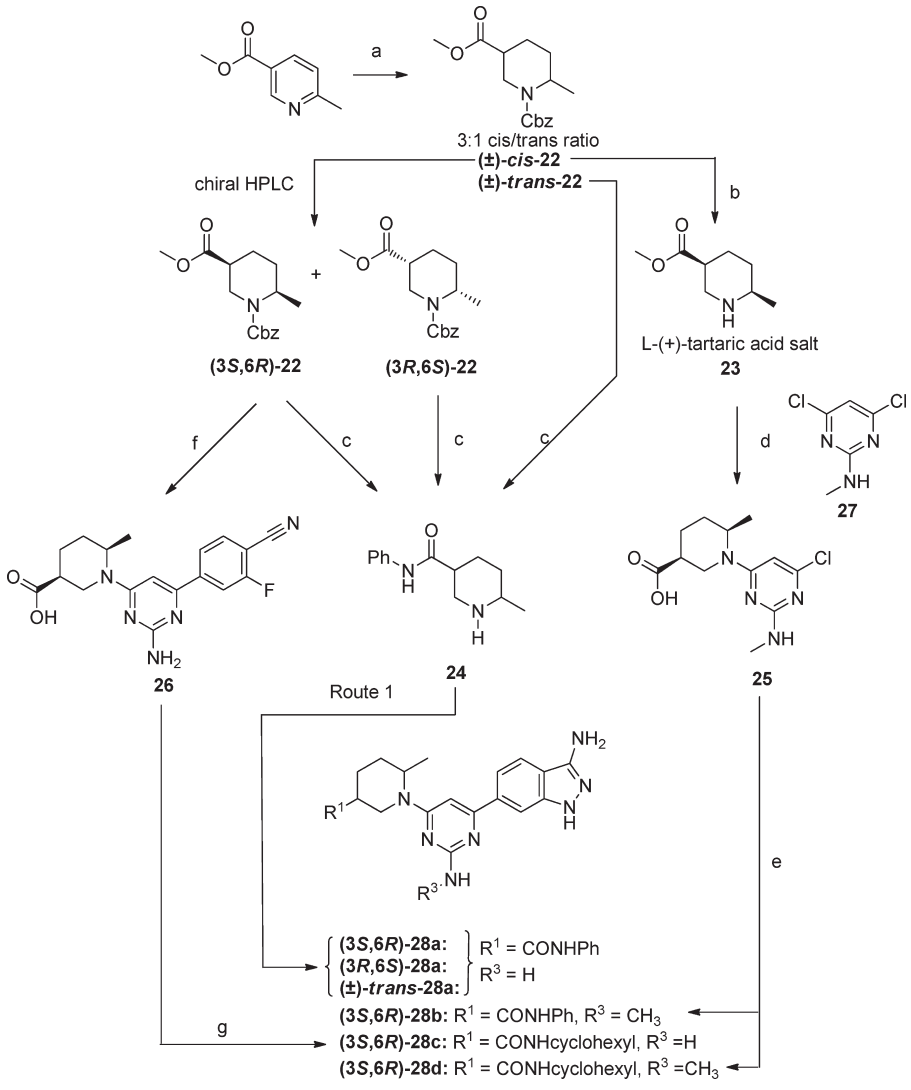
Scheme 4. Synthesis of Compounds of General Structure 21[a]



[a] Reagents and conditions: (a) amine, EDC, HOBt, *N*-methylmorpholine, rt; (b) TFA, CH_2Cl_2, rt.

Scheme 5. Synthesis of Bis-substituted Piperidine Analogues 28[a]



[a] Reagents and conditions: (a) (i) HCl, PtO_2, CH_3OH, (ii) CbzCl, DMAP, Et_3N, CH_2Cl_2, 15−25 °C; (b) (i) 10% Pd/C, H_2 (65 psi), EtOAc, EtOH, rt, (ii) crystallization as the tartrate salt (L-(+)-tartaric acid, IPA, water); (c) (i) $LiOH \cdot H_2O$, THF, water, CH_3OH, rt, (ii) aniline, EDC, HOBt, Hünig's base, DMF, rt, (iii) 10% Pd/C, H_2, EtOAc, EtOH, rt; (d) (i) $LiOH \cdot H_2O$, water, rt, (ii) **27**, $NaHCO_3$, 1,4-dioxane, 100 °C; (e) (i) amine, HATU, Hünig's base, CH_2Cl_2, 0 °C, (ii) route 1 or 2; (f) (i) 1,4-dioxane, conc HCl, 100 °C, (ii) **9a**, $NaHCO_3$, 1,4-dioxane, water, 120 °C, (iii) **3**, $Pd(PPh_3)_4$, $NaHCO_3$, 1,4-dioxane, water, 120 °C; (g) (i) cyclohexylamine, HATU, Hünig's base, DMF, rt, (ii) H_2NNH_2, EtOH, 110 °C.

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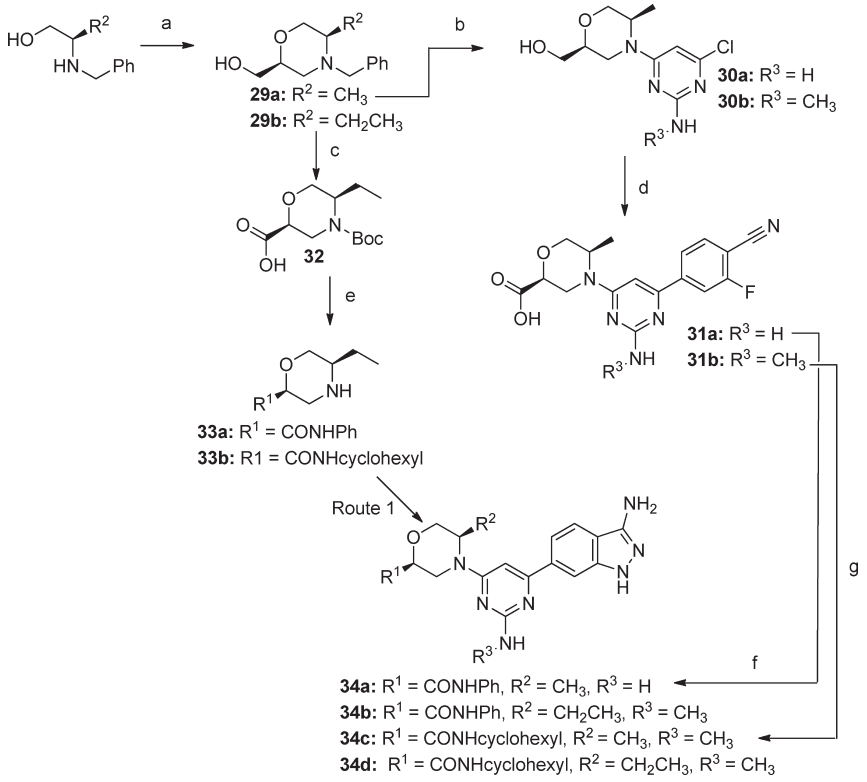
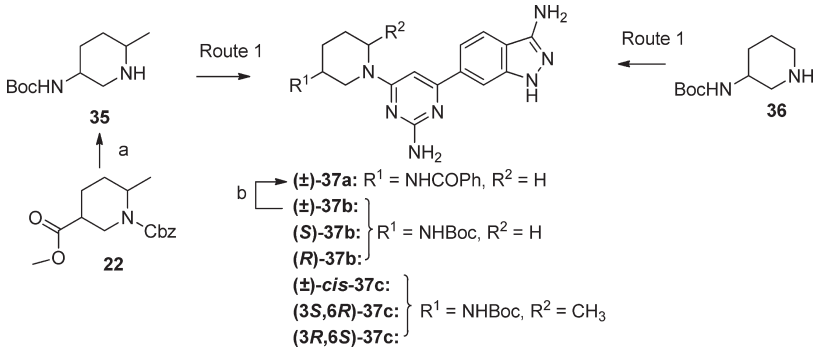
Scheme 6. Synthesis of Bis-substituted Morpholine Analogues 34[a]



[a] Reagents and conditions: (a) (i) (*R*)-(−)-epichlorohydrin, $LiClO_4$, toluene, or 1,2-dichloroethane, rt, (ii) $NaOCH_3$ in CH_3OH or NaOEt in EtOH, rt; (b) (i) HCl, EtOH, H_2, 10% Pd/C (Degussa type), rt, (ii) for **30a** (**9a**, Hünig's base, CH_3CN, 160 °C (MW)), for **30b** (**27**, K_2CO_3, EtOH, reflux); (c) (i) 10% Pd/C, H_2, CH_3OH, rt, (ii) Boc_2O, Hünig's base, THF, 40 °C, (iii) TEMPO, iodobenzene diacetate, CH_2Cl_2, 0 °C−rt; (d) (i) **3**, 1,4-dioxane, $NaHCO_3$(aq), $Pd(PPh_3)_4$, 100 °C, (ii) for **31a** (H_5IO_6/CrO_3, wet CH_3CN, 0−15 °C), for **31b** (TEMPO, iodobenzene diacetate, CH_2Cl_2, water, 0 °C−rt); (e) (i) EDC, HOAt, amine, CH_2Cl_2, rt, (ii) HCl, dioxane, rt; (f) (i) aniline, EDC, HOBT, DMF, rt, (ii) $H_2NNH_2 \cdot H_2O$, EtOH, 100 °C; (g) (i) cyclohexylamine, HATU, Hünig's base, DMF, rt, (ii) $H_2NNH_2 \cdot H_2O$, 1,4-dioxane, 100 °C.

Scheme 7. Synthesis of Compounds 37[a]



[a] Reagents and conditions: (a) (i) $LiOH \cdot H_2O$, THF, water, CH_3OH, rt, (ii) DPPA, *t*-butyl alcohol, Et_3N, 100 °C, (iii) Pd/C, H_2(50 psi), EtOAc, rt; (b) (i) conc HCl, 0 °C−rt, (ii) benzoyl chloride, $NaHCO_3$, THF, water, 0 °C−rt, (iii) HCl, CH_3OH, 65 °C.

Scheme 2. Route 1 involved the preparation of fluoronitrile intermediate **10** prior to the final cyclization, leading to the desired indazole analogues **12**. 6-Substituted aminopyrimidine compounds **9** were either commercially available or prepared by reaction of dichloropyrimidine **9a** with the corresponding nucleophile. Suzuki coupling of **9** with boronic acid **3** or the corresponding pinacol ester followed by hydrazine cyclization afforded compounds **12b**, **12e**−**j**, and **12l**−**m**. Compound **12c** was prepared by sequential Suzuki coupling of dichloropyrimidine **9a** with boronic acid **3** and then phenyl boronic acid, followed by hydrazine cyclization. Alternatively (route 2), intermediates **9k**−**m** were coupled with indazole boronic ester **11** under Suzuki conditions followed by acetyl group deprotection to provide the corresponding indazole analogues **12a**, **12d**, and **12k**.

Representative α-alkyl substituted cyclic amines were prepared as described in Scheme 3. The α-methylmorpholine amine

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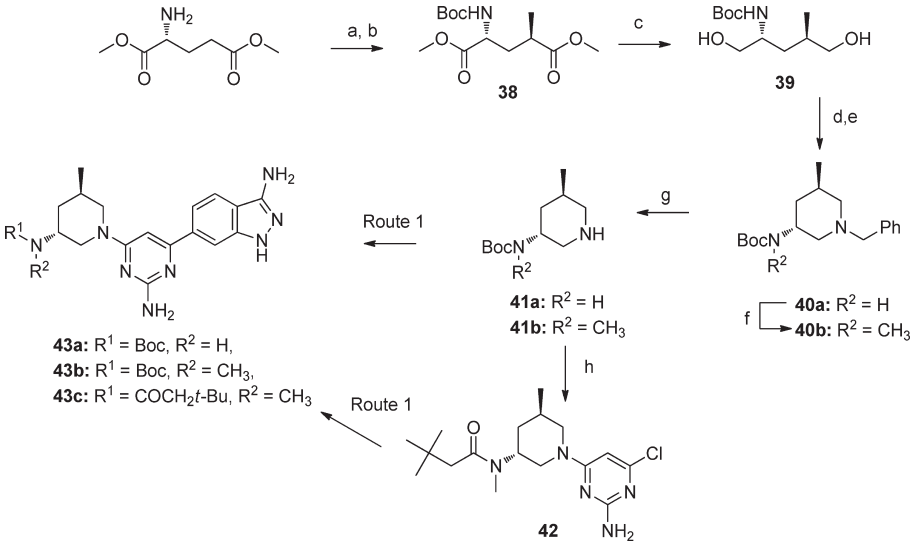
Scheme 8. Synthesis of Analogues 43[a]



[a] Reagents and conditions: (a) Boc_2O, Et_3N, CH_3OH, rt; (b) (i) LiHMDS, THF, −78 °C, (ii) CH_3I, −78 °C; (c) $CaCl_2$, $NaBH_4$, EtOH:THF (1:1), 0 °C; (d) MsCl, Et_3N, CH_2Cl_2, 0 °C; (e) benzylamine, 70 °C; (f) (i) NaH, DMF, rt, (ii) CH_3I, rt; (g) Pd/C (Degussa type), H_2, EtOH, rt; (h) (i) **9a**, Et_3N, EtOH, 100 °C, (ii) TFA, CH_2Cl_2, rt, (iii) 3,3-dimethylbutanoyl chloride, Hünig's base, CH_2Cl_2, rt.

individual enantiomers **17a** were prepared from the corresponding optically pure 2-amino-1-propanols by reductive amination with benzaldehyde followed by cyclization with chloroacetyl chloride to provide the enantiomerically pure morpholinone intermediates **14**. Reduction with Red-Al provided the benzyl protected morpholines **15**, which upon debenzylation under hydrogenolysis conditions afforded the desired α-methylmorpholines **17a**. The α-ethyl and α-isopropyl morpholine amines ((*R*)-**17b** and (*R*)-**17c**, respectively) were prepared by direct cyclization of the corresponding aminoalcohol with chloroacetyl chloride to provide morpholinones **16a** and **16b**, respectively, followed by reduction with $LiAlH_4$. These amines were then used to prepare analogues **18a**−**c** (Table 3) following the synthetic routes described in Scheme 2.

Compounds **21** were synthesized from their corresponding amines **20** following either synthetic routes 1 or 2 described in Scheme 2. Amines **20** were readily prepared from the Boc protected 3-piperidine or 2-morpholineamine carboxylic acids **19** by coupling with the corresponding amines followed by Boc deprotection (Scheme 4).

Several synthetic routes were used to prepare bis-substituted analogues of general structure **28** (Scheme 5). Hydrogenation of methyl 6-methylnicotinate using PtO_2 as catalyst,[15] followed by Cbz protection of the resulting secondary amine, afforded a 3/1 ratio of *cis*- and *trans*-2,5-bis-substituted piperidine isomers **22**, which were separated by flash chromatography. Chiral preparative HPLC separation afforded individual optically pure cis isomers (3*S*,6*R*)-**22** and (3*R*,6*S*)-**22**.[16] Compound **23** was obtained after removal of the Cbz protecting group of (±)-*cis*-**22** and crystallization of the resulting secondary amine as the L-(+)-tartrate salt. Hydrolysis of esters (3*S*,6*R*)-**22**, (3*R*,6*S*)-**22**, and (±)-*trans*-**22**, and subsequent coupling of the resulting acid with aniline followed by Cbz deprotection, provided the corresponding amines **24**. Compounds **28a** were prepared from amines **24** following synthetic route 1 described in Scheme 2. Analogues (3*S*,6*R*)-**28b** and (3*S*,6*R*)-**28d** were prepared from **23**. Saponification of **23**, followed by reaction of the resulting piperidine acid derivative with **27**, gave **25**. Reaction of compound **25** with the corresponding amines provided the amides, which were converted to compounds (3*S*,6*R*)-**28b** and (3*S*,6*R*)-**28d** following the synthetic routes described in Scheme 2. Compound (3*S*,6*R*)-**28c** was prepared from intermediate **26**, which was prepared from simultaneous ester hydrolysis and Cbz deprotection of (3*S*,6*R*)-**22** under acidic conditions, followed by reaction of the resulting piperidine acid derivative with pyrimidine **9a** and in situ Suzuki cross-coupling with boronic acid **3**. Reaction of intermediate **26** with cyclohexylamine, followed by cyclization with hydrazine, provided compound (3*S*,6*R*)-**28c**.

The syntheses of the morpholine analogues **34** are illustrated in Scheme 6. Bis-substituted morpholines **29** were prepared according to the reported protocol for similar compounds.[17] Removal of the benzyl protecting group in **29a**, followed by reaction with dichloroaminopyrimidines **9a** and **27**, afforded intermediates **30a** and **30b**, respectively. Suzuki coupling of **30** with boronic acid **3**, followed by oxidation[18] of the primary alcohol to the carboxylic acid, provided intermediates **31**. Reaction of intermediates **31** with an amine, followed by hydrazine cyclization, furnished the desired analogues **34a** and **34c**. Alternatively, replacement of the benzyl protecting group in **29b** with Boc, followed by oxidation of the primary alcohol, provided carboxylic acid **32**. Compound **32** was then converted to the amines **33** by reaction with the corresponding amine followed by deprotection of the Boc group. Final compounds **34b** and **34d** were synthesized from amines **33** by following synthetic route 1 described in Scheme 2.

Compounds **37b** and **37c** were prepared from amines **36** and **35**, respectively, following the general procedures (Scheme 7). While amine **36** was commercially available, amine **35** was prepared from compound **22** by ester hydrolysis, followed by Curtius rearrangement and subsequent Cbz deprotection. Compound (±)-**37a** was synthesized from (±)-**37b**.

A series of enantiomerically pure 5-methyl-3-aminopiperidine compounds of general structure **43** was prepared according to

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Scheme 8. Boc protection of dimethyl D-glutamate, followed by stereoselective α-methylation, provided compound **38**.[19] Reduction of the esters, followed by mesylation of the resulting diol **39** and subsequent treatment with neat benzylamine at 70 °C, provided piperidine **40a** with the desired regio- and stereochemistry. Methylation of the BocNH functionality on **40a** provided compound **40b**. Debenzylation of compounds **40** afforded amines **41**, which were converted to compounds **43a**−**b** by following the general synthetic route 1. Additionally, **41b** was converted to **42** by reaction with **9a**, Boc deprotection, and amide formation, which was then elaborated to **43c**.

RESULTS AND DISCUSSION

An X-ray crystal structure of compound **1** bound to PDK1 (Figure 2a) shows key hydrogen bonding interactions between the aminoindazole and Ser160 and Ala162 in the hinge region. Preliminary SAR at the 3-position of the indazole ring confirmed the importance of the amino group as a hydrogen bond donor for optimum binding (data not shown). In addition, the aminopyrimidine ring is engaged in a tight hydrogen bond network with the protein, with the pyrimidine ring nitrogens acting as acceptors for the catalytic Lys111 and Thr222 at the floor of the binding pocket. The amino group of the pyrimidine is a hydrogen bond donor to the catalytic residue Glu130. In agreement with the X-ray structural information, we previously demonstrated by SAR the importance that each nitrogen functionality of the aminopyrimidine contributes to PDK1 binding and inhibition of kinase activity.[14] Further inspection of the X-ray crystal structure revealed a small lipophilic pocket in the region occupied by the exocyclic amino group of the pyrimidine ring and defined by amino acid residues Met134, Val143, and Leu159. To further understand the SAR in this region, we varied substituents at the 2-position of the pyrimidine ring (Table 1). Consistent with the X-ray crystal structure, replacement of one NH_2 hydrogen with CH_3 (**8a**) was tolerated, causing only a minor reduction (0.4 log units) in enzyme potency relative to **1**, but increasing the size of the alkyl group to ethyl (**8c**) resulted in a significant reduction in potency. In addition, substituting both hydrogens with CH_3 (**8b**) or replacing the pyrimidine NH_2 with a methoxy group (**8d**) resulted in a significant reduction in potency, confirming the importance of a hydrogen bond donor at this position for favorable contact with Glu 130. This excellent agreement between the X-ray structural information and preliminary SAR provided a basis for further structure-based optimization of PDK1 activity.

Further examination of the crystal structure suggested that substitution at the pyrimidine 6-position could fill the lipophilic pocket under the G-loop (Figure 2b). Substitution with either alkyl, aryl, alkoxy, thiolate, alkylamine, or arylamine was tolerated and, in many instances, improved PDK1 potency (Table 2). Additionally, many of these substituents conferred good-to-excellent selectivity over other kinases such as Aurora A, Aurora B, ALK5, and ROCK1. These kinases were chosen as representative specificity targets based on profiling of **1**.[14] Compound **12a**, with a 6-methyl group, exhibited a 0.5 log increase in potency and maintained ligand efficiency (LE)[20,21] relative to compound **1**. Increasing the size of the alkyl substituent to *i*-Pr (**12b**) retained potency relative to **12a** with a concomitant decrease in LE. The methoxy compound **12d** showed similar potency to **12b**, whereas methylamine substitution (**12e**) increased potency and LE. Interestingly, although dimethylamine

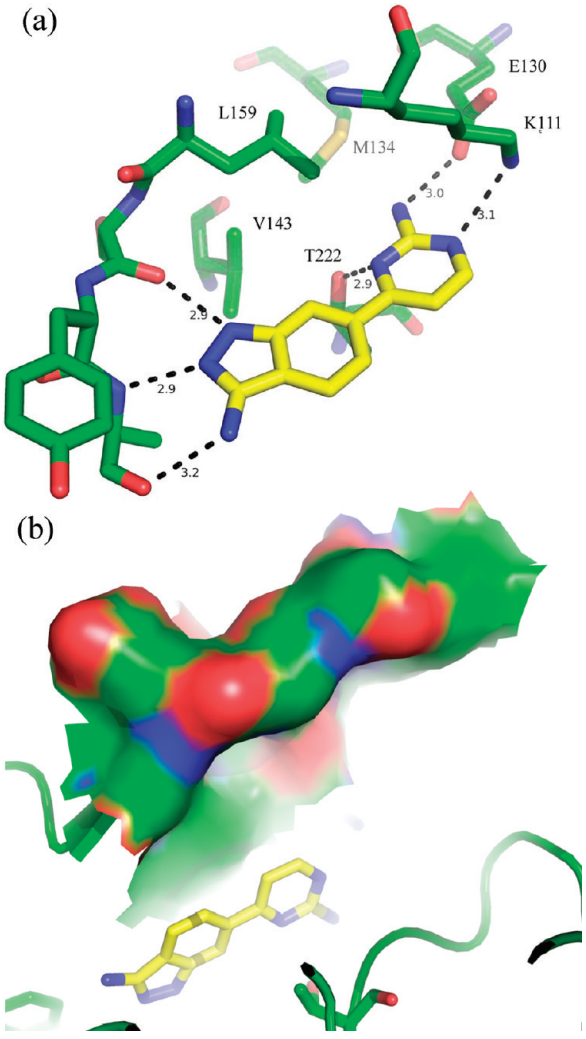
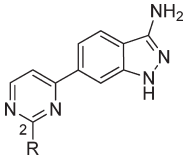


Figure 2. X-ray crystal structure of compound **1** bound to PDK1 (PDB code 3NUN). (a) Hydrogen bond distances in Å. (b) Surface of G-loop illustrating small hydrophobic pocket.

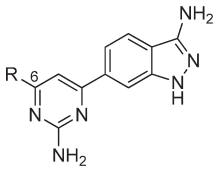
Table 1. SAR of the Hydrogen Bond Substitution at the Pyrimidine 2-Position

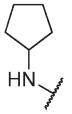


compd	R	PDK1 pIC_{50}	LE
1	NH_2	6.4	0.52
8a	$NHCH_3$	6.0	0.46
8b	$N(CH_3)_2$	4.7	0.34
8c	$NHCH_2CH_3$	5.1	0.37
8d	OCH_3	4.9	0.37

substitution (**12j**) did not result in an increase in potency relative to **1**, it significantly increased selectivity over other kinases such as ALK5, Aurora A, Aurora B, and ROCK1. These observations proved to be quite general, with monosubstituted amines

Table 2. Selected SAR of 6-Substituted Pyrimidines



Compd	R	Kinase pIC_{50}					LE	Compd	R	Kinase pIC_{50}					LE
		PDK1	AurA	AurB	ALK5	ROCK1				PDK1	AurA	AurB	ALK5	ROCK1	
1	H	6.4	6.4	5.5	5.8	5.8	0.52	**12g**		6.9	5.7	4.8	6.3	<5	0.45
12a	CH_3	6.9	5.4	4.9	5.1	5.4	0.53	**12h**		7.3	5.6	5.1	6.4	5.2	0.43
12b	*i*-Pr	6.6	5.5	5.1	5.7	<5	0.45	**12i**	PhNH	7.7	6.7	5.5	7.6	5.6	0.44
12c	Ph	6.7	5.8	5.7	5.5	<5	0.40	**12j**	$(CH_3)_2N$	6.3	<5	<5	5.3	<5	0.43
12d	CH_3O	6.6	5.2	5.1	5.5	5.3	0.48	**12k**		6.5	<5	<5	<5	<5	0.39
12e	CH_3NH	7.1	5.5	4.9	6.4	5.1	0.51	**12l**		6.6	<5	<5	<5	<5	0.41
12f	CH_3CH_2NH	6.8	5.6	5.0	6.3	<5	0.47	**12m**		6.6	<5	<5	<5	<5	0.39

providing a higher level of potency and LE (**12e**−**i**) and tertiary amines (**12j**−**m**) conferring a higher level of kinase selectivity.

The X-ray crystal structure of **12k** bound to PDK1 (Figure 3a) showed that the morpholine group was easily accommodated by PDK1 and situated under the G-loop. Interestingly, the crystal structure of the 6-ethylamino derivative **12f** with PDK1 (Figure 3b) revealed that the ethyl group is oriented toward the G-loop and is accommodated by a small lipophilic pocket. An overlay of **12f** and **12k** (Figure 3c) suggested that an axial alkyl group α to the amine on a cyclic amine could also fill this pocket. This substitution had potential to provide the higher potency observed for monoalkylamines as well as the high degree of kinase selectivity associated with tertiary amine substitution. We surmised that the axial conformation would be preferred based on allylic strain principles.[22]

As predicted from the X-ray crystal structure overlay of **12f** and **12k**, methyl substitution at the α-position of the morpholine ring ((*R*)-**18a**) increased enzyme potency (~0.5 log units) and LE relative to unsubstituted morpholine **12k** and also maintained an excellent level of kinase selectivity (Table 3). The α-methyl substituted piperidine analogue **18d** exhibited similar potency to (*R*)-**18a**. The predicted binding mode was confirmed by a crystal structure of (*R*)-**18a** bound to PDK1 (Figure 4). Consistent with the structural data, the enantiomer (*S*)-**18a** was less active than the unsubstituted morpholine derivative **12k**. Additionally, while ethyl substitution at the α-position of the morpholine ring increased enzyme potency (**18b**) relative to **12k**, increasing the size further to isopropyl (**18c**) had only a marginal effect.

Further examination of the X-ray crystal structure of **12k** bound to PDK1 suggested that additional favorable interactions with another lipophilic pocket along the G-loop could be accessed through carboxamide substitution at the β-position of either a morpholine or piperidine ring (Figure 5a). Both optically pure *N*-phenylcarboxamide substituted morpholine (*S*)-**21a** and piperidine (*S*)-**21b** analogues showed a significant increase in potency (~10-fold) relative to their unsubstituted counterparts **12k** and **12m**, with good kinase selectivity for PDK1 (Table 4). The binding mode was confirmed by a crystal structure of (*S*)-**21a** bound to PDK1 (Figure 5b), wherein interactions extending beyond the morpholine ring under the G-loop are observed. The carboxamide offers good contacts for H-bonding with a water molecule and serves as a linker for the phenyl ring to extend into the lipophilic pocket. Consistent with the binding mode, the corresponding enantiomers (*R*)-**21a** and (*R*)-**21b** were less potent. Cyclohexylcarboxamide substituted compound (*S*)-**21c** exhibited comparable potency to (*S*)-**21b**. Cyclopentylcarboxamide (*S*)-**21d** showed a slight reduction in potency, while compounds containing smaller alkylcarboxamide substituents were significantly less active (data not shown).

Examination of the X-ray crystal structures of (*R*)-**18a** and (*S*)-**21a** revealed that the morpholine ring adopts the same conformation with either the β-carboxamide or the α-alkyl substituent. Therefore, we hypothesized that incorporating both groups on a cis-disubstituted six-membered ring scaffold would be beneficial. This requires that the carboxamide occupies an equatorial position while the alkyl substituent occupies an axial position, filling the lipophilic G-loop pockets. Indeed, the combination of substituted carboxamides with alkyl substituents provided an additive effect (Table 5). For instance, the bis-substituted piperidine (3*S*,6*R*)-**28a** and morpholine **34a** analogues

dx.doi.org/10.1021/jm101527u |*J. Med. Chem.* 2011, 54, 1871–1895

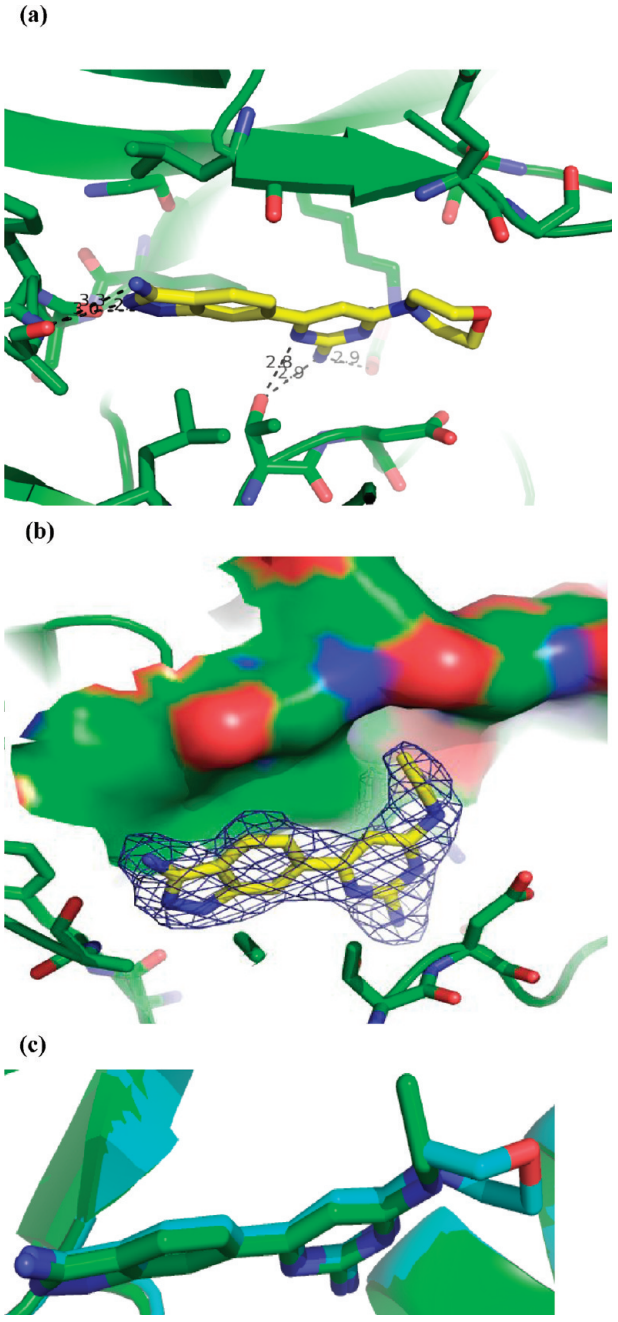


Figure 3. X-ray crystal structures and overlay of compounds **12k** and **12f** bound to PDK1.

showed an approximately 100-fold increase in potency relative to the corresponding unsubstituted amines **12m** and **12k**, respectively. The X-ray crystal structure of (3*S*,6*R*)-**28a** with PDK1 (Figure 6) confirmed the expected binding mode, where the α-methyl substituent occupies a small pocket under the G-loop and the phenyl carboxamide substituent extends beyond the piperidine ring under the G-loop. Consistent with this binding mode, the corresponding enantiomer (3*R*,6*S*)-**28a** and its racemic trans-stereoisomer (±)-*trans*-**28a** showed >100-fold lower potency.

Having achieved high enzyme potency and selectivity, we evaluated several compounds in a mechanistic cellular assay in PC-3 cells to determine the effect of inhibiting PDK1 on PI3-kinase dependent and independent pathways. PDK1-mediated phosphorylation of Thr^{308}-AKT (PI3K-dependent) and Ser^{221}-RSK (PI3K-independent) were evaluated as well as the PDK1-independent phosphorylation state of Ser^{473}-AKT. Consistent with potent and selective inhibition of PDK1, compounds (3*S*,6*R*)-**28a** and **34a** exhibited submicromolar inhibition of the phosphorylation of both Thr^{308}-AKT and Ser^{221}-RSK and did not affect the phosphorylation of Ser^{473}-AKT (IC_{50} > 29300 nM). Interestingly, methyl substitution on the pyrimidine 2-amino group provided compounds with increased cellular potency and kinase selectivity ((3*S*,6*R*)-**28b** vs (3*S*,6*R*)-**28a** and (3*S*,6*R*)-**28d** (GSK-2334470)[23] vs (3*S*,6*R*)-**28c**). A similar trend was observed for **34b**−**d**.

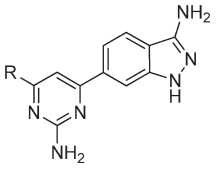
As part of our optimization process, we also investigated functionalities at the β-position to the nitrogen of the six-membered ring other than carboxamide. The activities of selected representatives are depicted in Table 6. The reverse amide (±)-**37a** and the carbamate (±)-**37b** exhibited similar potency to phenylcarboxamide (*S*)-**21b**. Although the expected increase in potency when adding the α-methyl group was observed ((±)-*cis*-**37c**), it was not as pronounced as in the carboxamide series. Surprisingly, an X-ray crystal structure of (±)-*cis*-**37c** with PDK1 (Figure 7) revealed that the opposite antipode occupied the active site (compared to the carboxamide series). To accommodate this stereoisomer, the piperidine adopted the alternate chair conformation, which places the methyl group away from the pocket in the G-loop. Consistent with the X-ray results, (3*R*,6*S*)-**37c** exhibited approximately 100-fold higher potency relative to its enantiomer (3*S*,6*R*)-**37c**, although it showed only a modest increase in potency (~0.5 log units) relative to its corresponding nonmethylated analogue (*R*)-**37b**.

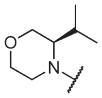
Analysis of the X-ray crystal structure of (3*R*,6*S*)-**37c** in PDK1 suggested that moving the methyl group to the β-position of the piperidine ring with a trans relationship to the carbamate functionality could potentially fill the lipophilic pocket under the G-loop. Thus a series of piperidine compounds of general structure **43** was investigated (Table 7). Indeed, compound **43a** exhibited high potency in the PDK1 enzyme assay as well as excellent potency in the mechanistic cellular assays. The binding mode was confirmed by an X-ray crystal structure of **43a** bound to PDK1 (Figure 8). The β-methyl substituent points toward the lipophilic pocket under the G-loop, and a hydrogen bond network involving the carbamate carbonyl group and both the catalytic lysine 111 and serine 94 is observed. The *N*-Me carbamate **43b** exhibited higher potency in the PDK1 enzyme assay (~10-fold), and the cellular mechanistic assay (~5-fold), relative to the NH carbamate **43a**. In addition, replacement of the oxygen atom in carbamate **43b** with carbon afforded amide **43c**, which also exhibited a similar potency profile in the PDK1 enzyme and mechanistic assays.

Selected compounds with potent mechanistic cellular activity were tested for antiproliferative activity against a panel of leukemia cell lines. We found that AML cell lines were more sensitive to PDK1 inhibition (Table 8), which is consistent with published data in which overexpression of PDK1 was found to be a common feature of acute myeloid leukemia (45% of patients), promoting monocyte colony formation and translocation of PKC.[24] However, we found that AML cell lines were not uniformly inhibited and only a subset of AML cell lines was sensitive to growth inhibition by our PDK1 inhibitors (data not shown).[25]

Compound (3*S*,6*R*)-**28d** was further evaluated through an in vitro kinase selectivity panel of 285 protein and lipid kinases.[26]

Table 3. SAR Results of α-Alkyl Substituted Cyclic Amines



Compd	R	Kinase pIC$_{50}$ PDK1	AurA	AurB	ALK5	ROCK1	LE	Compd	R	Kinase pIC$_{50}$ PDK1	AurA	AurB	ALK5	ROCK1	LE
(*R*)-**18a**		7.2	4.9	4.6	<5	4.8	0.41	**18c**		6.7	4.9	5.1	<5	4.8	0.35
(*S*)-**18a**		5.9	<5	4.6	<5	4.8	0.34	**18d**		6.9	<5	4.6	5.1	<5	0.39
18b		7.5	5.3	<5	<5	<5	0.41								

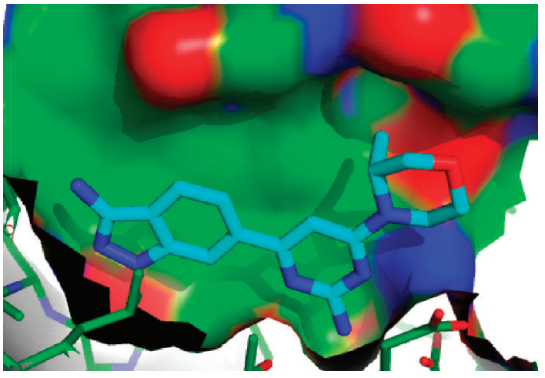


Figure 4. X-ray crystal structure of compound (*R*)-**18a** bound to PDK1.

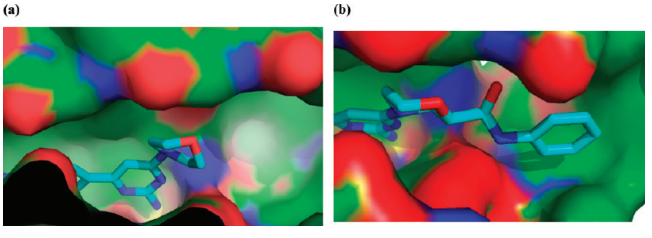
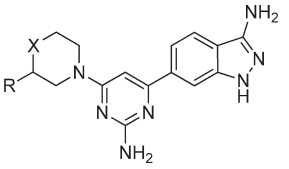


Figure 5. X-ray crystal structures of compounds **12k** and (*S*)-**21a** bound to PDK1.

Only 24 kinases were inhibited >50% in the presence of 10 μM (**3*S*,6*R*)-28d** (Table 9). IC$_{50}$ values available from our internal kinase panel for a subset of kinases including members from the AGC family are shown in Table 10. (**3*S*,6*R*)-28d** exhibited >1000-fold selectivity against each of these kinases compared to potency against PDK1.

The ability of (**3*S*,6*R*)-28d** to inhibit PDK1 signaling in vivo was evaluated using SCID mice bearing OCI-AML2 xenografts. At its MTD dose (100 mg/kg, ip, single dose), which afforded high exposure (AUC = 31362 ng·h/mL; C_{max} = 4510 ng/mL), (**3*S*,6*R*)-28d** exhibited 58% and 29% reduction of AKT^{T308} phosphorylation at 3 and 6 h, respectively, and 57% and 71% reduction of RSK^{S221} phosphorylation at 3 and 6 h, respectively (Figure 9). Consistent with the in vitro findings, we observed no inhibition of AKT^{S473} phosphorylation.

Table 4. SAR of Carboxamide Substitution on Morpholine and Piperidine Analogues



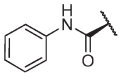
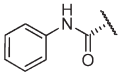
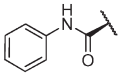
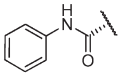
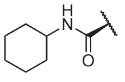
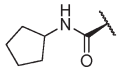
Compd	R	X	Kinase pIC$_{50}$ PDK1	AurA	AurB	ALK5	ROCK1	LE
(*S*)-**21a**		O	7.7	5.9	5.5	<5	6.1	0.33
(*R*)-**21a**		O	6	<5	<5	<5	<5	0.26
(*S*)-**21b**		CH_2	7.5	5.1	5.9	<5	5.7	0.32
(*R*)-**21b**		CH_2	6.9	5.1	5.8	<5	5.5	0.30
(*S*)-**21c**		CH_2	7.2	<5	5.4	<5	5.3	0.31
(*S*)-**21d**		CH_2	6.9	<5	<5	<5	<5	0.30

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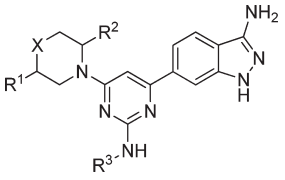
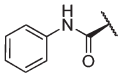
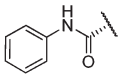
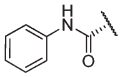
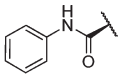
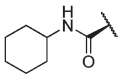
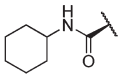
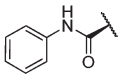
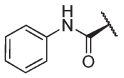
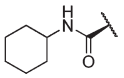
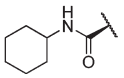
Table 5. SAR of Bis-substituted Morpholine and Piperidine Analogues



Compd	R^1	R^2	R^3	X	Kinase pIC_{50} PDK1	AurA	AurB	ALK5	ROCK1	LE	pAKT (T308) IC_{50} (nM)	pRSK (S221) IC_{50} (nM)
(3*S*,6*R*)-28a			H	CH_2	8.5	5.9	6.0	5.2	6.7	0.35	327	333
(3*R*,6*S*)-28a			H	CH_2	5.9	<5	5.3	<5	5.5	0.24	>29,300	>29,300
(±)-*trans*-28a			H	CH_2	6.1	<5	5.7	<5	<5	0.25	ND[a]	ND
(3*S*,6*R*)-28b			CH_3	CH_2	8.8	<5	5.7	<5	5.5	0.35	165	655
(3*S*,6*R*)-28c			H	CH_2	8.1	5.3	6.0	<5	6.2	0.34	472	316
(GSK2334470) **(3*S*,6*R*)-28d**			CH_3	CH_2	8.6	<5	5.5	<5	5.1	0.35	113	293
34a			H	O	8.3	6.7	6.5	<5	6.8	0.34	264	594
34b			CH_3	O	8.5	5.1	5.8	<5	5.5	0.33	57	88
34c			CH_3	O	8.5	<5	5.3	<5	<5	0.34	24	30
34d			CH_3	O	8.2	<5	5.8	<5	<5	0.32	85	102

[a] ND = not determined.

CONCLUSION

The optimization of compound **1** into highly potent and selective PDK1 inhibitors was accomplished via a structure-based design strategy targeting specific binding opportunities presented by the PDK1 G-loop to increase potency and selectivity. Interestingly, the majority of kinase inhibitors do not exploit the potential interactions presented by the G-loop, probably due to its typical high flexibility, which could pose a challenge for achieving high ligand binding affinity.[27] However, X-ray crystallography revealed distinct pockets in the PDK1 G-loop, which enabled optimization of the morpholine and piperidine derivatives through proper introduction of small alkyl groups on the six-membered ring. The most potent and selective inhibitors demonstrated submicromolar mechanistic cellular activity as measured by inhibition of phosphorylation of PDK1 substrates as well as antiproliferative activity against a subset of AML cell lines. Experiments with compound (**3*S*,6*R*)-28d** did confirm the ability of a PDK1 inhibitor to modulate downstream signaling in vivo. However, preliminary results from an efficacy study (SCID mice bearing OCI-AML2 tumors) indicated that compound (**3*S*,6*R*)-28d** only modestly inhibits tumor growth inhibition in vivo (data not shown). Taken together, these results advise that further biology studies will be necessary to determine the utility of a PDK1 inhibitor in the treatment of cancer either as a single agent or in combination therapies. The discovery of potent and highly selective PDK1 inhibitors, exemplified by (**3*S*,6*R*)-28d**, permits further delineation of PDK1 mediated signal transduction and potential pharmacological uses of a PDK1 inhibitor.[23,28]

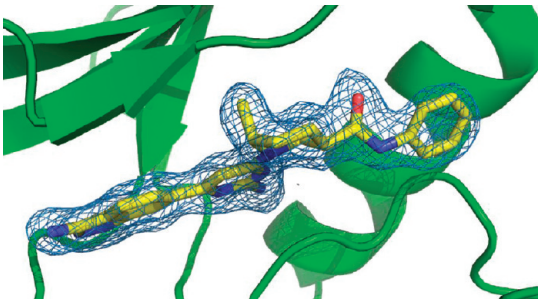
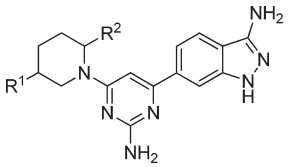
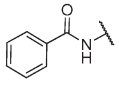
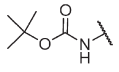
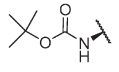
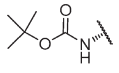
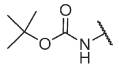
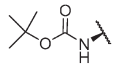
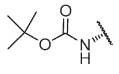


Figure 6. X-ray crystal structure of compound (**3*S*,6*R*)-28a** bound to PDK1.

Table 6. Selected SAR Data for Aminopiperidine Derivatives of General Structure 37



Compd	R^1	R^2	Kinase pIC_{50}					LE
			PDK1	AurA	AurB	ALK5	ROCK1	
(±)-**37a**		H	7.2	5.9	7.6	<5	<5	0.31
(±)-**37b**		H	7.3	5.9	6.4	<5	<5	0.32
(*S*)-**37b**		H	6.0	5.2	6.5	<5	<5	0.27
(*R*)-**37b**		H	7.6	5.8	5.3	<5	<5	0.34
(±)-*cis*-**37c**			7.8	6.1	6.8	<5	<5	0.33
(**3*S*,6*R***)-**37c**			6.2	5.7	7.3	<5	<5	0.27
(**3*R*,6*S***)-**37c**			8.0	6.3	6.0	<5	<5	0.34

EXPERIMENTAL SECTION

Chemistry. *General Methods.* Unless otherwise noted, commercially available materials were used without further purification. Air- or moisture-sensitive reactions were carried out under a nitrogen or argon atmosphere. Anhydrous solvents were obtained from Sigma-Adrich. Flash chromatography was performed using silica gel under standard techniques or using silica gel cartridges on an Analogix instrument. NMR spectra were recorded on a Bruker 400 MHz spectrometer. Chemical shifts (δ) are quoted in parts per million (ppm) relative to an internal solvent referance. Coupling constants (J) are recorded in hertz. LC-MS data were collected from a Sciex or Agilent instrument. Reverse phase HPLC purifications were conducted on a Gilson HPLC (monitoring at 215 and 254 nm) with a C18 column eluting with an acetonitrile/water gradient with 0.1% TFA in each solvent unless otherwise noted. Analytical HPLC data were generated by injecting 5 μL of very dilute sample solution in the appropriate solvent to a reverse phase HPLC system run over 4 min (5−95% acetonitrile/water with 0.1% TFA in each solvent). The products were detected by UV (254, 214, and 333 nm). All tested compounds were determined to be ≥95% purity by LC-MS and analytical HPLC unless otherwise noted.

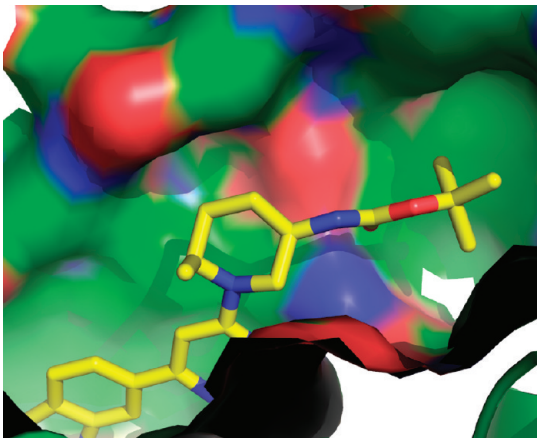


Figure 7. X-ray crystal structure of compound (**3*R*,6*S***)-**37c** bound to PDK1.

*4-(2-Amino-4-pyrimidinyl)-2-fluorobenzonitrile (**4**).* In a 25 mL sealed tube under argon were combined 4-chloro-2-pyrimidinamine (100 mg, 0.772 mmol) and (4-cyano-3-fluorophenyl)boronic acid (127 mg, 0.772 mmol) in 1,4-dioxane (5 mL) and saturated aqueous $NaHCO_3$ (1.25 mL). This mixture was degassed for 10 min with argon. $Pd(PPh_3)_4$ (50 mg, 0.04 mmol) was then added, and the reaction sealed and heated at 95 °C in an oil bath. After 3 h, the reaction was allowed to cool to room temperature and then poured onto water (50 mL) and EtOAc (50 mL). The organic layer was separated, dried ($MgSO_4$), filtered through a pad of Celite503, and concentrated to dryness. The resulting light-yellow solid was dissolved in $CHCl_3$ and purified by flash chromatography on SiO_2 (eluent: 90/10/1 $CHCl_3/CH_3OH/NH_4OH$) to afford the title compound (138 mg, 83%). LC-MS (ES) m/z = 215 $[M + H]^+$. ^1H NMR (400 MHz, DMSO-d_6): δ 6.88 (s, 2H), 7.29 (d, J = 5.1 Hz, 1H), 8.04−8.22 (m, 3H), 8.43 (d, J = 5.1 Hz, 1H).

*N-[4-(4-Cyano-3-fluorophenyl)-2-pyrimidinyl]-N-methylacetamide (**5**).* To 4-(2-amino-4-pyrimidinyl)-2-fluorobenzonitrile (230 mg, 1.07 mmol) was added acetic anhydride (10 mL), and the reaction mixture was stirred overnight at 80 °C. LCMS analysis showed a ~2:1 mixture of mono- and diacetate products. The solvent was partially removed under vacuum. Ethanol was added, and the solvents were evaporated. This process was repeated 2 more times until most of the acetic anhydride and acetic acid was removed. Methanol was added, and an insoluble white solid was observed which was immediately filtered to afford *N*-[4-(4-cyano-3-fluorophenyl)-2-pyrimidinyl]acetamide (145 mg, 53%) as a white solid. LC-MS (ES) m/z = 279 $[M + Na]^+$. ^1H NMR (400 MHz, DMSO-d_6): δ 2.26 (s, 3H), 7.90 (d, J = 5.3 Hz, 1H), 8.12−8.19 (m, 1H), 8.22−8.28 (m, 1H), 8.28−8.34 (m, 1H), 8.83 (d, J = 5.1 Hz, 1H), 10.72 (s, 1H).

To *N*-[4-(4-cyano-3-fluorophenyl)-2-pyrimidinyl]acetamide (140 mg, 0.546 mmol) and Cs_2CO_3 (196 mg, 0.60 mmol) in DMF (5 mL) was added iodomethane (0.038 mL, 0.60 mmol), and the reaction mixture was stirred for 3 h at room temperature. Water was added (ca. 20 mL), and a white precipitate was formed. The mixture was filtered, and the solid was washed with more water. The solid was dissolved in EtOAc, and the solution was dried ($MgSO_4$), filtered, and concentrated to afford the title compound (135 mg, 91%) as an off-white solid. LC-MS (ES) m/z = 293 $[M + Na]^+$. ^1H NMR (400 MHz, DMSO-d_6): δ 2.45 (s, 3H), 3.44 (s, 3H), 8.02 (d, J = 5.3 Hz, 1H), 8.15 (dd, J = 8.1, 6.8 Hz, 1H), 8.26 (dd, J = 8.1, 1.5 Hz, 1H), 8.33 (dd, J = 10.7, 1.4 Hz, 1H), 8.95 (d, J = 5.3 Hz, 1H).

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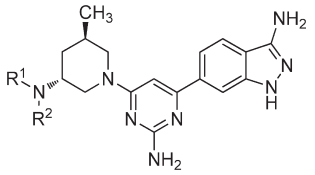
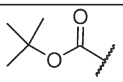
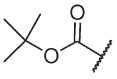
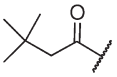
Table 7. SAR of 3-Methyl-5-aminopiperidine Analogues of General Structure 43



Compd	R^1	R^2	Kinase pIC_{50} PDK1	AurA	AurB	ALK5	ROCK1	LE	pAKT (T308) IC_{50} (nM)	pRSK (S221) IC_{50} (nM)
43a		H	8.2	6.4	5.8	<5	<5	0.35	74	151
43b		CH_3	9.1	6.9	6.6	<5	<5	0.38	9	19
43c		CH_3	9.2	7.2	6.7	<5	<5	0.38	22	116

6-[2-(Methylamino)-4-pyrimidinyl]-1H-indazol-3-amine (***8a***). To *N*-[4-(4-cyano-3-fluorophenyl)-2-pyrimidinyl]-*N*-methylacetamide (135 mg, 0.500 mmol) in ethanol (8 mL) was added hydrazine monohydrate (2 mL, 41 mmol), and the reaction mixture was stirred overnight at 95 °C. The solution was poured onto water. A precipitate started to form. The mixture was filtered, and the solid was washed with water followed by Et_2O. The residual solvent was removed under vacuum to afford the title compound (85 mg, 71%) as a light-yellow solid. LC-MS (ES) $m/z = 241$ $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.89 (d, 3H), 5.43 (s, 2H), 7.05−7.14 (m, 1H), 7.16 (d, $J = 5.3$ Hz, 1H), 7.62 (d, $J = 8.1$ Hz, 1H), 7.77 (d, $J = 8.3$ Hz, 1H), 8.02 (br s, 1H), 8.34 (d, $J = 4.8$ Hz, 1H), 11.61 (s, 1H).

N-Acetyl-N-(1-acetyl-6-bromo-1H-indazol-3-yl)acetamide (***6***). In a 100 mL flask under argon were combined 6-bromo-1*H*-indazol-3-amine (2.47 g, 11.7 mmol), acetic anhydride (22 mL, 233 mmol), and DMAP (0.071 g, 0.58 mmol), and the resulting mixture was heated at 120 °C for 5 h. The reaction was allowed to cool to room temperature, stirred overnight, and then concentrated to dryness. The resulting residue was dry loaded onto SiO_2 using acetone and purified by flash chromatography on SiO_2 using EtOAc/Hex as the eluent to afford the title compound (2.84 g, 68%) as a white solid. LC-MS (ES) $m/z = 360, 362$ $[M + Na]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.31 (s, 6H), 2.70 (s, 3H), 7.67 (dd, $J = 8.6$, 1.8 Hz, 1H), 7.83 (d, $J = 8.3$ Hz, 1H), 8.53 (d, $J = 1.5$ Hz, 1H).

N-[6-(2-Chloro-4-pyrimidinyl)-1H-indazol-3-yl]acetamide (***7***). To a mixture of *N*-acetyl-*N*-(1-acetyl-6-bromo-1*H*-indazol-3-yl)acetamide (0.439 g, 1.299 mmol), *N*-(1-acetyl-6-bromo-1*H*-indazol-3-yl)acetamide (0.063 g, 0.211 mmol), bis(pinacolato)diboron (0.384 g, 1.51 mmol), and KOAc (0.445 g, 4.53 mmol) was added 1,4-dioxane (8 mL), and nitrogen gas was bubbled through the mixture for 10 min. $PdCl_2$-(dppf)·CH_2Cl_2 (0.062 g, 0.076 mmol) was added, and the reaction mixture was stirred for 2 h at 100 °C in a sealed tube. The reaction mixture was cooled to room temperature and treated with 2,4-dichloropyrimidine (0.225 g, 1.510 mmol) and saturated aqueous $NaHCO_3$ (3 mL). Nitrogen gas was bubbled through the mixture for 10 min. $PdCl_2$(dppf)·CH_2Cl_2 (0.062 g, 0.076 mmol) was added, and the reaction mixture was stirred overnight at 100 °C in a sealed tube. The mixture was cooled to room temperature and poured into EtOAc and water and then filtered. The filtrate was poured into a separatory funnel, and the organic layer was separated, washed with brine, dried ($MgSO_4$), filtered, and concentrated. Flash chromatography on SiO_2 (gradient: $CHCl_3$ to 90:10:1 $CHCl_3$/CH_3OH/NH_4OH) afforded the title compound (84 mg, 19%) as a yellow solid. LC-MS (ES) $m/z = 288$ $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.13 (s, 3H), 7.85 (d, $J = 8.8$ Hz, 1H), 7.97 (d, $J = 8.6$ Hz, 1H), 8.25 (d, $J = 5.3$ Hz, 1H), 8.28−8.36 (m, 1H), 8.84 (d, $J = 5.3$ Hz, 1H), 10.51 (s, 1H), 13.00 (br. s., 1H).

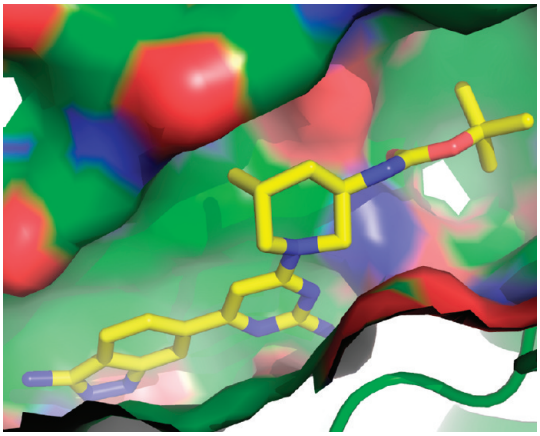


Figure 8. X-ray crystal structure of carbamate **43a** bound to PDK1.

6-[2-(Dimethylamino)-4-pyrimidinyl]-1H-indazol-3-amine (***8b***). To *N*-[6-(2-chloro-4-pyrimidinyl)-1*H*-indazol-3-yl]acetamide (20 mg, 0.070 mmol) was added dimethylamine (2.0 M in THF, 4 mL, 8.00 mmol), and the reaction mixture was stirred overnight at 100 °C. The mixture was poured into EtOAc and water, and the organic layer was separated, washed with brine, dried ($MgSO_4$), filtered, and concentrated. To the resulting residue was added CH_3OH (6 mL), followed by concentrated HCl (0.3 mL, 3.60 mmol), and the reaction mixture was stirred for 3 h at 60 °C. The solution was concentrated and evaporated from toluene twice. The resulting residue was triturated with Et_2O to afford an HCl salt of the desired product (20 mg, 88%) as a yellow solid. LC-MS (ES) $m/z = 255$ $[M + H]^+$. ^{1}H NMR (400 MHz, CD_3OD): δ 3.39−3.56 (m, 6H), 7.68 (d, $J = 6.8$ Hz, 1H), 8.15 (s, 2H), 8.41−8.47 (m, 2H).

6-[2-(Ethylamino)-4-pyrimidinyl]-1H-indazol-3-amine (***8c***). The title compound was prepared following synthetic procedures similar to the preparation of **8b**. LC-MS (ES) $m/z = 255$ $[M + H]^+$. ^{1}H NMR (400 MHz, CD_3OD): δ 1.40 (t, $J = 7.2$ Hz, 3H), 3.55−3.85 (m, 2H), 7.69 (d, $J = 6.6$ Hz, 1H), 8.09−8.23 (m, 2H), 8.43 (d, $J = 6.6$ Hz, 1H), 8.45 (s, 1H).

6-[2-(Methyloxy)-4-pyrimidinyl]-1H-indazol-3-amine (***8d***). To *N*-[6-(2-chloro-4-pyrimidinyl)-1*H*-indazol-3-yl]acetamide (8.5 mg, 0.030 mmol)

Table 8. Antiproliferative Activity of Selected PDK1 Inhibitors against a Panel of AML Cell Lines

	cell IC_{50} (μM)				
compd	K562 (chronic myelogenous leukemia)	OCI-AML2 (acute myeloid leukemia)	OCI-AML3 (acute myeloid leukemia)	HEL 92.1.7 (erythroleukemia)	F-36P (acute myeloid leukemia)
(3*S*,6*R*)-**28b**	8	0.58	0.65	12	0.46
(3*S*,6*R*)-**28d**	18	0.35	0.52	>30	0.28
34c	>30	0.46	0.69	19	0.39
34d	>30	0.89	0.76	16	0.46
43a	24	0.69	0.40	18	0.82
43b	17	0.38	0.24	18	0.19

Table 9. Kinase Selectivity Data for (3*S*,6*R*)-28d Screened at 10 μM[a]

kinase	%RA	kinase	%RA
SGK2(h)	−2	Rsk2(h)	28
PDK1(h)	0	CHK2(h)	29
PrKX(h)	4	PKCζ(h)	29
Rsk4(h)	12	BrSK1(h)	33
SGK(h)	13	Rsk1(r)	34
Rsk3(h)	14	NLK(h)	38
ARK5(h)	16	Met(D1246H)(h)	43
ROCK-II(r)	17	BrSK2(h)	44
ZIPK(h)	21	Aurora-B(h)	46
PKBγ(h)	22	Ret(h)	46
DRAK1(h)	23	Rsk1(h)	46
ROCK-II(h)	26	Met(Y1248H)(h)	49

[a] h = human; r = rat; RA = remaining activity.

Table 10. Inhibitory Activity of (3*S*,6*R*)-28d against Other Kinases

kinase	IC_{50} (nM)
PDK1	2.5
AKT1	>10000
ALK5	>10000
ASK1	>27542
Aurora A	39810
Aurora B	3162
EGFR	>10000
GSK3b	>25118
IKK1	>25118
PI3Kγ	25118
ROCK1	7943
SYK	>25118
VEGFR2	>10000

in CH_3OH (4 mL) was added concentrated HCl (0.2 mL, 2.400 mmol), and the reaction mixture was stirred overnight in a sealed tube at 50 °C. The solution was poured into saturated aqueous $NaHCO_3$ and EtOAc. The organic layer was separated, washed with brine, dried ($MgSO_4$), filtered, and concentrated to afford the crude desired product (6.5 mg) as a yellow solid. LC-MS (ES) m/z = 242 $[M + H]^+$. ^1H NMR (400 MHz, CD_3OD): δ 4.13 (s, 3H), 7.65 (d, J = 5.3 Hz, 1H), 7.74−7.89 (m, 2H), 8.18 (s, 1H), 8.59 (d, J = 5.3 Hz, 1H).

*6-Chloro-N^4-ethyl-2,4-pyrimidinediamine (**9d**).* In a 50 mL flask under argon were combined 4,6-dichloro-2-pyrimidinamine (0.50 g, 3.05 mmol) and ethylamine (2.0 M in CH_3OH, 15.24 mL, 30.5 mmol), and the reaction mixture was stirred at 50 °C for 3 h. The mixture was cooled to room temperature and concentrated. The resulting yellow solid was dissolved in EtOAc, and the solution was washed with water. The organic layer was dried ($MgSO_4$), filtered, and concentrated to afford the title compound (368 mg, 69%) as a yellow solid. LC-MS (ES) m/z = 173 $[M + H]^+$. ^1H NMR (400 MHz, DMSO-d_6): δ 1.08 (t, J = 7.2 Hz, 3H), 3.11−3.30 (m, 2H), 5.70 (s, 1H), 6.37 (bs, 2H), 7.08 (bs, 1H).

*4-[2-Amino-6-(ethylamino)-4-pyrimidinyl]-2-fluorobenzonitrile (**10d**).* In a 25 mL sealable tube under nitrogen were added (4-cyano-3-fluorophenyl)boronic acid (0.29 g, 1.74 mmol) and 6-chloro-N^4-ethyl-2,4-pyrimidinediamine (0.30 g, 1.74 mmol), followed by 1,4-dioxane (11.1 mL) and a saturated aqueous solution of $NaHCO_3$ (2.8 mL). The mixture was degassed with nitrogen for 10 min. $Pd(Ph_3P)_4$ (0.10 g, 0.087 mmol) was added, the vial was sealed, and the reaction mixture was stirred for 16 h at 95 °C. The reaction was cooled to room temperature, filtered, and concentrated. The resulting residue was partitioned between EtOAc and water. The organic layer was separated, dried ($MgSO_4$), filtered, and concentrated. The resulting yellow oil was dissolved in CH_3CN (5 mL w/5 drops TFA). DMSO (3 drops) and water (0.5 mL) were added, and the solution was purified by reverse-phase HPLC (CH_3CN/H_2O w/0.1%TFA). The fractions containing the desired product were combined and concentrated until most of the CH_3CN was removed (∼half the original volume). The resulting precipitate was filtered, washed with water, and dried under vacuum to afford a TFA salt of the title compound (164 mg) as a white solid. LC-MS (ES) m/z = 258 $[M + H]^+$. ^1H NMR (400 MHz, DMSO-d_6): δ 1.17 (t, J = 7.2 Hz, 3H), 3.37−3.50 (m, 2H), 6.38 (bs, 1H), 7.77 (bs, 1H), 7.94 (bs, 1H), 8.13−8.25 (m, 1H), 8.73 (bs, 1H).

*6-(3-Amino-1H-indazol-6-yl)-N^4-ethyl-2,4-pyrimidinediamine (**12f**).* To a 25 mL flask under argon was added 4-[2-amino-6-(ethylamino)-4-pyrimidinyl]-2-fluorobenzonitrile (0.164 g, 0.442 mmol), followed by EtOH (3.5 mL). Hydrazine monohydrate (0.87 mL, 17.7 mmol) was added, and the reaction mixture was stirred for 6 h at 80 °C. The reaction was cooled to room temperature and concentrated to near dryness. Water (3 mL) was added (orange oil was formed), followed by CH_3CN (8 drops), and the mixture was sonicated. A light-yellow precipitate formed. The suspension was cooled on an ice bath and the solid was filtered, washed with water, and dried under vacuum at 40 °C to afford the title compound (82 mg, 68%) as a light-yellow solid. LC-MS (ES) m/z = 270 $[M + H]^+$. ^1H NMR (400 MHz, DMSO-d_6): δ 1.13 (t, J = 7.2 Hz, 3H), 3.20−3.40 (m, 2H), 5.36 (s, 2H), 5.97 (s, 2H), 6.25 (s, 1H), 6.82 (bs, 1H), 7.41 (d, J = 8.3 Hz, 1H), 7.69 (d, J = 8.3 Hz, 1H), 7.84 (s, 1H), 11.50 (s, 1H).

Compounds **12b**, **12e**, **12g**−**j**, and **12l**−**m** were synthesized by following synthetic procedures similar to the preparation of **12f** (route 1).

*6-[2-Amino-6-(1-methylethyl)-4-pyrimidinyl]-1H-indazol-3-amine (**12b**).* LC-MS (ES) m/z = 269 $[M + H]^+$. ^1H NMR (400 MHz, DMSO-d_6): δ 1.24 (d, J = 6.8 Hz, 6H), 2.83 (m, 1H), 5.42 (s, 2H), 6.54 (s, 2H), 7.07 (s, 1H), 7.60 (dd, J = 8.5, 1.4 Hz, 1H), 7.76 (d, J = 8.3 Hz, 1H), 7.99 (s, 1H), 11.59 (s, 1H).

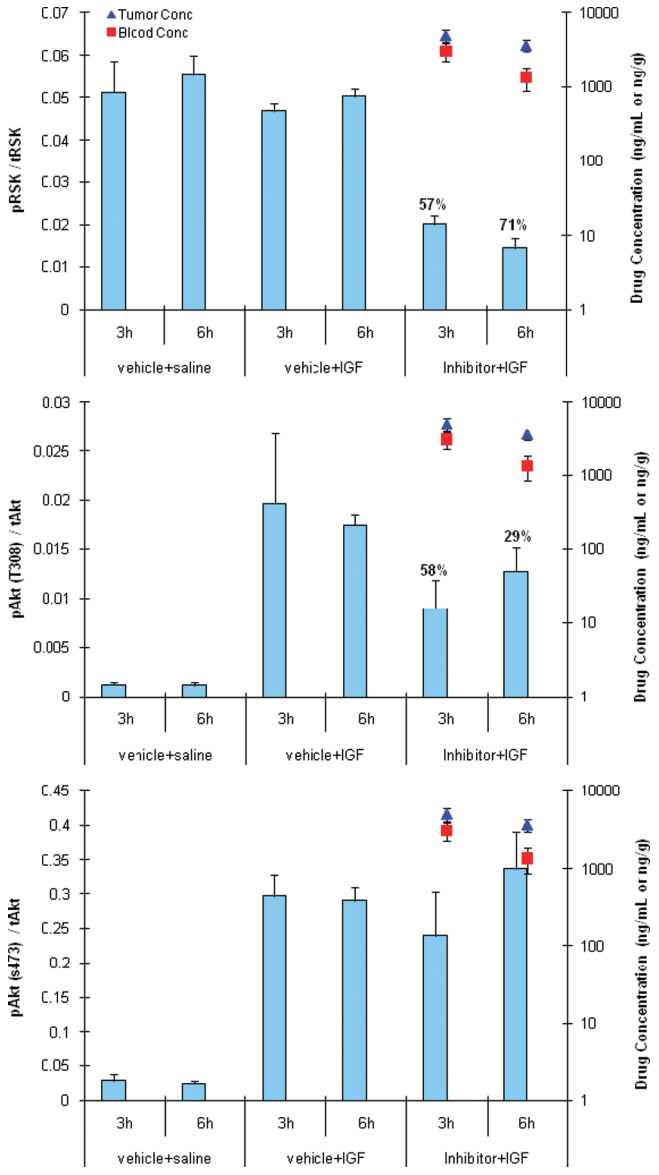


Figure 9. Effect of (3*S*,6*R*)-**28d** on the pharmacodynamic markers in OCL-AML2 xenografts.

6-(3-Amino-1H-indazol-6-yl)-N^4*-methyl-2,4-pyrimidinediamine* (***12e***). LC-MS (ES) m/z = 256 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.81 (d, 3H), 5.37 (s, 2H), 6.00 (s, 2H), 6.25 (s, 1H), 6.80 (bs, 1H), 7.43 (d, J = 8.3 Hz, 1H), 7.69 (d, J = 8.6 Hz, 1H), 7.85 (s, 1H), 11.50 (s, 1H).

6-(3-Amino-1H-indazol-6-yl)-N^4*-(1-methylethyl)-2,4-pyrimidinediamine* (***12g***). LC-MS (ES) m/z = 284 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.15 (d, J = 6.6 Hz, 6H), 4.12 (bs, 1H), 5.36 (s, 2H), 5.94 (s, 2H), 6.24 (s, 1H), 6.71 (d, J = 7.6 Hz, 1H), 7.40 (d, J = 8.6 Hz, 1H), 7.69 (d, J = 8.3 Hz, 1H), 7.82 (s, 1H), 11.50 (bs, 1H).

6-(3-Amino-1H-indazol-6-yl)-N^4*-cyclopentyl-2,4-pyrimidinediamine* (***12h***). LC-MS (ES) m/z = 310 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.38−1.61 (m, 4H), 1.61−1.78 (m, 2H), 1.92 (m, 2H), 4.22 (bs, 1H), 5.37 (s, 2H), 5.97 (bs, 2H), 6.26 (s, 1H), 6.87 (bs, 1H), 7.40 (d, J = 8.6 Hz, 1H), 7.70 (d, J = 8.4 Hz, 1H), 7.82 (s, 1H), 11.51 (s, 1H).

6-(3-Amino-1H-indazol-6-yl)-N^4*-phenyl-2,4-pyrimidinediamine* (***12i***). LC-MS (ES) m/z = 318 $[M + H]^+$. ^{1}H NMR (400 MHz, CD_3OD) δ 6.53 (s, 1H), 7.00−7.13 (m, 1H), 7.27−7.38 (m, 2H), 7.48 (dd, J = 8.6, 1.3 Hz, 1H), 7.64−7.72 (m, 2H), 7.77 (dd, J = 8.3, 0.8 Hz, 1H), 7.81−7.85 (m, 1H).

6-(3-Amino-1H-indazol-6-yl)-N^4*,N*^{4}*-dimethyl-2,4-pyrimidinediamine* (***12j***). LC-MS (ES) m/z = 270 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.07 (s, 6H), 5.37 (s, 2H), 6.02 (s, 2H), 6.44 (s, 1H), 7.56 (dd, J = 8.3, 1.3 Hz, 1H), 7.71 (d, J = 8.6 Hz, 1H), 7.94 (s, 1H), 11.50 (s, 1H).

6-[2-Amino-6-(1-pyrrolidinyl)-4-pyrimidinyl]-1H-indazol-3-amine (***12l***). LC-MS (ES) m/z = 296 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.93 (bs, 4H), 3.45 (bs, 4H), 5.37 (s, 2H), 6.00 (s, 2H), 6.27 (s, 1H), 7.54 (dd, J = 8.6, 1.3 Hz, 1H), 7.70 (d, J = 8.6 Hz, 1H), 7.92 (s, 1H), 11.49 (s, 1H).

6-[2-Amino-6-(1-piperidinyl)-4-pyrimidinyl]-1H-indazol-3-amine (***12m***). LC-MS (ES) m/z = 310 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.53 (m, 4H), 1.64 (m, 2H), 3.47−3.79 (m, 4H), 5.37 (s, 2H), 6.03 (s, 2H), 6.58 (s, 1H), 7.57 (dd, J = 8.3, 1.3 Hz, 1H), 7.70 (d, J = 8.6 Hz, 1H), 7.94 (s, 1H), 11.49 (s, 1H).

4-(2-Amino-6-phenyl-4-pyrimidinyl)-2-fluorobenzonitrile (***10a***). In a 25 mL sealable tube under argon were combined (4-cyano-3-fluorophenyl)-boronic acid (0.50 g, 3.03 mmol), 4,6-dichloro-2-pyrimidinamine (0.497 g, 3.03 mmol), saturated aqueous $NaHCO_3$ (4.04 mL), and 1,4-dioxane (16.2 mL), and the mixture was degassed with argon for 5 min. $Pd(Ph_3P)_4$ (0.175 g, 0.152 mmol) was added, the vial was sealed, and the reaction mixture was stirred for 16 h at 95 °C. The reaction was cooled to room temperature, filtered through a plug of Celite503, and concentrated. The resulting orange tar was partitioned between EtOAc and water. The organic layer was separated, washed with brine, dried ($MgSO_4$), filtered, and concentrated. The resulting orange solid was sonicated in $CHCl_3$ (50 mL) for 2 min. The mixture was filtrated, the solid was washed with $CHCl_3$, and the filtrate was concentrated. Flash chromatography on SiO_2 (40 g) of the resulting residue (gradient: $CHCl_3$ to 5% EtOAc/$CHCl_3$) afforded intermediate 4-(2-amino-6-chloro-4-pyrimidinyl)-2-fluorobenzonitrile (267 mg) as a yellow solid. LC-MS (ES) m/z = 249, 251 $[M + H]^+$.

In a 25 mL sealable tube under argon were combined 4-(2-amino-6-chloro-4-pyrimidinyl)-2-fluorobenzonitrile (0.196 g, 0.788 mmol) and phenylboronic acid (0.096 g, 0.79 mmol) in 1,4-dioxane (5.0 mL) and saturated aqueous $NaHCO_3$ (1.3 mL), and the mixture was degassed with argon for 5 min. $Pd(Ph_3P)_4$ (0.046 g, 0.04 mmol) was added, the vial was sealed, and the reaction mixture was stirred overnight at 95 °C. The reaction was cooled to room temperature and filtered, and the filtrate was concentrated to dryness. The resulting solid was partitioned between EtOAc and water. The organic layer was separated, washed with brine, dried ($MgSO_4$), filtered, and concentrated. Purification of the resulting solids on reverse phase HPLC (CH_3CN/H_2O w/ 0.1% TFA) afforded a TFA salt of the title compound (52 mg) as a white solid. LC-MS (ES) m/z = 291 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.95 (s, 2H), 7.49−7.64 (m, 3H), 7.90 (s, 1H), 8.08−8.16 (m, 1H), 8.26 (dd, J = 8.1, 1.5 Hz, 2H), 8.30 (dd, J = 8.1, 1.5 Hz, 1H), 8.36 (dd, J = 11.1, 1.3 Hz, 1H).

6-(2-Amino-6-phenyl-4-pyrimidinyl)-1H-indazol-3-amine (***12c***). Into a 25 mL sealable tube under argon were added 4-(2-amino-6-phenyl-4-pyrimidinyl)-2-fluorobenzonitrile (0.052 g, 0.18 mmol) and EtOH (5 mL). Hydrazine monohydrate (0.35 mL, 7.17 mmol) was added, the vial was sealed, and the reaction mixture was stirred overnight at 95 °C. The reaction was cooled to room temperature and concentrated. The resulting solid was sonicated in a mixture of EtOH (1 mL) and water (10 mL), filtered, washed with water, and dried under vacuum at 40 °C to afford the title compound as a yellow solid (44 mg, 77%). LC-MS (ES) m/z = 303 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.43 (s, 2H), 6.74 (s, 2H), 7.46−7.59 (m, 3H), 7.69−7.84 (m, 3H), 8.12 (s, 1H), 8.23 (m, 2H), 11.63 (s, 1 H).

6-[2-Amino-6-(4-morpholinyl)-4-pyrimidinyl]-1H-indazol-3-amine (***12k***). In a 25 mL sealable tube under argon were combined *N*-acetyl-*N*-(1-acetyl-6-bromo-1*H*-indazol-3-yl)acetamide (0.25 g, 0.74 mmol),

Table 11. Crystallographic Data Collection and Refinement Statistics

compd	**12f**	**12k**	(*R*)-**18a**	(*S*)-**21a**	(3*S*,6*R*)-**28a**	(3*R*,6*S*)-**37c**	**43a**
PDB code	3QCQ	3QCS	3QCX	3QCY	3QD0	3QD3	3QD4
				Data Collection			
space group	$P3_221$	$P3_221$	$P3_221$	$P3_221$	$P3_221$	$P3_221$	$P3_221$
cell dimensions							
a, *b*, *c* (Å)	123.7, 123.7, 47.1	123.9, 123.9, 47.0	123.4, 123.4, 47.0	123.7, 123.7, 47.1	123.9, 123.9, 47.0	123.5, 123.5, 46.9	124.3, 124.3, 47.0
α, β, γ (deg)	90, 90, 120	90, 90, 120	90, 90, 120	90, 90, 120	90, 90, 120	90, 90, 120	90, 90, 120
resolution (Å)[a]	99−2.5 (2.6−2.5)	50−2.5 (2.6−2.5)	30−2.3 (2.38−2.3)	30−2.2 (2.28−2.2)	30−2.0 (2.03−2.0)	30−2.0 (2.07−2.0)	30−2.3 (2.38−2.3)
R_{sym} or R_{merge}	0.114 (0.524)	0.091 (0.484)	0.077 (0.537)	0.086 (0.483)	0.082 (0.668)	0.10 (0.573)	0.078 (0.582)
$I/\sigma I$	26.8 (3)	22.1 (3.3)	18.8 (2.7)	21.5 (4.2)	25.7(4.1)	20.0 (3.7)	23.9 (3.3)
completeness (%)	99.6 (99.0)	99.8 (98.8)	99.9(99.7)	99.9 (100)	100 (100)	100 (100)	99.9 (100)
redundancy	10 (5.3)	10.2 (6.9)	4.9 (4.3)	6.2 (6.1)	8.7 (8.6)	7.4 (7.1)	7.1 (6.5)
				Refinement			
resolution (Å)	43 − 2.5	41 − 2.5	28 − 2.3	30 − 2.2	28 − 2.0	29 − 2.0	30 − 2.3
no. reflections	14029	14265	17623	20458	28628	26925	17921
R_{work}/R_{free}	0.182/0.251	0.182/0.251	0.180/0.225	0.178/0.212	0.189/0.231	0.183/0.211	0.194/0.244
no. atoms							
protein	2328	2261	2244	2233	2226	2280	2245
ligand	20	23	24	32	33	32	32
water/ion	53	68	109	132	133	121	30
B factors							
protein	41.1	43.4	41.6	29.6	31.0	25.4	39.9
ligand	29.8	29.1	30.0	21.7	22.1	18.2	28.6
water/ion	63.5	69.9	51.5	43.0	38.3	31.2	42.3
rms deviations							
bond lengths (Å)	0.008	0.005	0.010	0.005	0.013	0.007	0.008
bond angles (deg)	1.13	0.96	1.35	0.97	1.4	1.17	1.22

[a] Values in parentheses are for highest-resolution shell.

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bis(pinacolato)diboron (0.197 g, 0.776 mmol), and potassium acetate (0.145 g, 1.48 mmol) in 1,4-dioxane (4.9 mL). The mixture was degassed with argon for 5 min. $PdCl_2(dppf)\cdot CH_2Cl_2$ (0.024 g, 0.03 mmol) was added, the tube was sealed, and the reaction mixture was stirred for 5 h at 100 °C. The mixture was cooled to room temperature, the tube was unsealed, and 4-chloro-6-(4-morpholinyl)-2-pyrimidinamine (0.175 g, 0.813 mmol), $NaHCO_3$ (0.25 g, 2.96 mmol), water (1.64 mL), and $PdCl_2(dppf)\cdot CH_2Cl_2$ (0.024 g, 0.03 mmol) were added. The tube was resealed under argon, and the reaction mixture was stirred overnight at 100 °C. The mixture was cooled to room temperature, diluted with CH_3CN (10 mL), filtered through a pad of Celite503, and concentrated. The resulting brown solid was dissolved in 10 mL of solvent (30/70 CH_3CN/H_2O w/0.25 mL TFA), and the solution was filtered through a 0.45 μ filter disk. The filtrate was purified by reverse-phase HPLC (CH_3CN/H_2O w/0.1% TFA) to afford a TFA salt of the acetylated Suzuki coupling intermediate (151 mg) as a white solid. LC-MS (ES) m/z = 354 $[M + H]^+$. This intermediate was then dissolved in CH_3OH (10 mL). HCl (12 M, 0.69 mL, 8.3 mmol) was added, and the reaction mixture was stirred at 60 °C for 4 h. The mixture was cooled to room temperature and filtered. The solid was washed with hexanes to afford an HCl salt of the title compound (75 mg) as a light-yellow solid. LC-MS (ES) m/z = 312 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.73 (m, 4H), 3.89 (m, 4H), 6.98 (s, 1H), 7.66 (d, J = 8.6 Hz, 1H), 8.08 (m, 2H).

Compounds **12a** and **12d** were synthesized following synthetic procedures similar to the preparation of **12k** (route 2).

*6-(2-Amino-6-methyl-4-pyrimidinyl)-1H-indazol-3-amine (**12a**).* HCl salt. LC-MS (ES) m/z = 241 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.51 (s, 3H {assumed to be hidden beneath DMSO peak}), 7.59 (s, 1H), 7.81 (dd, J = 8.6, 1.3 Hz, 1H), 7.99 (d, J = 8.6 Hz, 1H), 8.21 (s, 1H), 12.72 (bs, 1H).

*6-[2-Amino-6-(methyloxy)-4-pyrimidinyl]-1H-indazol-3-amine (**12d**).* LC-MS (ES) m/z = 257 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.86 (s, 3H), 5.40 (s, 2H), 6.58 (s, 1H), 6.64 (s, 2H), 7.54 (dd, J = 8.6, 1.0 Hz, 1H), 7.72 (d, J = 8.3 Hz, 1H), 7.95 (s, 1H), 11.57 (s, 1H).

*(2R)-2-[(Phenylmethyl)amino]-1-propanol ((**R**)-**13**).* To (2*R*)-2-amino-1-propanol (4.5 g, 60 mmol) in toluene (120 mL) was added benzaldehyde (636 mL). A Dean−Stark trap was placed on the flask, and the reaction mixture was heated to reflux until no further water evolved. The reaction was cooled down to room temperature and concentrated. The resulting residue was dissolved in ethanol (120 mL) and treated with $NaBH_4$ (5.67 g, 150 mmol) at 0 °C, followed by sufficient 4N HCl in dioxane to adjust the pH to 2. The reaction mixture was stirred overnight at room temperature and then concentrated in vacuo. The resulting residue was dissolved in 1N aq HCl (200 mL) and washed with CH_2Cl_2 (2 × 100 mL). The aqueous phase was then adjusted to pH > 13 with 6N aqueous NaOH and extracted with CH_2Cl_2 (2 × 150 mL). The combined organic phase was dried over anhydrous Na_2SO_4, filtered, and concentrated to afford the title compound (9.44 g, 95%) as a colorless oil, which solidified under high vacuum. LC-MS (ES) m/z = 166 $[M + H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.12 (d, J = 6.6 Hz, 3H), 2.07 (bs, 2H), 2.78−2.96 (m, 1H), 3.30 (dd, J = 10.6, 6.8 Hz, 1H), 3.63 (dd, J = 10.6, 4.0 Hz, 1H), 3.73−3.81 (m, 1H), 3.86−3.95 (m, 1H), 7.24−7.32 (m, 1H), 7.35 (m, 4H).

*(3R)-3-Methyl-4-(phenylmethyl)morpholine ((**R**)-**15**).* To (2*R*)-2-[(phenylmethyl)amino]-1-propanol (8.43 g, 51 mmol) in THF (50 mL) was added a solution of K_2CO_3 (21.15 g, 153 mmol) in water (50 mL). To the resulting mixture at 0 °C was added slowly via syringe chloroacetyl chloride (5.7 mL, 71.4 mmol) with vigorous stirring, and the reaction mixture was stirred for 1 h at 0 °C. A 50% aqueous NaOH solution was added to adjust the pH > 13, and the resulting mixture was warmed up overnight to room temperature. The solution was extracted with CH_2Cl_2 (2 × 200 mL), and the organic layer was dried (Na_2SO_4), filtered, and concentrated to afford (5*R*)-5-methyl-4-(phenylmethyl)-3-morpholinone ((*R*)-**14**) as a colorless oil. LC-MS (ES) m/z = 206 $[M + H]^+$.

To a solution of (5*R*)-5-methyl-4-(phenylmethyl)-3-morpholinone (11.7 g, 57 mmol) in toluene (140 mL) at 0 °C was added sodium bis(2-methoxyethoxy)aluminumhydride (Red-Al, 35 mL, 3 mL/g of morpholinone) slowly via addition funnel, and the reaction mixture was stirred overnight at 60 °C. The reaction was cooled down to 0 °C and quenched by dropwise addition of 1N aqueous NaOH (15 mL). The resulting mixture was partitioned between Et_2O (100 mL) and 1N aqueous NaOH (100 mL). The organic layer was separated, and the aqueous layer was further extracted with Et_2O (50 mL). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated. The resulting residue was azeotroped with CH_3OH (50 mL) to afford the title compound (10.69 g) as a colorless oil. LC-MS (ES) m/z = 192 $[M + H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.11 (d, J = 6.1 Hz, 3H), 2.14−2.32 (m, 1H), 2.45−2.56 (m, 1H), 2.61 (d, J = 11.9 Hz, 1H), 3.16 (d, J = 13.1 Hz, 1H), 3.26−3.42 (m, 1H), 3.57−3.66 (m, 1H), 3.68−3.79 (m, 2H), 4.08 (d, J = 13.1 Hz, 1H), 7.23−7.30 (m, 1H), 7.30−7.40 (m, 4H).

*(3R)-3-Methylmorpholine ((**R**)-**17a**).* To (3*R*)-3-methyl-4-(phenylmethyl)morpholine (10.7 g, 56 mmol) in CH_3OH (110 mL) were added 6N aqueous HCl (9.3 mL) and Pd/C (1.07 g, 10 wt %), and the reaction mixture was stirred overnight at room temperature under a H_2 atmosphere (balloon setup). The mixture was filtered through a glass fiber filter, and the filter cake was washed with CH_3OH. The combined filtrate was concentrated and azeotroped with CH_3OH (4 × 100 mL) to afford the HCl salt of the title compound as a yellow oil that solidified under high vacuum (7.91 g). ^{1}H NMR (400 MHz, CD_3OD): δ 3.95−4.05 (m, 2H), 3.77 (m, 1H), 3.47−3.54 (m, 1H), 3.38−3.47 (m, 1H), 3.30−3.34 (m, 1H), 3.18−3.28 (m, 1H), 1.29 (d, J = 6.3 Hz, 3H).

*6-{2-Amino-6-[(3R)-3-methyl-4-morpholinyl]-4-pyrimidinyl}-1H-indazol-3-amine ((**R**)-**18a**).* The title compound was prepared from amine (*R*)-**17a** by following synthetic route 1. LC-MS (ES) m/z = 326 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.19 (d, J = 6.8 Hz, 3H), 3.11 (m, 1H), 3.44 (m, 1H), 3.56−3.63 (m, 1H), 3.68−3.74 (m, 1H), 3.92 (dd, J = 11.1, 3.0 Hz, 1H), 4.05−4.14 (m, 1H), 4.49 (bs, 1H), 5.38 (s, 2H), 6.11 (s, 2H), 6.55 (s, 1H), 7.57 (dd, J = 8.6, 1.3 Hz, 1H), 7.71 (d, J = 8.6 Hz, 1H), 7.95 (s, 1 H), 11.52 (s, 1H). Compound (*S*)-**18a** was prepared similarly.

*(5R)-5-Ethyl-3-morpholinone (**16a**).* NaH (1.51 g, 37.9 mmol) in dry toluene (10 mL) was cooled to 0 °C under nitrogen, and (2*R*)-2-amino-1-butanol (1.5 g, 16.8 mmol) in toluene (5 mL) was added dropwise. The mixture was stirred for 20 min, warming up to room temperature, and chloroacetyl chloride (1.5 mL, 18.9 mmol) in toluene (5 mL) was added dropwise. An exotherm was observed, so the mixture was cooled in an ice bath during the addition. After the addition was completed, the reaction mixture was heated to 110 °C overnight. The mixture was cooled to room temperature, and 5 g of ammonium chloride were added portionwise. The mixture was stirred for 20 min and filtered. The filter cake was washed with toluene and discarded. The filtrate was concentrated to give an orange oil that was dissolved in CH_2Cl_2 and purified by SiO_2 chromatography (gradient: CH_2Cl_2 to 90:10:1 $CH_2Cl_2/CH_3OH/NH_4OH$) to afford the title compound (1.2 g, 55%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.96 (t, J = 7.6 Hz, 3H), 1.52−1.63 (m, 2H), 3.40−3.51 (m, 2H), 3.85−3.94 (m, 1H), 4.07−4.20 (m, 2H), 7.55 (bs, 1H).

*(3R)-3-Ethylmorpholine ((**R**)-**17b**).* A solution of 1 M $LiAlH_4$ in THF (7.7 mL, 7.7 mmol) was cooled in an ice bath under nitrogen. A solution of (5*R*)-5-ethyl-3-morpholinone (500 mg, 3.87 mmol) in THF (10 mL) was added dropwise, and the solution was heated to 70 °C for 16 h. After aproximately 2 h, a thick white precipitate had formed. The reaction mixture was cooled down to room temperature and carefully quenched with water (1 mL), 2 M NaOH (1 mL), and water (4 mL). The resulting slurry was stirred at room temperature for 1 h and then filtered through Celite. The filter cake was washed with EtOAc and discarded. The filtrate was washed with brine, dried ($MgSO_4$), and filtered. HCl in ether (3.87 mL, 3.87 mmol) was added, producing a

cloudy solution, and the solvent was evaporated to afford the HCl salt of the title compound (251 mg, 56%) as an orange solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.88−0.97 (m, 3H), 1.46−1.72 (m, 2H), 2.91−3.18 (m, 3H), 3.46 (dd, J = 12.3, 10.2 Hz, 1H), 3.71 (td, J = 11.8, 2.7 Hz, 1H), 3.82−3.97 (m, 2H), 9.68 (bs, 2H).

*6-{2-Amino-6-[(3R)-3-ethyl-4-morpholinyl]-4-pyrimidinyl}-1H-indazol-3-amine (**18b**).* The title compound was prepared from amine (*R*)-**17b** following synthetic route 1. LC-MS (ES) m/z = 340 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.88 (t, J = 7.5 Hz, 3H), 1.63−1.80 (m, 2H), 3.09 (td, J = 12.8, 3.2 Hz, 1H), 3.37−3.46 (m, 2H), 3.51 (dd, J = 11.4, 2.8 Hz, 1H), 3.80−3.91 (m, 2H), 4.30 (bs, 1H), 5.38 (s, 2H), 6.09 (bs, 2H), 6.56 (s, 1H), 7.56 (dd, J = 8.6, 1.0 Hz, 1H), 7.71 (d, J = 8.3 Hz, 1H), 7.94 (s, 1H), 11.52 (s, 1H).

*6-{2-Amino-6-[(3R)-3-(1-methylethyl)-4-morpholinyl]-4-pyrimidinyl}-1H-indazol-3-amine (**18c**).* The title compound was synthesized following synthetic procedures similar to the preparation of **18b**. LC-MS (ES) m/z = 354 $[M + H]^+$. ^{1}H NMR (400 MHz, CD_3OD): δ 0.88 (d, J = 6.8 Hz, 3H), 1.10 (d, J = 6.6 Hz, 3H), 2.47−2.54 (m, 1H), 3.21−3.30 (m., 1H), 3.51−3.58 (m, 2H), 3.93 (dd, J = 11.2, 3.41 Hz, 1H), 4.10 (d, J = 11.6 Hz, 1H), 6.46 (s, 1H), 7.47 (dd, J = 8.5, 1.4 Hz, 1H), 7.76 (d, J = 8.6 Hz, 1H), 7.79 (s, 1H).

*6-{2-Amino-6-[(2R)-2-methyl-1-piperidinyl]-4-pyrimidinyl}-1H-indazol-3-amine (**18d**).* The title compound was prepared from commercially available (2*R*)-2-methylpiperidine following synthetic route 1. LC-MS (ES) m/z = 324 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.14 (d, J = 6.8 Hz, 3H), 1.29−1.44 (m, 1H), 1.52−1.82 (m, 5H), 2.80−2.98 (m, 1H), 4.35 (d, J = 12.6 Hz, 1H), 4.80 (bs, 1H), 5.38 (s, 2H), 6.02 (s, 2H), 6.53 (s, 1H), 7.56 (dd, J = 8.5, 1.1 Hz, 1H), 7.70 (d, J = 8.3 Hz, 1H), 7.93 (s, 1 H), 11.50 (s, 1H).

*(3S)-N-Phenyl-3-piperidinecarboxamide ((**S**)-**20b**).* To a mixture of (3*S*)-1-{[(1,1-dimethylethyl)oxy]carbonyl}-3-piperidinecarboxylic acid (500 mg, 2.181 mmol), HOBt (668 mg, 4.36 mmol), and EDC (502 mg, 2.62 mmol) in DMF (5 mL) was added *N*-methylmorpholine (1.20 mL, 10.90 mmol), and the solution was stirred for 10 min. Aniline (223 mg, 2.399 mmol) was added, and the reaction mixture was stirred overnight at room temperature. The reaction was diluted with EtOAc (50 mL) and then washed with water and brine. The organic layer was then dried (Na_2SO_4), filtered, and concentrated to give a light-yellow oil. The oil was purified on Biotage SP1 25 m column with a gradient of 0−40% EtOAc in hexane over 14 column volumes. The residue was taken up in a premixed 2:1 CH_2Cl_2:TFA solution (5 mL), and the resulting mixture was stirred for 15 min and then concentrated to isolate the crude TFA salt of the title compound. LC-MS (ES) m/z = 205 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.61−1.73 (m, 2H), 1.79−1.89 (m, 1H), 2.00−2.07 (m, 1H), 2.77−2.85 (m, 1H), 2.92 (d, J = 10.6 Hz, 1H), 3.08 (dd, J = 10.7, 5.9 Hz, 1H), 3.20 (d, J = 11.9 Hz, 1H), 3.33 (d, J = 10.9 Hz, 1H), 7.06 (t, J = 7.3 Hz, 1H), 7.31 (t, J = 8.0 Hz, 2H), 7.59 (d, J = 7.6 Hz, 2H), 8.61 (bs, 1H), 10.18 (s, 1H).

*(3S)-1-[2-Amino-6-(3-amino-1H-indazol-6-yl)-4-pyrimidinyl]-N-phenyl-3-piperidinecarboxamide ((**S**)-**21b**).* The title compound was prepared from amine (*S*)-**20b** following synthetic route 1. LC-MS (ES) m/z = 429 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.43−1.59 (m, 1H), 1.75−1.94 (m, 2H), 2.03−2.16 (m, 1H), 2.57−2.69 (m, 1H), 3.12−3.31 (m, 2H), 3.43−3.54 (m, 2H), 4.40−4.58 (m, 1H), 4.72−5.07 (m, 1H), 6.94−7.11 (m, 2H), 7.30 (m, 2H), 7.37−7.43 (m, 1H), 7.61 (d, J = 8.1 Hz, 2H), 7.75 (bs, 1H), 7.90 (d, 1H), 10.08 (d, J = 12.9 Hz, 1H), 12.10 (bs, 2H). Compound ((*R*)-**21b**) was prepared similarly.

*4-[2-Amino-6-(3-amino-1H-indazol-6-yl)-4-pyrimidinyl]-N-phenyl-2-morpholinecarboxamide ((±)-**21a**).* The title compound was synthesized from (±)-**19a** following synthetic procedures similar to the preparation of ((*S*)-**21b**). LC-MS (ES) m/z = 431 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.99−3.16 (m, 2H), 3.70 (td, J = 11.4, 2.5 Hz, 1H), 4.09 (d, J = 11.4 Hz, 1H), 4.15−4.29 (m, 2H), 4.65 (bs, 1H), 5.38 (s, 2H), 6.23 (bs, 2H), 6.68 (s, 1H), 7.09 (t, J = 7.45 Hz, 1H), 7.33 (t, J = 8.0 Hz, 2H), 7.60 (dd, J = 8.6, 1.3 Hz, 1H), 7.69−7.74 (m, 3H), 7.97 (s, 1H), 9.84 (s, 1H), 11.53 (s, 1H).

The individual enantiomers (*S*)-**21a** and (*R*)-**21a** were separated by preparative HPLC on a Chiralpak AD-H (250 mm × 20 mm) column: 5:95 = CH_3OH:CH_3CN (+0.1% isopropylamine) at 20 mL/min; 23 °C; UV 254 nm.

*(3S)-1-[2-Amino-6-(3-amino-1H-indazol-6-yl)-4-pyrimidinyl]-N-cyclohexyl-3-piperidinecarboxamide ((**S**)-**21c**).* Amine (*S*)-**20c** was prepared according to the synthetic procedures described for (*S*)-**20b**, and converted to (*S*)-**21c** following synthetic route 2. LC-MS (ES) m/z = 435 $[M + H]^+$. NMR spectrum observed a large water peak. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.08−1.30 (m, 4H), 1.36−1.59 (m, 2H), 1.59−1.77 (m, 4H), 1.91 (d, J = 3.3 Hz, 1H), 3.09 (d, J = 11.9 Hz, 1H), 3.20 (d, J = 11.4 Hz, 1H), 4.38 (bs, 1H), 4.57−4.91 (m, 1H), 6.99 (d, J = 14.2 Hz, 1H), 7.57 (dd, J = 8.0, 4.7 Hz, 1H), 7.85 (d, J = 7.3 Hz, 1H), 7.94−8.01 (m, 2H), 12.62 (d, J = 1.0 Hz, 1H).

*(3S)-1-[2-Amino-6-(3-amino-1H-indazol-6-yl)-4-pyrimidinyl]-N-cyclopentyl-3-piperidinecarboxamide ((**S**)-**21d**).* Amine (*S*)-**20d** was prepared according to the synthetic procedures described for (*S*)-**20b** and converted to (*S*)-**21d** following synthetic route 2. LC-MS (ES) m/z = 421 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.37 (m, 2H), 1.42−1.55 (m, 2H), 1.55−1.70 (m, 2H), 1.78 (m, 2H), 1.90 (m, 1H), 2.36 (m, 1H), 3.03−3.12 (m, 1H), 3.17 (m, 1H), 3.36−3.50 (m, 1H), 3.98 (m, 1H), 4.33 (m, 2H), 4.67 (d, J = 12.9 Hz, 1H), 4.86 (d, J = 11.4 Hz, 1H), 6.96−7.09 (m, 1H), 7.66 (m, 1H), 7.98 (d, J = 7.3 Hz, 1H), 8.02−8.10 (m, 2H), 12.83 (bs, 1H).

*cis-3-Methyl 1-(Phenylmethyl)-6-methyl-1,3-piperidinedicarboxylate ((±)-cis-**22**).* A solution of methyl 6-methylnicotinate (50 g, 331 mmol, 1 equi.) in CH_3OH (400 mL) and conc HCl (26 mL) was added to a slurry of platinum(IV) oxide (2.0 g) in 50 mL of CH_3OH/water (4/1) in a Parr bottle. The mixture was hydrogenated at room temperature under 60 psi of hydrogen gas for 4.5 h. The mixture was then filtered through Celite. The filtrate was concentrated in vacuo, after chasing with 500 mL of toluene, to about 77 g of syrup. This residue was dissolved in CH_2Cl_2 (500 mL) and chilled in an ice bath. To this stirred solution was added DMAP (0.4 g, 3.31 mmol), followed by TEA (101 mL, 728 mmol, 2.2 equiv) portionwise. A suspension formed when TEA was added. This mixture was chilled to 15 °C. To the resulting suspension was added benzylchloroformate (52 mL, 364 mmol, 1.1 equiv) dropwise over a 25 min period such that the temperature of the mixture was kept at 15−20 °C. After completion of benzylchloroformate addition, the mixture was stirred chilled with an ice bath for another 30 min and then at ambient temperature for 1 h. This mixture was washed with 300 mL of cold 1N HCl. The organic was concentrated in vacuo. The residue was partitioned between toluene (400 mL), MTBE (200 mL), and water (250 mL). The organic was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give an oil (97 g) as the crude (NMR showed ~3:1 cis/trans ratio of isomers). Silica gel column chromatography using gradient elution of EtOAc in hexane gave 64.4 g (65% yield) of the title compound. LC-MS (ES) m/z = 292 $[M + H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.19 (d, J = 6.8 Hz, 3H), 1.51 (m, 1H), 1.65−1.84 (m, 2H), 1.90−2.00 (m, 1H), 2.44 (t, J = 11.2 Hz, 1H), 2.89−3.11 (m, 1H), 3.71 (s, 3H), 4.29 (bs, 1H), 4.51 (bs, 1H), 5.16 (s, 2H), 7.32−7.50 (m, 5H).

(±)-*trans*-**22**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.18 (d, J = 6.8 Hz, 3H), 1.37−1.42 (m, 1H), 1.78−1.91 (m, 2H), 2.04−2.10 (m, 1H), 2.61 (m, 1H), 3.13 (dd, J = 13.9, 4.3 Hz, 1H), 3.62 (s, 3H) 4.45 (m, 1H), 4.50 (d, J = 14.1 Hz, 1H), 5.14 (m, 2H), 7.25−7.40 (m, 5H).

(±)-*cis*-**22** was resolved by chiral stationary phase HPLC, supercritical fluid chromatography (SFC) or by crystallization of a tartrate salt. The following conditions were used for the HPLC analysis and preparative resolution:

Analytical Separation Method. A ChiralPak AD-3 column (150 mm × 4.6 mm I.D.) was used eluting with a mixture of 15% ethanol and 85% hexane containing 0.1% diethylamine at a flow rate of 1 mL/min.

Preparative Separation Method. The separation was run in a Berger Multi-Gram III preparative SFC instrument. The column used was a ChiralPak IC (250 mm × 30 mm I.D.) eluting with a mixture of 75% SFC CO_2 and 25% 2-propanol at a flow rate of 90 mL/min. (**3*S*,6*R*)-22**. $[\alpha]^{23}_D$ = +14.5 (*c* 1.35, $CHCl_3$). (**3*R*,6*S*)-22**. $[\alpha]^{23}_D$ = −13.1 (*c* 0.95, $CHCl_3$).

*Resolution by Crystallization of the Tartrate Salt: Methyl (3S,6R)-6-Methyl-3-piperidinecarboxylate L-(+)-Tartaric Acid Salt (**23**).* A solution of *cis*-3-methyl 1-(phenylmethyl)-6-methyl-1,3-piperidinedicarboxylate (69 g, 237 mol) in EtOH (50 mL) and EtOAc (300 mL) was added to a slurry of 10% Pd/C (3.7 g) in EtOAc (30 mL) and EtOH (10 mL) under nitrogen in a Parr shaker bottle. The mixture was hydrogenated under 65 psi at room temperature for 4 h. The mixture was filtered through Celite and washed with EtOAc. The filtrate was concentrated in vacuo to give 37 g of *cis*-3-methyl 1-(phenylmethyl)-6-methyl-1,3-piperidinedicarboxylate as a liquid. LC-MS (ES) m/z = 158 $[M + H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.08 (d, J = 6.32 Hz, 3H), 1.15−1.33 (m, 1H), 1.53−1.64 (m, 1H), 1.64−1.78 (m, 1H), 2.13−2.25 (m, 1H), 2.31 (bs, 1H), 2.48−2.57 (m, 1H), 2.63−2.77 (m, 1H), 2.86 (dd, J = 13.1, 3.8 Hz, 1H), 3.39−3.51 (m, 1H), 3.74 (s, 3H).

A suspension of L-(+)-tartaric acid (39 g, 260 mmol, 1.05 equiv) in 2-propanol (200 mL) and water (13 mL) was heated in a water bath at 60 °C until all dissolved. To this hot stirred solution was added *cis*-3-methyl 1-(phenylmethyl)-6-methyl-1,3-piperidinedicarboxylate (39 g, 248 mmol), followed by addition of 25 mL of 2-propanol rinse. The resulting mixture was heated to 60 °C, resulting in a clear solution, and then cooled to room temperature while the hot water bath was removed. This hot solution was seeded with a sample of methyl (3*S*,6*R*)-6-methyl-3-piperidinecarboxylate L-(+)-tartaric acid salt that had a chiral purity of 98% ee and aged at ambient temperature (with the water bath removed) for 20 min. The mixture turned into an oily texture with seeds still present. To the mixture was added 5 mL of water and heated in the warm water bath at 43 °C. The mixture became clear with the seeds still present. The heating was stopped, and the mixture was stirred in the warm water bath. After 20 min, the mixture gradually turned into a paste. After another 10 min, the water bath was removed, and the mixture was stirred at ambient temperature for another 1 h. The resulting paste was filtered. The cake was washed with 50 mL of 2-propanol, giving 62 g of wet solids. This cake was taken up in 150 mL of 2-propanol and 8 mL of water and stirred as a slurry while being heated in a water bath to 60 °C (internal temp 55 °C) for 5 min. The heating was turned off while the mixture was still stirred in the warm water bath. After 30 min, the mixture was filtered. The cake was washed with 100 mL of 2-propanol. Drying under house vacuum at room temperature for 48 h gave 46.7 g of solids. An analytical sample was derivatized to the corresponding *N*-Cbz derivative, which was determined by chiral HPLC (methods used to analyze the resolution of (±)-*cis*-**22** above) to have 85% ee. This material was taken up in 2-propanol (420 mL) and water (38 mL) as a suspension. The mixture was heated in a water bath to 65 °C, at which time the mixture became a clear solution. The heating bath was removed. The mixture was seeded and aged at ambient temperature for 20 h. The solids formed were filtered and then washed with 100 mL of 2-propanol. The solids collected were dried under house vacuum at room temperature for 24 h and then under vacuum at room temperature for another 24 h to give 28.5 g of the title compound. An analytical sample was converted to the *N*-Cbz derivative. The ee was determined to be 97.7%. LC-MS (ES) m/z = 158 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.13 (d, J = 6.4 Hz, 3H), 1.24−1.43 (m, 1H), 1.59−1.79 (m, 2H), 1.93−2.06 (m, 1H), 2.77−2.88 (m, 1H), 2.98−3.14 (m, 2H), 3.38 (dd, J = 12.9, 2.8 Hz, 1H), 3.89 (s, 2H).

*(3S,6R)-6-Methyl-N-phenyl-3-piperidinecarboxamide ((**3S,6R**)-**24**).* To a solution of 3-methyl 1-(phenylmethyl) (3*S*,6*R*)-6-methyl-1,3-piperidinedicarboxylate (15 g, 51.5 mmol) in THF/water/CH_3OH (10/5/1, 480 mL) was added LiOH·H_2O (2.59 g, 61.8 mmol), and the reaction mixture was stirred at room temperature for about 1 h. The mixture was acidified with 1N HCl until the pH was ∼5 and then concentrated in vacuo. The resulting residue was extracted with EtOAc (2 × 300 mL) and $CHCl_3$ (2 × 300 mL), and the combined organic layers were dried ($MgSO_4$), filtered, and concentrated in vacuo to afford (3*S*,6*R*)-6-methyl-1-{[(phenylmethyl)oxy]carbonyl}-3-piperidinecarboxylic acid (17 g). LC-MS (ES) m/z = 278 $[M + H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.19 (d, J = 7.1 Hz, 3H), 1.57−1.91 (m, 3H), 1.94−2.05 (m, 1H), 2.48 (m, 1H), 3.02 (t, J = 12.8 Hz, 1H), 4.30 (bs, 1H), 4.53 (bs, 1H), 5.16 (s, 2H), 7.32−7.50 (m, 5H).

To a solution of (3*S*,6*R*)-6-methyl-1-{[(phenylmethyl)oxy]carbonyl}-3-piperidinecarboxylic acid (17 g, 61.3 mmol) in DMF (400 mL) was added aniline (8.56 g, 92 mmol), Hünig's base (32.1 mL, 184 mmol), HOBt (14.08 g, 92 mmol), and EDC (17.63 g, 92 mmol) at room temperature. The reaction mixture was stirred overnight at room temperature then diluted with H_2O (∼100 mL) and extracted with EtOAc (3 × 250 mL). The combined organic layers were washed with 1N HCl (100 mL), H_2O (3 × 100 mL), and brine (100 mL), and then dried ($MgSO_4$), filtered, and concentrated in vacuo to afford crude phenylmethyl (2*R*,5*S*)-2-methyl-5-[(phenylamino)carbonyl]-1-piperidinecarboxylate (21 g). LC-MS (ES) m/z = 353 $[M + H]^+$.

A solution of phenylmethyl (2*R*,5*S*)-2-methyl-5-[(phenylamino)carbonyl]-1-piperidinecarboxylate (21 g, 59.6 mmol) in EtOAc (250 mL) and EtOH (250 mL) was degassed. Pd/C (10%, 6.34 g, 5.96 mmol) was added, and then H_2 was bubbled through the mixture. The reaction mixture was stirred at room temperature under H_2 balloon overnight. The mixture was filtered through a pad of Celite and concentrated in vacuo to afford the crude title compound (12 g). LC-MS (ES) m/z = 219 $[M + H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.18 (d, J = 6.1 Hz, 3H), 1.36−1.51 (m, 1H), 1.55−1.65 (m, 1H), 1.68−1.83 (m, 1H), 2.10−2.21 (m, 1H), 2.54−2.62 (m, 1H), 2.70−2.84 (m, 1H), 2.96 (dd, J = 12.0, 3.2 Hz, 1H), 3.40 (m, 1 H), 7.05−7.12 (m, 1H), 7.30−7.37 (m, 2H), 7.56−7.65 (m, 2H), 11.16 (bs, 1H).

*(3S,6R)-1-[2-Amino-6-(3-amino-1H-indazol-6-yl)-4-pyrimidinyl]-6-methyl-N-phenyl-3-piperidinecarboxamide ((**3S,6R**)-**28a**).* The title compound was prepared from amine (**3*S*,6*R*)-24** following synthetic route 1. LC-MS (ES) m/z = 443 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.18 (d, J = 6.8 Hz, 3H), 1.75 (s, 2H), 1.83 (m, 1H), 1.92 (m, 1H), 5.37 (s, 2H), 6.10 (s, 2H), 6.64 (s, 1H), 7.03 (s, 1H), 7.32 (s, 2H), 7.29−7.34 (m, 2H), 7.64 (d, J = 7.3 Hz, 4H), 7.96 (s, 1H), 8.32 (s, 1H), 10.06 (s, 1H), 11.49 (s, 1H). Compound (**3*R*,6*S*)-28a** was prepared similarly.

*trans-1-[2-Amino-6-(3-amino-1H-indazol-6-yl)-4-pyrimidinyl]-6-methyl-N-phenyl-3-piperidinecarboxamide ((±)-trans-**28a**).* The title compound was synthesized following synthetic procedures similar to the preparation of (**3*S*,6*R*)-28a**. LC-MS (ES) m/z = 443 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.21 (d, J = 6.6 Hz, 3H), 1.42−1.55 (m, 1H), 1.90−2.06 (m, 3H), 2.83 (bs, 1H), 3.35−3.41 (m, 1H), 4.61 (bs, 1H), 4.70 (d, J = 6.6 Hz, 1H), 5.37 (s, 2H), 6.07 (s, 2H), 6.55 (s, 1H), 6.90−7.06 (m, 1H), 7.23 (t, J = 8.0 Hz, 2H), 7.44−7.52 (m, 1H), 7.58 (d, J = 7.6 Hz, 2H), 7.67 (d, J = 8.3 Hz, 1H), 7.90 (s, 1H), 9.83 (s, 1H), 11.50 (s, 1H).

*4,6-Dichloro-N-methyl-2-pyrimidinamine (**27**).* Methylamine (2 M solution in THF, 113 mL, 217 mmol, 2.05 equiv) was charged to a 1 L three-neck flask fitted with a magnetic stirrer and a thermometer. The mixture was chilled in an ice bath. To this stirred solution was added via addition funnel a solution of 4,6-dichloro-2-(methylsulfonyl)pyrimidine (25 g, 110 mmol) in EtOAc (250 mL) portionwise over a 25 min period. The temp was between 5−10 °C. After completion of addition, the ice bath was removed, and the mixture was stirred for 1 h at ambient temperature. LC-MS showed complete conversion. The suspension was filtered and washed with EtOAc. The filtrate was concentrated in vacuo. The residue was partitioned between water (100 mL) and EtOAc (450 mL). The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give white solids, which were triturated in 150 mL of CH_2Cl_2. These solids were collected by filtration

and washed with cold CH_2Cl_2 (50 mL). Drying under house vacuum at room temperature for 20 h and then high vacuum at room temperature for 3 h gave the title compound (9.31 g) as a solid. LC-MS (ES) m/z = 179 $[M + H]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 3.04 (s, 3H), 5.57 (bs, 1H), 6.63 (s, 1H).

*(3S,6R)-1-[6-(3-Amino-1H-indazol-6-yl)-2-(methylamino)-4-pyrimidinyl]-6-methyl-N-phenyl-3-piperidinecarboxamide ((**3S,6R**)-**28b**).* Methyl (3S,6R)-6-methyl-3-piperidinecarboxylate L-tartaric acid salt (4.0 g, 13.02 mmol) was dissolved in water (25 mL), to which was added $LiOH \cdot H_2O$ (1.80 g, 43.0 mmol, 3.3 equiv). The mixture was stirred at room temperature for 20 h. LC-MS showed complete ester hydrolysis. To this mixture was added $NaHCO_3$ (4.81 mg, 57.3 mmol, 4.4 equiv), 4,6-dichloro-*N*-methyl-2-pyrimidinamine (2.32 g, 13.02 mmol, 1 equiv), and 1,4-dioxane (25 mL). The reaction mixture was heated under reflux at 100 °C for 24 h. The mixture was concentrated in vacuo. The resulting residue was suspended in 40 mL of water, to which was added cold 2N HCl until pH = 3. The resulting mixture was filtered, and the solids were washed with water, dried under house vacuum at room temperature for 18 h, and then under vacuum over P_2O_5 at room temperature for 24 h to give (3S,6R)-1-[6-chloro-2-(methylamino)-4-pyrimidinyl]-6-methyl-3-piperidinecarboxylic acid (**25**) (3.23 g) as a solid. LC-MS (ES) m/z = 285 $[M + H]^+$.

To (3S,6R)-1-[6-chloro-2-(methylamino)-4-pyrimidinyl]-6-methyl-3-piperidinecarboxylic acid (880 mg, 3.09 mmol) in CH_2Cl_2 (15 mL) at room temperature was added Hünig's base (1.62 mL, 9.27 mmol, 3 equiv) and aniline (0.56 mL, 6.18 mmol, 2 equiv), and the resulting mixture was chilled in an ice bath. To this stirred solution was added HATU (1.29 g, 3.40 mmol, 1.1 equiv) in one portion. The resulting suspension was stirred in the ice bath for 45 min. LC-MS showed complete conversion. The mixture was filtered, and the filtrate was concentrated in vacuo. Silica gel column chromatography with gradient elution of 1% EtOAc in $CHCl_3$ to 35% EtOAc in $CHCl_3$ gave (3S,6R)-1-[6-chloro-2-(methylamino)-4-pyrimidinyl]-6-methyl-*N*-phenyl-3-piperidinecarboxamide (700 mg). LC-MS (ES) m/z = 360 $[M + H]^+$. ^1H NMR (400 MHz, CD_3OD): δ 1.27 (d, J = 6.8 Hz, 3H), 1.71−1.95 (m, 3H), 2.03−2.14 (m, 1H), 2.49−2.62 (m, 1H), 2.87 (s, 3H), 3.08−3.23 (m, 1H), 6.11 (s, 1H), 7.06−7.16 (m, 1H), 7.26−7.38 (m, 2H), 7.52−7.62 (m, 2H).

The title compound was prepared from (3S,6R)-1-[6-chloro-2-(methylamino)-4-pyrimidinyl]-6-methyl-*N*-phenyl-3-piperidinecarboxamide following route 2. LC-MS (ES) m/z = 457 $[M + H]^+$. ^1H NMR (400 MHz, CD_3OD): δ 1.33 (d, J = 6.8 Hz, 3H), 1.82−1.97 (m, 3H), 2.06−2.16 (m, 1H), 2.57−2.65 (m, 1H), 2.98 (s, 3H), 3.15−3.25 (m, 1H), 6.51 (s, 1H), 7.12 (t, J = 7.5 Hz, 1H), 7.34 (t, J = 8.1 Hz, 2H), 7.54 (dd, J = 8.5, 1.1 Hz, 1H), 7.60 (dd, J = 8.6, 1.3 Hz, 2H), 7.76 (d, J = 8.3 Hz, 1H), 7.87 (s, 1H).

*(3S,6R)-1-[6-(3-Amino-1H-indazol-6-yl)-2-(methylamino)-4-pyrimidinyl]-N-cyclohexyl-6-methyl-3-piperidinecarboxamide ((**3S,6R**)-**28d**).* To a suspension of (3S,6R)-1-[6-chloro-2-(methylamino)-4-pyrimidinyl]-6-methyl-3-piperidinecarboxylic acid (3.05 g, 10.71 mmol) in CH_2Cl_2 (50 mL) at room temperature was added Hünig's base (2.70 mL, 15.43 mmol, 1.3 equiv) and cyclohexylamine (1.60 mL, 14.2 mmol, 1.2 equiv), and the resulting mixture was chilled in an ice bath. To this stirred solution was added HATU (4.96 g, 13.1 mmol, 1.1 equiv) in one portion, and the resulting suspension was stirred in the ice bath for 30 min. LC-MS showed complete conversion. The mixture was diluted with CH_2Cl_2 (50 mL) and filtered through Celite. The filtrate was washed water (2 × 25 mL) and then brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. Silica gel column chromatography using gradient elution of 1% EtOAc in $CHCl_3$ to 50% EtOAc in $CHCl_3$ afforded (3S,6R)-1-[6-chloro-2-(methylamino)-4-pyrimidinyl]-*N*-cyclohexyl-6-methyl-3-piperidinecarboxamide (4.26 g) as a foam. LC-MS (ES) m/z = 366 $[M + H]^+$.

The title compound was prepared from (3S,6R)-1-[6-chloro-2-(methylamino)-4-pyrimidinyl]-*N*-cyclohexyl-6-methyl-3-piperidinecarboxamide following synthetic route 1. LC-MS (ES) m/z = 463 $[M + H]^+$. ^1H NMR (400 MHz, CD_3OD): δ 1.16−1.32 (m, 3H), 1.29 (d, J = 6.8 Hz, 3H), 1.34−1.45 (m, 2H), 1.65−1.68 (m, 1H), 1.76−1.81 (m, 5H), 1.85−1.92 (m, 2H), 1.97−2.05 (m, 1H), 2.35−2.42 (m, 1H), 2.97 (s, 3H), 3.11−3.15 (m, 1H), 3.64−3.70 (m, 1H), 4.45−4.65 (bs, 1H), 4.72−4.92 (bs, 1H), 6.45 (s, 1H), 7.52 (dd, J = 8.5, 1.1 Hz, 1H), 7.75 (d, J = 8.3 Hz, 1H), 7.85 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 14.6, 22.9, 24.6, 25.3, 27.9, 29.0, 32.5, 42.6, 47.2, 89.1, 107.7, 114.7, 116.6, 119.9, 136.7, 141.7, 149.2, 162.5, 163.0, 163.3, 172.3

*(3S,6R)-1-[2-Amino-6-(4-cyano-3-fluorophenyl)-4-pyrimidinyl]-6-methyl-3-piperidinecarboxylic acid (**26**).* Into a 75 mL sealable tube were added 3-methyl 1-(phenylmethyl) (3S,6R)-6-methyl-1,3-piperidinedicarboxylate (10 g, 34.3 mmol), 1,4-dioxane (20 mL), and concentrated HCl (20 mL), and the mixture was stirred at 100 °C for 3 h. The mixture was then cooled to room temperature. The solution was transferred to a 500 mL round-bottom flask and concentrated to dryness. Trituration with Et_2O and CH_3CN afforded the HCl salt of (3S,6R)-6-methyl-3-piperidinecarboxylic acid (6.17 g) as a colorless oil that turned into a white solid after standing overnight. ^1H NMR (400 MHz, CD_3OD): δ 1.34 (d, J = 6.6 Hz, 3H), 1.46−1.63 (m, 1H), 1.83−2.03 (m, 2H), 2.13−2.33 (m, 1H), 2.88−3.01 (m, 1H), 3.18 (dd, J = 13.1, 4.0 Hz, 1H), 3.22−3.31 (m, 1H), 3.58−3.71 (m, 1H).

A mixture of 4,6-dichloro-2-pyrimidinamine (3.0 g, 18.3 mmol), (3S,6R)-6-methyl-3-piperidinecarboxylic acid (3.98 g, 20.12 mmol), and $NaHCO_3$ (7.68 g, 91 mmol) in 1,4-dioxane (100 mL) and water (50 mL) was stirred overnight at 117 °C in a sealed tube. The reaction was allowed to cool to room temperature. LC-MS showed that most of the 4,6-dichloro-2-pyrimidinamine had been consumed. Then 4-cyano-3-fluorobenzeneboronic acid (3.32 g, 20.12 mmol) and $Pd(Ph_3P)_4$ (0.423 g, 0.366 mmol) were added, and the reaction mixture was stirred for 24 h at 117 °C. The mixture was poured into water (300 mL) and EtOAc (200 mL). The pH of the solution was 9, and the desired product was in the aqueous layer. The aqueous layer was separated from the organic layer, and then it was filtered. 6N HCl was added dropwise to the filtrate to make the pH = 4. A precipitate formed. The precipitate was filtered, washed with water, and dried to afford the crude title compound (3.6 g) as a light-yellow solid. LC-MS (ES) m/z = 356 $[M + H]^+$. ^1H NMR (400 MHz, DMSO-d_6): δ 1.13 (d, J = 6.8 Hz, 3H), 1.56−1.94 (m, 5H), 2.26−2.39 (m, 1H), 2.86 (bs, 1H), 6.26 (s, 2H), 6.77 (s, 1H), 7.97−8.05 (m, 1H), 8.10−8.24 (m, 2H), 12.47 (bs, 1H).

*(3S,6R)-1-[2-Amino-6-(3-amino-1H-indazol-6-yl)-4-pyrimidinyl]-N-cyclohexyl-6-methyl-3-piperidinecarboxamide ((**3S,6R**)-**28c**).* To a solution of (3S,6R)-1-[2-amino-6-(4-cyano-3-fluorophenyl)-4-pyrimidinyl]-6-methyl-3-piperidinecarboxylic acid (415 mg, 1.128 mmol) and HATU (533 mg, 1.401 mmol) in DMF (3 mL) in a 10 mL round-bottom flask was added Hünig's base (0.408 mL, 2.34 mmol), and the resulting mixture was stirred at room temperature for 15 min. Cyclohexylamine (0.16 mL, 1.401 mmol) was added, and the reaction mixture was stirred at room temperature for 0.5 h. LC-MS showed the reaction was complete. The reaction was poured into water, and EtOAc was added to extract the product. The organic layer was concentrated, and the formed solid was recrystallized from CH_3CN to afford (3S,6R)-1-[2-amino-6-(4-cyano-3-fluorophenyl)-4-pyrimidinyl]-*N*-cyclohexyl-6-methyl-3-piperidinecarboxamide (245 mg) as a light-yellow solid. LC-MS (ES) m/z = 437 $[M + H]^+$.

In a microwave vial, (3S,6R)-1-[2-amino-6-(4-cyano-3-fluorophenyl)-4-pyrimidinyl]-*N*-cyclohexyl-6-methyl-3-piperidinecarboxamide (235 mg, 0.538 mmol), EtOH (5 mL), Hünig's base (0.376 mL, 2.153 mmol), and anhydrous hydrazine (0.101 mL, 3.23 mmol) were combined, and the yellow suspension was heated overnight at 110 °C in an oil bath. When the temperature of the reaction reached 100 °C all of the solids were dissolved. After overnight, there was a yellow suspension as well as some black-colored solid formed. LCMS showed mainly product. The black

solid and the yellow solid were carefully separated due to the black solid being heavier than yellow solid in the CH_3OH solvent. The yellow solid in CH_3OH was filtered and washed with CH_3OH to remove the color and afford the title compound (157 mg) as a white solid. LC-MS (ES) m/z = 449 $[M + H]^+$. ^1H NMR (400 MHz, DMSO-d_6): δ 1.05−1.35 (m, 8H), 1.50−1.88 (m, 9H), 2.24 (s, 1H), 2.90 (s, 1H), 3.46−3.60 (m, 1H), 5.38 (s, 2H), 6.07 (s, 2H), 6.57 (s, 1H), 7.57 (d, J = 8.3 Hz, 1H), 7.70 (d, J = 8.3 Hz, 1H), 7.79 (d, J = 7.6 Hz, 1 H), 7.94 (s, 1H), 11.49 (s, 1H).

*[(2S,5R)-5-Methyl-4-(phenylmethyl)-2-morpholinyl]methanol (**29a**).* To a stirred solution of (2*R*)-2-[(phenylmethyl)amino]-1-propanol (1.65 g, 9.99 mmol) in toluene (50 mL) was added (*R*)-(−)-epichlorohydrin (1.02 mL, 12.98 mmol), followed by lithium perchlorate (1.062 g, 9.99 mmol) under nitrogen. After stirring 2 days, TLC (5% methanol−dichloromethane) showed there was only a trace of starting material and a major product. A solution of sodium methoxide (25 wt % in CH_3OH) (5.71 mL, 24.96 mmol) was then added and the mixture was stirred for 3 days. Saturated aq NH_4Cl (75 mL) was added, and the product was extracted with EtOAc (3 × 75 mL). The combined organics were washed with brine, dried ($MgSO_4$), filtered, and evaporated to give the crude product, which was purified by chromatography (Analogix RS-120 silica cartridge) eluting with 20−50% EtOAc in hexanes to afford the title compound (1.11 g) as a colorless oil. LC-MS (ES) m/z = 222 $[M + H]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 1.12 (d, J = 6.6 Hz, 3H), 2.23−2.61 (m, 3H), 2.80 (m, 1H), 3.49−3.57 (m, 1H), 3.60−3.77 (m, 5H), 3.78−3.86 (m, 1H), 7.17−7.47 (m, 5H). $[\alpha]_D$ = −5.2 (c = 1.55, CH_3OH, 23.5 °C).

*[(2S,5R)-4-(2-Amino-6-chloro-4-pyrimidinyl)-5-methyl-2-morpholinyl]methanol (**30a**).* To a stirred solution of [(2*S*,5*R*)-5-methyl-4-(phenylmethyl)-2-morpholinyl]methanol (1.05 g, 4.74 mmol) in ethanol (15 mL) was added concentrated hydrochloric acid (0.435 mL, 5.22 mmol). The mixture was purged with nitrogen to degas, and then 10% palladium on carbon (Degussa Type E101 NE/W, 50% wet, 150 mg, 0.070 mmol) was added and the mixture was purged with hydrogen and then stirred under a balloon of H_2. After stirring 4 h, TLC (50% EtOAc−hexanes) showed no starting material and baseline product. The mixture was degassed with nitrogen, filtered through Celite, and evaporated to dryness to afford [(2*S*,5*R*)-5-methyl-2-morpholinyl]methanol (880 mg) as the hydrochloride salt. ^1H NMR (400 MHz, D_2O): δ 1.34 (d, J = 7.1 Hz, 3H), 3.04−3.12 (m, 1H), 3.14−3.24 (m, 1H), 3.50−3.67 (m, 3H), 3.74−3.83 (m, 2H), 3.83−3.89 (m, 1H).

In a microwave vessel, Hünig's base (0.59 mL, 3.35 mmol) was added to [(2*S*,5*R*)-5-methyl-2-morpholinyl]methanol hydrochloride (208 mg, 1.12 mmol) in CH_3CN (3 mL), and then 2-amino-4,6-dichloropyrimidine (174 mg, 1.06 mmol) was added. The mixture was heated with stirring in a microwave reactor at 160 °C for 1 h. HPLC indicated complete conversion. The mixture was filtered through a 0.45 μm filter disk, evaporated, and the product was purified further by chomatography (Analogix SF25−40 g silica column) eluting with 2−5% CH_3OH in $CHCl_3$ to afford the title compound (258 mg) as a white foam. LC-MS (ES) m/z = 260 $[M + H]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 1.28 (d, J = 6.8 Hz, 3H), 1.98 (bs, 1H), 2.99 (bs, 1H), 3.53−3.63 (m, 1H), 3.70 (bs, 1H), 3.75−3.82 (m, 2H), 3.83−3.89 (m, 1H), 4.87 (bs, 2H), 5.95 (s, 1H).

*(2S,5R)-4-[2-Amino-6-(3-amino-1H-indazol-6-yl)-4-pyrimidinyl]-5-methyl-N-phenyl-2-morpholinecarboxamide (**34a**).* In a sealable vessel was added (4-cyano-3-fluorophenyl)boronic acid (163 mg, 0.986 mmol), [(2*S*,5*R*)-4-(2-amino-6-chloro-4-pyrimidinyl)-5-methyl-2-morpholinyl]methanol (232 mg, 0.897 mmol) and 1,4-dioxane (5 mL), and saturated aqueous $NaHCO_3$ (2.5 mL). The mixture was purged with nitrogen to degas, and then $Pd(PPh_3)_4$ (104 mg, 0.090 mmol) was added and the vessel was sealed and heated at 100 °C overnight. HPLC and LC-MS indicated the product had formed but some starting pyrimidine remained. Another portion of (4-cyano-3-fluorophenyl)boronic acid (50 mg, 0.303 mmol) was added, and the reaction was stirred at 120 °C in a microwave for 30 min. The reaction was worked up by diluting with toluene and water. The aqueous layer was extracted with toluene (2 × 5 mL) and the combined organics were washed with water and brine, dried ($MgSO_4$), filtered, and evaporated. The product was purified by silica gel chromatography (Analogix SF25−40 g) eluting with 2−10% CH_3OH in $CHCl_3$ to give 4-{2-amino-6-[(2*S*,5*R*)-2-(hydroxymethyl)-5-methyl-4-morpholinyl]-4-pyrimidinyl}-2-fluorobenzonitrile (193 mg) as an off-white foam. LC-MS (ES) m/z = 344 $[M + H]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 1.32 (d, J = 6.8 Hz, 3H), 2.06 (d, J = 6.1 Hz, 1H), 3.05 (bs, 1H), 3.59−3.68 (m, 1H), 3.68−3.78 (m, 1H), 3.78−3.94 (m, 3H), 4.91 (bs, 2H), 6.31 (s, 1H), 7.70 (dd, J = 8.3, 6.6 Hz, 1H), 7.78−7.92 (m, 2H).

A stock solution of oxidant was prepared by dissolving H_5IO_6 (11.4 g, 50 mmol) and CrO_3 (23 mg, 1.15 mol %) in wet CH_3CN (0.75% water) to a volume of 114 mL (takes about 2 h to dissolve).

In a 100 mL RB was placed 4-{2-amino-6-[(2*S*,5*R*)-2-(hydroxymethyl)-5-methyl-4-morpholinyl]-4-pyrimidinyl}-2-fluorobenzonitrile (188 mg, 0.548 mmol) and wet CH_3CN (0.75% water, 4.5 mL). The stirred solution was cooled in an ice bath, and 4.37 mL of the above stock solution was added very slowly dropwise (over 10 min). After 2 h, there was a small amount of starting material observed by TLC ($NaHCO_3$ added to an aliquot, 5% CH_3OH−$CHCl_3$), so the reaction was placed in the refrigerator overnight. At this time, there was no starting material by TLC. The reaction was quenched by adding Na_2HPO_4 (0.5 g) in 5 mL of water. After stirring a few minutes, the mixture was cloudy and the pH = 5. The product was extracted with EtOAc (5 × 10 mL), and the combined organics were washed with 5% $NaHSO_3$, brine, dried ($MgSO_4$), filtered, and evaporated to afford the crude (2*S*,5*R*)-4-[2-amino-6-(4-cyano-3-fluorophenyl)-4-pyrimidinyl]-5-methyl-2-morpholinecarboxylic acid (**38a**) (102 mg) as an off-white solid. LC-MS (ES) m/z = 358 $[M + H]^+$.

A 5 mL vial was charged with (2*S*,5*R*)-4-[2-amino-6-(4-cyano-3-fluorophenyl)-4-pyrimidinyl]-5-methyl-2-morpholinecarboxylic acid (72 mg, 0.201 mmol), HOBt (29.9 mg, 0.222 mmol), and DMF (1 mL) under nitrogen. The mixture was stirred and cooled in an ice bath, and then EDC (42.5 mg, 0.222 mmol) was added. After stirring 10 min, aniline (0.020 mL, 0.222 mmol) was added and the mixture was allowed to warm to room temperature and stir. After 4 h, there was no change in progress. The mixture was diluted with EtOAc and washed with water, saturated $NaHCO_3$, brine, and dried ($MgSO_4$). After filtering, the filtrate was concentrated, and the product was purified by silica gel chromatography (Analogix SF-4 g) eluting with 20−50% EtOAc in $CHCl_3$ to afford (2*S*,5*R*)-4-[2-amino-6-(4-cyano-3-fluorophenyl)-4-pyrimidinyl]-5-methyl-*N*-phenyl-2-morpholinecarboxamide (59 mg) as a light-orange oil. LC-MS (ES) m/z = 433 $[M + H]^+$.

(2*S*,5*R*)-4-[2-amino-6-(4-cyano-3-fluorophenyl)-4-pyrimidinyl]-5-methyl-*N*-phenyl-2-morpholinecarboxamide (57 mg, 0.132 mmol) was dissolved in ethanol (3 mL) with stirring in a 5 mL microwave vessel. Hydrazine monohydrate (150 μL, 3.09 mmol) was added, and the mixture was capped and heated at 100 °C in an oil bath for 24 h. The mixture was partitioned between EtOAc and water, and the aqueous layer was extracted with EtOAc. The combined organics were washed with brine, dried ($MgSO_4$), filtered, and evaporated. Purification by silica gel chromatograpy (Analogix RS-4 g) eluting with 90:10:1 $CHCl_3$:CH_3OH:conc aq NH_4OH afforded the title compound (19 mg, 0.041 mmol, 30.8% yield) as a colorless film/foam. NMR showed a residual solvent peak with singlets at 1.9 and 3.36, so the material was azeotroped with CH_3CN and then triturated to give a white powder. ^1H NMR (400 MHz, DMSO-d_6): δ 1.24 (d, J = 6.3 Hz, 3H), 3.04 (bs, 1H), 3.82 (d, J = 9.8 Hz, 1H), 3.93 (d, J = 11.1 Hz, 1H), 4.07−4.23 (m, 1H), 4.53 (bs, 2H), 5.39 (bs, 2H), 6.22 (bs, 2H), 6.61 (s, 1H), 7.10 (t, J = 7.1 Hz, 1H), 7.34 (t, J = 7.7 Hz, 2H), 7.60 (d, J = 8.3 Hz, 1H), 7.69−7.78 (m, 3H), 7.96 (s, 1H), 9.87 (bs, 1H), 11.52 (s, 1H).

*{(2S,5R)-4-[6-Chloro-2-(methylamino)-4-pyrimidinyl]-5-methyl-2-morpholinyl}methanol (**30b**).* The title compound was prepared similarly to **30a** but using pyrimidine **27**. LC-MS (ES) m/z = 273, 275 $[M + H]^+$. ^1H NMR (400 MHz, DMSO-d_6): δ 1.15 (d, J = 6.8 Hz, 3H), 2.76

(s, 3H), 2.85 (bs, 1H), 3.31−3.55 (m, 3H), 3.56−3.66 (m, 1H), 3.67−3.78 (m, 1H), 6.19 (bs, 1H).

*(2S,5R)-4-[6-(3-amino-1H-indazol-6-yl)-2-(methylamino)-4-pyrimidinyl]-N-cyclohexyl-5-methyl-2-morpholinecarboxamide (**34c**).* The title compound was prepared from **30b** following similar synthetic procedures to the synthesis of **34a**. LC-MS (ES) m/z = 465 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.04−1.16 (m, 1H), 1.19 (d, J = 6.6 Hz, 3H), 1.23−1.37 (m, 4H), 1.52−1.63 (m, 1H), 1.63−1.79 (m, 4H), 2.76−2.99 (m, 4H), 3.55−3.68 (m, 1H), 3.74 (dd, J = 11.4, 2.8 Hz, 1H), 3.86 (d, J = 11.1 Hz, 1H), 3.91 (dd, J = 11.4, 2.8 Hz, 1H), 4.20−4.83 (bs, 2H), 5.39 (s, 2H), 6.50−6.71 (m, 2H), 7.56−7.69 (m, 2H), 7.72 (d, J = 8.3 Hz, 1H), 7.99 (s, 1H), 11.50 (s, 1H).

*[(2S,5R)-5-Ethyl-4-(phenylmethyl)-2-morpholinyl]methanol (**29b**).* To (*R*)-(−)-2-amino-1-butanol (5 g, 56.1 mmol) in CH_3OH (120 mL) was added benzaldehyde (6.24 mL, 61.7 mmol), and the reaction mixture was stirred under nitrogen for 15 min. The mixture was then cooled in an ice bath, and sodium borohydride (2.33 g, 61.7 mmol) was added portionwise. The mixture was stirred in the ice bath for 1.5 h. NaOH (6 N, 25 mL) was added, and the mixture was concentrated. The resulting residue was taken up in 100 mL of H_2O and extracted with Et_2O (2×). The organics were combined, dried over Na_2SO_4, and concentrated to afford (2*R*)-2-[(phenylmethyl)amino]-1-butanol (10.92 g) as a fluffy white solid. LC-MS (ES) m/z = 180 $[M + H]^+$.

To a solution of (2*R*)-2-[(phenylmethyl)amino]-1-butanol (10.06 g, 56.1 mmol) in 1,2-dichloroethane (DCE, 250 mL) was added (*R*)-(−)-epichlorohydrin (6.75 g, 72.9 mmol), followed by lithium perchlorate (5.97 g, 56.1 mmol) under nitrogen. The reaction was stirred for 2 days at room temperature, then sodium ethoxide (21 wt % in ethanol, 52.4 mL, 140 mmol) was added and the reaction continued to stir for 3 days. Saturated NH_4Cl was added, and the product was extracted with EtOAc. The organic layers were combined, dried over Na_2SO_4, and concentrated to afford the title compound (16.2 g) as a colorless oil. LC-MS (ES) m/z = 236 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.80 (t, J = 7.5 Hz, 3H), 1.44−1.71 (m, 2H), 2.26−2.45 (m, 3H), 3.19−3.50 (m, 4H), 3.49−3.80 (m, 3H), 4.60 (bs, 1H), 7.19−7.27 (m, 1H), 7.28−7.37 (m, 4H).

*(2S,5R)-4-[6-(3-Amino-1H-indazol-6-yl)-2-(methylamino)-4-pyrimidinyl]-5-ethyl-N-phenyl-2-morpholinecarboxamide (**34b**).* [(2*S*,5*R*)-5-Ethyl-4-(phenylmethyl)-2-morpholinyl]methanol (0.5 g, 2.125 mmol) was dissolved in CH_3OH (20 mL) and placed under a nitrogen atmosphere. Palladium on carbon (10 wt %, 0.023 g, 0.212 mmol) was added, and the flask was flushed with nitrogen and evacuated (3×). Then the reaction was placed under an atmosphere of hydrogen (balloon) and stirred at room temperature overnight. The reaction mixture was filtered through Celite and concentrated to afford [(2*S*,5*R*)-5-ethyl-2-morpholinyl]methanol (0.309 g) as a colorless oil. LC-MS (ES) m/z = 146 $[M + H]^+$.

To [(2*S*,5*R*)-5-ethyl-2-morpholinyl]methanol (0.309 g, 2.125 mmol) in THF (10 mL) were added Boc_2O (0.493 mL, 2.125 mmol) and Hünig's base (0.371 mL, 2.125 mmol), and the reaction mixture was heated to 40 °C and stirred overnight. The reaction was then cooled to room temperature and concentrated to afford 1,1-dimethylethyl (2*S*,5*R*)-5-ethyl-2-(hydroxymethyl)-4-morpholinecarboxylate (0.488 g). LC-MS (ES) m/z = 246 $[M + H]^+$.

To a vigorously stirred solution of 1,1-dimethylethyl (2*S*,5*R*)-5-ethyl-2-(hydroxymethyl)-4-morpholinecarboxylate (2.52 g, 10.27 mmol) in CH_2Cl_2 (50 mL) that was cooled to 0 °C were added TEMPO (0.321 g, 2.054 mmol) and (diacetoxyiodo)benzene (7.28 g, 22.60 mmol). The ice bath was removed, and the reaction was allowed to warm to room temperature and stirred overnight. The reaction was quenched with CH_3OH and then concentrated to afford crude (2*S*,5*R*)-4-{[(1,1-dimethylethyl)-oxy]carbonyl}-5-ethyl-2-morpholinecarboxylic acid (**32**) (0.711 g) as a yellow oil. LC-MS (ES) m/z = 260 $[M + H]^+$.

To (2*S*,5*R*)-4-{[(1,1-dimethylethyl)oxy]carbonyl}-5-ethyl-2-morpholinecarboxylic acid (0.250 g, 0.964 mmol) in CH_2Cl_2 (10 mL) was added aniline (0.088 mL, 0.964 mmol), HOAt (0.131 g, 0.964 mmol), and EDC (0.222 g, 1.157 mmol), and the reaction mixture was stirred at room temperature overnight. The reaction mixture was washed with water (2×), and the organics were dried over Na_2SO_4 and concentrated to afford 1,1-dimethylethyl (2*S*,5*R*)-5-ethyl-2-[(phenylamino)carbonyl]-4-morpholinecarboxylate (0.247 g) as a brown oil. LC-MS (ES) m/z = 240 $[M + H]^+$.

1,1-Dimethylethyl (2*S*,5*R*)-5-ethyl-2-[(phenylamino)carbonyl]-4-morpholinecarboxylate (0.269 g, 0.804 mmol) was taken up in HCl in 1,4-dioxane (4 M, 0.024 mL, 0.804 mmol) and stirred at room temperature overnight. The reaction was concentrated to yield an HCl salt of (2*S*,5*R*)-5-ethyl-*N*-phenyl-2-morpholinecarboxamide (**33a**) (0.198 g) as a brown oil. LC-MS (ES) m/z = 235 $[M + H]^+$.

The title compound was prepared from amine **33a** following route 1. LC-MS (ES) m/z = 473 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.92 (t, 3H), 1.74−1.86 (m, 2H), 3.34 (s, 3H), 3.71−3.74 (m, 1H), 3.74−3.77 (m, 1H), 4.03 (bs, 1H), 4.06 (bs, 1H), 4.10−4.16 (m, 1H), 4.64−4.85 (m, 1H), 5.39 (bs, 2H), 6.60−6.67 (m, 2H), 7.07−7.13 (m, 1H), 7.31−7.36 (m, 2H), 7.60−7.64 (m, 1H), 7.70−7.74 (m, 3H), 8.00 (s, 1H), 9.87 (bs, 1H), 11.50 (bs, 1H).

*(2S,5R)-4-[6-(3-Amino-1H-indazol-6-yl)-2-(methylamino)-4-pyrimidinyl]-N-cyclohexyl-5-ethyl-2-morpholinecarboxamide (**34d**).* The title compound was synthesized following synthetic procedures similar to the preparation of **34b**. TFA salt. LC-MS (ES) m/z = 479 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.85−0.92 (m, 3H), 1.07−1.15 (m, 2H), 1.20−1.35 (m, 4H), 1.52−1.61 (m, 2H), 1.65−1.80 (m, 4H), 2.81−2.88 (m, 5H), 3.56−3.63 (m, 1H), 3.63−3.69 (m, 1H), 3.86−3.92 (m, 1H), 3.93−3.99 (m, 1H), 4.57−4.83 (m, 2H), 5.38 (bs, 1H), 6.53−6.64 (m, 2H), 7.58−7.67 (m, 2H), 7.70−7.74 (m, 1H), 7.97 (bs, 1H), 11.49 (bs, 1H).

*1,1-Dimethylethyl {1-[2-Amino-6-(3-amino-1H-indazol-6-yl)-4-pyrimidinyl]-3-piperidinyl}carbamate ((±)-**37b**).* The title compound was prepared from amine **36** following synthetic route 1. LC-MS (ES) m/z = 425 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.38 (s, 9H), 1.43 (m, 2H), 1.66−1.79 (m, 1H), 1.80−1.95 (m, 1H), 2.78−2.93 (m, 1H), 2.95−3.10 (m, 1H), 3.29−3.40 (m, 1H), 4.11 (d, J = 12.6 Hz, 1H), 4.26 (bs, 1H), 5.38 (s, 2H), 6.08 (bs, 2H), 6.57 (s, 1H), 6.93 (d, J = 7.3 Hz, 1H), 7.55 (d, J = 8.3 Hz, 1H), 7.70 (d, J = 8.3 Hz, 1H), 7.93 (s, 1H), 11.51 (s, 1H).

Each pure enantiomer of **37b** was prepared following the same synthetic route as described for ((±)-**37b**), starting from the corresponding pure enantiomer of **36**.

*N-{1-[2-Amino-6-(3-amino-1H-indazol-6-yl)-4-pyrimidinyl]-3-piperidinyl}benzamide ((±)-**37a**).* 1,1-Dimethylethyl {1-[2-amino-6-(3-amino-1*H*-indazol-6-yl)-4-pyrimidinyl]-3-piperidinyl}carbamate (865 mg, 2.04 mmol) was added portionwise to ice-cooled concentrated hydrochloric acid (8 mL, 96 mmol) with stirring. A solid yellow mass formed. The ice bath was removed, and the reaction was allowed to warm to room temperature with stirring. HPLC indicated complete conversion. The mixture was diluted with ice−water (20 mL), and the solution was concentrated under reduced pressure. The residue was dissolved in ethanol (15 mL) and evaporated, followed by suspending in CH_3CN (15 mL) and evaporating. The residue was then triturated with 2-propanol (10 mL) and filtered, washed with 2-propanol followed by hexanes, and dried under vacuum to afford 6-[2-amino-6-(3-amino-1-piperidinyl)-4-pyrimidinyl]-1*H*-indazol-3-amine trihydrochloride (761 mg) as a yellow powder. A 140 mg portion was suspended in CH_3OH (2 mL) and water (1 mL) and was brought to pH 10 with 1 N NaOH. The solution was filtered and purified by Gilson automated reverse phase HPLC (Gemini Phenomenex C18 5 μ, 100 mm × 300 mm), eluting with 5−90% CH_3CN in water + 0.1%NH_4OH). Pure fractions were combined, evaporated, and dried to afford the free base (72 mg) as a white solid. LC-MS (ES) m/z = 325 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.15−1.29 (m, 1H), 1.31−1.46 (m, 1H), 1.51 (bs, 2H), 1.62−1.75 (m, 1H), 1.87 (d,

J = 10.6 Hz, 1H), 2.53−2.68 (m, 2H), 2.83 (t, J = 12.1 Hz, 1H), 4.30 (m, 2H), 5.38 (s, 2H), 6.03 (bs, 2H), 6.58 (s, 1H), 7.56 (d, J = 8.6 Hz, 1H), 7.70 (d, J = 8.3 Hz, 1H), 7.94 (s, 1H), 11.52 (s, 1H).

Sodium bicarbonate (145 mg, 1.73 mmol) was added to a solution of 6-[2-amino-6-(3-amino-1-piperidinyl)-4-pyrimidinyl]-1*H*-indazol-3-amine trihydrochloride (150 mg, 0.346 mmol) in water (1.5 mL) with stirring. Tetrahydrofuran (1.5 mL) was added, the mixture was cooled in an ice bath, and benzoyl chloride (0.044 mL, 0.380 mmol) was added dropwise with stirring. The mixture was then warmed to room temperature and stirred for 2 h. LC-MS showed starting material, product, and bis-benzoylation. Additional portions of benzoyl chloride (0.040 mL, 0.345 mmol) and $NaHCO_3$ (145 mg, 1.73 mmol) were added, and the mixture was stirred for 1 h. HPLC showed complete conversion to multibenzoylated product. The reaction was diluted with EtOAc, washed with water, dried (Na_2SO_4), filtered, and evaporated. The crude residue was suspended in CH_3OH (8 mL), concentrated aqueous HCl (1 mL, 12 mmol) was added, and the mixture was stirred for 3 days at 65 °C. HPLC showed conversion to the desired product. The mixture was concentrated to ca. 2 mL and then diluted with CH_3CN (ca. 8 mL) and heated. Additional CH_3CN was added to the hot solution until turbid. The mixture was allowed to cool slowly to room temperature with strring over 2 h. The precipitate was collected by filtration and washed with CH_3CN, then Et_2O, and finally hexanes. Drying afforded the title compound (156 mg) dihydrochloride as an off-white solid. A 100 mg portion was purified further by reverse phase HPLC (Gemini Phenomenex C18 5 μ, 100 mm × 300 mm), eluting with 5−90% CH_3CN in water + 0.1%NH_4OH) to give the title compound (55 mg) as a white solid. LC-MS (ES) m/z = 429 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.43−1.60 (m, 1H), 1.60−1.74 (m, 1H), 1.76−1.86 (m, 1H), 1.92−2.03 (m, 1H), 2.87−3.00 (m, 2H), 3.83−3.95 (m, 1H), 4.27−4.39 (m, 1H), 4.49 (bs, 1H), 5.38 (s, 2H), 6.12 (s, 2H), 6.64 (s, 1H), 7.46 (t, J = 7.3 Hz, 2H), 7.50−7.54 (m, 1H), 7.54−7.61 (m, 1H), 7.71 (d, J = 8.6 Hz, 1H), 7.87 (d, J = 7.1 Hz, 2H), 7.94 (s, 1H), 8.39 (d, J = 7.6 Hz, 1H), 11.52 (s, 1H).

*1,1-Dimethylethyl [(3R,6S)-6-Methyl-3-piperidinyl]carbamate ((**3R,6S**)-**35**).* (3*R*,6*S*)-6-Methyl-1-{[(phenylmethyl)oxy]carbonyl}-3-piperidinecarboxylic acid (prepared as described in the experimental procedure for (3*S*,6*R*)-**28b**, 85 g, 307 mmol) in a 2 L RB flask was azeotroped with toluene (3 × 200 mL). The residue (an oil) was dissolved in 500 mL of *t*-butanol (anhydrous grade). To this solution was added triethylamine (64.1 mL, 460 mmol, 1.5 equiv) at room temperature in one portion. Diphenyl azidophosphate (110 g, 398 mmol, 1.3 equiv) was added via an addition funnel at room temperature portionwise into this stirred mixture. Addition took 20 min. The resulting mixture (a light-yellow clear solution) was stirred at room temperature for 30 min, followed by heating in an oil bath to 100 °C under reflux for 20 h. LC-MS showed complete conversion. The mixture was concentrated in vacuo to remove as much *t*-butanol as possible. The residue was diluted with EtOAc (1 L), which was washed with 200 mL of cold 2N HCl, followed by 200 mL of 2.5 N NaOH. The NaOH portion was salted with NaCl and back-extracted with EtOAc (400 mL). All the organic portions were combined, washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was taken up in hexane (500 mL) and EtOAc (20 mL). A waxy paste developed. The mixture was chilled in the refrigerator for 20 h, resulting in a two-phase mixture. The top layer was decanted off and gave 42 g as an oil after concentration in vacuo, which would undergo further silica gel column purification. The waxy bottom component was taken up in $CHCl_3$ (300 mL) and EtOAc (30 mL). The mixture was stirred for 5 min and filtered. The filtrate was concentrated in vacuo to give an oil (150 g), which would undergo further silica gel column purification. The waxy solids (30 g) collected were identified as $(PhO)_2POOH$ and were discarded. Silica gel column chromatography of the above two oils on multiple runs using gradient elution of 1−50% EtOAc in $CHCl_3$ gave phenylmethyl (2*S*,5*R*)-5-({[(1,1-dimethylethyl)oxy]carbonyl}amino)-2-methyl-1-piperidinecarboxylate (88.28 g) as a thick clear syrup. LC-MS (ES) m/z = 349 $[M + H]^+$. ^{1}H NMR (400 MHz, CD_3OD): δ 1.17 (d, J = 7.1 Hz, 3H), 1.45 (s, 9H), 1.56−1.66 (m, 2H), 1.73−1.78 (m, 2H), 2.66 (t, J = 12.0 Hz, 1H), 4.41−4.44 (m, 1H), 5.14 (s, 2H), 7.31−7.39 (m, 5H).

Pd/C (6.30 g) was added to a 2 L Parr bottle, followed by addition of EtOAc (50 mL) under nitrogen. The mixture was stirred as a slurry, followed by addition of a solution of phenylmethyl (2*S*,5*R*)-5-({[(1,1-dimethylethyl)oxy]carbonyl}amino)-2-methyl-1-piperidinecarboxylate (58 g, 166 mmol) in EtOAc (330 mL). The mixture was shaken under 50 psi of hydrogen at room temperature for 4 h. LC-MS showed complete conversion. The mixture was filtered through Celite and rinsed with EtOAc. The filtrate was concentrated in vacuo to give the title compound (35.47 g) as an oil. LC-MS (ES) m/z = 215 $[M + H]^+$. ^{1}H NMR (400 MHz, CD_3OD): δ 1.13 (d, J = 6.3 Hz, 3H), 1.34−1.40 (m, 1H), 1.47 (s, 9H), 1.56−1.62 (m, 1H), 1.66−1.75 (m, 1H), 1.79−1.86 (m, 1H), 2.71−2.78 (m, 1H), 2.87 (m, 1H),) 2.98−3.05 (m, 1H), 3.61 (bs, 1H).

*1,1-Dimethylethyl {(3R,6S)-1-[2-Amino-6-(3-amino-1H-indazol-6-yl)-4-pyrimidinyl]-6-methyl-3-piperidinyl}carbamate ((**3R,6S**)-**37c**).* The title compound was prepared from (3*R*,6*S*)-**35** following synthetic route 1. LC-MS (ES) m/z = 439 $[M + H]^+$. ^{1}H NMR (400 MHz, CD_3OD): δ 1.25 (d, J = 7.1 Hz, 3H), 1.48 (s, 9H), 1.64−1.77 (m, 2H), 1.78−1.87 (m, 2H), 2.73 (t, J = 12.1 Hz, 1H), 3.41−3.48 (m, 1H), 4.50−4.60 (bs, 1H), 4.75−4.85 (bs, 1H), 6.52 (s, 1H), 7.48 (d, J = 8.3 Hz, 1H), 7.77 (d, J = 8.3 Hz, 1H), 7.80 (s, 1H).

Compounds (±)-*cis*-**37c** and (3*S*,6*R*)-**37c** were prepared from (±)-*cis*-**35** and (3*S*,6*R*)-**35**, respectively, following the synthetic procedures described for (2*S*,5*R*)-**37c**.

*Dimethyl (4R)-N-{[(1,1-Dimethylethyl)oxy]carbonyl}-4-methyl-D-glutamate (**38**).* In a 20 mL RB flask was dissolved dimethyl D-glutamate (25 g, 118 mmol) in CH_3OH (150 mL). Triethylamine (36.2 mL, 260 mmol) was added, followed by Boc_2O (33.5 g, 154 mmol), and the reaction mixture was stirred at room temperature for 18 h. The reaction was concentrated and then dissolved in 200 mL of CH_2Cl_2. The resulting organic solution was washed with 1N HCl (2 × 50 mL), followed by brine, dried over Na_2SO_4, filtered, and concentrated. Purification was done on a 350 g Biotage SNAP column with gradient of 0−35% EtOAc in hexane over 35 min to isolate dimethyl *N*-{[(1,1-dimethylethyl)oxy]-carbonyl}-D-glutamate (30.7 g) as a clear oil. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.38 (s, 9H), 1.79 (ddd, J = 14.5, 8.8, 6.1 Hz, 1H), 1.94 (t, J = 13.4 Hz, 1H), 2.33−2.43 (m, 2H), 3.59 (s, 3H), 3.62 (s, 3H), 4.00 (dd, J = 9.4, 7.6 Hz, 1H), 7.29 (d, J = 7.8 Hz, 1H).

Dimethyl *N*-{[(1,1-dimethylethyl)oxy]carbonyl}-D-glutamate (4 g, 14.53 mmol) in THF (40 mL) was placed in a 250 mL RB flask, and it was cooled to −78 °C. LiHMDS (1.0 M in THF, 30.5 mL, 30.5 mmol) was added dropwise, and the mixture was stirred at −78 °C for 0.5 h. Methyl iodide (1.817 mL, 29.1 mmol) was then added as rapidly as possible, and the solution was stirred at −78 °C for 4.5 h. The reaction was then quenched with 1N HCl solution (40 mL) and extracted with EtOAc (3 × 50 mL). The organics were combined and washed with saturated $NaHCO_3$ followed by brine, then dried over Na_2SO_4 and concentrated to give a red−amber colored oil. The oil was purified on a Biotage SNAP 50 g column with a gradient of 0 to 35% EtOAc in hexane over 35 min to give the title compound (1.37 g) as a clear oil. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.08 (d, J = 7.1 Hz, 3H), 1.38 (s, 9H), 1.71 (ddd, J = 13.6, 8.3, 5.1 Hz, 1H), 1.94 (ddd, J = 13.9, 10.1, 6.1 Hz, 1H), 2.43−2.49 (m, 1H), 3.60 (s, 3H), 3.62 (s, 3H), 4.01 (ddd, J = 9.9, 8.4, 5.2 Hz, 1H), 7.29 (d, J = 8.3 Hz, 1H).

*1,1-Dimethylethyl [(1R,3R)-4-hydroxy-1-(hydroxymethyl)-3-methylbutyl]carbamate (**39**).* To a solution of dimethyl (4*R*)-*N*-{[(1,1-dimethylethyl)oxy]carbonyl}-4-methyl-D-glutamate (1.79 g, 6.19 mmol) in ethanol (15 mL) and THF (15 mL) was added $CaCl_2$ (2.83 g, 24.8 mmol), and the resulting mixture was cooled in an ice bath. Sodium borohydride (1.873 g, 49.5 mmol) was added portionwise, and the white

cloudy mixture was stirred for 0.5 h in an ice bath. The reaction was then allowed to stir overnight at room temperature. A 10% Na_2CO_3 solution was added to quench the reaction, but the solution was too thick and an additional 20 mL of water was added. The white slurry was extracted with EtOAc (3 × 60 mL). The organics were combined and washed with saturated NaCl solution, dried over Na_2SO_4, filtered, and concentrated to afford the crude title compound (1.56 g) as a clear oil. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.82 (d, J = 6.6 Hz, 3H), 1.18 (t, J = 7.1 Hz, 1H), 1.31 (d, J = 3.0 Hz, 1H), 1.37 (s, 9H), 1.49−1.60 (m, 1H), 3.09−3.33 (m, 4H), 3.45 (td, J = 7.0, 5.1 Hz, 2H), 4.37 (dt, J = 9.2, 5.2 Hz, 1H), 4.56 (t, J = 5.7 Hz, 1H).

*1,1-Dimethylethyl [(3R,5R)-5-Methyl-1-(phenylmethyl)-3-piperidinyl]carbamate (**40a**).* 1,1-Dimethylethyl [(1*R*,3*R*)-4-hydroxy-1-(hydroxymethyl)-3-methylbutyl]carbamate (1.56 g, 6.69 mmol) was dissolved in CH_2Cl_2 (30 mL) in a 250 mL RB flask capped with a rubber septa. The reaction flask was charged with N_2 and cooled in an ice bath. Triethylamine (3.73 mL, 26.7 mmol) was added, followed by dropwise addition of methanesulfonyl chloride (1.563 mL, 20.06 mmol). After the addition, the reaction was allowed to stir at 0 °C for 1 h. The reaction was then diluted with CH_2Cl_2 (30 mL) and washed with saturated $NaHCO_3$ (30 mL) then water, dried with Na_2SO_4, filtered, and concentrated to afford the crude (2*R*,4*R*)-2-({[(1,1-dimethylethyl)oxy]carbonyl}amino)-4-methyl-5-[(methylsulfonyl)oxy]pentyl methanesulfonate (2.61 g). LC-MS (ES) m/z = 390 $[M + H]^+$.

Into a 10 mL sealable vial was added (2*R*,4*R*)-2-({[(1,1-dimethylethyl)oxy]carbonyl}amino)-4-methyl-5-[(methylsulfonyl)oxy]pentyl methanesulfonate (1.05 g, 2.70 mmol), followed by benzylamine (5.88 mL, 53.9 mmol), and the reaction was capped and heated at 70 °C for 24 h. The reaction was allowed to cool, and then it was transferred into a 1N NaOH solution (20 mL). The resulting oily mixture was then extracted with hexane (3 × 20 mL). The combined extracts were washed with saturated NaCl solution, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography on a 20 g Biotage SNAP column with gradient elution of 0−20% EtOAc in hexane over 20 min to afford the title compound (452 mg) as a clear oil. LC-MS (ES) m/z = 305 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.85−0.92 (m, 3H), 1.22−1.28 (m, 1H), 1.37 (s, 9H), 1.50 (m, 1H), 1.90 (bs, 2H), 2.29 (bs, 2H), 2.35−2.44 (m, 1H), 3.43 (m, 2H), 3.64 (bs, 1H), 6.44 (d, J = 6.8 Hz, 1H), 7.24 (m, 1H), 7.31 (m, 4H).

*1,1-Dimethylethyl [(3R,5R)-5-Methyl-3-piperidinyl]carbamate (**41a**).* Into a Parr shaker jar was added 10% Pd/C (316 mg, 0.148 mmol, Degussa type), followed by a solution of 1,1-dimethylethyl [(3*R*,5*R*)-5-methyl-1-(phenylmethyl)-3-piperidinyl]carbamate (452 mg, 1.485 mmol) in ethanol (20 mL). The Parr shaker jar was then placed into the shaker, flushed with N_2, and then pressurized with hydrogen to 30 psi. The reaction was shaken at room temperature overnight. The reaction was filtered and concentrated to give the crude title compound (310 mg). LC-MS (ES) m/z = 215 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.79 (d, J = 6.6 Hz, 3H), 1.16−1.27 (m, 1H), 1.39 (s, 9H), 1.65 (m, 2H), 2.11 (dd, J = 12.4, 8.6 Hz, 1H), 2.60 (m, 2H), 2.71 (dd, J = 12.1, 3.0 Hz, 1H), 3.27 (d, J = 7.1 Hz, 1H), 6.66 (m, 1H).

*1,1-Dimethylethyl{(3R,5R)-1-[2-amino-6-(3-amino-1H-indazol-6-yl)-4-pyrimidinyl]-5-methyl-3-piperidinyl}carbamate (**43a**).* The title compound was prepared from amine **41a** following synthetic route 1. LC-MS (ES) m/z = 439 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.88 (d, J = 6.8 Hz, 3H), 1.29 (s, 9H), 1.37−1.52 (m, 2H), 1.68 (m, 1H), 1.93−2.07 (m, 1H), 2.83 (bs, 1H), 3.38 (bs, 1H), 3.63 (bs, 1H), 3.89 (m, 1H), 5.37 (s, 2H), 6.04 (bs, 2H), 6.49 (s, 1H), 6.82 (d, J = 6.3 Hz, 1H), 7.50 (d, J = 8.6 Hz, 1H), 7.69 (d, J = 8.3 Hz, 1H), 7.89 (s, 1H), 11.49 (s, 1H).

*1,1-Dimethylethyl Methyl[(3R,5R)-5-methyl-1-(phenylmethyl)-3-piperidinyl]carbamate (**40b**).* To 1,1-dimethylethyl [(3*R*,5*R*)-5-methyl-1-(phenylmethyl)-3-piperidinyl]carbamate (196 mg, 0.644 mmol) in DMF (3 mL) was added NaH (60% dispersion in mineral oil, 38.6 mg, 0.966 mmol), and the resulting mixture was vigorously stirred for 30 min. CH_3I (0.044 mL, 0.708 mmol) was added, and the reaction mixture was stirred for 3 h at room temperature. Saturated aqueous $NaHCO_3$ was added carefully (ca. 5 mL, initial vigorous bubbling), and the resulting mixture was poured onto water and EtOAc. The organic layer was separated, washed with brine, dried ($MgSO_4$), filtered, and concentrated. Flash chromatography on SiO_2 (0−30% EtOAc in hexane gradient) afforded the title compound (162 mg) as a colorless oil. LC-MS (ES) m/z = 319 $[M + H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.10 (d, J = 7.1 Hz, 3H), 1.39−1.54 (m, 10H), 1.64−1.75 (m, 1H), 2.01 (dt, J = 7.0, 3.7 Hz, 1H), 2.09−2.24 (m, 2H), 2.32 (bs, 1H), 2.68 (dd, J = 10.5, 3.9 Hz, 1H), 2.85 (s, 3H), 3.32−3.60 (m, 2H), 7.21−7.28 (m, 1H), 7.29−7.36 (m, 4H).

*1,1-Dimethylethyl {(3R,5R)-1-[2-Amino-6-(3-amino-1H-indazol-6-yl)-4-pyrimidinyl]-5-methyl-3-piperidinyl}methylcarbamate (**43b**).* A solution of 1,1-dimethylethyl methyl[(3*R*,5*R*)-5-methyl-1-(phenylmethyl)-3-piperidinyl]carbamate (160 mg, 0.502 mmol) in ethanol (6 mL) was degassed with N_2 for 10 min. Pd/C 10% (Degussa type, 54 mg) was added, and the resulting mixture was stirred for 3 days at room temperature under a hydrogen atmosphere (balloon setup). The mixture was degassed with N_2 and filtered through an Acrodisk, rinsing with ethanol (ca. 15 mL). The filtrate was concentrated under vacuum to afford crude 1,1-dimethylethyl methyl[(3*R*,5*R*)-5-methyl-3-piperidinyl]carbamate (**41b**). LC-MS (ES) m/z = 229 $[M + H]^+$. The residue (**41b**) was taken up into 1,4-dioxane (6 mL), and to that solution were added 4,6-dichloro-2-pyrimidinamine (79 mg, 0.477 mmol) and satd aq $NaHCO_3$ (3 mL). The mixture was stirred overnight at 100 °C in a sealed tube and then allowed to cool to room temperature. (4-Cyano-3-fluorophenyl)boronic acid (124 mg, 0.754 mmol) was added, and N_2 gas was bubbled through the mixture for 10 min. $Pd(PPh_3)_4$ (29.0 mg, 0.025 mmol) was added, the vessel was sealed, and the reaction mixture was stirred for 4 h at 100 °C. The mixture was then poured into water and EtOAc. The organic layer was separated, and the aqueous layer was further extracted with EtOAc. The combined organic layers were washed with brine, dried ($MgSO_4$), filtered, and concentrated. Flash chromatography on SiO_2 (0−70% EtOAc in hexane gradient) afforded 1,1-dimethylethyl {(3*R*,5*R*)-1-[2-amino-6-(4-cyano-3-fluorophenyl)-4-pyrimidinyl]-5-methyl-3-piperidinyl}methylcarbamate (170 mg) as a thick oil. LC-MS (ES) m/z = 441 $[M + H]^+$.

To 1,1-dimethylethyl {(3*R*,5*R*)-1-[2-amino-6-(4-cyano-3-fluorophenyl)-4-pyrimidinyl]-5-methyl-3-piperidinyl}methylcarbamate (170 mg, 0.386 mmol) in ethanol (6 mL) was added hydrazine monohydrate (0.70 mL, 14.3 mmol), and the reaction mixture was stirred overnight at 100 °C in a sealed tube. The mixture was poured into water (ca. 150 mL), and a white precipitate formed. It was filtered, and the solid was air-dried for 2 h. The resulting white solid was dried under vacuum at 45 °C for 1 h to afford the title compound (110 mg). LC-MS (ES) m/z = 453 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.92−1.02 (m, 3H), 1.41 (s, 9H), 1.46−1.55 (m, 1H), 1.85−1.99 (m, 1H), 2.07−2.19 (m, 1H), 2.76 (s, 3H), 3.02−3.14 (m, 1H), 3.12−3.23 (m, 1H), 3.90−4.15 (m, 2H), 4.35 (m, 1H), 5.38 (s, 2H), 6.07 (s, 2H), 6.60 (s, 1H), 7.57 (dd, J = 8.6, 1.3 Hz, 1H), 7.70 (d, J = 8.6 Hz, 1H), 7.95 (s, 1H), 11.50 (s, 1H).

*N-{(3R,5R)-1-[2-Amino-6-(3-amino-1H-indazol-6-yl)-4-pyrimidinyl]-5-methyl-3-piperidinyl}-N,3,3-trimethylbutanamide (**43c**).* In a 20 mL sealable vial was added 1,1-dimethylethyl methyl[(3*R*,5*R*)-5-methyl-3-piperidinyl]carbamate (**41b**, 443 mg, 1.94 mmol), 4,6-dichloro-2-pyrimidinamine (477 mg, 2.91 mmol), triethylamine (0.541 mL, 3.88 mmol), and ethanol (10 mL). The vial was sealed, and the reaction mixture was heated overnight at 100 °C. The reaction was concentrated, and the resulting residue was purified by flash chromatography on SiO_2 (gradient: 0−55% EtOAc in hexane) to afford 1,1-dimethylethyl [(3*R*,5*R*)-1-(2-amino-6-chloro-4-pyrimidinyl)-5-methyl-3-piperidinyl]methylcarbamate (678 mg). LC-MS (ES) m/z = 356, 358 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.91 (d, J = 7.1 Hz, 3H), 1.41 (s, 9H), 1.44−1.54 (m, 1H), 1.90 (td, J = 11.9, 4.8 Hz, 1H), 2.04−2.17 (m, 1H), 2.72 (s, 3H), 3.01 (t, J = 11.5 Hz,

1H), 3.10 (dd, J = 13.3, 2.9 Hz, 1H), 3.79 (bs, 1H), 4.03 (m, 1H), 6.08 (s, 1H), 6.47 (bs, 2H).

To 1,1-dimethylethyl [(3*R*,5*R*)-1-(2-amino-6-chloro-4-pyrimidinyl)-5-methyl-3-piperidinyl]methylcarbamate (75 mg, 0.211 mmol) in CH_2Cl_2 (2 mL) was added TFA (2 mL), and the reaction was allowed to sit at room temperature for 2 h. Toluene (~15 mL) was added, and the resulting mixture was evaporated under vacuum. To the resulting residue were added CH_2Cl_2 (2 mL) and Hünig's base (0.18 mL, 1.05 mmol), followed by 3,3-dimethylbutanoyl chloride (0.03 mL, 0.21 mmol), and the resulting mixture was stirred overnight at room temperature. Saturated aqueous $NaHCO_3$ (ca. 20 mL) was added, and the resulting mixture was extracted with EtOAc (ca. 50 mL). The organic layer was separated, washed with brine, dried ($MgSO_4$), filtered, and concentrated to afford crude *N*-[(3*R*,5*R*)-1-(2-amino-6-chloro-4-pyrimidinyl)-5-methyl-3-piperidinyl]-*N*,3,3-trimethylbutanamide (**42**, 80 mg). LC-MS (ES) m/z = 354 $[M + H]^+$.

The title compound was prepared from compound **42** following synthetic route 1 as a mixture of rotamers. LC-MS (ES) m/z = 451 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6 + 1 drop D_2O): δ 0.98 (m, 12H), 1.41 and 1.52 (m, 1H), 1.87−2.41 (m, 4H), 2.76 and 2.90 (s, 3H), 2.99−3.25 (m, 2H), 3.84−3.99 (m, 1H), 4.01−4.26 (m, 1H), 4.39−4.66 (m, 1H), 6.57 and 6.62 (s, 1H), 7.50−7.60 (m, 1H), 7.70 (m, 1H), 7.92 (s, 1H).

Biochemical Characterization of PDK1 Inhibitors and Crystallographic Studies. $pIC_{50} = -\log_{10}(IC_{50})$, where the IC_{50} is the molar concentration of compound required to inhibit the kinase activity by 50%. Experimental details describing (1) the in vitro PDK1 inhibition assays for pIC_{50} determination and (2) PDK1 crystallography (Table 11) are described in a previous publication.[14]

Phospho-AKT (S473, T308, Total AKT) and Phospho-RSK ELISA. PC3 cells (ATCC, Manassas, VA) were plated in 96-well flat bottom plates (Corning, Lowell, MA) at a density of 15000 cells/well in RPMI 1640 medium supplemented with 10% FBS. Cells were incubated at 37 °C, 5% CO_2, for 18−20 h. Compounds (dissolved in 100% DMSO) were diluted in an 11-point 3-fold dilution in DMSO. Compound dilution stocks were further diluted in RPMI 1640 with 10% FBS and added to each cell well. DMSO without compound was used in control wells. Final concentration of DMSO in each well was 0.15%. After 6 h at 37 °C, cells were washed with cold PBS (without calcium or magnesium) and lysed in lysis buffer (Meso Scale Discovery, Gaithersburg, MD) supplemented with 1 protease inhibitor tablet/10 mL (Roche, Indianapolis, IN), 10 mM NaF, and 200 μL/10 mL Sigma phosphatase inhibitors 1 and 2 (Sigma Aldrich, St Louis, MO) for 30 min at 4 °C. All washes were performed on a Bio Tek ELx405 plate washer (Bio Tek Instruments, Winooski, VT).

ELISA plates (Meso Scale Discovery; AKT Duplex, cat. N41100B-1; phospho AKT, cat. N411CAB-1; RSK, cat. N41ZB-1) were prepared by the addition of blocking buffer (3% Blocker A diluted in wash buffer (Meso Scale Discovery) for AKT duplex assay and phospho AKT assays; 5% Blocker A/1% Blocker B in Tris-buffered saline for RSK) for 1 h and washed with wash buffer. Lysates were transferred to assay wells and incubated overnight at 4 °C. Following washing with wash buffer, detection buffer (1% Blocker A in wash buffer for AKT duplex and phospho AKT or 1% Blocker A in TBS for RSK) with appropriate antibodies was added. Detection of AKT duplex and phospho AKT was carried out using a sulfo-tagged detection antibodies (Meso Scale Discovery). Detection of phospho-RSK was carried out sequentially with anti phospho-RSK1 (S221)/RSK2 (S227) (R&D Systems, Minneapolis, MN, cat. AF892) and goat antirabbit sulfo-tag antibody (cat R32AB-1; Meso Scale Discovery). Plates were incubated for 1 h at room temperature and washed with wash buffer. Plates were read on a SECTOR Imager 6000 (Meso Scale Discovery) using Workbench software (Meso Scale Discovery) following addition of read buffer (Meso Scale Discovery) to each well. For analysis, phospho-AKT (S473) signals were normalized to total AKT, while phospho-AKT (T308) and phospho-RSK signals were analyzed without normalization. All values were expressed as percent of the DMSO-treated controls. IC_{50}s were determined from inhibition curves using XLfit4 software (IDBS, Guildford, UK).

Pharmacodynamic Studies. Female SCID mice bearing subcutaneous OCI-AML2 tumor xenografts of ~500−600 mm^3 in size were treated with vehicle (1% DMSO, 20% PEG400, pH 4.2) or PDK1 inhibitor (100 mg/kg) by intraperitoneal administration. Three and six hours later, 10 μg of hIGF-I (via the tail vein) were administered, and animals were euthanized after 10 min for collection of tumor tissue and blood. Frozen tumors samples were homogenized in lysis buffer and analyzed for phosphor- and total AKT and RSK by ELISA, as described above. Concentration of compound in the blood and tumor samples was also analyzed by LC/MS/MS.

Accession Codes

†PDB codes: **12f**, 3QCQ; **12k**, 3QCS; (***R***)-**18a**, 3QCX; (***S***)-**21a**, 3QCY; (**3*S*,6*R***)-**28a**, 3QD0; (**3*R*,6*S***)-**37c**, 3QD3; **43a**, 3QD4.

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ABBREVIATIONS USED

PDK1, phosphoinositide-dependent protein kinase-1; PI3K, phosphatidylinositol 3-kinase; AKT, protein kinase B; PIP2, phosphatidylinositol 3,4,-diphosphate; PIP3, phosphatidylinositol 3,4,5-triphosphate; PKC, protein kinase C; SGK, serum- and glucocorticoid-induced protein kinase; S6K1, p70 ribosomal S6 kinase; RSK, p90 ribosomal S6 kinase; ALK5, TGF-β type I receptor; ROCK1, Rho-associated protein kinase-1; LE, ligand efficiency; G-loop, glycine rich loop; MTD, maximum tolerated dose; AUC, area under the curve; AML, acute myeloid leukemia

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