Bioorganic & Medicinal Chemistry Letters 18 (2008) 5648–5652

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Design and synthesis of 2-amino-pyrazolopyridines as Polo-like kinase 1 inhibitors

Raymond V. Fucini [a,*,†], Emily J. Hanan [b,†], Michael J. Romanowski [c], Robert A. Elling [c], Willard Lew [b], Kenneth J. Barr [b], Jiang Zhu [b], Joshua C. Yoburn [b], Yang Liu [b], Bruce T. Fahr [b], Junfa Fan [b], Yafan Lu [b], Phuongly Pham [b], Ingrid C. Choong [b], Erica C. VanderPorten [a], Minna Bui [b], Hans E. Purkey [b], Marc J. Evanchik [a], Wenjin Yang [b]

[a] Department of Biology, Sunesis Pharmaceuticals, Inc., 395 Oyster Point Boulevard Suite 400, South San Francisco, CA 94080, USA
[b] Department of Chemistry, Sunesis Pharmaceuticals, Inc., 395 Oyster Point Boulevard Suite 400, South San Francisco, CA 94080, USA
[c] Department of Structural Biology, Sunesis Pharmaceuticals, Inc., 395 Oyster Point Boulevard Suite 400, South San Francisco, CA 94080, USA

ARTICLE INFO

Article history:
Received 12 June 2008
Revised 25 August 2008
Accepted 26 August 2008
Available online 29 August 2008

Keywords:
2-Amino-pyrazolopyridine
Polo-like kinase (Plk)
SAR
Kinase inhibitor

ABSTRACT

A series of 2-amino-pyrazolopyridines was designed and synthesized as Polo-like kinase (Plk) inhibitors based on a low micromolar hit. The SAR was developed to provide compounds exhibiting low nanomolar inhibitory activity of Plk1; the phenotype of treated cells is consistent with Plk1 inhibition. A co-crystal structure of one of these compounds with zPlk1 confirms an ATP-competitive binding mode.

Polo-like kinases (Plks) belong to a highly conserved family of serine/threonine kinases that play critical roles during multiple stages of mitosis.[1–3] Four Plks have been identified in mammals (Plk1–4), each containing an N-terminal catalytic domain and a C-terminal domain containing one or two highly conserved sequences called polo box domains (PBDs).[1] The best-characterized human Plk is Plk1. Plk1 is expressed maximally during late G2 and M phases of the cell cycle and contributes to the regulation of centrosome maturation, bipolar spindle formation, and cytokinesis.[1–4] Inhibition of Plk1 activity with small molecules leads to the formation of aberrant mitotic spindle poles, which in turn triggers the spindle assembly checkpoint (SAC), resulting in mitotic arrest and strong induction of apoptosis.[1,5–7] Plk1 is overexpressed in a broad range of human tumors and this overexpression is positively correlated with aggressiveness and poor prognosis in many cancers.[1,4,5] Plk1 is thus considered a good target for chemotherapeutic intervention.[1,4,5]

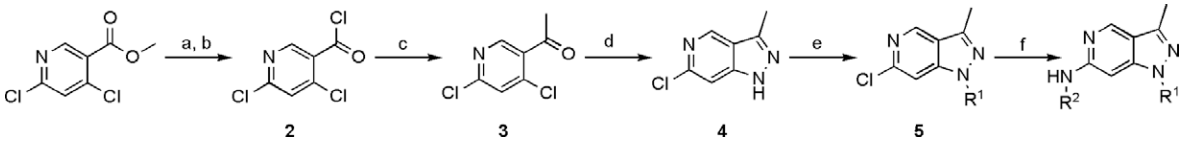
As part of our drug discovery efforts, we identified 2-amino-pyrazolopyridine **1** as a moderate inhibitor of Plk1. In this communication, we describe the synthesis and structure–activity relationship (SAR) development of this novel chemical series, and present an X-ray crystal structure of a small-molecule inhibitor in complex with the kinase domain of zebrafish Plk1 (zPlk1), a homolog that possesses a single conserved amino acid substitution (zPlk1 Ile118 = hPlk1 Leu132) for a residue lining the active site compared to human Plk1.[16]

The identification of **1** as a Plk1 inhibitor prompted further investigation into this class of compounds. A SAR study was initiated, with a particular focus on the phenyl group linked to the pyrazole nitrogen. Compounds **1**–**22** were synthesized as shown in Scheme 1. Commercially available 2,4-dichloro-nicotinic acid methyl ester was first hydrolyzed to the carboxylic acid and then converted to acyl chloride **2** by reaction with oxalyl chloride. Treatment of **2** with copper iodide and methyl lithium at low temperature yielded methyl ketone **3**.[8] Cyclization of **3** to pyrazole **4** was effected through treatment with hydrazine. Reaction of **4** with aryl halides in the presence of copper iodide, potassium carbonate, and *N*,*N*-dimethylglycine resulted in N-arylation of the pyrazole ring.[9] Standard Buchwald–Hartwig amination conditions provided the desired compounds **1** and **6**–**22**.[10]

The SAR of these compounds is detailed in Table 1. Moving a methyl or fluoro group around the phenyl ring indicated that substitution at the 3-position is preferred. However, the electron-donating methoxy group is not well tolerated except in the 4-position. Further exploration of the 3-position confirmed the preference for relatively small electron withdrawing groups, with the 3-chloro (compound **15**) being the most active as

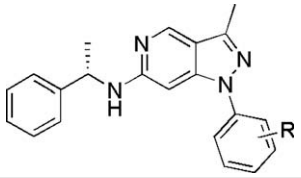
* Corresponding author. Tel.: +1 650 266 3500; fax: +1 650 266 3505.
E-mail addresses: info@sunesis.com, rfucini@sunesis.com (R.V. Fucini).
† These authors contributed equally to this work.

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.08.095



Scheme 1. Reagents and conditions: (a) LiOH, THF/water, 25 °C, 1 h, 100%; (b) oxalyl chloride, DMF/DCM, 25 °C, 1 h; (c) CuI, MeLi, THF, −78 °C, 95%; (d) hydrazine, Et_3N, DMA, 80 °C, 2 h, 75%; (e) R–I or R–Br, CuI, K_2CO_3, *N*,*N*-dimethylglycine, DMSO, 100 °C, 12–24 h; (f) R^2NH_2, Pd_2dba_3, *rac*-BINAP, NaOtBu, toluene, 100 °C, 2–15 h.

Table 1
SAR for phenyl substitution of **1**[a]



Compound	R	Plk1 IC_{50} (μM)
1	H	1.301
6	2-CH_3	7.685
7	3-CH_3	0.474
8	4-CH_3	4.528
9	2-OCH_3	14.451
10	3-OCH_3	5.928
11	4-OCH_3	0.703
12	2-F	4.516
13	3-F	0.464
14	4-F	3.241
15	3-Cl	0.121
16	3-*i*Pr	1.476
17	3-CN	0.412
18	3-$N(CH_3)_2$	0.977
19	3-SO_2CH_3	0.225
20	3,5-CH_3	0.149
21	3,5-Cl	0.641
22	3-Cl,5-Br	0.274

[a] See Supplementary data for assay details.

compared with isopropyl, cyano, *N*,*N*-dimethylamino, or methyl sulfone groups. Compounds with 3,5-bis-substitution (**20–22**) also showed good activity, with bis-methyl compound **22** having 10-fold better activity than compound **1**.

The observation that 3-chloro substitution on the right-hand phenyl ring provides an improvement in activity, and that 3,5-bis-substitution is tolerated, led us to maintain 3-chloro substitution while extending larger groups at the 5-position in an attempt to generate hydrogen-bonding interactions with the protein's catalytic machinery. A selection of this expanded series of compounds is shown in Table 2, and their syntheses are depicted in Schemes 2–4.

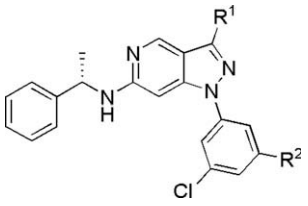
The bis-substituted phenyl compounds **28–30** were synthesized from intermediate **4** (Scheme 2). Copper-mediated arylation of **4** provided benzyl alcohol **23**. Dess–Martin oxidation to **24** followed by condensation with the appropriate phosphonate gave the α,β-unsaturated ester **25**.[11] Rhodium-catalyzed hydrogenation provided intermediate **26**. Amination conditions as described above generated compound **27**, and TFA hydrolysis of the *tert*-butyl ester yielded compound **28**. HATU-mediated amide bond formation yielded compounds **29** and **30**.

Tritylation of intermediate **4** followed by amination of the pyridine ring yielded **32** (Scheme 3). Subsequent detritylation to **33** and copper-mediated arylation with 1,3-dibromo-5-chlorobenzene provided compound **22** (an alternate synthesis to that shown in Scheme 1).[9] Palladium-catalyzed Heck coupling with *N*,*N*-bis-Boc-allylamine generated compound **34**.[12] Rhodium-catalyzed hydrogenation of the alkene followed by amino-deprotection yielded desired compound **36**.

Compounds **37** and **38** were synthesized from compound **22**, which was derivatized through Suzuki couplings with aryl boronic acids (Scheme 4).[13] Des-methyl compound **39** was synthesized in the same fashion, starting with 4,6-dichloropyridine-3-carbaldehyde.

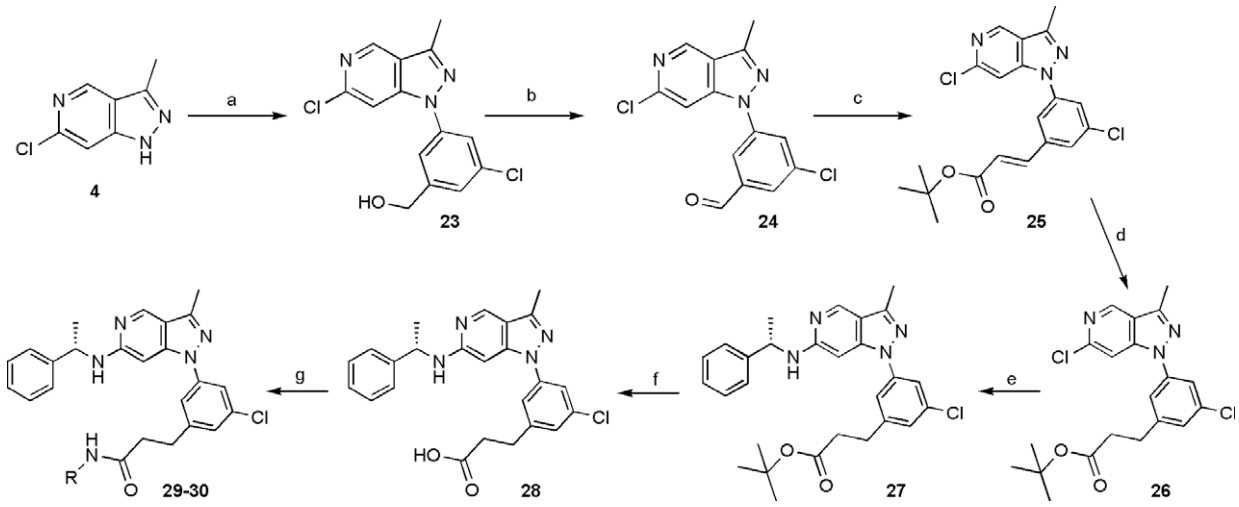
As shown in Table 2, propionic acid **28** was highly active against the Plk1 enzyme (0.021 μM) as was propionic amide **29** (0.032 μM). The secondary amide **30** retains most of this activity, as does the propyl-amine **36**. Rigidifying the alkyl chain with a phenyl ring also maintains activity, as long as the hydrogen-bond donor is in the proper position. For example, benzamide **38** provides 5-fold better enzymatic activity as compared to acetamide **37**, which has a one atom shift in hydrogen-bond donor position.

Table 2
SAR for bis-substitution of phenyl ring[a]

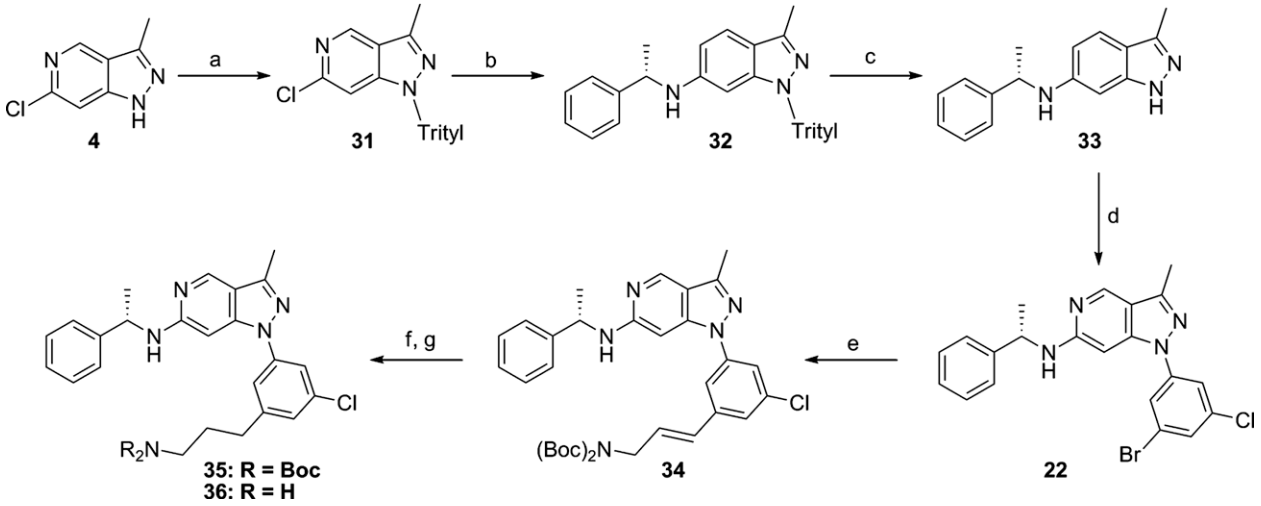


Compound	R^1	R^2	Plk1 IC_{50} (μM)	HCS EC_{50}[b] (μM)
15	CH_3	H	0.121	>20
28	CH_3	3-Propionic acid	0.021	>20
29	CH_3	3-Propionamide	0.032	9.35
30	CH_3	3-(*N*-2-Hydroxyethyl)-propionamide	0.048	9.20
36	CH_3	3-Propyl-amine	0.059	5.80
37	CH_3	Phenyl-2-*N*-acetamide	0.207	>20
38	CH_3	2-Benzamide	0.042	3.64
39	H	2-Benzamide	0.050	2.43

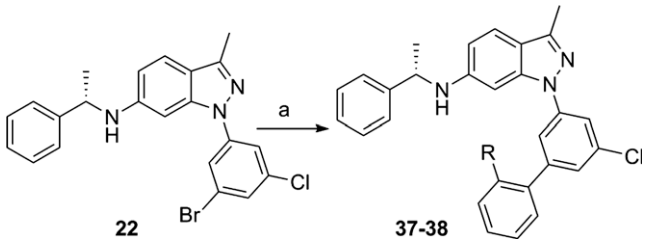
[a] See Supplementary data for assay details.
[b] Mitotic arrest as measured by doubling of DNA content in High Content Screening (HCS) of HCT116 cells.



Scheme 2. Reagents and conditions: (a) (3,5-dichlorophenyl)-methanol, CuI, K_2CO_3, *N*,*N*-dimethylglycine, DMSO, 100 °C, 16 h, 30%; (b) Dess–Martin, DCM, 12 h, 40%; (c) (diethoxy-phosphoryl)acetic acid *tert*-butyl ester, LiCl, DBU, THF, 6 h, 78%; (d) Rh/Al_2O_3, H_2, EtOAc/iPrOH, 4 h, 71%; (e) (*S*)-α-methylbenzylamine, Pd_2dba_3, *rac*-BINAP, NaOtBu, toluene, 100 °C, 18 h, 13%; (f) TFA/DCM, 1 h, 98%; (g) H_2NR, HATU, DIEA, DMF, 2 h, 80–90%.



Scheme 3. Reagents and conditions: (a) trityl chloride, NEt_3, DCM, 0–20 °C, 3 h, 89%; (b) (*S*)-α-methylbenzylamine, Pd_2dba_3, *rac*-BINAP, NaOtBu, toluene, 100 °C, 24 h; (c) HBr, HOAc, 20 °C, 1 h, 39% (2 steps); (d) 1,3-dibromo-5-chlorobenzene, CuI, *N*,*N*-dimethylglycine, K_2CO_3, DMSO, 100 °C, 18 h, 50%; (e) *N*,*N*-bis-Boc-allylamine, $Pd(OAc)_2$, $P(o\text{-}tol)_3$, NEt_3, DMA, 100 °C, 18 h; (f) Rh/Al_2O_3, H_2, EtOAc/iPrOH, 5 h, 75%; (g) TFA/DCM, 1 h, 90%.



Scheme 4. Reagent and condition: (a) aryl boronic acid, $Pd(PPh_3)_4$, Na_2CO_3, toluene/ethanol, 110 °C, 16 h, 28–65%.

Finally, removal of the methyl group on the pyrazole ring is tolerated with no loss of activity (compound **39**).

Several heterocyclic variations were explored, and it was found that a variety of substituted thiophene rings may be attached to the pyrazole 1-nitrogen with activity comparable to that of analogous phenyl compounds (data not shown). All other synthesized heterocyclic replacements for the phenyl ring were found to result in decreased activity.

Limited SAR studies were also performed on the left-hand amino group of compound **1** (data not shown). It was determined that neither the des-methyl-benzylamine nor the (*R*)-enantiomer of compound **1** retained any activity. Minimal substitution around the benzyl ring was tolerated, with most substitution resulting in loss of activity. While the (*S*)-α-methyl-benzyl amine could be exchanged for a variety of substituted anilines and result in comparable activity, no significant improvements were observed.

Plk1 inhibition leads to a mitotic arrest and cell death, thus we tested the effects of inhibitors on cell cycle progression and viability in the HCT 116 colorectal cancer cell line. Mitotic arrest was determined as a doubling of DNA content (4 N) and increased levels of phospho-histone H3, a marker of productive entry into mitosis. Monastrol, an inhibitor of the mitotic kinesin Eg5 that causes cells to arrest in mitosis with monopolar spindles, was used as a reference. As shown in Figure 1, administration of monastrol or compound **39** resulted in comparable EC_{50} values for both mitotic endpoints (Fig. 1a). Fluorescence microscopy imaging of cells treated with either monastrol or **39** revealed that DNA was organized in aster-like morphologies characteristic of monopolar spindle and

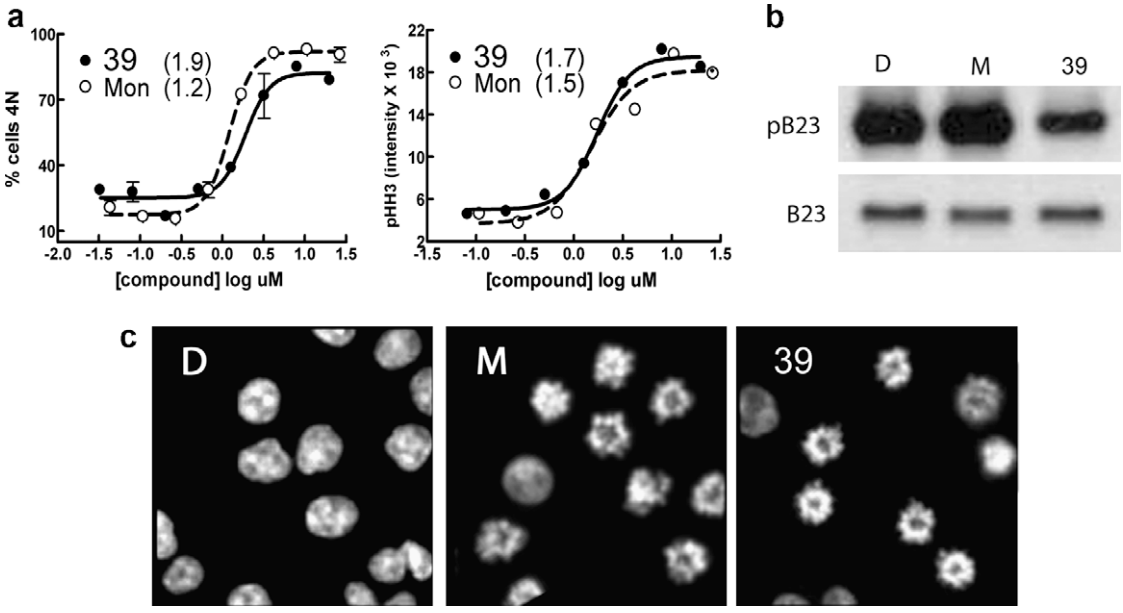


Figure 1. Phenotypic and mechanistic assessment of cellular Plk inhibition. (a) Dose–response curves from cell cycle assays for compound **39** and monastrol.[8] EC_{50} values are shown in parentheses (μM); (b) Western analysis of B23-nucleophosmin phosphorylation (pB23) as compared to total B23 in HeLa cells. Cells were synchronized with 300 nM nocodazole and treated with DMSO (D), 2 μM monastrol (M) or 5 μM **39** for 16 h; (c) fluorescence microscopy of Hoechst 3342-stained nuclei shows prometaphase arrested cells with monopolar spindles in monastrol-treated (M) and **39**-treated cells compared to control cells (D).

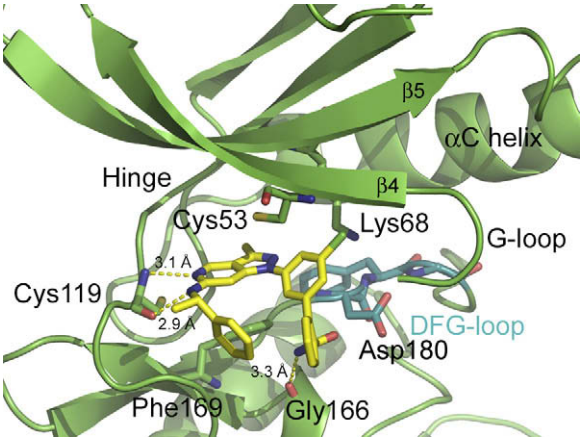


Figure 2. Structure of pyrazolopyridine **38** in the zPlk1 active site.

mitotic arrest[14] (Fig. 1c). Biochemical activity for **39** is greater than 50-fold selective for Plk1 over isoforms Plk2 and Plk3, and over 150-fold selective over the highly homologous kinase Aurora A (see Supplementary data, Table S1).

To further support the conclusion that these responses are a result of Plk inhibition and not another mitotic regulator, we monitored phosphorylation of B23-nucleophosmin, a known substrate for Plk1.[15] Western analysis of nocodazole-synchronized HeLa cells shows a marked decrease in B23 phosphorylation in **39**-treated cells compared to monastrol-treated or control cells (Fig. 1b). Together, these data support a mechanism of action for these 2-amino-pyrazolopyridines as functioning through inhibition of cellular Plk1. As shown in Table 2, several of the more active analogs induce mitotic arrest with an EC_{50} less than 10 μM. These compounds also exhibit a cytotoxic effect, consistent with Plk1 inhibition, as demonstrated by comparable activities observed in an MTT assay (data not shown).

A co-crystal structure of **38** with zPlk1 further elucidates the SAR of this series (Fig. 2).[16] The pyrazolopyridine ring is sandwiched between Phe169 (hPlk1 Phe183) at the bottom of the purine-binding pocket and Cys53 (hPlk1 Cys67) on the β4 strand in the back of the G-loop. The pyridine nitrogen forms a hydrogen-bond with the amide nitrogen of Cys119 (hPlk1 Cys133) (distance = 3.0 Å) located in the hinge region. Additionally, the proton of the pyrazolopyridine 2-amino group forms a direct hydrogen-bonding interaction with the carbonyl of the same amino acid residue (distance = 2.7 Å). The interaction of **38** with the protein appears to be stabilized by the tight packing of the 3-chloro group against a hydrophobic pocket formed by the β-strands on each side of the G-loop and the side chain of the catalytic lysine 68 (hPlk1 Lys82) on the β5 strand. This interaction explains the SAR for this position as seen in Table 1. The observed water-mediated hydrogen-bonding interaction between the 2-benzamide nitrogen and Asp180 (hPlk1 Asp194) of the DFG-loop explains the SAR described in Table 2, where precise placement of a hydrogen-bond donor significantly improves inhibitory activity. Additional lipophilic interactions are observed between the terminal benzamide ring of **38** and the bottom of the purine pocket. Co-crystal structures of three additional analogs with zPlk1 were obtained, all demonstrating similar interactions (see Supplementary data, Fig. S1).

In conclusion, we have disclosed a novel series of 2-amino-pyrazolopyridines that act as inhibitors of Plk1. Key SAR and binding elements have been identified, and initial hit **1** has been improved to achieve several compounds with Plk1 enzymatic activity <50 nM. Moreover, the primary mechanism of this series in cells has been shown to be that of Plk1 inhibition. Crystal structures of inhibitors in this series with zPlk1 have confirmed their binding mode and provided insight to inform future generations of Plk inhibitors.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.08.095.

References and notes

1. Eckerdt, F.; Yuan, J.; Strebhardt, K. *Oncogene* **2005**, *24*, 267.
2. Lowery, D. M.; Lim, D.; Yaffe, M. B. *Oncogene* **2005**, *24*, 248.
3. Xie, S.; Xie, B.; Lee, M. Y.; Dai, W. *Oncogene* **2005**, *24*, 277.
4. Takai, N.; Hamanaka, R.; Yoshimatsu, J.; Miyakawa, I. *Oncogene* **2005**, *24*, 287.
5. Strebhardt, K.; Ullrich, A. *Nat. Rev. Cancer* **2006**, *6*, 321.
6. Plyte, S.; Musacchio, A. *Curr. Biol.* **2007**, *17*, R280.
7. Peters, U.; Cherian, J.; Kim, J. H.; Kwok, B. H.; Kapoor, T. M. *Nat. Chem. Biol.* **2006**, *2*, 618.
8. Dubois, J. E.; Boussu, M.; Lion, C. *Tetrahedron Lett.* **1971**, *12*, 829.
9. (a) Klapars, A.; Antilla, J. C.; Huang, X.; Buchwald, S. L. *J. Am. Chem. Soc.* **2001**, *123*, 7727; (b) Zhang, H.; Cai, Q.; Ma, D. *J. Org. Chem.* **2005**, *70*, 5164.
10. Wolfe, J. P.; Buchwald, S. L. *J. Org. Chem.* **2000**, *65*, 1144.
11. Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4155.
12. Ziegler, C. B.; Heck, R. F. *J. Org. Chem.* **1978**, *43*, 2941.
13. Miyaura, N.; Suzuki, A. *Chem. Rev.* **1995**, *95*, 2457.
14. Lenart, P.; Petronczki, M.; Steegmaier, M.; Di Fiore, B.; Jesse, L.; Hoffmann, M.; Rettig, W.; Kraut, N.; Peters, J.-M. *Curr. Biol.* **2007**, *17*, 4.
15. Zhang, H.; Shi, X.; Paddon, H.; Hampong, M.; Dai, W.; Pelech, S. *J. Biol. Chem.* **2004**, *279*, 34.
16. Expression, purification, and crystallization of Plk1 Elling, R. A.; Fucini, R. V.; Romanowski, M. J. *Acta Crystallogr.* **2008**, *64*, 909. Details for this X-ray crystal structure are provided as Supplementary data.