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

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ABSTRACT

A series of 2-amino-isoxazolopyridines was designed and synthesized as Polo-like kinase (Plk) inhibitors. Key SAR and crystallographic data are discussed. More advanced analogues inhibit Plk1 with good enzymatic activity and modest cell-based activity. Differential selectivity among the three Plk isoforms is observed.

Polo-like kinases (Plks) are a family of serine-threonine kinases that play fundamental roles in regulating cell division.[1–3] Exposure to Plk inhibitors leads to multiple defects in mitosis, causing cell death.[1,4–6] These enzymes present promising targets for cancer therapy due to frequent overexpression in various human tumors.[1,4,7] As part of our drug discovery efforts, we identified and developed pyrazolopyridine-based compounds **1** and **2** as a new class of Plk inhibitors (Fig. 1).[8] Additional work on developing the core structure of this series led to the identification of several alternate series; in this communication we report the synthesis and SAR of novel 2-amino-isoxazolopyridine-based compounds. A co-crystal structure of an isoxazolopyridine inhibitor was obtained in complex with zebrafish Plk1 (zPlk1), a homolog possessing a single conserved substitution for a residue lining the active site compared to human Plk1. The phenotype of isoxazolopyridine inhibitor-treated cells is consistent with Plk1 inhibition.

After the discovery of **1**, compound **3** was designed as a variation on the core structure, with the goal of identifying a distinct chemical series that could provide improvements in activity and physicochemical properties. With the observation that **3** exhibits low micromolar biochemical activity, the compounds in Table 1 were synthesized to develop initial structure–activity relationships (SAR) around the right hand phenyl ring. Compounds **4**–**9** were prepared from 2,5-dichloroisonicotinic acid as shown in Scheme 1. Following esterification of the carboxylic acid, ketones were generated using aryl Grignard reagents at low temperature. The isoxazole ring was formed by heating the ketones in the presence of hydroxylamine and potassium hydroxide.[9] Amination of the pyridine ring took place under standard Buchwald–Hartwig conditions.[10]

Compounds **13** and **15** were prepared as shown in Scheme 2. A Heck reaction of 1,3-dibromo-5-chlorobenzene and allyloxy-*tert*-butyl-dimethylsilane followed by rhodium-catalyzed reduction provided compound **10**.[11] Halogen–lithium exchange and reaction with 2,5-dichloro-isonicotinic acid methyl ester gave ketone **11**.[12] Cyclization and amination proceeded as described above, and deprotection of the alcohol with tetrabutylammonium fluoride provided compound **13**. Oxidation to the corresponding acid **14**, followed by acyl chloride formation and reaction with ammonia gave the desired amide **15**.

The data in Table 1 indicate that simple substitution around the right hand phenyl ring is tolerated, with substitution favored at the 3-position (compounds **4** and **7**). Bis-methoxy substitution at the

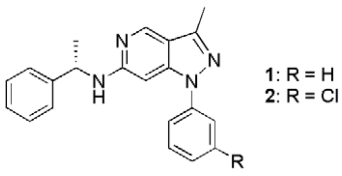

Figure 1. Plk1 inhibitors that served as a starting point for **3**. Biochemical Plk1 activity: **1** = 1.301 μM; **2** = 0.121 μM.

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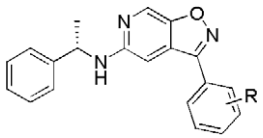
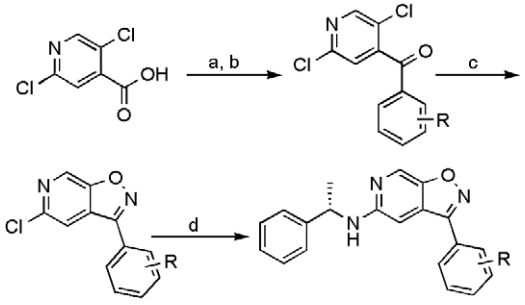
Table 1
SAR for phenyl substitution of **3**[a]



Compound	R	Plk1 IC_{50} (μM)	HCS IC_{50} (μM)[b]
3	H	1.616	>20
4	3-CH_3	0.549	6.69
5	4-CH_3	1.170	>20
6	3,5-CH_3	0.330	>20
7	3-Cl	0.625	>20
8	3,4-OCH_3	4.927	>20
9	3,4-Methylenedioxy	0.214	7.32
13	3-Cl-5-(3-propanol)	0.099	10.16
15	3-Cl-5-(3-propionamide)	0.051	8.35

[a] See Supplementary Data for assay details.
[b] Mitotic arrest as measured by doubling of DNA content in high-content screening (HCS) of HCT 116 cells.



Scheme 1. Reagents and conditions: (a) $TMSCHN_2$, $MeOH/CH_2Cl_2$, 25 °C, 0.5 h, 100%; (b) ArMgBr or ArMgCl, THF, −78 °C, 25–90%; (c) $NH_2OH{\cdot}HCl$, KOH, $^iPrOH/H_2O$, 80 °C, 40–60%; (d) (*S*)-α-methyl-benzylamine, Pd_2dba_3, 1,3-bis(2,6-diisopropylphenyl)imidazolium chloride, NaO^tBu, toluene, 110 °C, 15–24 h, 20–50%.

3- and 4-positions (compound **8**) results in a slight decrease in activity compared to the parent compound, but tying the methylene groups together (as in compound **9**) results in a 7.5-fold improvement in Plk1 inhibition over the parent compound **3**. Simple bis-substitution at the 3- and 5-positions is also well tolerated (compound **6**), which led us to maintain chloro-substitution at the 3-position and extend the substituent at the 5-position.

Based on a co-crystal structure of a pyrazolopyridine analog derived from **1** with zPlk1, we theorized that this strategy would allow us to engage Asp180 (hPlk1 Asp194) of the DFG-loop.[8] As shown by **13** and **15**, this strategy delivered good improvements in biochemical activity (15–30-fold improvement over parent **3**). A co-crystal structure of **13** with zPlk1 confirms that the propanol chain is situated in a hydrophobic pocket contributes additional affinity through lipophilic interactions, and the terminal hydroxyl group is clearly within distance to engage Asp180 through water-mediated hydrogen-bonding (Fig. 2).[13]

Plk1 inhibition leads to a mitotic arrest and cell death, thus we tested the effects of inhibitors on cell cycle progression in the HCT 116 colorectal cancer cell line. Mitotic arrest was determined as a doubling of DNA content (4N) as measured by a high-content cell screening (HCS) assay. As indicated in Table 1, compounds (e.g., **4** and **7**) with comparable enzymatic activity show variable ability to arrest the cell cycle as measured in the HCS assay. Physicochemical or pharmacokinetic properties such as solubility, cell-permeability or efflux are not entirely sufficient to explain these observations. All compounds tested were moderately to highly cell permeable in an MDCK assay and moderately to highly stable in human liver microsomes. It is possible, at least in part, that biochemical potency <1 μM prerequisites cell activity for this series.

Compounds **18**–**23** were synthesized to determine if any analogs without a direct phenyl attachment could be identified with

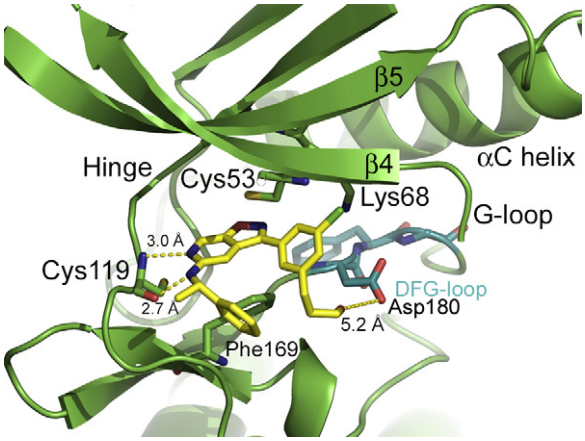
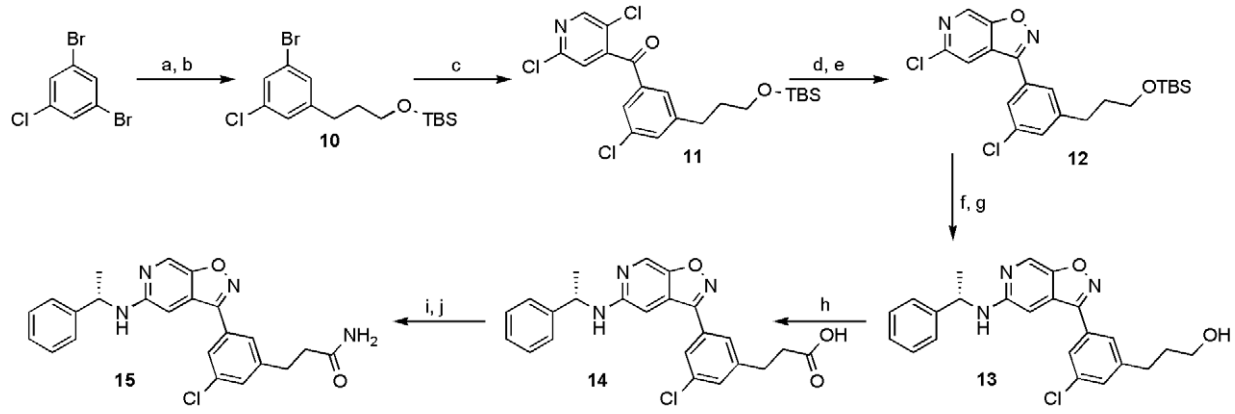
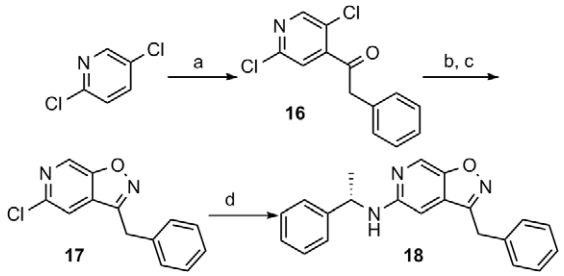


Figure 2. Structure of isoxazolopyridine **13** in the zPlk1 active site.



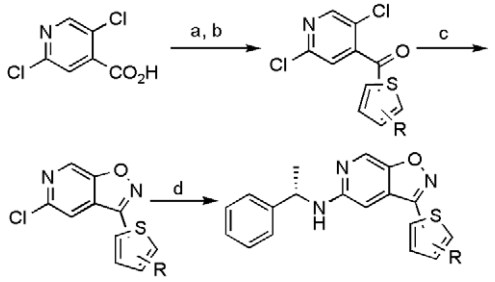
Scheme 2. Reagents and conditions: (a) allyloxy-*tert*-butyl-dimethylsilane, $Pd(OAc)_2$, PPh_3, TEA, DMF, 80 °C; (b) H_2, Rh/Al_2O_3, $EtOAc/^iPrOH$, 25 °C, 24 h, 85% (2 steps); (c) ^{t}BuLi, 2,5-dichloro-isonicotinic acid methyl ester, −78 °C, 78%; (d) $NH_2OH{\cdot}HCl$, KOH, $^iPrOH/H_2O$, 80 °C; (e) TBSCl, Et_3N, DCE, 60 °C, 78% (2 steps); (f) (*S*)-α-methyl-benzylamine, Pd_2dba_3, 1,3-bis(2,6-diisopropylphenyl)imidazolium chloride, NaO^tBu, toluene, 110 °C, 7 h, 70%; (g) TBAF, THF, 25 °C, 2 h, 70%; (h) Jones Reagent, acetone, 0 °C, 1 h, 56%; (i) oxalyl chloride, DMF, DCM, 25 °C, 0.5 h; (j) ammonia, Et_3N, dioxane, 25 °C, 1 h, 51% (2 steps).



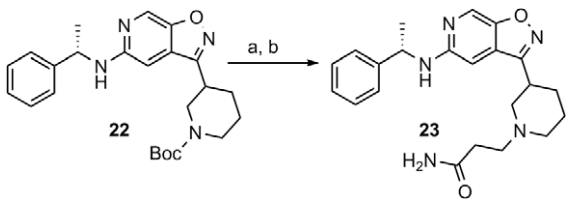
Scheme 3. Reagents and conditions: (a) i—LDA, THF, 0.5 h, −78 °C; ii—methyl-phenyl acetate, 0.5 h, −78 °C, 45%; (b) $NH_2OH{\cdot}HCl$, pyridine, 2 h, 100 °C; (c) NaOH, H_2O, 3 h, 100 °C, 10% (2 steps); (d) (*S*)-α-methyl-benzylamine, Pd_2dba_3, Peppsi-^{i}Pr, NaO^{t}Bu, toluene, 80 °C, 15 h, 5%.

improved activity. The synthesis of compound **18** is described in Scheme 3. 2,5-Dichloropyridine was lithiated and quenched with methylphenylacetate to provide **16**.[12] Treatment with hydroxylamine in pyridine generated the oxime, which cyclized to the isoxazole **17** when treated with aqueous sodium hydroxide.[9] Amination under standard conditions provided the desired compound **18** (Scheme 3). Thiophenes **19** and **20** were generated as described in Scheme 4; reaction of lithiated thiophenes with pyridine methyl esters gave the desired ketones, which were cyclized and aminated to provide the final compounds. Compound **21** was synthesized in an analogous manner to **18**, starting with 2-methyl-1-cyclohexane-carboxylic acid. Intermediate **22** was also synthesized in the same fashion. Deprotection of **22** followed by alkylation with 3-bromopropionamide provided compound **23** (Scheme 5).

Table 2 summarizes the structure–activity relationships for the inhibition of Plk1 for these non-phenyl analogs. Appending a benzyl group to the isoxazole ring ablated all activity (compound **18**). The heterocyclic compounds **19** and **20** demonstrate that the phenyl ring may be exchanged for a thiophene with minimal loss of

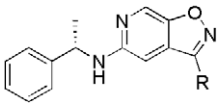


Scheme 4. Reagents and conditions: (a) $TMSCHN_2$, $MeOH/CH_2Cl_2$, 25 °C, 0.5 h, 100%; (b) bromo-thiophene, ^{t}BuLi, THF, −78 °C, 70–85%; (c) $NH_2OH{\cdot}HCl$, KOH, ^{i}PrOH/H_2O, 80 °C, 10–40%; (d) (*S*)-α-methyl-benzylamine, Pd_2dba_3, 1,3-bis(2,6-diisopropylphenyl)imidazolium chloride, NaO^{t}Bu, toluene, 110 °C, 15–24 h, 10–30%.



Scheme 5. Reagents and conditions: (a) HCl, dioxane, 25 °C, 3 h, 100%; (b) 3-bromopropionamide, Et_3N, THF, 40 °C, 24 h, 14%.

Table 2
SAR for phenyl replacements[a]



Compound	R	Plk1 IC_{50} (μM)
18	Benzyl	>20
19	2-Thiophene	3.007
20	3-(4-Methyl)thiophene	0.779
21	2-Methyl-cyclohexane	5.689
23	3-(*N*-3-Propionamide)piperidinyl	7.479

[a] See Supplementary Data for assay details.

activity (**19** is comparable in activity to **3**, **20** is comparable to **4**). Saturated rings were not as well tolerated, neither compound **21** nor **23** providing any improvement over initial hit **3**. None of the compounds described in Table 2 exhibited measurable cell-based activity (>20 μM in the cell-cycle assay).

An examination of alternate amino groups appended to the pyridine ring revealed that limited substitution on the benzyl ring was tolerated, with only fluoro substituents exhibiting comparable activity and all other substitution resulting in inactive compounds. A variety of substituted anilines provided activity within a few fold of that seen with the benzyl amine, including typical solubilizing group appendages. None of these variations provided a significant advantage over the (*S*)-α-methyl-benzyl amine (data not shown).

Next, we assessed Plk-isoform selectivity of this series by profiling biochemical activities for several compounds against human Plk1, Plk2, and Plk3. As shown in Table 3, three structurally similar compounds all show distinct selectivity profiles. Compound **4** inhibits Plk1 and Plk2 in the 1 μM range, but shows no inhibition against Plk3 up to 10 μM. The 3,4-methylenedioxy version (compound **9**) is highly active against Plk2 (0.004 μM), with 6-fold selectivity against Plk3 and 50-fold selectivity against Plk1. The most active compound against Plk1 (**15**, 0.051 μM), is 3-fold less active against Plk2 and 27-fold less active against Plk3. Interestingly, all three of these compounds result in comparable inhibition in the cell-cycle assay, consistent with the role of these isoforms in mediating cell cycle progression. (Comparable activity is also seen in MTT proliferation assays; data not shown.) Further confirmation for inhibition of Plk1 is seen in HCT 116 cells treated with compound **15**, where the expected monopolar spindle phenotype is observed (Supplementary Data, Fig. S1).

In conclusion, we have developed a novel 2-amino-isoxazolo-pyridine series as Plk inhibitors. Starting from compound **3** with modest biochemical potency we identified a series of Plk inhibitors with good biochemical potency and modest cell-based activity. A co-crystal structure confirmed that these compounds bind to Plk1 in an ATP-competitive manner. The phenotype observed in cells treated with these inhibitors is consistent with inhibition of Plk1. Lastly, we identified compounds differentially selective for Plk1, 2, and 3, which could serve as useful tools for elucidating the specific cellular functions of these Plk isoforms.

Table 3
Plk isoform profiles of selected compounds[a]

Compound	Plk1 IC50 (μM)	Plk2 IC50 (μM)	Plk3 IC50 (μM)
4	0.549	1.449	>10
9	0.214	0.004	0.024
15	0.051	0.172	1.382

[a] See Supplementary Data for assay details.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.08.091.

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