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Heteroaryl-linked 5-(1*H*-benzimidazol-1-yl)-2-thiophenecarboxamides: Potent inhibitors of polo-like kinase 1 (PLK1) with improved drug-like properties

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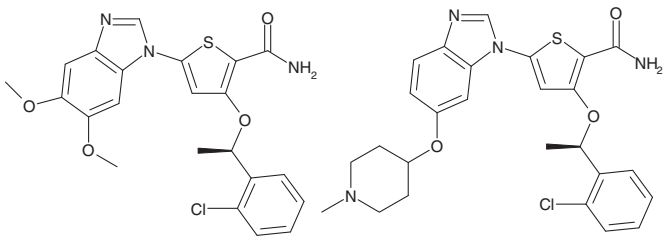
ABSTRACT

Potent inhibitors of PLK1 with acceptable solubility, mouse iv clearance, and reduced CYP450 inhibition were identified. Drug-like properties were improved using a heteroaryl ring as a functional handle for manipulation of inhibitors' physiochemical and DMPK properties.

Cancer cells are able to grow through continuous mitotic division as a result of misregulated cell cycle control.[1] Interruption of the cell cycle through the use of anti-mitotics such as taxanes and vinca alkaloids has been established as an effective approach to cancer therapy.[2] Polo-like kinase 1 (PLK1) has been shown to play an integral role in the regulation of mitotic progression of cells, including mitotic entry, spindle assembly, chromosome segregation, and cytokinesis.[3,4] Given its central role in mitosis, PLK1 has generated much interest as an anticancer target, and a wealth of preclinical target validation data has been reported in the literature. For example, PLK1 depletion though various means has been shown to inhibit tumor cell proliferation in vitro[5] and in vivo.[6] In contrast, normal cells have been shown to survive PLK1 depletion.[7] Finally, PLK1 has been found to be overexpressed in a variety of tumors, and has been shown to correlate with poor clinical outcomes.[8] Small molecule inhibitors of PLK1[9] offer the potential advantages of selectively interfering with aberrant cancer cell mitosis while mitigating the severe dose-limiting toxicities currently associated with anti-mitotic therapies.[10]

As part of an effort aimed at developing intravenous, ATP-competitive inhibitors of PLK1, the thiophene amide series shown in Figure 1 was identified and initial SAR efforts around this series were recently described.[11] Compound **1** was found to be a potent inhibitor of both PLK1 and PLK3, while compound **2** was shown to be more than 300-fold selective for inhibition of PLK1 over PLK3, giving these compounds each distinct profiles for evaluating inhibition of polo-like kinases as an approach to cancer treatment.



1
PLK1 IC_{50} = 0.8 nM
PLK3 IC_{50} = 6 nM
HCT116 IC_{50} = 25 nM

2
PLK1 IC_{50} = 2 nM
PLK3 IC_{50} = 630 nM
HCT116 IC_{50} = 11 nM

Figure 1. Novel inhibitors of PLK1 and PLK3.

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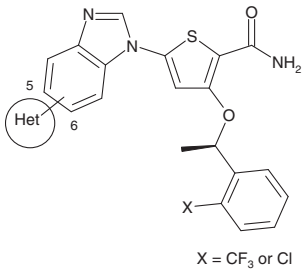
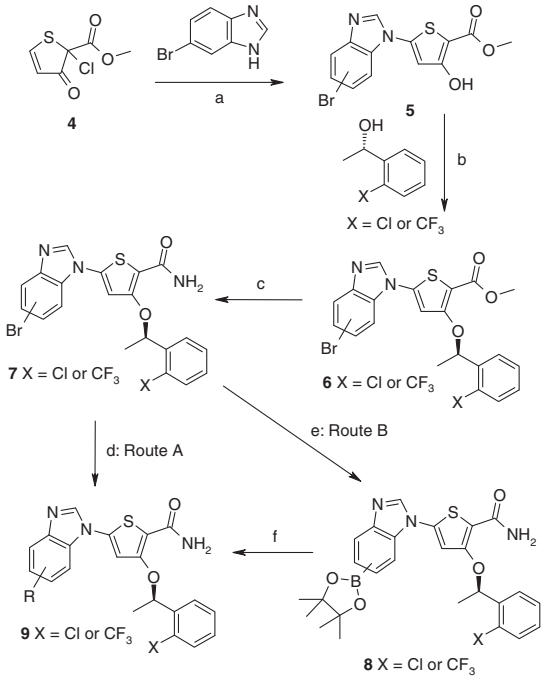


Figure 2. Heteroaryl-linked (2-thienyl)-1*H*-benzimidazole template.

While compound **1** had a desirable in vitro profile, its aqueous solubility was poor for an intravenous formulation required for the target product profile. In turn, while aqueous solubility was dramatically improved in **2**, DMPK issues prevented progression of this ether-linked series. Further SAR on the thiophene amide template quickly identified that aryl and heteroaryl substitution at the 5- or 6-position of the benzimidazole maintained potent inhibition of PLK1 and PLK3 (Fig. 2). With the discovery of this novel functional handle to potentially manipulate physiochemical and DMPK properties, an SAR effort was undertaken in order to fully explore this series.

Compounds were prepared according to Scheme 1. Conjugate addition of 5-bromo-1*H*-benzimidazole to thiophene precursor **4**[11a,18b] resulted in *N*-aryl benzimidazole derivative **5** as a mixture of 5- and 6-regioisomeric products. Mitsunobu coupling of the regioisomeric mixture **5** with a chiral benzyl alcohol[18] provided **6** with inversion of absolute stereochemistry at the chiral center. The regioisomeric mixture **6** was then heated in a sealed tube with methanolic ammonia to afford the final regioisomeric mixture of amide products **7**. The 5- and 6-regioisomers can be easily separated at



Scheme 1. Reagents and conditions: (a) $CHCl_3$, $NaHCO_3$, (regioisomeric mixture, 60–69%); (b) PS-PPh_3, DTBAD, CH_2Cl_2, (regioisomeric mixture, 50-83%); (c) 7 N ammonia in methanol, 80 °C, sealed tube, (54–88%); (d) aryl/heteroaryl boronate ester or acid, 1 M Na_2CO_3, $PdCl_2$(dppf), DMA, 80 °C, (67–97%); (e) bis(pinacolato)diboron, KOAc, $PdCl_2(PPh_3)_2$, microwave, 150 °C (52–70%); (f) aryl/heteroaryl halide, 1 M Na_2CO_3, $PdCl_2$(dppf), DMF, 80 °C, (20–88%).

either the ester stage (**6**) or the amide stage (**7**) by flash chromatography for use in further SAR studies. Alternatively, the regiospecific isomers of both **6** and **7** can be prepared using a regioselective route previously described.[12]

Heteroaryl moieties were added by Suzuki–Miyaura reaction either directly from bromides **7** when the boronate ester or boronic acid coupling partners were readily available (Route A), or by preparation of boronate esters **8**, followed by reaction with the appropriate heteroaryl halide as recently reported (Route B).[13]

A series of five- and six-membered aryl- and heteroaryl-linked benzimidazoles were prepared and screened for activity against PLK1 and PLK3 enzymes.[14] Additionally, compounds were tested in a cellular proliferation assay in HCT116 cells.[15] Data in Table 1 show that substitution of both the 5- and 6-positions of the benzimidazole were well tolerated, as all compounds exhibited potent inhibition of PLK1. However, the 5-substituted benzimidazoles exhibited a 2–3-fold increased cellular potency over their 6-substituted counterparts (Table 1, compounds **10–17, 23, 24**, **26** and **27**). Several examples also displayed potent activity against PLK3, although this activity was reduced in the presence of a heteroatom at the ortho-position of the heteroaryl ring (Table 1, compounds **18–20**). In general, there was little difference observed in enzyme potency between compounds with 2-Cl or 2-CF_3 substitution on the benzyl ether, although the 2-Cl benzyl ethers were slightly more potent at the cellular level (Table 1, compounds **12**, **14**, **22** and **23**). While it appeared that dual inhibitors of PLK1 and 3 potently inhibit cellular proliferation, it is unclear to what extent PLK3 activity might be contributing to cellular potency (Table 1, compounds **12**, **22**, **23**, and **29**). The data suggests that potent PLK1 inhibition is required to have a dramatic impact on cellular proliferation, and in this case, a lower potency threshold in the PLK1 enzyme assay may have helped to distinguish between nanomolar and potentially sub-nanomolar compounds.

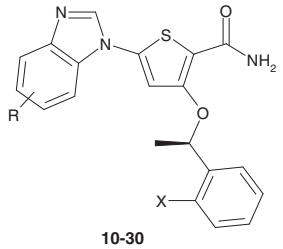
Compounds **14**, **23**, **28** and **29** were further profiled for developability and in vivo pharmacokinetic properties (Table 2). As previously mentioned, an intravenous dosing route was desired for a potential anti-mitotic PLK inhibitor. It was determined that the minimally acceptable solubility in the dosing formulation of 1.0 mg/mL was required for development. Compounds from this first iteration of SAR showed generally poor solubility and potent P450 inhibition.[16] On the other hand, the addition of the heteroaryl ring occasionally resulted in improved mouse iv clearance (Table 2). Compounds **23** and **28** showed reduced iv clearance in mouse (15 and 38 mL/min/kg, respectively) compared to **1** and **2** (91 and 99 ml/min/kg, respectively).

Our strategy for improving the aqueous solubility and reducing CYP450 inhibition of this heteroaryl-linked series revolved around adding polar functionality to the more metabolically stable pyrazole and aminopyridine heteroaryl rings found in **23** and **28**, and to the pyridimidine ring in **29**. A model of pyridine **12** docked into a homology model of PLK1[17] shows the pyridine ring surrounded by hydrophilic arginine residues 57 and 136. These residues flank both faces of the heteroaryl ring as it extends into the solvent exposed region of the ATP binding site (Fig. 3). Based on this model, we hypothesized that hydrophilic functionality would be well tolerated in this area.

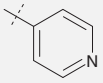
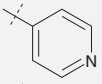
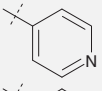
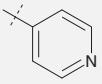
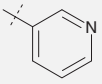
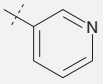
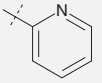
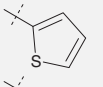
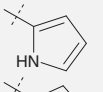
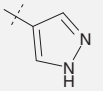
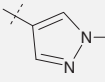
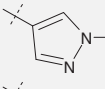
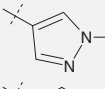
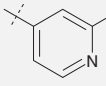
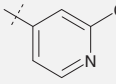
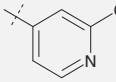
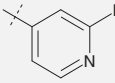
Aminoalkyl pyridine and pyrimidine compounds (**31**–**40**) were typically prepared via nucleophilic aromatic substitution according to Scheme 2. Substituted pyrazoles (**41**–**43**) were prepared by SN_2 alkylation with the appropriate alkyl halide.[18]

As predicted, hydrophilic functionality was well tolerated on the pyridine, pyrimidine and pyrazole rings (Table 3). In addition, potent inhibition of cellular proliferation was also consistently observed with the addition of polar functionalities. Inhibition of PLK1 was not sensitive to the presence of a secondary or tertiary amine at the 2-position of the pyridine (Table 3, compare

Table 1
PLK1 and PLK3 enzyme inhibition and cancer cell proliferation assay results for compounds **10–30**

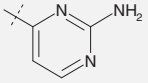
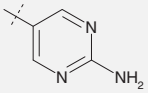


10-30

Compd	X	Position	R	PLK1 IC_{50}[a] (nM)	PLK3 IC_{50}[a,d] (nM)	HCT116 IC_{50}[a,b,c] (nM)
10	CF_3	5	Ph	6	–	1052
11	CF_3	6	Ph	18*	–	1975[c]
12	CF_3	5		1	8	87
13	CF_3	6		2	16*	125[c]
14	Cl	5		1	10*	53[c]
15	Cl	6		2	25*	172[c]
16	CF_3	5		1	16*	167
17	CF_3	6		2	40	178[c]
18	CF_3	5		6	79*	635
19	CF_3	5		3	50*	3638
20	CF_3	5		5	126*	236
21	CF_3	5		1	–	31
22	CF_3	5		1	6*	71
23	Cl	5		1	6	20
24	Cl	6		1	13	54[c]
25	CF_3	5		1	–	143
26	CF_3	5		4	–	365
27	CF_3	6		53*	–	813[c]
28	CF_3	5		1	–	24

(*continued on next page*)

Table 1 (*continued*)

Compd	X	Position	R	PLK1 IC_{50}[a] (nM)	PLK3 IC_{50}[a,d] (nM)	HCT116 IC_{50}[a,b,c] (nM)
29	CF_3	5		1	5*	15
30	CF_3	5		1	—	202

[a] Values are means of at least two experiments unless marked with (*) which denotes $n = 1$.
[b] All values were obtained using CTG assay format unless otherwise marked.
[c] MEB assay format.
[d] (—) indicates data not determined.

compounds **31**–**34** with **35**–**38**), or to the presence of a pendant non-basic amine (compounds **36** and **37**). Similarly, the potency of substituted pyrazoles was not sensitive to the length of the linker chain or the type of basic amine attached (compounds **41**–**44**).

Substitution adjacent to the pyridine nitrogen also resulted in an improved P450 inhibition profile compared to **14**. Compounds having this substitution pattern had equal or better CYP450 inhibition profiles compared to **28** (Table 3, compounds **31**–**39**). In the N-substituted pyrazole series (**41**–**44**), reduced P450 inhibition was also generally observed compared to N-methylated **23**. Interestingly, compounds containing a morpholine ring showed an increased liability with CYP2C9 (Table 3, compounds **34** and **41**).

The main goals of prosecuting this SAR were to improve solubility in the iv dosing formulation and to improve the iv pharmacokinetic properties encumbering **1** and **2**. Compared to **28**, pyridines with additional cyclic basic amine groups did not show significantly improved solubility (Table 3, compounds **32**, **33**, and **38**). However, 3-pyridine isomer **39** did offer a solubility advantage over the corresponding 4-pyridyl analog **38**. Pyrazoles with straight-chain alkyl linkers (**42–44**) showed a notable improvement in solubility over pyrazole **23** and morpholine analog **41**. Addition of non-basic polar functionality, as in **36** and **37**, actually resulted in a decrease in solubility compared to **28**. Finally, with the exception of **37** and **41**, compounds in the pyridyl and pyrazole series showed a notable improvement in mouse iv clearance compared to **1** and **2** (Tables 2 and 3).

This thiophene series proved to be a fairly selective template for inhibition of PLK1 over other kinases. Representative compound **44** showed 10-fold selectivity at the enzyme level over PLK3 and VEGFR2. Notably, greater than 100-fold selectivity was observed for Aurora B, CDK2, EGFR, SRC1, GSK3β and p38α.

In conclusion, utilization of a heteroaryl ring as a functional handle for SAR to improve mouse iv clearance, solubility, and P450 proved to be a worthwhile strategy. Compounds in both the pyridine and pyrazole series were identified that offered improved potencies, P450 profiles, solubility and iv clearance in mouse.

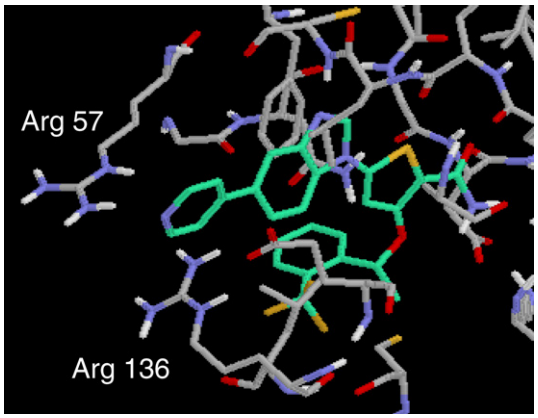
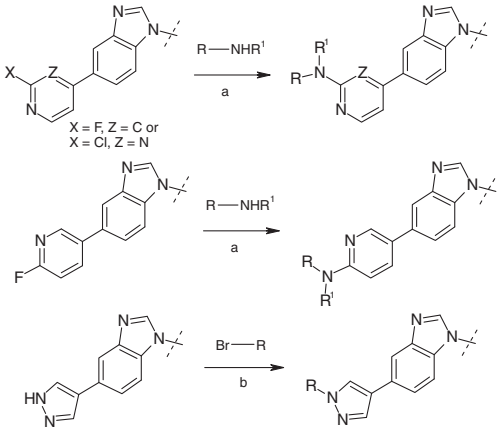


Figure 3. Compound **12** (green) docked into a homology model of PLK1.



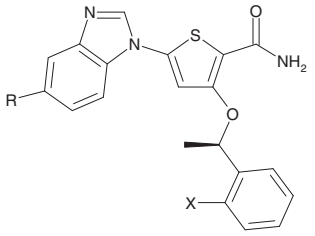
Scheme 2. Reagents and conditions: (a) excess amine, neat, microwave, 180 °C, (46–86%); (b) Cs_2CO_3, DMF, 80 °C (30–53%).

Table 2
Developability properties for selected PLK1 inhibitors

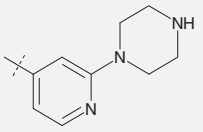
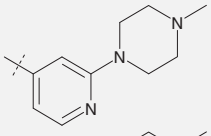
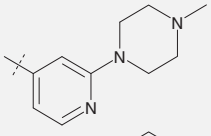
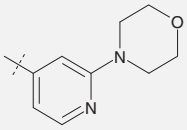
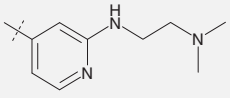
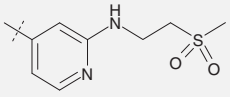
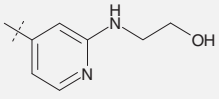
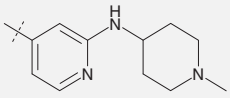
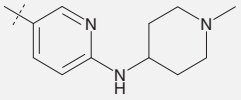
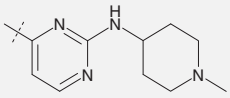
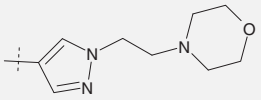
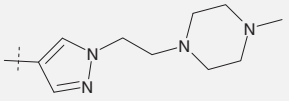
Compd	Solubility[a] (mg/mL)	CYP2C9 FCA[b,c] IC_{50} μM	CYP3A4 DEF[b,c] IC_{50} μM	CYP3A4 7BQ[b,c,e] IC_{50} μM	Mouse iv Cl ml/min/kg
23	0.1	<0.1	0.20	0.20	15
14	0.003	0.1[d]	<0.1[d]	<0.1[d]	70
28	1.0	0.2[d]	6.5[d]	—	38
29	0.2	<0.1	6.8	>100	70

[a] Solubility was determined in 20% SBE β-cyclodextrin at pH 6 (equilibrium).
[b] P450 determination using Cypex recombinant human enzyme unless otherwise stated.
[c] FCA: 7-methoxy-4-trifluoromethylcoumarin-3-acetic acid; DEF: diethoxyfluorescein; 7BQ: 7-benzyloxyquinoline.
[d] P450 determination using Gentest recombinant human enzyme and PPR as fluorescent substrate instead of 7BQ (PPR: 7-{3-(4-phenylpiperazin-1-ylmethyl)benzyl}resorufin).
[e] (—) indicates data not determined.

Table 3
PLK1 enzyme inhibition, cancer cell proliferation assay, P450 inhibition, solubility and iv clearance results for compounds **31**–**44**

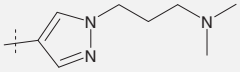
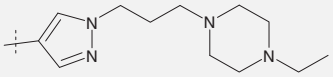


31-44

Compd	X	R	PLK1 IC_{50} nM[a]	HCT116 IC_{50} nM[a,b]	CYP3A4 IC_{50} DEF/7BQ μM[d,f]	CYP2C9 IC_{50} FCA μM[d]	Solubility mg/mL[c,f]	Mouse iv Cl mL/min/Kg[f]
31	Cl		8	25	1.4/5.9	21.0[e]	–	58
32	Cl		2	21	1.1/27.0	1.6[e]	0.50	52
33	CF_3		3	30	0.4/1.9	0.2	0.87	32
34	CF_3		5	68	20.0/2.8	0.1	–	–
35	CF_3		3	21	0.8/27.0	0.9	3.23	62
36	Cl		1	39	–/3.5	1.7	0.03	–
37	Cl		1	14	–/3.7	0.2	0.30	109
38	Cl		1	11	1.1/4.7	4.3	1.05	48
39	Cl		2	36	3.4/16	8.9	2.93	19
40	Cl		1	6	1.0/15.0	1.0[e]	–	–
41	Cl		1	20	–/2.1	0.1[e]	0.53	82
42	Cl		1	16	0.2/1.9	0.7	4.55	54

(*continued on next page*)

Table 3 (*continued*)

Compd	X	R	PLK1 IC_{50} nM[a]	HCT116 IC_{50} nM[a,b]	CYP3A4 IC_{50} DEF/7BQ μM[d,f]	CYP2C9 IC_{50} FCA μM[d]	Solubility mg/mL[c,f]	Mouse iv Cl mL/min/Kg[f]
43	Cl		2	15	0.9/5.0	0.4	5.83	44
44	Cl		1	15	1.0/4.5	2.4	3.12	44

[a] Values are means of at least two experiments.
[b] Cell assay data obtained using the CTG assay format.
[c] Solubility was determined in 20% SBE β-cyclodextrin at pH 6 (equilibrium).
[d] P450 determination using Cypex recombinant human enzyme unless otherwise stated.
[e] P450 determination using Gentest recombinant human enzyme and PPR as fluorescent substrate instead of 7BQ.
[f] (—) indicates data not determined.

Pyridines **38** and **39** and pyrazole **44** met all of these criteria and were advanced into further biological studies.

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