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Design of potent thiophene inhibitors of polo-like kinase 1 with improved solubility and reduced protein binding

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ABSTRACT

A series of thiophene PLK1 inhibitors was optimized for increased solubility and reduced protein binding through the appendage of basic amine functionality. Interesting selectivity between PLK1 and PLK3 was also obtained through these modifications.

A relationship between cancer proliferation and an improperly controlled cell cycle has been well established.[1] Clinical use of anti-mitotics to disrupt the cell cycle as a method of cancer therapy has proven effective in certain instances.[2] The polo-like kinases (PLK) represent an attractive target for cancer treatment through interference with the cell cycle.[3] These proteins are an evolutionarily conserved family of serine/threonine kinases characterized by an amino-terminal kinase domain and carboxy-terminal polo box domain(s) and include PLK1, PLK2 (SNK), PLK3 (PRK/FNK), and PLK4 (SAK). PLK1 represents the most studied member of this family and has known roles throughout mitosis, including mitotic entry, spindle formation, chromosome segregation, and cytokinesis.[4] Further preclinical validation of PLK1 as a cancer target has been demonstrated through the use of small interfering RNA in cell culture[5] and antisense oligonucleotides in mouse tumor xenograft models.[6] Finally, a correlation between elevated PLK1 expression and poor prognosis in the clinic has been established.[7] In light of the aforementioned evidence, a program directed toward the discovery of small molecule inhibitors of PLK1 was initiated.

Prior work in a thiophene series of PLK inhibitors that led to the discovery of compound **1** was recently communicated (Fig. 1).[8] Further in vitro characterization of compound **1** was also recently disclosed.[9] Compound **1** is a potent inhibitor of PLK1 and PLK3[10] and moderately inhibits the growth of tumor cells in vitro.[11] Compound **1** served as a useful tool compound for understanding the consequences of inhibiting PLK in vitro; however, the compound lacked many of the features considered desirable in a clinical candidate. In order to discover a PLK1 inhibitor suitable for clinical studies, work continued in the thiophene series and focused on improved cellular potency, solubility, and protein binding.

Subsequent to the work described previously, a minor modification to the template resulted in a significant improvement in

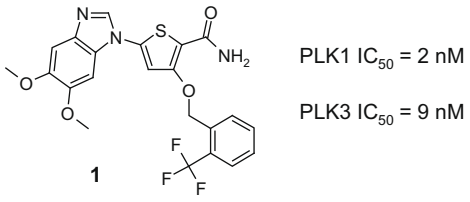

Figure 1. PLK tool compound.

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cellular potency (Table 1). Installation of a methyl group at the benzylic carbon in the *R*-configuration (**3**) resulted in a more than 40-fold improvement in the ability to inhibit the growth of HCT116 (colorectal carcinoma) cells over the unsubstituted analog (**2**). The enantiomer (**4**) was a less potent inhibitor of PLK1 at the enzyme level; however, its potency in the cellular assay was similar to **2**. The effect of benzylic substitution on PLK3 potency was similar to that observed with PLK1. An explanation for the improvement in cell potency remains unclear. Since the concentration of PLK1 in the enzyme assay is 2 nM, the potency of **3** may have been underestimated in that assay. It was also noted that compound **3** maintained an excellent kinase selectivity profile among more than 40 kinases tested in house, which is comparable to **1**.

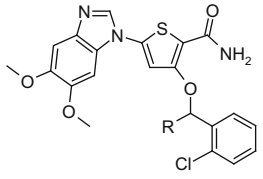
With this marked improvement in cellular potency, attention was next directed to additional areas for lead optimization. Since the target product profile for a PLK1 inhibitor included an intravenous formulation, solubility was an important attribute in any potential clinical candidate. Compounds were routinely screened in a high throughput manner for their solubility in a phosphate buffered solution at pH 7.4 and were generally poorly soluble (**3** = 2 μM).[12] It was also noted that compounds in this series were typically highly bound to human plasma proteins.[13] As a means to improve the solubility and reduce the protein binding of compounds, the installation of more polar functional groups was examined. Analysis of a homology model of **3** bound in the ATP-binding site of PLK1 indicated that the 6-position of the benzimidazole might be a good location for appending such groups (Fig. 2). With the nitrogen of the benzimidazole contacting the β-strand at Cys_{133}, the 6-position is oriented toward solvent. In addition, there are residues such as Glu_{140} and Ser_{137}, which are potentially disposed to favorably interact with such polar functional groups.

In order to effectively prosecute this hypothesis, efficient syntheses of versatile phenol intermediates **14** and **15** were desired (Scheme 1). The syntheses began with Buchwald coupling of aryl bromide **5** with amines **6** or **7**.[14] Reduction of nitro-anilines **8** and **9** was accomplished by hydrogenation using a sulfided platinum catalyst. Upon treatment with pyridinium *p*-toluensulfonate and trimethyl orthoformate, diamines **10** and **11** cyclized to afford benzimidazoles **12** and **13**. Subsequent reaction with trifluoroacetic acid cleanly removed the 4-methoxybenzyl protecting group to provide phenols **14** and **15**. These phenols served as versatile intermediates for evaluation of solubility enhancing groups at this position. Functionalization was typically accomplished through Mitsunobu couplings[15] or standard S_N2 alkylations, followed by additional routine transformations.[16]

Methyl ether analogs **16** and **17** were both highly protein bound and poorly soluble (Table 2). Although a modest improvement in cellular potency was observed, incorporation of a diol (**18**) only minimally improved solubility. The installation of flexible amine chains (**19**–**22**) improved solubility and reduced protein binding relative to **16** and **17**. While compounds such as **21** and **22** were quite potent, potency in the cellular assay could be further enhanced by elimination of the methylene spacer between the oxygen atom and the piperidine ring (**23**–**25**). These analogs exhibited an excellent combination of potency, solubility, and reduced protein binding. The azepine analogs (**26** and **27**) were similar in potency and solubility to their piperidine counterpart (**25**); however, protein binding increased in each case. It should also be noted that methylation of the piperidine nitrogen led to both improved solubility and reduced protein binding (**24** vs **23**).

Perhaps the most interesting observation with regard to these new analogs with basic amine functionality was their improved selectivity versus PLK3. Examination of **23** in a homology model bound in the ATP-binding site of PLK1 provided some rationale

Table 1
Benzylic methyl group



Compound	R	PLK1 IC_{50}[a] (nM)	PLK3 IC_{50}[a] (nM)	HCT116 IC_{50}[a] (nM)
2	H	2	9	1140
3	Me (*R*)	0.8	6	25
4	Me (*S*)	30	44*	994

[a] Data are the average of $n \geqslant 2$, except where noted with (*), which is $n = 1$.

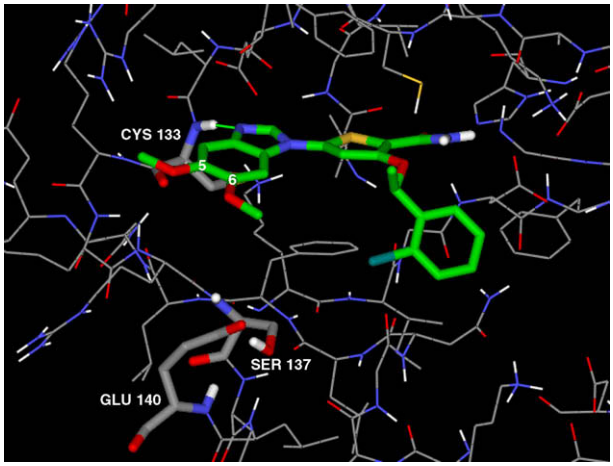
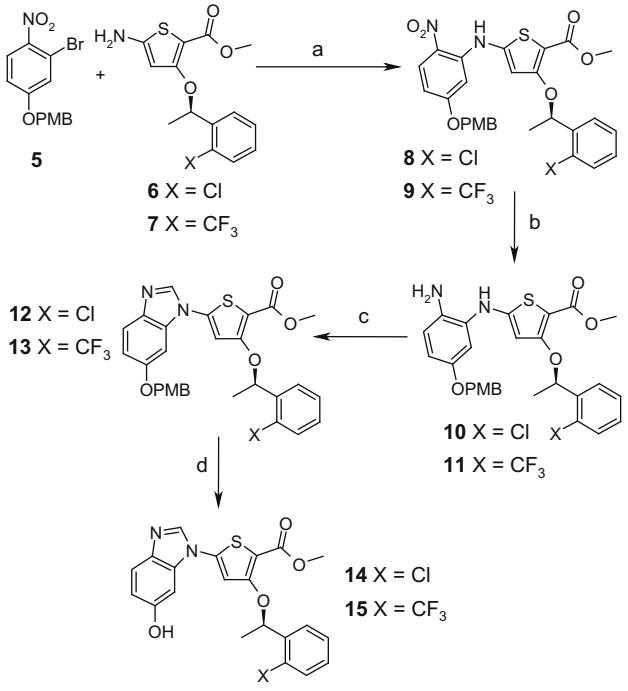
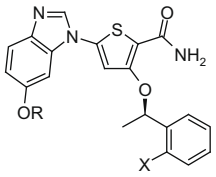


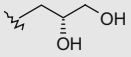
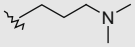
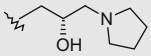
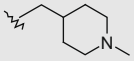
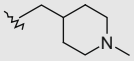
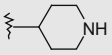
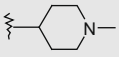
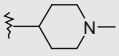
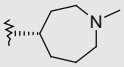
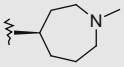
Figure 2. Homology model of **3** in PLK1.



Scheme 1. Reagents and conditions: (a) $Pd_2(dba)_3$, Cs_2CO_3, XANTPHOS, dioxane, 60 °C (80% for **8**, 74% for **9**); (b) H_2 (1 atm), Pt(sulfided)/C, EtOAc (97% for **10**); (c) PPTS, $CH(OMe)_3$, Et_2O (97% over 2 steps for **12**); (d) TFA, CH_2Cl_2, 0 °C (75% for **14**, 92% for **15** over 2 steps).

Table 2
Analogs with solubility enhancing groups



Compound	X	R	PLK1 IC_{50}[a] (nM)	PLK3 IC_{50}[a] (nM)	HCT116 IC_{50}[a] (nM)	Solubility PBS, pH 7.4[a] (μM)	Eq. dialysis PB[a] (%)
16	Cl	Me	1	35*	321	3*	99.5*
17	CF_3	Me	3	41*	476	1*	99.5*
18	Cl		1	61*	73	15*	—
19	Cl		4	460*	32	161*	97.4*
20	Cl		3	610*	45	170*	96.2*
21	CF_3		7	550	30	109	97.7
22	Cl		6	1100*	34	⩾172	97.9*
23	CF_3		3	350	15	104	96.6
24	CF_3		2	270*	12	179	94.8*
25	Cl		2	630*	11	⩾190	91.5*
26	Cl		3	240*	17	⩾200*	96.9*
27	Cl		4	250	13	200*	98.0*

[a] Data are the average of $n \geqslant 2$, except where noted with (*), which is $n = 1$.

for this observation (Fig. 3). In this model, it appears that there is a hydrogen bond interaction between Glu_{140} and the piperidine nitrogen. The corresponding residue in both PLK3 and PLK2 is a histidine. The interaction of the charged amine with the histidine residue is likely not favorable, resulting in the reduced potency observed against PLK3.

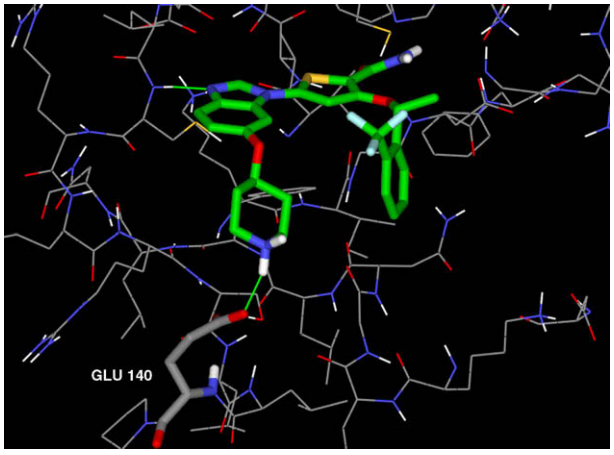


Figure 3. Homology model of **23** in PLK1.

Table 3
Expanded cellular proliferation panel

Cell line	Tumor type	IC_{50} (nM)[a]	
		24	**25**
COLO205	Colon	5	5
HT29	Colon	3	3
A549	Lung	14	11
MX-1	Breast	10	9
SKOV-3	Ovarian	32	11

[a] Data are the average of $n \geqslant 2$.

Compounds **24** and **25** were considered to have a good balance of potency, selectivity, solubility, and lower protein binding. Each was evaluated against a broader panel of cell lines in order to further determine its ability to inhibit cellular proliferation (Table 3).[11] Both proved to be potent inhibitors of cell growth in vitro across a wide range of cell lines representing multiple tumor types.

The kinase selectivity of early compounds within this chemical series was considered excellent.[8] Examination of **24** and **25** against an expanded panel of more than 50 human kinases showed these new analogs have maintained good selectivity (Table 4).[17] Both compounds have activity against Nek2, which is also a serine/threonine kinase with important roles throughout mitosis, including centrosome separation, microtubule organization, and mitotic exit.[18] Selectivity against other kinases compared to PLK1 is greater

Table 4
Expanded kinase selectivity panel

Kinase	IC_{50}[a] (nM)	
	24	**25**
PLK1	2	2
PLK3	270*	630*
Nek2	25	21*
PDGFR1β	110*	420*
PIM-1	340*	310*
PI3K-γ	390*	—
PI3K-δ	530*	440*
VEGFR2	800	1200*
Nuak1	840*	2000*
Others	>1000 (48 kinases)	>1000 (50 kinases)

[a] Data are the average of $n \geqslant 2$, except where noted with (*), which is $n = 1$.

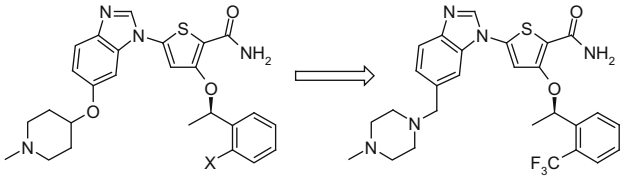


Figure 4. GSK461364.

than 100-fold in all other cases, with the exception of PDGFR1β for **24**, which is approximately 50-fold selective versus that enzyme.

In summary, the appendage of cyclic amine functionality to the 6-position of the benzimidazole of thiophene inhibitors was used to afford compounds with suitable potency, selectivity, solubility, and protein binding for progression into advanced assays. Subsequent work within this chemical template resulted in the discovery of GSK461364 (Fig. 4), a close structural analog of **24** and **25**.[19] GSK461364 is currently under evaluation in clinical trials. Further communications regarding the discovery and profile of GSK461364 are planned.

Acknowledgments

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10. The PLK1 enzyme assay was conducted using the kinase domain only. The PLK3 assay was conducted using full length enzyme. Both were in an SPA format. For a complete description see Ref. 9.
11. Cell proliferation assays: Cells were seeded in 96-well dishes, incubated overnight at 37 °C, and treated with various concentrations of inhibitors for 72 h. Cell proliferation was quantified using the CellTiter-Glo Luminescent Cell Viability Assay (Promega, Madison, WI), following the manufacturer's recommendations. IC_{50} values were determined from using a 4-parameter curve fit software package (XLfit4).
12. The compound was first dissolved in DMSO in a concentrated form (10 or 20 mM) followed by spiking a fixed volume of the DMSO stock solution into the final buffer. Two samples were prepared for each compound. One (the standard sample) contained the compound at a fixed concentration of 20 μM in an aqueous/organic mixed solvent cocktail. The other (test sample) contained the compound at a maximum total concentration of 200 μM in, pH 7.4, 0.05 M phosphate buffer. The test sample was spun for 15 min to remove any undissolved solid. HPLC analyses were performed on these samples. The peak areas were used for computing the solubility.
13. Stock solutions of compound were spiked into human plasma at target concentrations of 2000 ng/mL. The mixtures were inverted gently several times to insure homogeneity and triplicate 50 μL aliquots were collected to verify initial concentrations. Following assembly of dialysis plate (HTDialysis membrane strips, molecular weight cut off limit of 12,000–14,000 daltons), spiked plasma (150 μL) was placed in the donor compartment of the well and phosphate buffered saline, pH 7.4 (150 μL) in the receiver compartment. Eight wells were set up per compound and plasma type. Plate was placed in a 37 °C incubator on a plate shaker. Following the 6-h incubation period, the plate was removed. Single 50 μL aliquots from each donor and receiver compartment (per well) were analyzed. Sample analysis was by LC/MS/MS (results reported as Drug Peak Area/Internal Standard Peak Area ratios).
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