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Discovery of thiophene inhibitors of polo-like kinase

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ABSTRACT

The discovery and development of a series of thiophenes as potent and selective inhibitors of PLK is described. Identification and characterization of **2**, a useful in vitro PLK inhibitor tool compound, is also presented.

The lack of proper cell cycle control and regulation is a hallmark of cancer cells.[1] Inhibition of the cell cycle through the use of antimitotics has been established as an effective approach to cancer therapy.[2] Polo-like kinases (PLK) are an evolutionarily conserved family of serine/threonine kinases characterized by an amino-terminal serine/threonine kinase domain and carboxy-terminal polo box domain(s). The PLK family includes PLK1, PLK2 (SNK), PLK3 (PRK/FNK), and PLK4 (SAK). PLK1 plays important roles throughout mitosis and is involved in the regulation of mitotic progression, including mitotic entry, spindle formation, chromosome segregation, and cytokinesis.[3] Depletion of PLK1 with small interfering RNA in vitro has been shown to induce mitotic arrest, and can lead to apoptosis.[4] In addition, the inhibition of PLK1 using antisense oligonucleotides has shown activity in mouse tumor xenograft models.[5] PLK1 expression is elevated in numerous cancer types and has been correlated with poor clinical prognosis.[6] Such features make PLK1 a novel and attractive target for cancer treatment.[7] Herein the discovery and development of small molecule PLK1 inhibitors is reported, as exemplified by **2** (Fig. 1). Compound **2** is a potent inhibitor of PLK1 and is a useful tool compound for further understanding the role(s) of PLK1 in mitosis.

Ongoing efforts directed toward the design of novel kinase inhibitor templates led to the identification of thiophene **1**, a promising lead with good PLK1 enzyme potency.[8] The synthesis of this class of compounds began with methyl 2-chloro-3-oxo-2,3-dihydro-2-thiophenecarboxylate **3** (Scheme 1).[9] Conjugate addition of a benzimidazole to **3** gave *N*-aryl benzimidazole derivative **4**. In the case of 5-substituted benzimidazoles, this reaction produced a regioisomeric mixture of products. Alkylation with a benzyl bromide or Mitsunobu coupling with the corresponding benzyl alcohol provided **5**. Treatment of **5** with methanolic ammonia under sealed tube conditions with heating afforded the final amide products **6**.

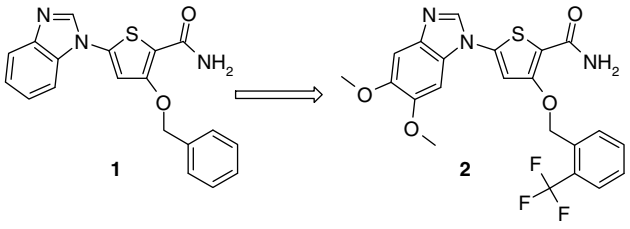

Figure 1. Evolution of PLK1 tool compound **2**.

A study to investigate the effects of changing the electronic nature of the benzyl ether portion of the inhibitor was undertaken (Table 1).[10] Single-point substitutions to vary the electronic character of the phenyl ring were made. Most substitutions were well tolerated with substitution at the 2-position generally preferred.

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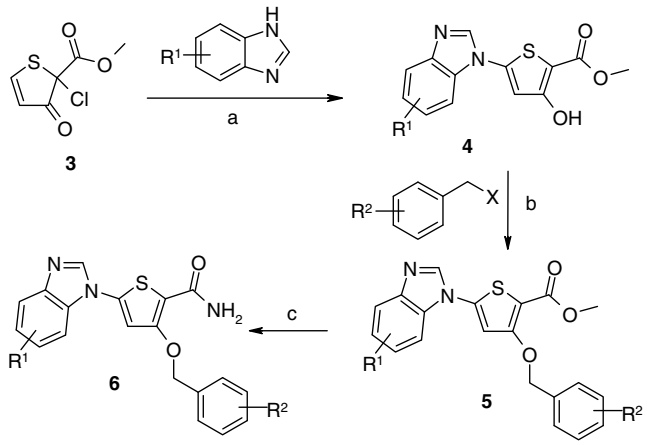
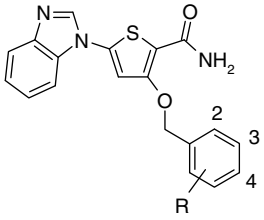


Scheme 1. Reagents and conditions: (a) $CHCl_3$, $NaHCO_3$ or *N*-methylimidazole; (b) K_2CO_3, DMF (X = Br) or PPh_3, DIAD, CH_2Cl_2 (X = OH); (c) 7 N ammonia in methanol, 80 °C, sealed tube.

Table 1
Initial SAR of the benzyl ether



Compound	R	PLK1 IC_{50} (nM)
1	H	61
7	2-Me	12
8	3-Me	12
9	4-Me	70
10	2-OMe	12
11	3-OMe	28
12	4-OMe	35
13	2-Cl	12
14	3-Cl	22
15	4-Cl	100
16	2-CF_3	15
17	2-Br	11
18	2-F	14

Based on **1** docked into a homology model of PLK1 (Fig. 2), it was believed that the benzimidazole portion of the molecule offered an area for further SAR exploration. The benzimidazole nitrogen was thought to contact the hinge at cysteine residue 133. Analysis of the model indicated that there was likely room for the attachment of functional groups at both the 5- and 6-position of the benzimidazole. In search of a more significant potency boost, attention was turned to modification of this area (Table 2). Compounds with promising enzyme data were also screened in a 3-day cellular proliferation assay using the human colon carcinoma cell line HCT116.[11] Benzimidazoles substituted at the 6-position were more potent than the corresponding five substituted analogs. Comparison of the PLK1 kinase data for **20** and **26** indicated that the electronic nature of the 6-position substituent may not be of primary importance for potency. Overall, the dimethoxy analog **2** proved to be the most potent compound from this set with respect to both the enzyme and cellular assay. In addition, the symmetrical benzimidazole was desirable from a synthetic standpoint since it did not produce regioisomers in the previously described conjugate addition reaction. Therefore, the SAR around the 5,6-dimethoxybenzimidazole was further explored.

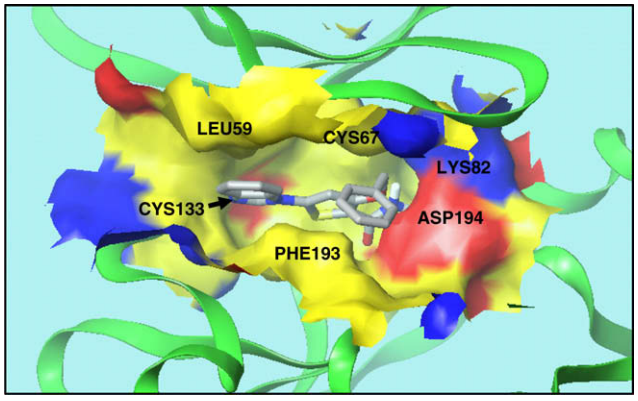
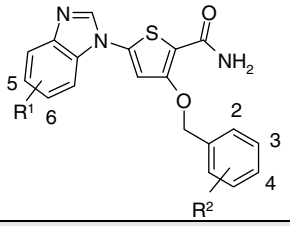


Figure 2. Homology model of **1** docked into PLK1.

Table 2
Benzimidazole substitution SAR



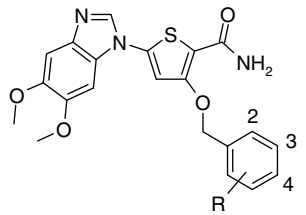
Compound	R^1	R^2	PLK1 IC_{50} (nM)	HCT116 IC_{50} (nM)
19	5-Cl	2-Me	21	>28,000
20	6-Cl	2-Me	4	1690
21	5-CF_3	2-Br	300	>30,000
22	6-CF_3	2-Br	9	3590
23	5-SO_2Me	2-CF_3	250	>30,000
24	6-SO_2Me	2-CF_3	12	2960
25	5-OMe	2-CF_3	8	2700
26	6-OMe	2-CF_3	2	1230
2	5,6-Di-OMe	2-CF_3	2	699

At this point, the effort returned to the benzyl ether to further evaluate this portion of the molecule (Table 3). Halogens at the 2-position (**28** and **29**) provided similar potency to **2** at the cellular level although the effect was subtle compared to the unsubstituted benzyl ether (**27**). Larger groups at the 2-position (**31** and **32**) gave analogs that were less potent. Substitution at either the 3- or 4-position also resulted in a reduction in potency regardless of the size of the substituent (**35**–**39**).

To expand the SAR of this template, a series of analogs was prepared in which the benzyl ether moiety was replaced with other ethers (Table 4). This series included saturated alkyl-, aryl-, and heteroaryl-containing ethers. Extending the length of the alkyl chain between the oxygen and the aryl ring was not effective at enhancing potency (**40** and **41**). Although saturated alkyl ethers **42** and **43** maintained similar enzyme potency to benzyl ether **27**, there was an approximately threefold drop in the cellular potency. Thiophenes linked by a methylene unit were tolerated (**44**–**46**), while furans proved less effective (**47** and **48**). Finally, while an unsubstituted pyridine analog (**49**) had reduced potency, incorporation of a bromide at the 2-position (**50**) restored potency.

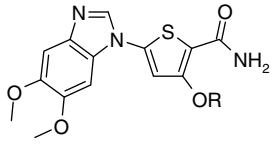
To determine if the primary amide was the optimal group for the 2-position of the thiophene, modifications were made at this position (Table 5). Only the acid **52** and the thioamide **55** maintained similar enzyme potency to **2**; however, both molecules suffered from poor cellular potency. It may be that these compounds lack the necessary properties to penetrate the cell. It is clear that

Table 3
Benzyl ether SAR with the 5,6-dimethoxybenzimidazole



Compound	R	PLK1 IC_{50} (nM)	HCT116 IC_{50} (nM)
2	2-CF_3	2	699
27	H	12	1090
28	2-Br	2	926
29	2-Cl	2	999
30	2-CN	6	2630
31	2-SO_2Me	31	5430
32	2-COMe	17	2640
33	2-OCF_3	5	1150
34	2-OMe	8	2110
35	3-NMe_2	31	3470
36	3-NH_2	21	2270
37	3-CN	99	>30,000
38	4-OMe	13	12,100
39	4-SO_2Me	75	>30,000

Table 4
Benzyl ether replacement SAR



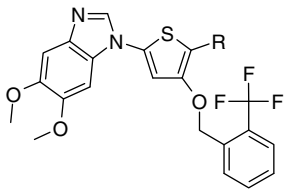
Compound	R	PLK1 IC_{50} (nM)	HCT116 IC_{50} (nM)
2	2-Trifluoromethylbenzyl	2	699
27	benzyl	12	1090
40	CH_2CH_2Ph	130	24,100
41	$CH_2CH_2CH_2Ph$	61	7750
42	Cyclopentylmethyl	34	3380
43	Cyclohexylmethyl	17	3090
44	2-Thienylmethyl	11	1540
45	3-Thienylmethyl	17	1520
46	(3-Chloro)-2-thienyl)methyl	2	1480
47	2-Furylmethyl	27	>25,000
48	3-Furylmethyl	190	19,000
49	4-Pyridinylmethyl	130	21,000
50	(2-Bromo-4-pyridinyl)methyl	14	1770

the primary amide is preferred over substituted amides (**57** and **58**).

Compound **2** was selected for profiling against additional cancer cell lines in a 3-day proliferation assay (Table 6).[11] Most of the cell lines profiled showed similar sensitivity to compound **2** as the HCT116 cells, with the exception of the PC-3 cell line. The reason for this is unclear at the moment as PLK1 expression in PC-3 cells is roughly equivalent to that found in HCT116 and MCF7 cells as measured using PCR.[13]

Compound **2** is representative of the selectivity observed with this series (Table 7). Not surprisingly, **2** is a potent inhibitor of PLK3, as there is a high homology in the ATP-binding site of PLK1 and PLK3. The consequences of inhibiting PLK3 are not as well understood as those for PLK1; however, PLK3 is also involved in the cell cycle, and its highest levels are found during S phase through M phase. With regard to non-familial kinases, these compounds possess some noteworthy activity against VEGFR2, VEGFR3, C-FMS, and PDGFR1β; however, the overall selectivity profile for **2** appears excellent.[14]

Table 5
Primary amide replacement SAR



Compound	R	PLK1 IC_{50} (nM)	HCT116 IC_{50} (μM)	Synthesis
2	$CONH_2$	2	699	—
51	CO_2Me	>1000	—	—
52	CO_2H	2	7660	a
53	CN	700	—	b
54	1*H*-Tetrazol-5-yl	73	>30,000	c
55	$CSNH_2$	6	>30,000	d
56	COMe	85	—	e
57	CONHMe	>1000	—	f
58	$CONMe_2$	>1000	—	g

Synthesis: (a) **51**, 1 N LiOH, dioxane; (b) **2**, 2-Chloro-1,3-dimethylimidazolinium chloride[12], Et_3N, TFA, CH_2Cl_2; (c) **53**, NaN_3, NH_4Cl, DMF, microwave; (d) **2**, Lawesson's reagent, dioxane, 80 °C; (e) i—**52**, EDC, HATU, DIEA, HNMe(OMe)·HCl; ii—MeMgCl, THF; (g) i—**52**, $SOCl_2$, PhMe, 90 °C; ii—NH_2Me, CH_2Cl_2; (g) i—**52**, $SOCl_2$, PhMe, 90 °C; $HNMe_2$, CH_2Cl_2.

Table 6
Cellular proliferation inhibition activity for **2**

Cell line	Tumor type	IC_{50} (nM)
A549	Lung adenocarcinoma	411
H460	Lung adenocarcinoma	380
HCT116	Colorectal carcinoma	699
HN5	Head and neck squamous cell carcinoma	678
MCF7	Breast adenocarcinoma	558
N87	Gastric carcinoma	601
PC-3	Prostate adenocarcinoma	6820
RKO	Colorectal carcinoma	398

Table 7
Enzyme inhibition profile of **2**

Kinase	IC_{50} (nM)
PLK1	2
PLK3	9
VEGFR3	92
PDGFR1β	160
VEGFR2	360
C-FMS	620
AURORA A	4800
EGFR	>48,000
GSK3	>15,000
P38α	>15,000
SRC	>30,000

Table 8
Mouse pharmacokinetic profile of **2**

Route	Dose (mg/kg)	Cl (mL/min/kg)	V_{SS} (L/kg)
iv	6.7	47.4	1.5
		AUC (ng h/mL)	*F*%
po	10	489	14

A pharmacokinetic study of **2** in mice was conducted to gauge its clearance and oral bioavailability (Table 8).[15] Compound **2** proved to be a moderate clearance molecule with a moderate volume of distribution. Although an iv route of administration for a

PLK1 inhibitor in the clinic was preferred, oral exposure data was also gathered. The oral bioavailability of **2** was low.

In summary, a novel series of thiophenes were developed as potent and selective inhibitors of PLK1. This work led to the identification of **2**, a useful tool compound for evaluation of PLK1 biology. Further description of the biological properties of **2** have recently been disclosed supporting its role as an effective anti-mitotic agent in vitro.[16] In addition, the work within this chemical template that resulted in the discovery of GSK461364 was recently disclosed.[17] GSK461364 is currently under evaluation in clinical trials. Subsequent communications regarding the extension of the SAR within this chemical series will be forthcoming.

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