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Imidazopyridine derivatives as potent and selective Polo-like kinase (PLK) inhibitors

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ABSTRACT

A novel class of imidazopyridine derivatives was designed as PLK1 inhibitors. Extensive SAR studies supported by molecular modeling afforded a highly potent and selective compound **36**. Compound **36** demonstrated good antitumor efficacy in xenograft nude rat model.

Polo-like kinases (PLKs) are categorized as serine-threonine kinases and this family includes three kinases, PLK1, PLK2 and PLK3. Among them, PLK1 participates in multiple steps in mitosis by phosphorylating various substrates. Inhibition of PLK1 activity in cancer cell causes mitotic arrest and finally induces strong cell-killing effect.[1–3] It is also reported that PLK1 is overexpressed in many clinical cancer samples, such as colon cancer, non-small cell lung cancer and others.[1,4,5] From these facts, PLK1 is thought to be a promising target for anti-cancer drug. In fact, several pharmaceutical companies have disclosed PLK1 inhibitors.[6–8] Of these, dihydropteridinone derivative *BI 2536* and thiophene amide *GSK 461364* are being evaluated in clinical studies (Fig. 1).[7,8]

Initially, we screened Merck chemical collection to identify a hit compound for starting the PLK1 program. Unfortunately, no hit compound with PLK1 potency (IC_{50}) of less than 1 μM was obtained. At the same time, another effort was to explore a new structure class based on known PLK1 inhibitors by chemical modifications such as bioisosteric replacement and/or scaffold hopping. For this purpose, benzimidazole derivative **1**[9] disclosed by GSK was selected as a starting point for our efforts because of its unique structure and strong enzyme potency. Here we report the design and SAR study of novel imidazopyridine PLK1 inhibitors.

Before starting the modification, we investigated the binding mode of compound **1** in ATP binding pocket of PLK1.[10] Compound **1** was selected for the docking study in terms of simple

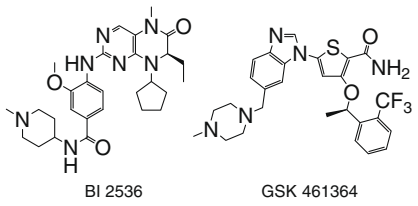

Figure 1. Structures of BI 2536 and GSK 461364.

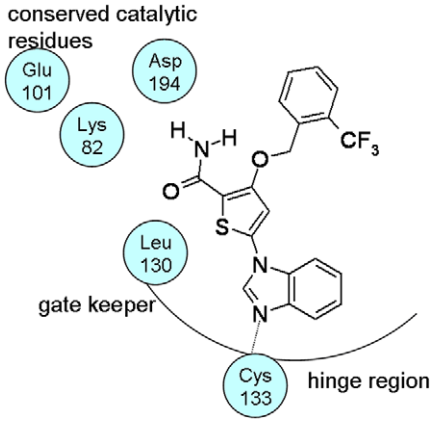

Figure 2. Docking study of compound **1**.

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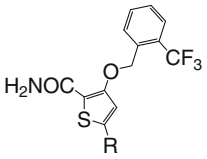
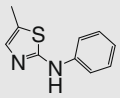
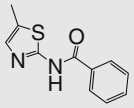
structure and good enzyme potency. As a result, it seemed that Cys133 in the hinge region may interact with the nitrogen of the benzimidazole by a hydrogen bond and the amide function may bind to the conserved catalytic residues (Fig. 2). This information prompted us to convert the benzimidazole into other heterocycles which potentially bind to Cys133. Encouragingly, pyrazolopyridine (**2**) and imidazopyridine (**3**) were found to replace the benzimidazole (Table 1). Deletion of the phenyl moiety in **3** (**4**) resulted in the reduction in potency, suggesting that the bicyclic aromatics could well occupy the ATP binding pocket. Aminothiazoles (**5–7**) which are known as a hinge binder of the reported kinase inhibitors,[11] were also tested. However, they completely lost PLK1 potency. Compound **3** was selected for further modification based on the highest enzyme potency in this series.

Next, the effect of the substituents around the benzyl moiety in compound **3** was investigated (Table 2). According to the patent information[9] reported by GSK, *ortho*-substituted compounds (**3, 8**) and methylated compounds (**9, 10**) at the benzylic position were tested. Replacement of CF_3 to Cl (**8**) significantly improved cell potency[12] with maintained enzyme potency. Introduction of a methyl group with (*R*)-configuration (**9**) enhanced both enzyme and cell potencies. However, compound **9** suffered from the large discrepancy between enzyme and cell potencies (44-fold). We speculated that high liphophilicity and low aqueous solubility of compound **9** may affect permeability, resulting in the reduction of the cell potency. Taking the relationship between cell potency, log *D* value and aqueous solubility into consideration, compound **9** was further optimized to identify analogs with better cell potency by incorporating hydrophilic substituents.

Docking of compound **9** into PLK1 model led to the proposed binding mode shown in Figure 3.[10,13] In this docking model, imi-

Table 1
Replacement of benzimidazole



Compound	R	PLK1 (IC_{50}, μM)	Solubility (μM)[a] pH 7.4 buffer	log *D*7.4[b]
1		0.035	<1.0	4.6
2		0.130	1.2	4.8
3		0.022	1.2	4.2
4		0.430	NT	NT
5		>3.00	NT	NT
6		>3.00	NT	NT
7		2.10	NT	NT

[a] Ref. 16.
[b] Ref. 17.

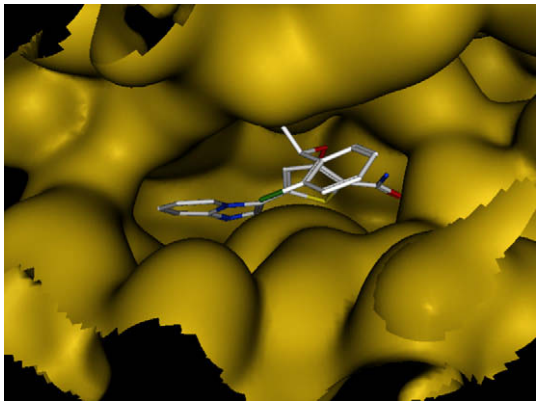
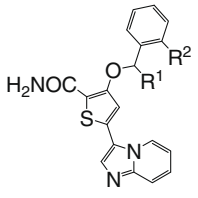


Figure 3. Docking study between homology model of PLK1 and compound **9**.

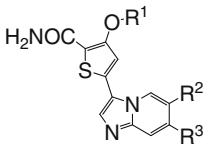
Table 2
Effect of substituents on *ortho*-position and benzylic position

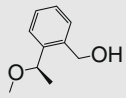
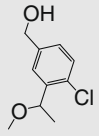
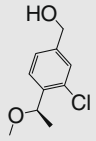


Compound	R^1	R^2	PLK1 (IC_{50}, nM)	Cell[a] (EC_{50}, μM)	Solubility (μM) pH 7.4 buffer	log *D*7.4
3	H	CF_3	22	5.00	1.2	4.2
8	H	Cl	35	1.15	1.3	4.1
9	(*R*)-Me	Cl	7.0	0.31	<1.0	4.8
10	(*S*)-Me	Cl	300	NT	NT	NT
1			35	2.00	<1.0	4.6

[a] Mitotic arrest in HeLaS3.

Table 3
Profile of hydroxymethyl analogs



Compound	R^1	R^2	R^3	PLK1 (IC_{50}, nM)	Cell (EC_{50}, μM)	Solubility (μM) pH 7.4 buffer	log $D_{7.4}$
11		H	H	88	3.75	50.1	3.2
12		H	H	39	1.16	26.1	3.7
13		H	H	4.9	0.08	13.8	3.8
14		CH_2OH	H	7.3	0.21	1.7	4.2
15[a]		H	CH_2OH	92	NT	1.7	4.1

[a] Diastereomer mixture.

dazopyridine 1-N was found in the position to interact with the hinge residue of Cys133, and the amide moiety was close to Lys82 and Asp194 as observed in the docking model of compound **1**. *Meta*- and *para*-positions of the benzyl group, and 5- and 6-positions of the imidazopyridine were facing towards the solvent accessible region, suggesting that hydrophilic substituents in these positions might be tolerated. Based on this model, we decided to explore the best position to incorporate hydrophilic substituents by introducing a hydroxymethyl group.

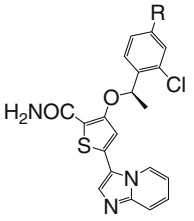
The result of the solvent region-directed modification is shown in Table 3. As expected by the modeling prediction, hydroxymethyl analogs (**13, 14**) maintained PLK1 enzyme potency, while compounds (**11, 12, 15**) decreased potency. In this modification, compound **13** showed the most promising data such as enzyme potency, cellular potency and chemical properties. Given the information that a hydroxymethyl at the *para*-position of the benzyl group in **13** may extend to the solvent region, we replaced the hydroxymethyl group by various amines to enhance the solubility and cell potency.

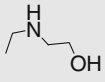
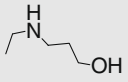
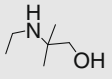
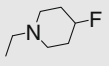
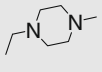
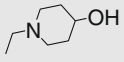
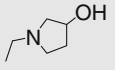
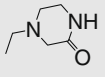
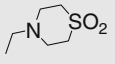
Table 4 summarizes the structure–activity relationship of amine derivatives. The amines we tested were generally tolerable for enzyme potency, suggesting that the solvent region accepted a wide range of structures regardless of the properties. On the other hand, improvement in cell potency was dependent on the nature of the amine groups such as bulkiness, lipophilicity and basicity. Especially, acyclic amines (**16**, **17**, **18**, **19**, **22**) exhibited better cell potency than **13** did. Unfortunately, these compounds were easily oxidized by air and light to generate the corresponding aldehyde. However, a *tert*-butyl group in **22** prevented the oxidation probably due to its steric bulkiness around the amine group. Moreover, aqueous solubility of compound **22** was greatly improved compared to **13**.

Additional enhancement in potency was observed by exploring the *ortho*-substituent of the benzyloxy group in **22** (Table 5). Since the patent information[9] implied that small and lipophilic substituents such as trifluoromethyl and chloro groups were tolerable, we replaced the chloro group in **22** by substituents with appropriate size and lipophilicity. As a result, compound **36** with a difluoromethoxy group showed better enzyme and cell potencies as well as good solubility. With these encouraging data, **36** was selected for further evaluation.

Compound **36** displayed high selectivity for PLK1 over a panel of 212 kinases in the KinaseProfiler assays from Millipore.[14] Of 212 tested kinases, only 2 isoform kinases (PLK2 and PLK3) showed more than 50% inhibition at 1 μM. IC_{50} values for PLK2, PLK3 were 21 nM and 178 nM, respectively.

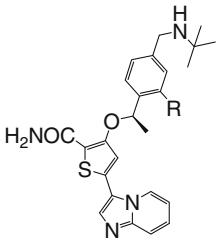
Table 4
Replacement of hydroxymethyl group in compound **13**



Compound	R	PLK1 (IC_{50}, nM)	Cell (EC_{50}, nM)	Solubility (μM) pH 7.4 buffer	log $D_{7.4}$
13		4.9	80	13.8	3.8
16		16	36	>170	1.9
17		22	48	>170	2.9
18		21	37	>170	2.8
19		28	68	>170	2.3
20		12	220	14.8	3.6
21		25	121	>170	3.5
22		21	48	>170	2.9
23		21	184	>170	2.4
24		19	219	>170	2.1
25		23	120	>170	2.9
26		13	400	11.2	3.7
27		17	120	NT	2.8
28		27	390	169	3.3
29[a]		20	227	>170	3.3
30		12	1700	45.7	2.8
31		16	400	2.9	3.2

[a] Diastereomer mixture.

Table 5
Effect of *ortho*-substituents in compound **22**



Compound	R	PLK1 (IC_{50}, nM)	Cell (EC_{50}, nM)	Solubility (μM) pH 7.4 buffer	log $D_{7.4}$
22	–Cl	21	48	>170	2.9
32	–F	46	290	>170	2.2
33	–Me	150	NT	NT	NT
34		210	NT	NT	NT
35	$-CHF_2$	20	82	>170	2.6
36	$-OCHF_2$	9.8	19	>170	2.7

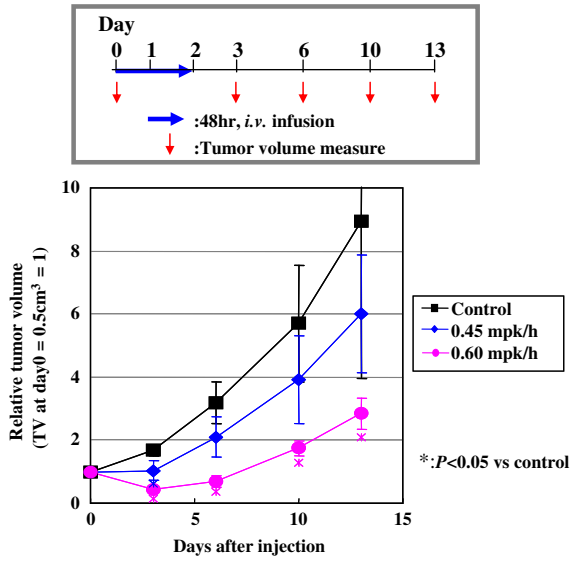
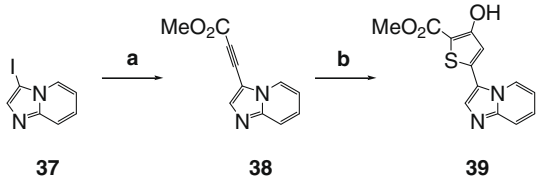


Figure 4. In vivo antitumor efficacy of compound **36**.

Table 6
Tumor growth inhibition (%) after dosing of compound **36**

	Plasma concentration at 48 h (nM)	Day 3 (%)	Day 6 (%)	Day 10 (%)	Day 13 (%)
0.45 mpk/h	50	38	34	31	33
0.60 mpk/h	80	74	78	69	68

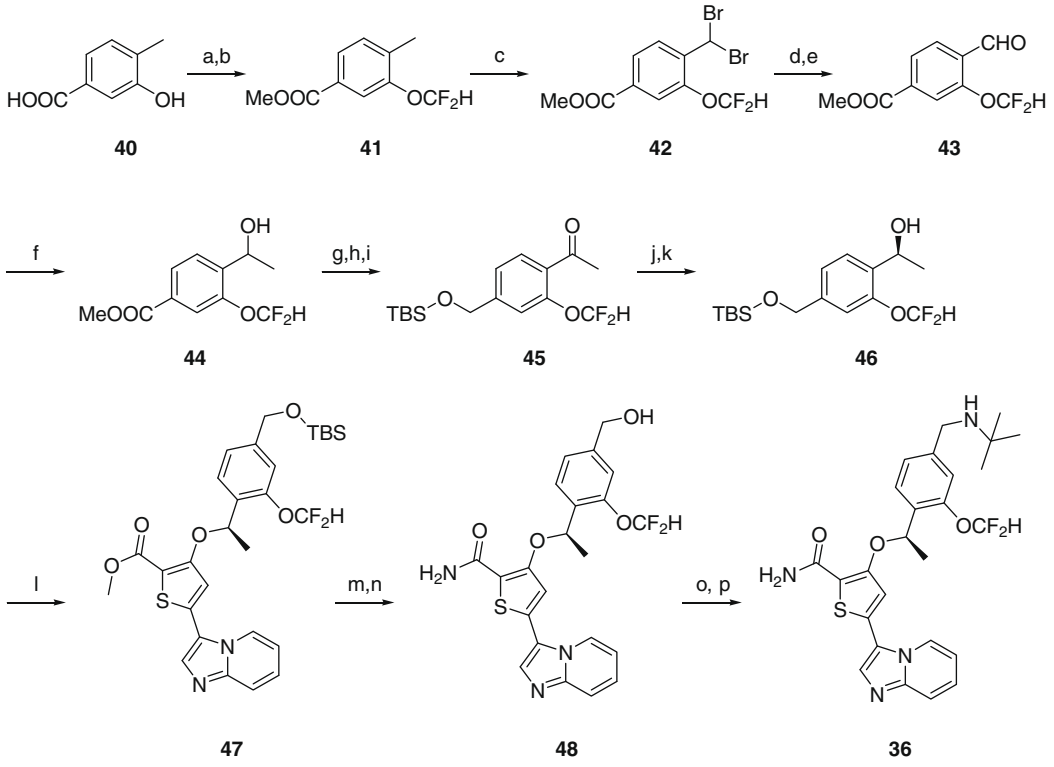
In vivo antitumor efficacy of compound **36** was examined in Hela-luc xenograft bearing rats (Fig. 4 and Table 6). Iv infusion of compound **36** for 48 h demonstrated significant tumor growth inhibition. 38% and 74% growth inhibition were observed on day 3 after infusion at 0.45 mpk/h and 0.60 mpk/h, respectively. In addition, administration induced mild and reversible white blood cell reduction, but did not cause severe toxicity such as body weight loss and diarrhea.



Scheme 1. Synthesis of compound **39**. Reagents and conditions: (a) methyl propiolate, $PdCl_2(PPh_3)_2$, CuI, K_2CO_3, 30 °C, THF; (b) methylthioglycolate, MeONa, MeOH, 58% in two steps.

The synthetic route for **36** is illustrated in Schemes 1 and 2. The preparation started with Sonogashira reaction of methyl propiolate with 3-iodoimidazopyridine **37**.[15] Michael addition of methyl thioglycolate to **38** and subsequent intramolecular cyclization gave a key intermediate **39** (Scheme 1). For the benzyl alcohol part, commercially available 3-hydroxy-4-methylbenzoic acid (**40**) was used as a starting material. Difluoromethylation of **40** followed by esterification produced ester **41**. Conversion of the methyl group in **41** to an aldehyde **43** was conducted in three steps. Dibromination of the methyl group and substitution with KOAc followed by hydrolysis provided the aldehyde **43**. Methylation of **43** with Grignard reagent afforded alcohol **44**. Reduction of an ester group in **44**, protection of the primary alcohol and oxidation of the secondary alcohol with SO_3/pyridine gave ketone **45**. Chiral reduction of the ketone **45** using (*R*)-2-methyl-CBS-oxazaborolidine gave **46** (95%ee) which was treated with Lipase PS-C to enrich the optical purity, providing enantiopure **46** (>99%ee). Mitsunobu reaction of **46** with the key intermediate **39** afforded ester **47**. Treatment of **47** with methanolic ammonia and deprotection of TBS group provided amide **48**. Finally, mesylation of the resulting alcohol in **48** followed by substitution with *t*-butylamine furnished compound **36**. Other derivatives were prepared in a similar method to **36**.

In summary, we identified a highly potent and selective PLK1 inhibitor **36** with an imidazopyridine scaffold based on molecular modeling with compound **1** and following extensive chemical modifications. In addition, compound **36** displayed good in vivo antitumor efficacy. Thus **36** represents a potential lead for the treatment of cancer. Further studies of this class are ongoing and the results will be reported elsewhere.



Scheme 2. Synthesis of compound **36**. Reagents and conditions: (a) $SOCl_2$, MeOH, reflux; (b) $ClF_2CCOONa$, K_2CO_3, MeCN, reflux, 58% in two steps; (c) NBS, $(PhCOO)_2$, CCl_4, 70 °C; (d) KOAc, MeCN, reflux; (e) NaOMe, MeOH/THF, rt, 81% in three steps; (f) MeMgCl, THF, −20 °C; (g) $LiBH_4$, THF, 50 °C; (h) TBSCl, imidazole, DMF, rt; (i) SO_3/pyridine, DMSO, 70% in four steps. (j) BH_3–DMS, (*R*)-2-methyl-CBS-oxazaborolidine, THF, 0 °C; (k) lipase PS-C, vinyl acetate, hexane, 40 °C, 69% in two steps; (l) **39**, *n*-Bu_3P, DIAD, THF, rt; (m) NH_3, MeOH, 70 °C in sealed tube; (n) TBAF, THF, rt, 85% in three steps; (o) MsCl, *i*-Pr_2NEt, $CHCl_3$; (p) *t*-$BuNH_2$, DMSO, 68% in two steps.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.06.084.

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12. It is well-known that inhibition of PLK1 leads to mitotic arrest. Therefore, mitotic arrest was measured to assess cell potency of the compounds. See Supplementary data.
13. The Conolly molecular surface of the binding pocket is colored in gold with Molecular Operating Environment (MOE) 2006.08 (Chemical Computing Group Inc., Montreal, Quebec, Canada)
14. Compound **36** was tested against a panel of kinases. Notably, it had <50% inhibition @ 1 μM against Abl, Abl(T315I), ALK, ALK4, AMPK, Arg, ARK5, ASK1, Aurora A, Axl, Blk, Bmx, BRK, BrSK1, BrSK2, BTK, CaMKI, CaMKII, CaMKIV, CDK1/cyclinB, CDK2/cyclinA, CDK2/cyclinE, CDK3/cyclinE, CDK5/p25, CDK5/p35, CDK6/cyclinD3, CDK7/cyclinH/MAT1, CDK9/cyclinT1, CHK1, CHK2, CK1γ1, CK1γ2, CK1γ3, CK1d, CK1, CK2, cKit(D816V), cKit, c-RAF, CSK, cSRC, DAPK1, DAPK2, DCAMKL2, DDR2, DMPK, DRAK1, DYRK2, eEF-2K, EGFR, EGFR(L858R), EGFR(L861Q), EphA1, EphA2, EphA3, EphA4, EphA5, EphA6, EphA7, EphA8, EphB1, EphB2, EphB3, EphB4, ErbB4, Fer, Fes, FGFR1, FGFR2, FGFR3, FGFR4, Fgr, Flt1, Flt3(D835Y), Flt3, Flt4, Fms, Fyn, GRK5, GRK6, GSK3α, GSK3β, Hck, HIPK1, HIPK2, HIPK3, IGF-1R, IKKα, IKKβ, IR, IRR, IRAK1, IRAK4, Itk, JAK2, JAK3, JNK1α1, JUK2α2, JNK3, Lck, LIMK1, LKB1, LOK, Lyn, MAPK1, MAPK2, MAPKAP-K2, MAPKAP-K3, MARK1, MEK1, Met, MINK, MKK4, MKK6, MKK7β, MLCK, MLK1, Mnk2, MRCKα, MRCKβ, MSK1, MSK2, MSSK1, MST1, MST2, MST3, MuSK, NEK2, NEK3, NEK6, NEK7, NEK11, NLK, p70S6K, PAK2, PAK3, PAK4, PAK5, PAK6, PAR-1Bα, PASK, PDGFRα, PDGFRβ, PDK1, PhKγ2, PI3-kinaseβ, PI3-kinaseγ, PI3-kinaseδ, Pim-1, Pim-2, PKA, PKBα, PKBβ, PKBγ, PKCa, PKCβI, PKCβII, PKCγ, PKCδ, PKCε, PKCη, PKCι, PKCμ, PKCθ, PKCζ, PKD2, PKG1α, PKG1β, PRAK, PRK2, PrKX, PTK5, Pyk2, Ret, RIPK2, ROCK-I, ROCK-II, Ron, Ros, Rse, Rsk1, Rsk2, Rsk3, SAPK2a, SAPK2a(T106M), SAPK2b, SAPK3, SAPK4, SGK, SGK2, SGK3, SIK, SRPK1, SRPK2, STK33, Syk, TAK1, TBK1, Tie2, TrkA, TrkB, TSSK1, TSSK2, WNK2, WNK3, Yes, ZAP-70 and ZIPK.
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