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Short communication

Structure-based design and SAR development of novel selective polo-like kinase 1 inhibitors having the tetrahydropteridin scaffold

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ABSTRACT

Polo-like kinase 1 (Plk1) is a validated target for the treatment of cancer. In this report, by analyzing amino acid residue differences among the ATP-binding pockets of Plk1, Plk2 and Plk3, novel selective Plk1 inhibitors were designed based on BI 2536 and BI 6727, two Plk1 inhibitors in clinical studies for cancer treatments. The Plk1 inhibitors reported herein have more potent inhibition against Plk1 and better isoform selectivity in the Plk family than these two lead compounds. In addition, by introducing a hydroxyl group, our compounds have significantly improved solubility and may target specific polar residues Arg57, Glu69 and Arg134 of Plk1. Moreover, most of our compounds exhibited antitumor activities in the nanomolar range against several cancer cell lines in the MTT assay. Through this structure-based design strategy and SAR study, a few promising selective Plk1 inhibitors having the tetrahydropteridin scaffold, for example, **L34**, were identified and could be for further anticancer research.

1. Introduction

The family of Polo-like kinases (Plks) has five members, Plk1-5, which are serine/threonine kinases of the cell cycle in mammalian cells and play important roles in cell cycle regulation and are critical targets for therapeutic invention, mainly in the field of cancer [1–4]. Plk1 and Plk4 are targets for cancer therapy, and Plk3 is considered to be a tumor suppressor, whereas, Plk2 has been debated as a target to treat cancer or a tumor suppressor [5–7]. In addition, Plk2 is considered as a possible target for Parkinson's disease [8].

Plk1-3, possessing two different functionally targeted sites: *N*-terminal catalytic domain (NCD) and C-terminal noncatalytic polo-box domain (PBD) [9,10], share a high level of structural and sequential similarity. Whereas, Plk4-5 are structurally the most distinct members of the family [11].

Plk1, a key mitotic regulator, is the most extensively studied and characterized among Plk1-5 and has been validated as an broad-spectrum anticancer target [12,13]. Plk1 overexpression is found in up to 80% of malignancies including breast, non-small cell lung, colorectal, prostate, pancreatic, papillary thyroid, ovarian, head and neck and non-Hodgkin's lymphoma. A few Plk1 inhibitors, such as BI 2536 [14,15], BI 6727 (volasertib) [16], GSK 461364, GW843682X [17,18], have advanced into clinical trials and showed encouraging anticancer effects in many kinds of tumors [2,13,19]. Among these promising anticancer agents, BI 6727 has reached phase III trials and showed inspiring results and therefore was awarded breakthrough drug status in 2013 and orphan drug status for acute myeloid leukemia in 2014. Nevertheless, no Plk1 inhibitors have been licensed by the FDA for cancer treatment.

Drug solubility is a major challenge for drug development. Especially, anticancer drugs are notorious for their poor solubility and are hard to design oral dosage forms [20]. BI 2536 and BI 6727

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have been taken via intravenous drip infusion in the clinical trials and their solubilities have limited their applications. Therefore, developing potent selective Plk1 inhibitors with satisfactory solubility is extremely desired in this field.

Structure-based drug design (SBDD) has played great roles for drug discovery [21,22]. In our previous work, we have successfully obtained novel selective Plk2 inhibitors with high potency by employing a SBDD strategy, mainly by analyzing critical amino acid residue differences in the binding sites among Plk1, Plk2 and Plk3 [5]. This work reports our similar efforts of identifying more potent and more soluble selective Plk1 inhibitors than BI 2536 and BI 6727, two drugs serving for lead compounds herein. Compared with BI 2536, several Plk1 inhibitors with improved solubility, Plk1 inhibition, isoform selectivity, and antiproliferative activity were yielded through this strategy.

2. Results

2.1. Structure-based design of selective Plk1 inhibitors

It was reported that the $-OCH_3$ group of BI 2536 is an important specificity determinant against non-Plks by taking advantage of a small pocket generated by Leu132 in the hinge region of Plk1 [23]. However, we observed that in the close proximity of this $-OCH_3$ group in the ATP binding site of Plk1 (PDB ID: 2RKU [23]), there are three charged polar residues: Arg57, Glu69, and Arg134. Extending a polar group from the $-OCH_3$ of BI 2536 to interact with these three residues, the potency against Plk1 and isoform or kinome selectivity may be increased. We further aligned kinase-inhibitor complexes of Plk1 (PDB ID: 2RKU), Plk2 (PDB ID: 4I6F [8]) and Plk3 (PDB ID: 4B6L) and noticed there are differences for the residues around the $-OCH_3$ group (see Fig. 1A) among the ATP-binding pockets of Plk1-3. Two most noticeable variances are: (1) Tyr161 of Plk2, in which both of the equivalent ones of Plk1 and Plk3 are leucine respectively; (2) Arg134 of Plk1, in which both of the corresponding residues of Plk2 and Plk3 are serine. We assumed that an extended polar group from the $-OCH_3$ of BI 2536 may form hydrogen bonds with part or all of these three polar residues of Plk1, whereas, it may conflict with Tyr161 of Plk2, and does not form hydrogen bonds with Ser163 (Plk2) and Ser143 (Plk3) because of the distances. Hydroxyl is both a hydrogen bond donor and acceptor, and it has great impact on aqueous solubility of compounds. Therefore, it is reasonable to extend a $-OH$ from the $-OCH_3$ of BI 2536 and we designed novel Plk1 inhibitors and did SAR development according to Fig. 2 for the purpose to discover highly potent selective Plk1 inhibitors with improved solubility.

A supposed compound was docked into the ATP binding site of Plk1 by employing Glide 6.7 in the Schrödinger Suite. The modeling results verified our assumptions: the extended $-OCH_2CH_2OH$ group forms three possible hydrogen bonds with Arg57, Glu69 and Arg134 (Fig. 1B), whereas, this $-OCH_2CH_2OH$ group would conflict with the bulky Tyr161 of Plk2, and only forms one possible hydrogen bond with Glu78 of Plk3. This result inspired us and we synthesized many compounds and assayed their potency against Plk1-3.

2.2. Biological evaluation

Compounds were assayed for their Plk1-3 inhibitory activities and isoform selectivity among Plk1-3 using the FRET-based Z′-Lyte assay system. More details about this assay can be found in the Supporting Information.

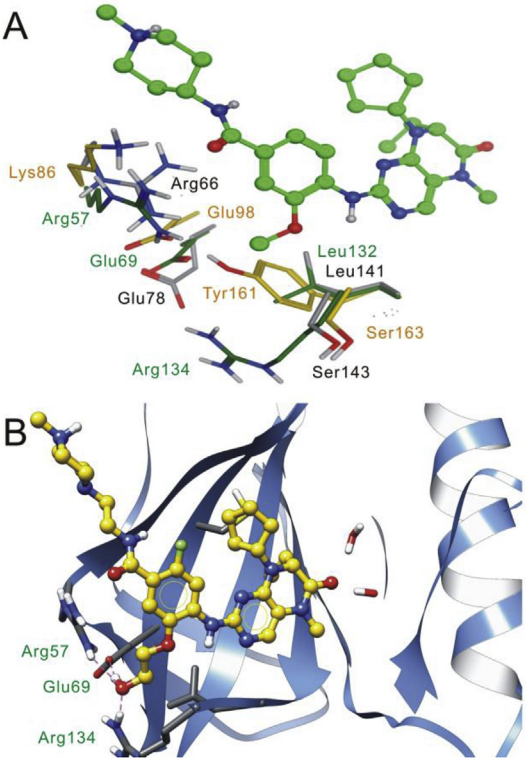


Fig. 1. (A) Differences of the residues among the active sites of Plk1 (green), Plk2 (orange) and Plk3 (grey). BI 2536 binding to Plk1 is shown as green ball and stick mode. (B) Modeling results indicated that the extended $-OCH_2CH_2OH$ group could form three possible hydrogen bonds with Arg57, Glu69 and Arg134 of Plk1. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

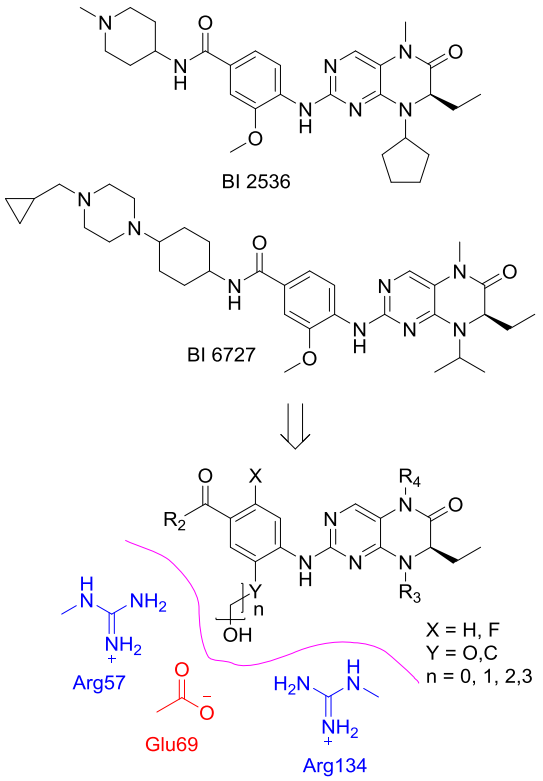
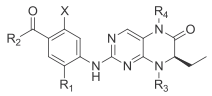


Fig. 2. Design strategy and SAR development of novel Plk1 inhibitors which may interact with three proximal polar residues, Arg57, Glu69 and Arg134 of Plk1, around the $-OCH_3$ of BI 2536.

Table 1
Enzyme inhibition and isoform selectivity of compounds **L1** − **L39** against Plk1-3.[a].



Compd	R_1	R_2	R_3	R_4	X	IC_{50} (nM) for Plks			Selectivity against Plk1	
						Plk1	Plk2	Plk3	Plk2/Plk1	Plk3/Plk1
L1	$-OCH_3$			Me	-H	13.97	3.98	6.04	0.28	0.43
L2	$-OCH_3$			$-CH_2CH_2OH$	-H	291.6	3474	>10 μM	11.73	>33.77
L3	$-OCH_3$			$-CH_2OCH_3$	-H	1200	>10 μM	>10 μM	>8.33	>8.33
L4	$-OCH_3$			-H	-H	52.06	75.77	3011	1.46	57.84
L5	-OH			Me	-H	5.89	7.98	30.40	1.35	5.16
L6	$-CH_3$			Me	-H	5.75	4.72	18.91	0.82	3.29
L7				Me	-H	884.6	1796.9	1093	2.03	1.24
L8				Me	-H	344.7	898.4	1252	2.61	3.63
L9				Me	-H	10.17	36.04	112.8	3.54	11.09
L10				Me	-H	9.31	152.0	452.0	16.32	48.55
L11				Me	-H	4.27	338.7	554.5	79.32	129.86
L12				Me	-H	9.42	230.9	150.2	24.43	15.95
L13				Me	-H	17.29	743.9	205.2	43.02	11.87
L14		CH_3-		Me	-H	5.76	131.7	91.93	22.86	15.96
L15		NH_2-		Me	-H	20.72	290.5	162.8	14.02	7.86
L16				Me	-H	15.66	195.9	157.4	12.51	10.05
L17				Me	-H	21.38	314.2	406.9	14.70	19.03
L18				Me	-H	6.86	85.11	142.5	12.41	20.78
L19				Me	-H	30.31	640.8	476.7	21.14	15.73
L20				Me	-H	40.74	626.2	385.3	15.37	9.45
L21				Me	-H	34.94	498.0	440.6	14.25	12.61
L22				Me	-H	21.21	873.6	1351	41.19	63.70
L23				Me	-H	17.90	385.3	166.1	21.53	9.28
L24				Me	-H	38.02	265.1	699.6	6.974	18.40

(*continued on next page*)

Table 1 (*continued*)

Compd	R_1	R_2	R_3	R_4	X	IC_{50} (nM) for Plks			Selectivity against Plk1	
						Plk1	Plk2	Plk3	Plk2/Plk1	Plk3/Plk1
L25				Me	-H	126.3	>10 μM	>10 μM	>79.18	>79.18
L26				Me	-H	123.8	>10 μM	>10 μM	>80.78	>80.78
L27				Me	-H	177.0	>10 μM	>10 μM	>56.50	>56.50
L28				Me	-F	3.58	122.2	116.6	34.13	32.57
L29				Me	-F	5.14	61.17	84.80	11.90	16.50
L30				Me	-F	9.97	107.2	43.78	10.75	4.39
L31				Me	-F	7.99	107.0	46.93	13.39	5.87
L32				Me	-F	7.20	33.83	45.07	4.70	6.26
L33				Me	-F	7.28	643.0	436.2	88.32	59.92
L34				Me	-F	3.89	30.51	22.14	7.84	5.69
L35				Me	-F	10.17	167.3	196.0	16.45	19.27
L36				Me	-F	20.00	397.3	366.3	19.87	18.32
L37				Me	-F	126.8	3180	2977	25.08	23.48
L38				Me	-F	26.77	718.3	749.7	26.83	28.01
L39				Me	-F	52.61	868.7	974.33	16.51	18.51
Staurosporine	–	–	–	–	–	864.6	957.2	>10 μM	1.107	>11.57
BI 2536	$-OCH_3$			Me	-H	7.06	13.55	20.78	1.92	2.94

[a] Each IC_{50} is the mean ± SEM from three experiments.

In our assay, BI 2536, as the positive control compound, received IC_{50} values of 7.06, 13.55, and 20.78 nM against Plk1-3 respectively (see Table 1), which is consistent with the reported values [23], implying that BI 2536 is a potent but not so selective Plk1 inhibitor. Another control compound, staurosporine, a natural prototypical ATP-competitive kinase inhibitor, inhibited Plk1-3 with IC_{50}s of 864.6 nM, 957.2 nM and more than 10 μM. We initiated our project by doing a simple SAR by modifying R_1, R_2, R_3 and R_4 of the scaffold (see Table 1). When 1-methylpiperidinyl (R_2) of BI 2536 was altered to cyclopentyl, the activity of compound **L1** against Plk1 dropped ~2 folds, and **L1** completely lost selectivity. When changing the methyl group (R_4) to $-CH_2CH_2OH$ (**L2**), $-CH_2OCH_3$ (**L3**), or just chopping it (**L4**), the activities against Plk1 dropped a lot. When altering the $-OCH_3$ group (R_1) to smaller groups such as $-OH$ (**L5**) or $-CH_3$ (**L6**), the inhibition was recovered. Bulky apolar groups (**L7** and **L8**) in this position led to dramatically dropped inhibitory activities against all of Plk1-3. This is not surprised because these groups may conflict with the surrounding residues in the active sites of all Plk1-3. When R_1 is $-CH_2CH_2OH$ (**L9**) and $-OCH_2CH_2OH$ (**L10**), these two compounds received improved Plk1 inhibition and isoform selectivity. Compound **L11** is the corresponding compound of BI 2536 in which the $-OCH_3$ is modified to $-OCH_2CH_2OH$. It got its IC_{50} value of 4.27 nM against Plk1, ~1.7 folds increment over BI 2536. It impressed us more with its remarkably improved isoform selectivity to Plk2 (79.32 versus 1.92) and Plk3 (129.86 versus 2.94) when compared with BI 2536, which confirms our design strategy is reasonable. Based on **L11**, we then further modified R_2 to different rings or linear fatty chains and got compounds **L12** – **L24**, among which, compound **L14** demonstrated the best Plk1 inhibition (IC_{50} = 5.76 nM) and good isoform selectivity. Compounds **L25** – **L27** were yielded by modifying R_3 from cyclopentyl to isopropyl based on compounds **L10** – **L12**, respectively. BI 6727 contains an isopropyl group, however, this modification led to dramatic dropping of the inhibition against all of Plk1-3 in our study.

Incorporation of fluorine into molecules can modulate their pharmacokinetics properties, including lipophilicity, electrophilicity, metabolic stability, chemical stability, etc., and the strategic incorporation of fluorine to improve drug potency has become gradually prevalent in drug discovery [24]. The benzene ring of BI 2653 is sandwiched by Leu59 and Arg136 in the active site of Plk1. Introducing a fluorine atom into the 2-position of the benzene may increase the hydrophobic interactions of the designed compounds

with Leu59 and Arg136 of Plk1. In addition, the 2-F would form a possible weak hydrogen bond with the 1-amide attached into this benzene, and thus configurate a more suitable binding conformation, which will result in improved potency. Hence, we incorporated a fluorine in the 2-position of the core, and yielded compounds **L28** – **L39**. Indeed, through this strategy, the inhibition against Plk1 was enhanced. For example, **L34** got an IC_{50} value of 3.89 nM against Plk1, in contrast to 21.21 nM of its precursor **L22**. Overall the isoform selectivity of **L28** – **L39** dropped a little bit.

Plk1 is overexpressed in up to 80% of malignancies, and was identified as a broad-spectrum anticancer target. Therefore, we investigated the antiproliferative activities of these 39 compounds, which were tested using six human tumor cell lines: K562 (human immortalized myelogenous leukemia cell line), MCF-7 (breast cancer cell line), HuH-7 (hepatocellular carcinoma cells), A549 (human lung cancer cell line), H1975 (human lung carcinoma cell line), and Hela (human cervical cancer cell line). As the control compound, BI 2536 received GI_{50} values of 60.5, 67.0, 28.4, 57.1, 130.0, 34.0 nM against each of them (see Table 2).

Generally, many of our compounds showed good cytotoxic activities in the nanomolar range. Among them, compounds **L5**, **L6**, **L9**, **L11**, **L12**, **L14**, **L16**, **L18**, **L28**, **L29**, **L32**, **L34** demonstrated equivalent or even better antiproliferative activities than BI 2536. The most impressive compound is **L34**, which showed GI_{50} values of 10.6, 28.2, 24.9, 20.2, 107.9, 8.03 nM against K562, MCF-7, HuH-7, A549, H1975 and Hela, respectively, 1–6 folds improvement over BI 2536 (see Fig. 3). In addition, **L34** got GI_{50} values of 9.47, 6.71, 21.0 nM against other three human cancer cell lines: DU145 (prostate cancer cell line), HT29 (colon adenocarcinoma cell line) and HL60 (leukemia cell line). The anticancer activities of our compounds were generally consistent with the IC_{50} values against Plk1, verifying the correlation between the *in vitro* Plk1 inhibitory activity and cellular cytotoxicity. Incorporation of a fluorine in the 2-position of benzene ring of the core not only improved the inhibition against Plk1, but also had good impact on the antiproliferative activities. For example, when comparing compound **L22** with **L34**, it is found that **L34** had 17–111 folds improvement to kill the six cell lines. The replacement of cyclopentyl in the position R_3 by an isopropyl (compounds **L25** – **L27**, **L37** – **L39**) led to seriously worsened antiproliferative activities in our study.

2.3. Chemistry

The synthesis of compounds **L1** – **L6** is illustrated in Scheme 1. Intermediates dihydropyridinone derivatives were synthesized according to the method in a previous study of ours [5]. Acylation of materials **1**–**3** using a cyclopentylamine afforded intermediates **a1** – **a3**. Nitro reduction of **b1** – **b3** using iron powder, followed by a Buchwald-Hartwig reaction, yielded compounds **L1** – **L6**.

The synthesis of compounds **L7** – **L27** is depicted in Scheme 2. Commercially available 3-hydroxy-4-nitrobenzoic acid (**4**) was converted to intermediates **c1** – **c14** by an amide reaction. Then, their nitro groups were reduced to offer intermediates **d1** – **d14**, which were converted to intermediates **e1** – **e17** using Buchwald-Hartwig coupling sequentially. **e1** – **e17** were further reacted with various substituted bromine derivatives, respectively, leading to compounds **L7** – **L27** by Mitsunobu reaction.

As shown in Scheme 3, compounds **L28** – **L39** were synthesized from 3-fluoro-4-nitrobenzonic acid (**5**), which was reacted with ethylene glycol to yield intermediate **6**, which then was converted to intermediates **f1** – **f9** by reacting with several various amides. **f1** – **f9** were further reduced to intermediates **g1** – **g9**. Compounds **L28** – **L39** were obtained from intermediates **g1** – **g9** by Buchwald–Hartwig coupling.

Several of our compounds were calculated for their aqueous

Table 2
Antiproliferative activities of compounds **L1** – **L39** against six different human cancer cell lines.[a,b,c,d]

Compd	GI_{50} (nM) to cancer cell lines					
	K562	MCF-7	HuH-7	A549	H1975	Hela
L1	84.0	85.5	70.4	64.6	NA	64.2
L2	4437	3722	4467	5020	3831	4820
L3	>10 μM	>10 μM	6757	8131	>10 μM	>10 μM
L4	809.7	744.3	429.5	729.4	1104	727.9
L5	10.7	6.25	12.2	10.3	20.2	9.74
L6	23.1	24.7	36.8	23.1	40.0	20.8
L7	958.4	722.6	322.5	572.9	NA	466.4
L8	1983	1802	1145	968.1	NA	1504
L9	57.4	64.9	25.5	76.8	196.6	45.1
L10	91.3	98.9	109.8	71.2	157.9	50.7
L11	69.6	61.5	71.3	36.0	130.9	29.2
L12	63.8	64.3	19.9	79.4	166.0	41.9
L13	65.8	140.4	216.2	138.3	170.6	122.5
L14	38.1	107	56.0	87.6	220	42.0
L15	132.3	251.8	128.6	446.7	1197	118.0
L16	46.9	135.1	52.4	84.4	361.3	25.4
L17	98.9	331.0	81.6	197.1	519.2	82.3
L18	47.4	162.3	65.9	79.3	677.1	24.7
L19	143.1	269.0	196.8	321.9	676.5	129.0
L20	239.1	196.8	NA	470.0	NA	152.9
L21	182.5	153.6	NA	240.4	NA	117.1
L22	187.2	871	876	540	3810	892.3
L23	188.3	207.7	NA	686.0	NA	187.1
L24	58.2	312.0	239.1	282.7	3200	132.8
L25	1249	1818	1449	2590	2366	1117
L26	6501	>10 μM	>10 μM	>10 μM	>10 μM	>10 μM
L27	772.6	1273	721.7	3441	3547	1748
L28	24.6	81.4	66.9	44.0	501.2	13.1
L29	60.0	43.8	79.3	67.9	164.9	33.4
L30	120.2	222.5	113.3	171.0	509.6	47.14
L31	86.3	130.0	140.6	98.3	252.6	39.4
L32	45.2	30.6	92.4	67.6	127.5	28.4
L33	265.2	158.4	349.1	352.1	567.2	123.1
L34	10.6	28.2	24.9	20.2	107.9	8.03
L35	1071	907.7	830.6	1193	2470	225.1
L36	100.1	404.7	231.9	172.2	1151	36.0
L37	730.7	1546	806.7	1717	5133	434.9
L38	540.2	833.1	884.9	852.0	2118	274.5
L39	915.1	931.2	1107	1170	1917	351.4
BI 2536	60.5	67.0	28.4	57.1	130.0	34.0

[a] BI 2536 was used as the positive control compound.
[b] Inhibition of cell growth by the listed compounds was determined by using CCK-8 assay.
[c] NA: not active up to the highest concentration tested.
[d] Standard error of the GI_{50} was generally less than 10%.

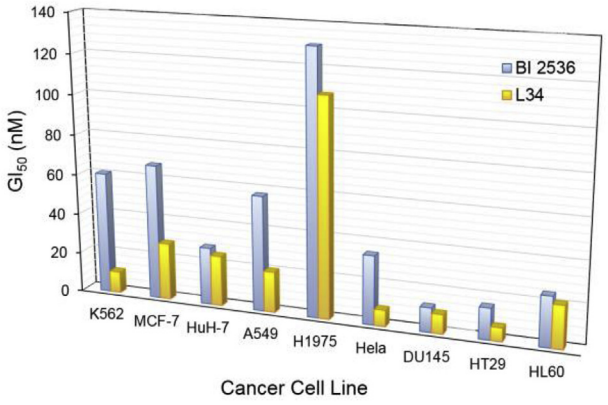
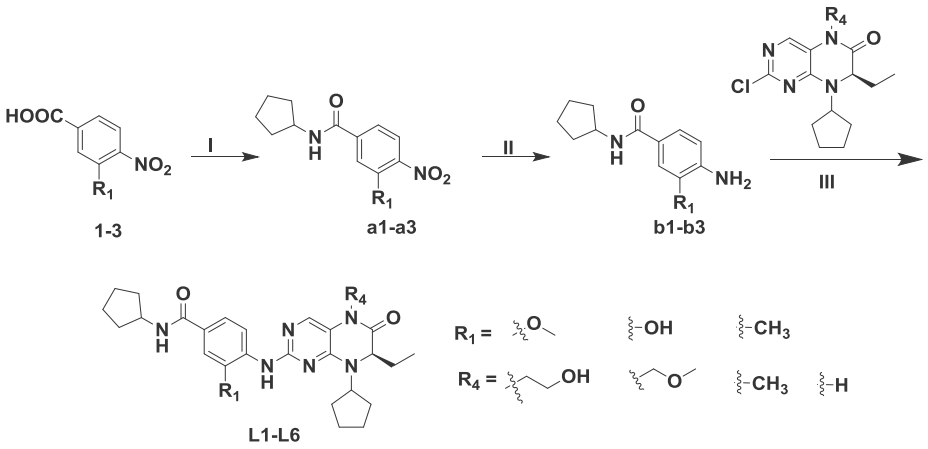
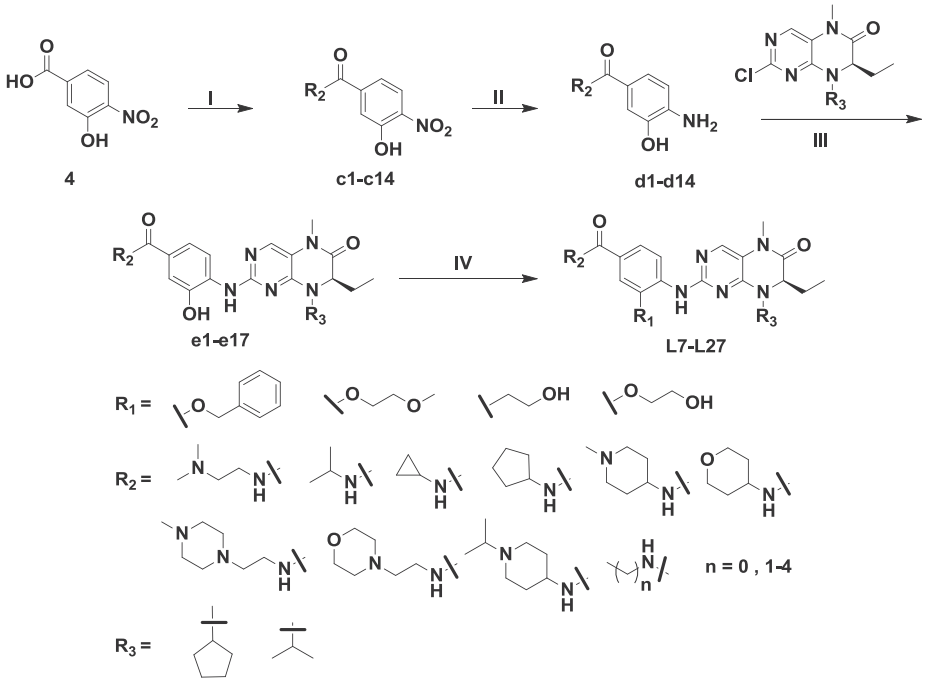


Fig. 3. Comparison of antiproliferative activities of BI 2536 (blue bars) and L34 (yellow bars) against nine human cancer cell lines. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)



Scheme 1. Synthesis of Compounds L1 − L6. *Regents and conditions*: I) Amine, HBTU, Et_3N, DMF, rt, 12 h; II) AcOH, Fe, 60 °C, 2 h; III) $Pd_2(dba)_3$, Xantphos, Cs_2CO_3, dioxane, 110 °C, 16 h.



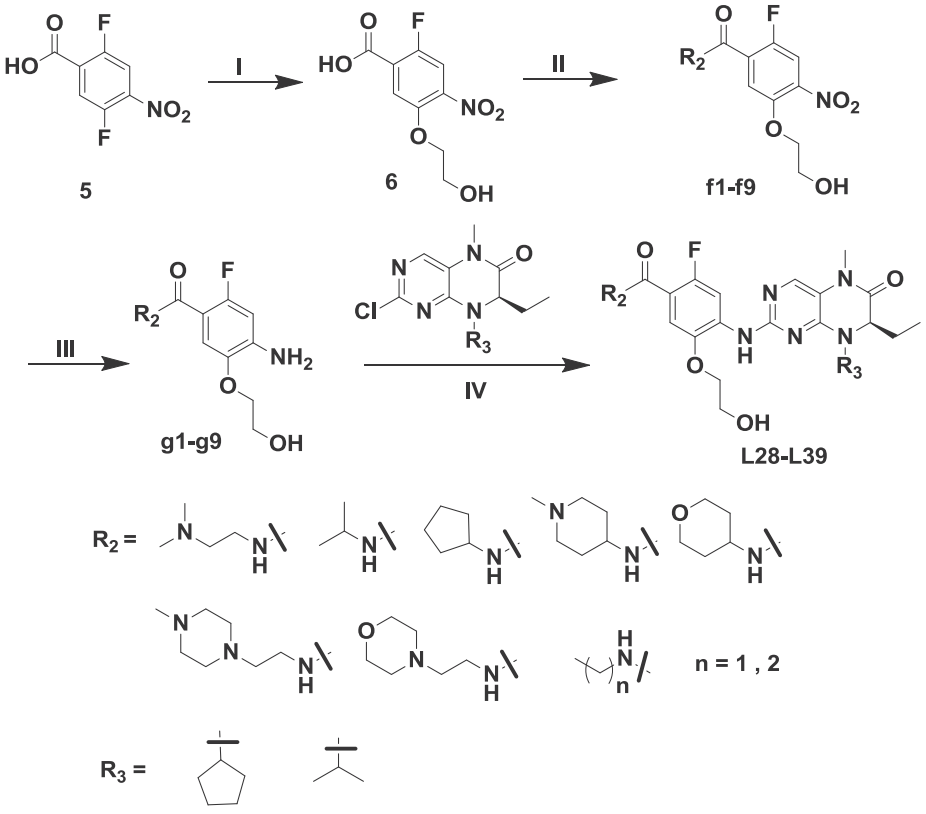
Scheme 2. Synthesis of Compounds L7 − L27. *Regents and conditions*: I) Amine, HBTU, Et_3N, DMF, rt, 12 h; II) AcOH, Fe, 60 °C, 2 h; III) $Pd_2(dba)_3$, Xantphos, Cs_2CO_3, dioxane, 110 °C, 16 h; IV) Benzyl bromide, 1-bromo-2-ethyl methyl ether or 2-bromoethanol, K_2CO_3, acetone, 60 °C, 12 h.

solubility (logS) and permeability (logP) using QikProp 4.4 and MOE 2014. It is clearly demonstrated that compounds containing an extra $-CH_2OH$ group, such as **L11**, have better aqueous solubility and lower logP values than the corresponding compounds, such as BI 2536 (see Table 3).

The calculation was verified by our further measurement of aqueous solubility of compounds **L5**, **L6**, **L11**, **L28**, **L29**, **L32**, **L34**, and BI 2536 by using UV−visible spectrophotometer in PBS buffer (pH 7.4). The UV−vis absorption spectra of the various concentration of four compounds are shown as examples in Fig. 4. BI 2536 has an aqueous solubility of 0.73 mg/mL. Introducing an extra $-CH_2OH$ (**L11**) from the methoxyl of BI 2536 remarkably improved the solubility by ~1.6 fold. Incorporation of a fluorine atom into the 2-position of **L11** dramatically deteriorated the solubility (**L28**, 0.53 mg/mL). However, **L34**, the compound having a fluorine atom and exhibiting the best biological activites both in the kinase and cell line assays, has an aqueous solubility value of 2.23 mg/mL, demonstrating our strategy to improve the Plk1 inhibition, selectivity among Plk1-3 and solublitiy is fruitful.

3. Conclusion

Plk1, a serine/threonine protein kinase, is an important regulator of cell cycle progression. Overexpression of Plk1 is often associated with oncogenesis, therefore, Plk1 is widely recognized as an oncogene and hence represents an attractive target for cancer



Scheme 3. Synthesis of Compounds L28 – L39. *Regents and conditions*: I) Ethylene glycol, KOH, 80 °C, 8 h; II) Amine, HBTU, Et_3N, DMF, rt, 12 h; III) AcOH, Fe, 60 °C, 2 h; IV) $Pd_2(dba)_3$, Xantphos, Cs_2CO_3, dioxane, 110 °C, 16 h.

Table 3
Predicted and measured aqueous solubility (logS[a]) and permeability (logP[b]).

Compd	QikProp 4.4			MOE 2014			Measured solubility (mg/mL)
	QPlogS	ClQPlogS	QPlogPo/w	logS	SlogP	logP(o/w)	
L5	−6.35	−6.00	3.83	−5.22	4.10	3.78	0.23
L6	−6.90	−6.31	4.69	−5.74	4.71	4.39	<0.07
L11	−4.87	−5.02	2.93	−4.92	2.92	2.35	1.14
L28	−5.15	−5.37	3.09	−5.22	3.06	2.53	0.53
L29	−5.73	−6.28	3.18	−5.46	2.57	3.14	0.18
L32	−5.90	−6.08	3.64	−5.42	3.37	2.81	<0.15
L34	−3.53	−4.17	2.18	−4.71	2.21	1.40	2.23
BI 2536	−5.36	−5.13	3.65	−5.13	3.56	3.04	0.73

[a] Predicted aqueous solubility. S in $mol \cdot dm^{-3}$.
[b] Predicted octanol/water partition coefficient.

intervention. A few Plk1 inhibitors have been entered into clinical trials, and among them, BI 6727 was awarded breakthrough drug status, however, no Plk1 inhibitors have been approved for cancer treatment yet.

To develop more soluble, potent and selective Plk1 inhibitors, in this report, by taking advantage of residue differences among the ATP-binding pockets of Plk1, Plk2 and Plk3, and employing a SBDD strategy and using BI 2536 and BI 6727 as the template compounds, we designed and synthesized a series of Plk1 inhibitors having the tetrahydropteridin scaffold, which may target three specific polar residues Arg57, Glu69 and Arg134 of Plk1.

Our strategy yielded several Plk1 inhibitors with better solubility, Plk1 inhibition, isoform selectivity, and antiproliferative activity than BI 2536, the most illustrious Plk1 inhibitor. Compound **L34** stood out from the compounds reported herein. It received an IC_{50} value of 3.89 nM against Plk1 and good selectivity over Plk2 and Plk3. This compound showed impressive GI_{50} values of 10.6, 28.2, 24.9, 20.2, 107.9, 8.03, 9.47, 6.71, 21.0 nM against nine cancer cell lines, K562, MCF-7, HuH-7, A549, H1975, Hela, DU145, HT29, and HL60 respectively in the antiproliferative activity assay. Remarkably, the aqueous solubility of this compound had ~3 folds improvement over BI 2536. All these imply that **L34** is a good candidate for further anticancer research.

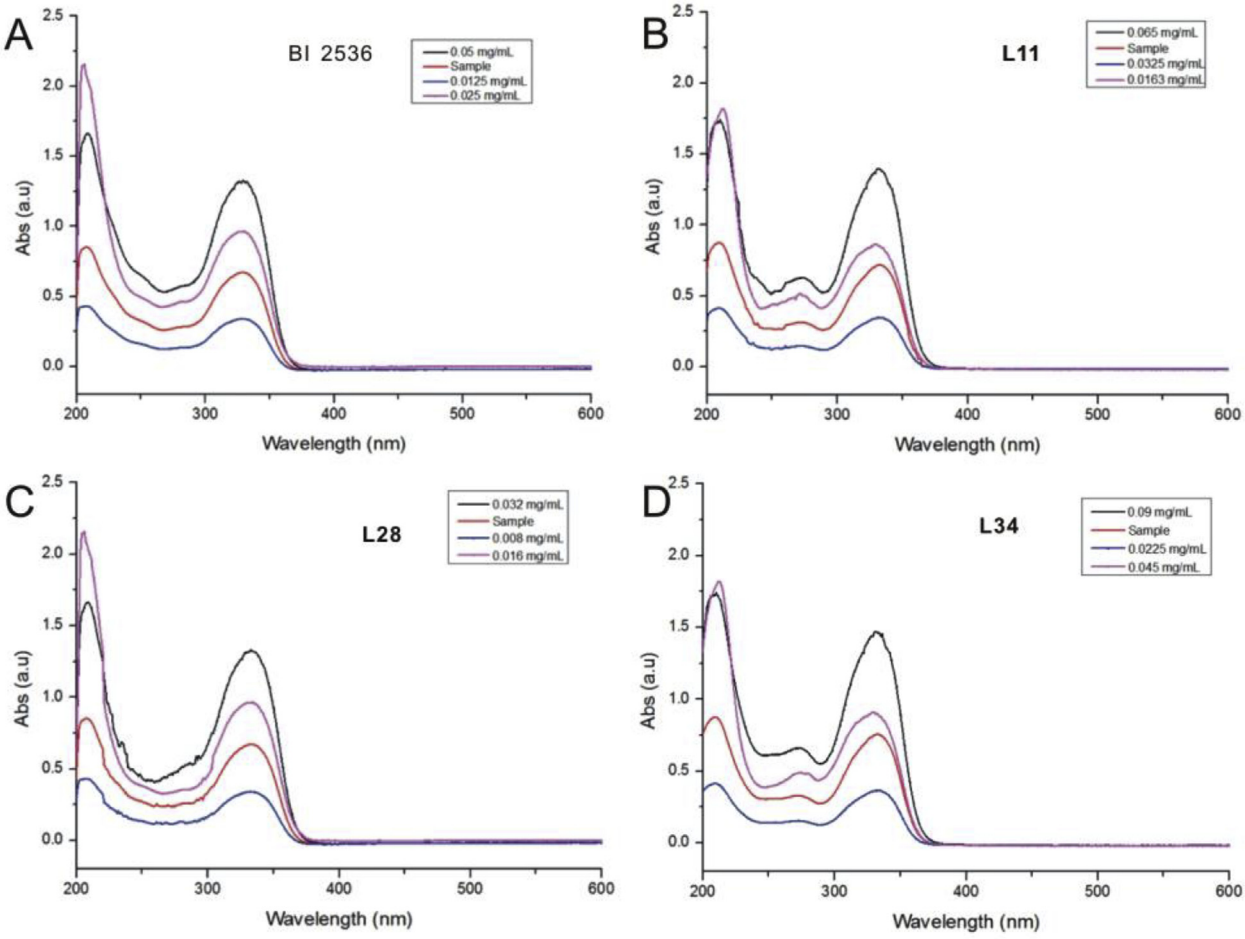


Fig. 4. UV–vis absorbance. (A)–(D): Absorption spectra of BI 2536, **L11**, **L28** and **L34** at various concentrations.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2019.111769.

References

[1] O. Goroshchuk, I. Kolosenko, L. Vidarsdottir, A. Azimi, C. Palm-Apergi, Polo-like kinases and acute leukemia, Oncogene 38 (2019) 1–16.
[2] C.Z. Liao, R.S. Yao, Diversity evolution and jump of Polo-like kinase 1 inhibitors, Sci. China Chem. 56 (2013) 1392–1401.
[3] N.D. Palmisiano, M.T. Kasner, Polo-like kinase and its inhibitors: ready for the match to start? Am. J. Hematol. 90 (2015) 1071–1076.
[4] K. Strebhardt, Multifaceted polo-like kinases: drug targets and antitargets for cancer therapy, Nat. Rev. Drug Discov. 9 (2010) 643–660.
[5] M.M. Zhan, Y. Yang, J. Luo, X.X. Zhang, X. Xiao, S. Li, K. Cheng, Z. Xie, Z. Tu, C. Liao, Design, synthesis, and biological evaluation of novel highly selective polo-like kinase 2 inhibitors based on the tetrahydropteridin chemical scaffold, Eur. J. Med. Chem. 143 (2018) 724–731.
[6] G. Cozza, M. Salvi, The acidophilic kinases PLK2 and PLK3: structure, substrate targeting and inhibition, Curr. Protein Pept. Sci. 19 (2018) 728–745.
[7] M.V. Reddy, B. Akula, S. Jatiani, R. Vasquez-Del Carpio, V.K. Billa, M.R. Mallireddigari, S.C. Cosenza, D.R. Venkata Subbaiah, E.V. Bharathi, V.R. Pallela, P. Ramkumar, R. Jain, A.K. Aggarwal, E.P. Reddy, Discovery of 2-(1H-indol-5-ylamino)-6-(2,4-difluorophenylsulfonyl)-8-methylpyrido[2,3-d] pyrimi din-7(8H)-one (7ao) as a potent selective inhibitor of Polo like kinase 2 (PLK2), Bioorg. Med. Chem. 24 (2016) 521–544.
[8] D.L. Aubele, R.K. Hom, M. Adler, R.A. Galemmo Jr., S. Bowers, A.P. Truong, H. Pan, P. Beroza, R.J. Neitz, N. Yao, M. Lin, G. Tonn, H. Zhang, M.P. Bova, Z. Ren, D. Tam, L. Ruslim, J. Baker, L. Diep, K. Fitzgerald, J. Hoffman, R. Motter, D. Fauss, P. Tanaka, M. Dappen, J. Jagodzinski, W. Chan, A.W. Konradi, L. Latimer, Y.L. Zhu, H.L. Sham, J.P. Anderson, M. Bergeron, D.R. Artis, Selective and brain-permeable polo-like kinase-2 (Plk-2) inhibitors that reduce alpha-synuclein phosphorylation in rat brain, ChemMedChem 8 (2013) 1295–1313.
[9] C. Liao, J.E. Park, J.K. Bang, M.C. Nicklaus, K.S. Lee, Probing binding modes of small molecule inhibitors to the polo-box domain of human polo-like kinase 1, ACS Med. Chem. Lett. 1 (2010) 110–114.
[10] J.E. Park, N.K. Soung, Y. Johmura, Y.H. Kang, C. Liao, K.H. Lee, C.H. Park, M.C. Nicklaus, K.S. Lee, Polo-box domain: a versatile mediator of polo-like kinase function, Cell. Mol. Life Sci. 67 (2010) 1957–1970.
[11] P.B. Sampson, Y. Liu, N.K. Patel, M. Feher, B. Forrest, S.W. Li, L. Edwards, R. Laufer, Y. Lang, F. Ban, D.E. Awrey, G. Mao, O. Plotnikova, G. Leung, R. Hodgson, J. Mason, X. Wei, R. Kiarash, E. Green, W. Qiu, N.Y. Chirgadze, T.W. Mak, G. Pan, H.W. Pauls, The discovery of polo-like kinase 4 inhibitors: design and optimization of spiro[cyclopropane-1,3'[3H]indol]-2'(1'H)-ones as orally bioavailable antitumor agents, J. Med. Chem. (2015) 130–146.
[12] E.G. Colicino, H. Hehnly, Regulating a key mitotic regulator, polo-like kinase 1 (PLK1), Cytoskeleton (Hoboken) 75 (2018) 481–494.
[13] R.E. Gutteridge, M.A. Ndiaye, X. Liu, N. Ahmad, Plk1 inhibitors in cancer therapy: from laboratory to clinics, Mol. Cancer Ther. 15 (2016) 1427–1435.
[14] P. Lenart, M. Petronczki, M. Steegmaier, B. Di Fiore, J.J. Lipp, M. Hoffmann, W.J. Rettig, N. Kraut, J.M. Peters, The small-molecule inhibitor BI 2536 reveals novel insights into mitotic roles of polo-like kinase 1, Curr. Biol. 17 (2007) 304–315.
[15] M. Steegmaier, M. Hoffmann, A. Baum, P. Lenart, M. Petronczki, M. Krssak, U. Gurtler, P. Garin-Chesa, S. Lieb, J. Quant, M. Grauert, G.R. Adolf, N. Kraut, J.M. Peters, W.J. Rettig, BI 2536, a potent and selective inhibitor of polo-like kinase 1, inhibits tumor growth in vivo, Curr. Biol. 17 (2007) 316–322.

[16] J. Van den Bossche, F. Lardon, V. Deschoolmeester, I. De Pauw, J.B. Vermorken, P. Specenier, P. Pauwels, M. Peeters, A. Wouters, Spotlight on volasertib: preclinical and clinical evaluation of a promising Plk1 inhibitor, Med. Res. Rev. 36 (2016) 749–786.
[17] Q.Y. Hu, Y.X. Chu, W.G. Hu, M. Peng, Q.B. Song, The cytotoxic effect of GW843682X on nasopharyngeal carcinoma, Anti Cancer Agents Med. Chem. 16 (2016) 1640–1645.
[18] K. Spaniol, J. Boos, C. Lanvers-Kaminsky, An in-vitro evaluation of the polo-like kinase inhibitor GW843682X against paediatric malignancies, Anti Canccer Drugs 22 (2011) 531–542.
[19] A. Berg, T. Berg, Inhibitors of the polo-box domain of polo-like kinase 1, Chembiochem 17 (2016) 650–656.
[20] K.T. Savjani, A.K. Gajjar, J.K. Savjani, Drug solubility: importance and enhancement techniques, ISRN Pharm 2012 (2012) 195727.
[21] C. Liao, M. Sitzmann, A. Pugliese, M.C. Nicklaus, Software and resources for computational medicinal chemistry, Future Med. Chem. 3 (2011) 1057–1085.
[22] T. Wang, M.B. Wu, R.H. Zhang, Z.J. Chen, C. Hua, J.P. Lin, L.R. Yang, Advances in computational structure-based drug design and application in drug discovery, Curr. Top. Med. Chem. 16 (2016) 901–916.
[23] M. Kothe, D. Kohls, S. Low, R. Coli, G.R. Rennie, F. Feru, C. Kuhn, Y.H. Ding, Selectivity-determining residues in Plk1, Chem. Biol. Drug Des. 70 (2007) 540–546.
[24] E.P. Gillis, K.J. Eastman, M.D. Hill, D.J. Donnelly, N.A. Meanwell, Applications of fluorine in medicinal chemistry, J. Med. Chem. 58 (2015) 8315–8359.