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Structure-based design and SAR development of 5,6-Dihydroimidazolo[1,5-*f*] pteridine derivatives as novel Polo-Like Kinase-1 inhibitors

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Abstract: Using structure-based drug design, we identified a novel series of 5,6-dihydroimidazolo[1,5-*f*]pteridine PLK1 inhibitors. Rational improvements to compounds of this class resulted in single-digit nanomolar enzyme and cellular activity against PLK1, and oral bioavailability. Compound **1** exhibits >7 fold induction of phosphorylated Histone H3 and is efficacious in an *in vivo* HT-29 tumor xenograft model.

Keywords:
PLK1 inhibitor
Structure-based drug design
Antitumor activity
Multidrug resistance

Polo-like kinase 1 (PLK1) is a serine/threonine kinase that controls entry into and progression through mitosis at multiple stages.[1] Over-expression of PLK1 is associated with poor prognosis and survival rates in a number of human cancers.[1d,2] Several reports have demonstrated that PLK1 depletion (by α-PLK1 antibody injection or siRNA interference) causes mitotic arrest and apoptosis in cancer cell lines, but not in normal diploid cells or non-dividing cells.[3] PLK1 was therefore considered an attractive target for treatment of proliferative disease.[4] Currently, small molecule PLK1 inhibitors are being evaluated in human clinical trials.[5] Among which, clinical responses have been reported with the investigational IV drug, Volasertib.[6]

Anti-mitotic agents that target tubulin, such as taxanes, have been clinically validated. However, these drugs have several drawbacks including development of peripheral neuropathy,[7] the need for pretreatment to reduce anaphylaxis,[8] and the potential for tumors to become refractory/resistant due to multidrug resistance.[2b] Consequently, there is a need for novel anti-mitotic drugs that target non-microtubule proteins such as the mitotic kinases.[3d] Herein, we report the discovery and development of a novel 5,6-dihydroimidazolo[1,5-*f*]pteridine series of PLK1 inhibitors.

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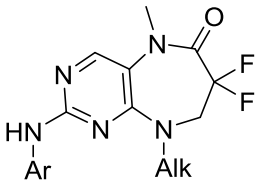

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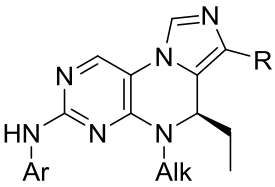

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In our previous publication[9] we reported the discovery of 7,7-difluoropyrimidodiazepinones (**2**) as PLK1 inhibitors. Based on PLK1 X-ray co-crystal information, 7,7-difluoropyrimidodiazepinones interact with Lys82 via a bound water molecule (Fig. 1)[9]. In an effort to provide a novel back up series, we decided to design compounds that would exhibit a direct interaction with Lys82 and examine how this interaction affects potency. In order to gain this direct bonding to Lys82, we designed and synthesized a novel 5,6-dihydroimidazolo[1,5-*f*]pteridine (**3**) scaffold.

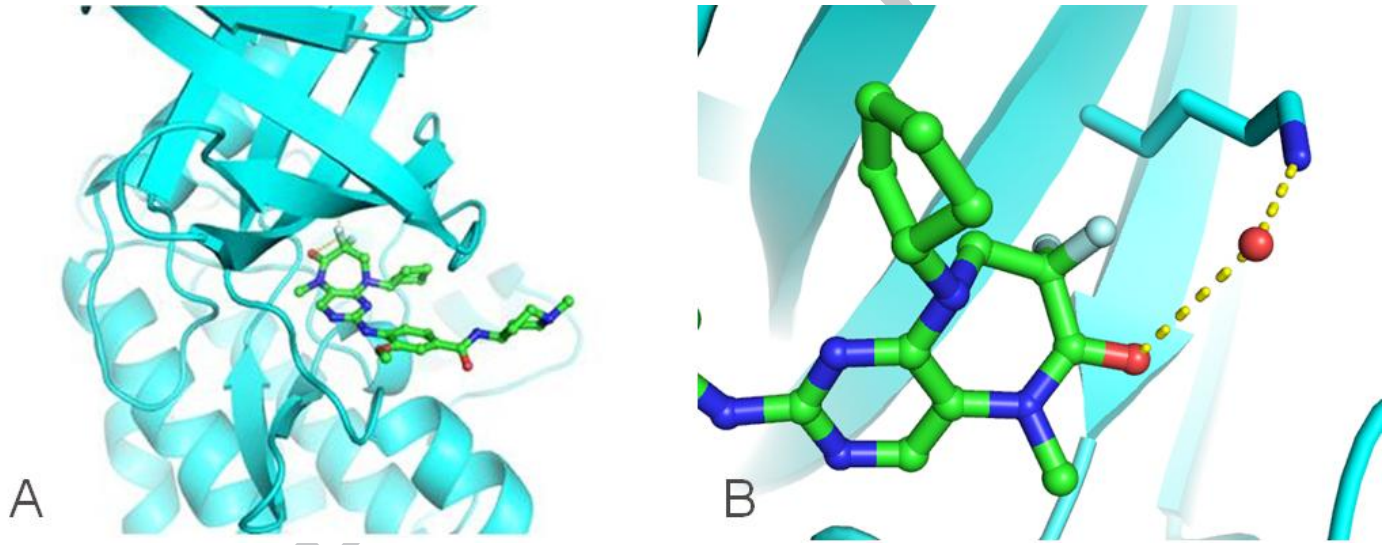
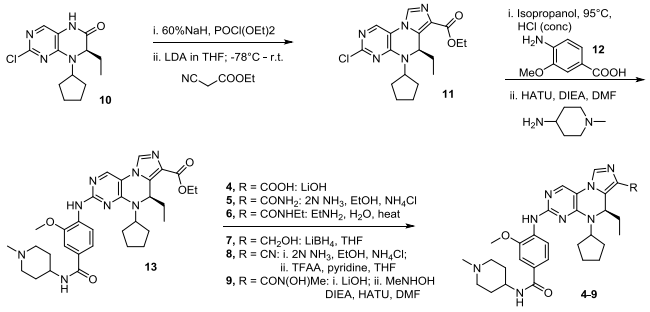


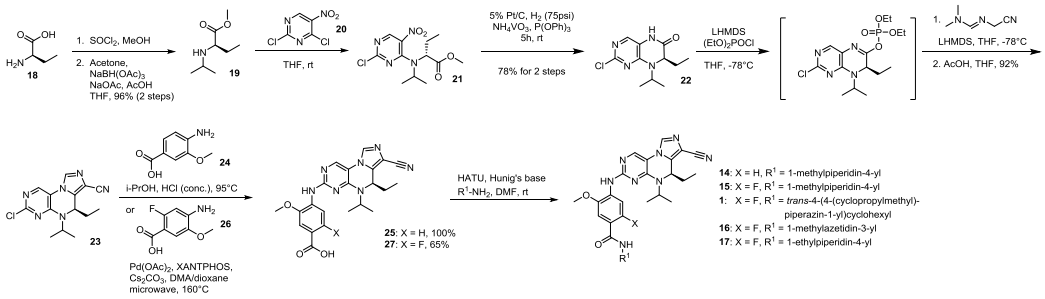
Figure 1. Co-crystal structure of a representative compound from 7,7-difluoropyrimidodiazepinone series with PLK1 where the amino pyrimidine is bound to the hinge residue (**A**) and the carbonyl at the 7-position makes a water-mediated interaction with Lys82 (**B**) (PDB: 5TA8).

Compounds (**4**-**9**) were prepared according to Scheme 1[9]. The carbonyl of compound **10** was activated with diethyl phosphorochloridate and reacted with ethyl 2-isocyanoacetate anion. This intermediate underwent an intramolecular cyclization to provide ester **11** in good yield. An S_NAr reaction at the 2-position of the core with aniline **12** followed by amide formation provided the key intermediate **13**, with the ester moiety serving as a versatile intermediate containing an appropriate vector for diversification. Finally, 7-substituted analogs **4**-**9** were obtained using conditions outlined in Scheme 1.

Scheme 1. Synthesis of 7-subsituted 5,6-dihydro-imidazolo[1,5-*f*]pteridines

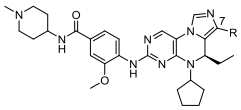


For compounds with the 7-nitrile functionality on the dihydroimidazolo[1,5-*f*]pteridine core, we sought a more direct synthetic route to access a key intermediate for their synthesis. The DMF adduct of aminoacetonitrile provided a stable synthon for this transformation and was used for larger scale production. The synthesis of compounds **1** and **14**-**17** is presented in Scheme 2. (R)-2-Aminobutanoic acid (**18**) was esterified under $SOCl_2$/MeOH conditions followed by a reductive alkylation of the amine moiety with acetone, which provided compound **19** in 96% yield over two steps. An S_NAr reaction with 2,4-dichloro-5-nitropyrimidine (**20**) then gave predominantly the 4-substitution product **21**. The nitro-group in **21** was hydrogenated under carefully controlled conditions to avoid the reduction of aryl chloride and also to prevent undesired intramolecular cyclization of the intermediate nitroso-compound. Concomitant intramolecular cyclization afforded (R)-2-chloro-7-ethyl-8-isopropyl-7,8-dihydropteridin-6(5H)-one (**22**) in 78% yield after two steps. Activation of the amide functionality in **22** with diethyl phosphorochloridate and subsequent reaction with N'-(cyanomethyl)-N,N-dimethylformimidamide anion was followed by cyclization under acidic conditions in a single pot to provide the key intermediate **23** in 92% yield. The chloride in **23** was then replaced by aniline **24** under acidic conditions to give acid **25**. These conditions were not successful for the fluorine-containing aniline **26**, however, and led to decarboxylation. Buchwald Pd-catalyzed coupling with amine **26** did give rise to the desired fluorine-containing acid **27**. Amide formation with HATU as a coupling reagent afforded the desired final compounds **1** and **14**-**17**. Compound **28** was also prepared according to this procedure, using cyclopentanone instead of acetone in the reductive alkylation step.



From the medicinal chemistry perspective, our initial goal was to find compounds that would exhibit a potent inhibition of PLK1 enzyme in vitro[10]. Our newly synthesized 5,6-dihydroimidazolo[1,5-*f*]pteridine scaffold **2** allowed for incorporation of various polar substituents at the 7-position of the core for the desired interaction with Lys82. We found that multiple groups were tolerated at this position and the level of PLK1 inhibitory activity was under 50nM for all compounds (**4**-**9**, **13**). Compounds **13** and **8** also demonstrated sub-10nM inhibition of PLK1 in HT-29 cell line,[11,12] with compound **8** exhibiting activity of 3 nM. This compound (**8**) also showed slow dissociation kinetics ($T_{1/2}$=476 min)[13].

Table 1. 7-Position substitution enzymatic and cellular potency data



No	R	PLK1_KD IC50 (nM)	HT29_ATP EC50 (uM)
13	-COOEt	2.1	0.009
4	-COOH	1.4	0.4
5	-CONH2	2.0	0.02
6	-CONHEt	45	0.1
7	-CH2OH	2.2	0.03
8	-CN	1.5	0.003
9	-CON(OH)Me	7.0	0.06

We were able to confirm our design concept with a co-crystal structure (Fig. 2). Indeed, the nitrile functionality extends into the back-pocket of PLK1 active site to replace one bound water molecule and interacts directly with Lys82.

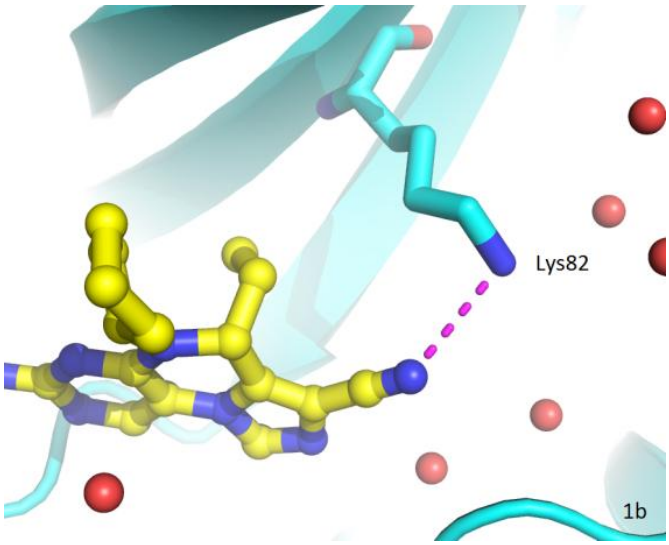
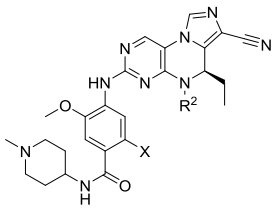


Figure 2. Direct hydrogen bonding to Lys82 is observed for 5,6-dihydro-imidazolo[1,5-*f*]pteridine 7-carbonitrile series of PLK1 inhibitors (PDB: 5TA6).

We chose this nitrile-containing scaffold for further optimization of ADME properties based on the attractive cellular potency.

While active in enzymatic and cellular assays, compound **8** had minimal bioavailability in rat (0%), likely a result of efflux (LLC-MDR/LLC ratio = 21)[14] and/or high clearance in rat (76 mL/min/kg). During our studies of the pyrimidodiazepinone series, we found that introduction of a fluorine *ortho* to the aniline could lower the MDR1 susceptibility[9]. As one can see from compound **28**, this strategy proved to be advantageous in this case as well, as we were able to reduce LLC-MDR/LLC ratio to 2.3.

Table 2. Selected R^2 and X substituents SAR: solving CYP inhibition and active transport problems.

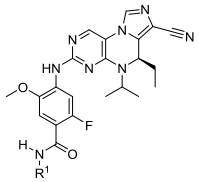


No.	R^2	X	PLK1_KD IC50 (nM)	HT29_ATP EC50 (nM)	LLC-MDR/LLC*	%F rat (po)	CYP inh. (pIC50>5)
8	cyclopentyl	H	1.5	2.7	21	0	3A4
28	cyclopentyl	F	2.2	1.0	2.3	49	3A4, 2C8
14	isopropyl	H	8.6	6.0	82	0.5	clean
15	isopropyl	F	1.9	4.0	7.6	47	clean
29	cyclohexyl	F	1.3	2.0	ND	ND	3A4, 2C8

Concurrently, this structural change also resulted in lower clearance (28 mL/kg/min), allowing us to gain bioavailability of 49% following oral administration in rats. However, compound **28** inhibited CYP isoforms 3A4 and 2C8. We hypothesized that this issue could be alleviated by lowering its cLogD (3.2). To this end, we replaced the N-9 cyclopentyl group with a less lipophilic isopropyl substituent resulting in compound **15** (cLogD of 2.4). This change was significant enough to eliminate the undesired CYP inhibition. Compound **15** possessed a good overall rodent ADME profile, and showed induction of phosphorylated Histone H3 (pHH3, 6.3 fold at 48 hours following a single IV dose of 30 mg/kg). However, we wanted to further improve its microsomal stability (HLM Eh=0.39). To accomplish this, we modified the nature of the amide substituent, leading to identification of compound **1**, which showed improved HLM Eh=0.28. Compound **1** demonstrated pHH3 induction (7.8) and showed high absorption in mouse, which was the species used for xenograft studies (Table 4).

In summary, we identified and optimized a novel series of 5,6-dihydroimidazolo[1,5-*f*]pteridine PLK1 inhibitors. Compound **1** showed a marked increase in pHH3 following a single administration (30 mpk po, 9 fold pHH3 increase after 48h) and showed efficacy (ms, 45 mg/kg iv, 1/time/week x 1 cycle: T/C 54.6%, BWL 5.5%).

Table 3. Selected R^1-substituent SAR: optimization for efficacy



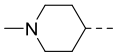
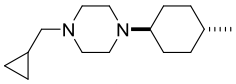
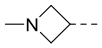
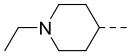
No.	R^1	PLK1_KD IC50 (nM)	HT29_ATP EC50 (nM)	LLC-MDR/LLC*	%F rat, po	CYP inh. (pIC50>5)	Fold pHH3 incr. (IV) 30 mpk	Notes
15		1.9	4.0	8	47	clean	6.3	
1		3.9	22	2	~100	clean	7.5	F~100% (ms)
16		1.7	3.0	ND	ND	clean	1.9	
17		0.4	3.0	4	ND	clean	5.2	hERG 37% inh. @ 10uM

Table 4. Pharmacokinetic parameters of compound **1** in mouse (female) following IV and PO administration[19]

Route (dose)	C_{max} ng/mL	T_{max} h	$T_{1/2}$ h	MRT h	AUC_{extrap} ng*h/mL	AUC_{0-t} ng*h/mL	CL mL/kg/min	V_{dss} L/kg	F %
IV (1mpk)	258	-	4.7	6.5	1007	713	22.7	8.9	-
Oral (5mpk)	354	4	8.5	13	4591	3900	-	-	101

Acknowledgments

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9. a) Synthesis of compound **10**: Nie, Z.; Feher, V.; Natala, S.; McBride, C.; Kiryanov, A.; Jones, B.; Lam, B.; Liu, Y.; Kaldor, S.; Stafford, J.; Hikami, K.; Uchiyama, N.; Kawamoto, T.; Hikichi, Yu.; Matsumoto, S.; Amano, N.; Zhang, L.; Hosfield, D.; Skene, R.; Zou, H.; Cao, X.; Ichikawa, T. *Bioorg. Med. Chem. Lett*. **2013,** *23*, 3662. b) Full experimental details for all compounds in this paper can be found in the following patent application: WO2010025073.
10. PLK1 enzyme assay: The inhibitory activities of the inhibitors were assessed by the TR-FRET assay, which the ATP-dependent phosphorylation of a biotinylated substrate peptide (1-μM) corresponding to residues 2470 through 2488 of mTOR protein (Biotin-AGAGTVPESIHSFIGDGLV) is measured.
11. Cell proliferation assay: HT-29 human colon adenocarcinoma cells were seeded into 96-well plates at 3,000 cells/well in DMEM (Dulbecco's Modified Eagle's Medium) plus 10% fetal calf serum (FCS). After 24 hours, cells were treated with serial dilutions of PLK1 inhibitors, and 72 hours later, the number of viable cells was assessed using the CellTiter-Glo Assay (Promega).
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13. The dissociation kinetics of PLK1 inhibitors was assessed by a method designed to determine the dissociation rate of a compound from an enzyme. Inhibitors were mixed with PLK1 and incubated at room temperature for 60 min. to allow for sufficient binding. The PLK1/inhibitor complex was then diluted into reaction buffer containing 1000-μM ATP and 1-μM FAM-mTOR peptide, and this initiation

of the enzymatic reaction was considered time zero (t_0). The dissociation rate was then measured on a Caliper LabChip 3000 (LC-3000) from Caliper Life Sciences Inc. with off-chip assays using a TC372 chip.

14. LLC – pig kidney cell line; LLC-MDR: MDR1 transfectant.
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19. Compounds were administered intravenously (IV vehicle: 30% b-cyclodextrin in 0.05M MSA, pH3) and orally (oral vehicle: 0.5% MC) at approximately 1.0/5.0 mg/kg for rat.

Graphical abstract