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Presentation Abstract

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Presentation Title:	Discovery, biological characterization and oral antitumor activity of polo-like kinase 1 (Plk1) selective small molecule inhibitors
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Author Block:	Jonathan J. Hollick, Michael G. Thomas, Claire J. Flynn, Stuart D. Jones, Janice McLachlan, Frederic Scaerou, Amy Emery, Nicola L. Rogers, Abhijeet Choudhary, Graeme Thomson, Simon R. Green, David M. Glover, David G. Blake. Cyclacel Ltd, Dundee, United Kingdom

Abstract Body:

We have identified novel inhibitors of Polo-like kinase 1 (Plk1) that exhibit strong single agent activity in solid tumor and leukemia xenograft models after oral dosing. Plk1 is a serine / threonine protein kinase thought to regulate cell division through promotion of mitotic entry, control of spindle assembly, orchestration of mitotic progression and initiation of cytokinesis. Plk1 has been reported to phosphorylate and deactivate the tumor suppressors p53, p63 and p73 thereby inhibiting apoptosis. Furthermore this repression of p53 family members may be responsible for the survival and tumorigenesis of liver cancer stem cells. Cancer cell proliferation is blocked *in vitro* and *in vivo* by antisense oligonucleotides and siRNAs to Plk1. Overexpression of Plk1 is associated with tumor development and many human cancers express elevated Plk1 levels compared to surrounding normal tissue. Numerous studies have shown that Plk1 expression levels correlate with disease progression, invasiveness and poor patient prognosis. Collectively, these observations support the selection of Plk1 as an attractive target for cell cycle-directed cancer therapy.

We have employed high throughput screening, *in silico* screening and *de novo* ligand design approaches to select an inhibitor scaffold for lead optimization. We have selected a set of ATP-competitive Plk1 inhibitors that exhibit high selectivity for Plk1 and inhibit growth of a broad range of tumor cell lines *in vitro* with IC_{50}s in the low nanomolar range. Structure-activity relationships (SAR) were determined through iterative targeted analogue synthesis and *in vitro* testing with SAR rationalized against x-ray crystallographic data. We observed selectivity of the inhibitor scaffold for Plk1 against a panel of over 200 kinases. Treatment of tumor cells with our Plk1 inhibitors induced a phenotype consistent with inhibition of Plk1, accumulation of cells in mitosis and induction of apoptosis. The extent of cytotoxicity was dependent on proliferation as determined by comparative viability of cells arrested in the G_1 phase versus proliferating cells. Compound evaluation using a cell-based activity assay for inhibition of Plk1 and pharmacokinetic profiling informed lead optimization studies to generate potential drug candidates with high oral bioavailability and which induce tumor stabilization, regression and tumor-free cures in solid tumor and leukemia xenograft models as single agents. For example, 6% T/C (p = 0.00001) was achieved in the HCT116 human colon carcinoma model.

In summary, we describe a set of Plk1 inhibitors which act as potent antiproliferative agents suitable for further development as oral Plk1 selective inhibitors for the treatment of human cancers.

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Discovery, biological characterization and oral antitumor activity of polo-like kinase 1 (Plk1) selective small molecule inhibitors

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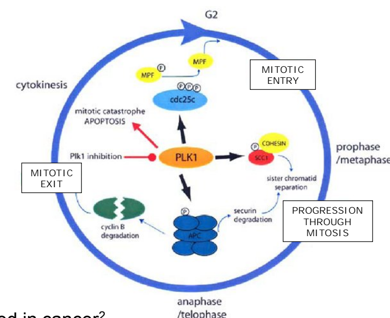
Abstract #4435

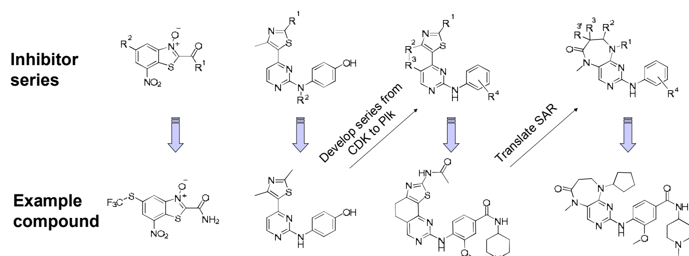
Introduction

Plk1, a serine / threonine kinase, is a key regulator of cell division controlling mitotic entry, bipolar spindle formation and mitotic exit.[1]



- Plk1 is frequently overexpressed in cancer[2]
- Level of expression correlates with aggressiveness and has prognostic value for predicting outcome[3-5]
- Cultured cells can be transformed by Plk1 overexpression and these cells can induce tumors in nude mice[6]
- Cancer cell proliferation is blocked *in vitro* and *in vivo* by small-molecule Plk1 inhibitors and Plk1 antisense / siRNA[7]
- Plk1 inhibitors cause mitotic arrest and subsequent induction of apoptosis in cancer cells[8]

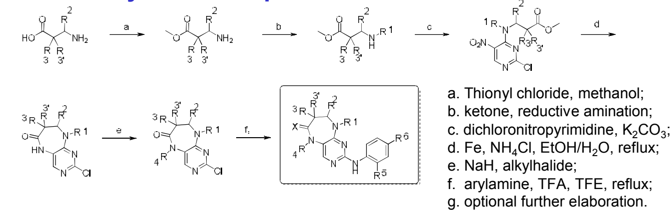
We have employed HTS, *in silico* screening[9,10] and *de novo* ligand design approaches to generate starting points for Plk1 inhibitor design. Characterization of early lead compounds supported selection of the pyrimidodiazepinone scaffold for further investigation.



Plk1 IC_{50}	20 nM	5 µM	0.29 µM	77 nM
Mode	ATP non-competitive	ATP competitive	ATP competitive	ATP competitive
Selectivity	High	Low	Moderate	High
Observation	Possibly reactive and covalently binding	Plk1 inhib^n secondary to CDK inhib^n	Poor cellular activity	Sub-micromolar cellular activity

Preliminary Scaffold Exploration

General synthetic sequence



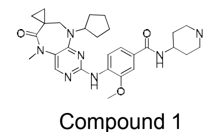
a. Thionyl chloride, methanol;
b. ketone, reductive amination;
c. dichloronitropyrimidine, K_2CO_3;
d. Fe, NH_4Cl, EtOH/H_2O, reflux;
e. NaH, alkylhalide;
f. arylamine, TFA, TFE, reflux;
g. optional further elaboration.

Summary of basic SAR against Plk1

R group	Modifications	Conclusion
R1	Alkyl, aryl, benzyl	Cyclic alkyl preferred; Cyclopentyl optimal
R2	Alkyl variants	H substituent optimal
R3	Alkyl variants	Spiropropyl optimal; *gem*-dimethyl, spirobutyl active
X	O, S	Carbonyl more potent and soluble
R4	H, Me, Et and ^{i}Pr	Me
R5	OMe, OCF_3, OEt, H, Hal, OH,	OMe optimal; F less selective
R6	CO_2H, OH, OMe, Amides	Secondary amides preferred; Range of amides tolerated

Characterization

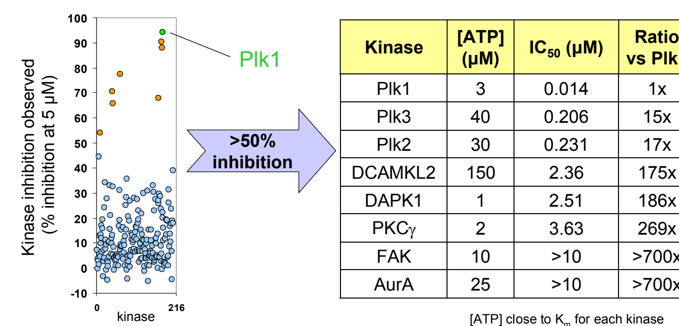
Compound 1, a lead-like Plk1 inhibitor, was selected as a representative tool compound from the series for characterization.



Compound 1

Kinase selectivity

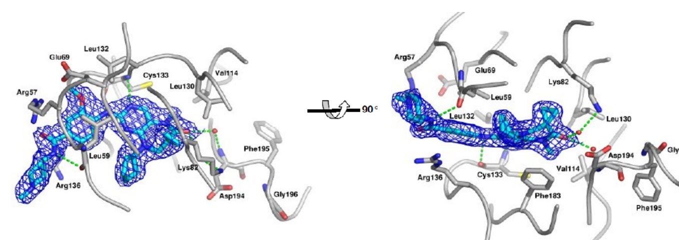
Compound 1 selectivity was tested in 216 kinase panel at 5 µM. IC_{50} values were determined against the 8 kinases inhibited by more than 50%. A high degree of selectivity for Plk1 was observed.



Kinase	[ATP] (µM)	IC_{50} (µM)	Ratio vs Plk1
Plk1	3	0.014	1x
Plk3	40	0.206	15x
Plk2	30	0.231	17x
DCAMKL2	150	2.36	175x
DAPK1	1	2.51	186x
PKCγ	2	3.63	269x
FAK	10	>10	>700x
AurA	25	>10	>700x

[ATP] close to K_m for each kinase

Crystal structure of representative compound



- Co-crystallization of compound 1 with Plk1 kinase domain solved to 1.95 Å
- Ligand binds the ATP-binding site and neighboring regions of the active site
- Various hydrophobic interactions (Phe183, Val114, Leu59 & others)
- Forms two specific hydrogen bonds to the hinge region
- Forms specific hydrogen bond to solvent-front exposed residue
- Polar back-pocket interactions bridged by water molecules

The crystal structure rationalized observed potency and selectivity data and identified regions of the inhibitor that project from the pocket.

Lead Optimization

During lead optimization, physicochemical and ADME properties were tuned through variation in the solvent exposed regions of the inhibitor. Leads were optimized for solubility, cellular activity and pharmacokinetic profiles.[11] Values for selected compounds are given below.

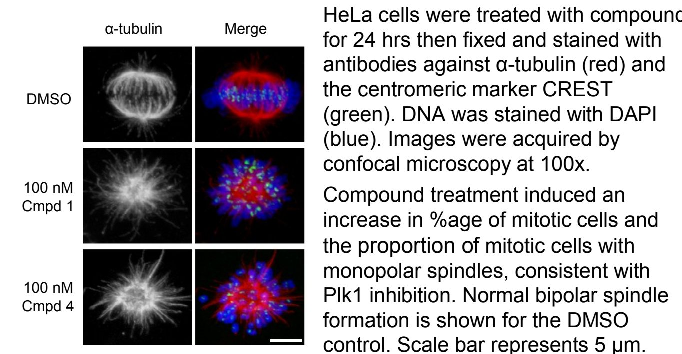
Compounds 2-4 compare favorably with compound 1 and have been further profiled in *in vitro*, cellular and *in vivo* studies.

Parameter	Route	Dose (mg/kg)	Observation			
			Cmpd 1	Cmpd 2	Cmpd 3	Cmpd 4
PK: T½ (hr)	iv	1	0.3	1.0	2.9	2.0
	oral	10	NT	1.1	3.9	2.6
PK: time with >1 µM exposure (hr)	oral	40	NT	1.5	NT	6.7
		80	2.9	4.4	NT	NT
Caco-2 Papp A-B (x 10^{-6} cm/s)	n/a	n/a	0.91	1.4	28	9.2
Efflux ratio BA/AB	n/a	n/a	7.0	4.6	0.22	1.2
hERG IC_{50} (µM)	n/a	n/a	NT	21	6	>30

NT = not tested; n/a = not applicable

Cellular Mechanism of Action

Treatment of proliferating cells with compounds results in accumulation of cells in mitosis. No effect on *in vitro* tubulin polymerization (with or without microtubule associated proteins) was observed with compound 1.



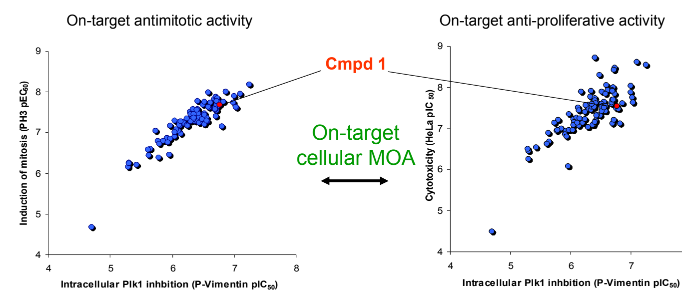
HeLa cells were treated with compound for 24 hrs then fixed and stained with antibodies against α-tubulin (red) and the centromeric marker CREST (green). DNA was stained with DAPI (blue). Images were acquired by confocal microscopy at 100x.

Compound treatment induced an increase in %age of mitotic cells and the proportion of mitotic cells with monopolar spindles, consistent with Plk1 inhibition. Normal bipolar spindle formation is shown for the DMSO control. Scale bar represents 5 µm.

	DMSO control	250 nM Cmpd 1	250 nM Cmpd 4
% Mitotic cells (n>250 cells/field)	2.4%	70.6%	69.0%
% of mitotic cells with monopolar spindles (n=50 mitotic cells)	4%	78%	74%

The mitosis-specific marker S10-phospho-histone H3 (PH3) and S82-phospho-vimentin (a cellular target of Plk1) were quantified in HeLa cells by high content immunofluorescence assays.

Across the inhibitor series induction of mitosis correlates with intracellular Plk1 inhibition, which in turn correlates with cytotoxicity.



Comparison of cell viability in actively proliferating cells and G1 arrested cells indicates a >50-fold window of selectivity for proliferating cells.

Low nM anti-proliferative activity, observed across a broad range of tumor cell lines, is independent of tissue origin, karyotype, spindle checkpoint or tumor suppressor (p53, Rb) and oncogene (Ras) status.

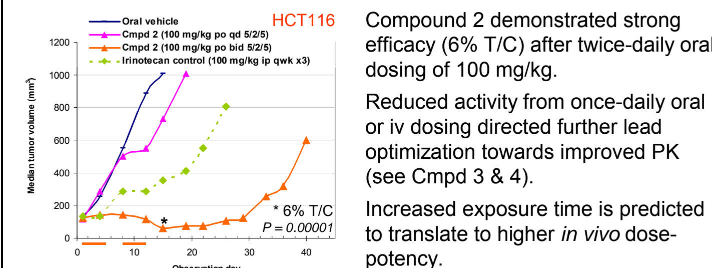
Cell line	Tumor origin	Ras status	Inhib^n of proliferation IC_{50} (nM)			
			Cmpd 1	Cmpd 2	Cmpd 3	Cmpd 4
MDA-MB-468	Breast	WT	5	12	7	NT
MCF7	Breast	WT	NT	12	4	8
HeLa	Cervix	WT	29	54	3	NT
HCT116	Colon	WT	11	29	23	NT
HCT116 p53-	Colon	Mut	12	30	37	NT
Colo-205	Colon	WT	8	16	8	2
HT-29	Colon	WT	2	10	3	NT
HL60	Leukemia	Mut	NT	43	41	55
NCI-H460	Lung	Mut	NT	146	53	32
NCI-H1299	Lung	Mut	NT	34	11	13
A549	Lung	Mut	NT	52	13	9
A2780	Ovary	WT	25	31	21	7
MIA PaCa-2	Pancreas	Mut	10	19	11	NT
BxPC3	Pancreas	WT	22	49	31	NT

NT = not tested

In Vivo Antitumor Efficacy

Preliminary xenograft studies demonstrate antitumor efficacy in solid tumor and leukemia models. The lines chosen are of intermediate sensitivity to Plk1 inhibitors based on *in vitro* cytotoxicity data.

Compound 2 – HCT116 colon carcinoma xenograft

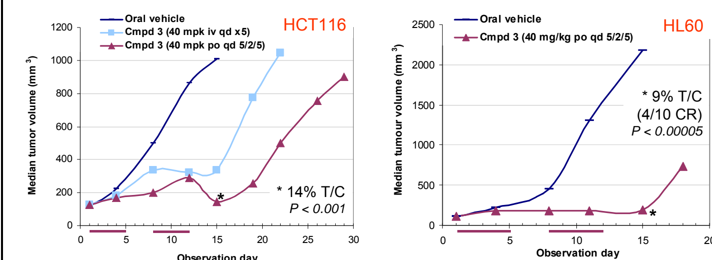


Compound 2 demonstrated strong efficacy (6% T/C) after twice-daily oral dosing of 100 mg/kg.

Reduced activity from once-daily oral or iv dosing directed further lead optimization towards improved PK (see Cmpd 3 & 4).

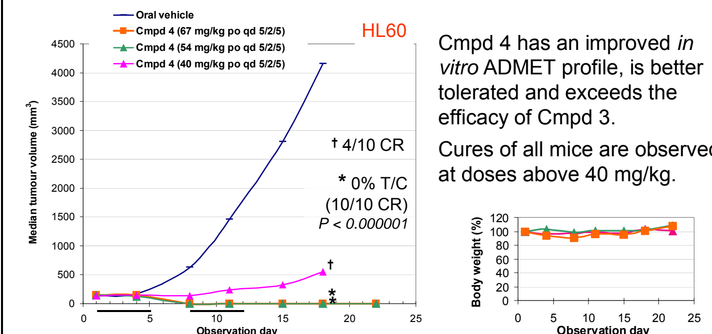
Increased exposure time is predicted to translate to higher *in vivo* dose-potency.

Compound 3 – HCT116 and HL60 xenografts



Cmpd 3 exhibits greater dose-potency, with once-daily dosing leading to significant efficacy in solid tumor and leukemia models at 40 mg/kg, including 4/10 complete regressions (CR), with these mice remaining tumor-free to end of study.

Compound 4 – HL60 promyelocytic leukemia xenograft



Cmpd 4 has an improved *in vitro* ADMET profile, is better tolerated and exceeds the efficacy of Cmpd 3.

Cures of all mice are observed at doses above 40 mg/kg.

The strong preliminary antitumor activity of these compounds justifies continued evaluation and optimization of dose and schedule.

Summary

- **Potent and highly selective inhibitors of the mitotic kinase Plk1 have been identified**
- **Compounds are highly active in preclinical xenograft models of human cancers upon repeated oral or intravenous treatments and across multiple dose levels**
- **Structure-rationalized drug design has been employed and lead optimization has delivered drug-like compounds with high oral bioavailability**
- **Intracellular biomarkers tracking inhibition of Plk1 specific events and general mitosis markers along with mitotic phenotype analysis have been used to ensure on-target mechanism of action**

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