Preclinical Pharmacokinetics of CASC-578 a Novel Selective Potent and Orally Bioavailable Small Molecule Checkpoint Kinase 1 Inhibitor

Dina Leviten[1], Teresa Sierra[1], Ashley Dozier[1], Richard Boyce[2], Robert Boyle[2], Scott Peterson[1], Alex Vo[1]

[1]Cascadian Therapeutics, Inc., Seattle, WA; [2]Sentinel Oncology, UK

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Checkpoint Kinase 1 (Chk1) Inhibition: Cancer Therapeutic Rationale

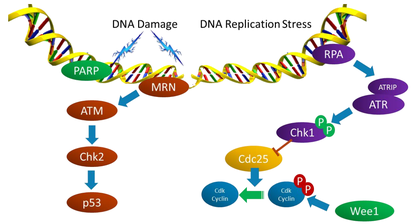


Figure 1. Chk1 inhibitors block cell cycle checkpoint activation by disrupting the control of Cdc25, leading to activation of Cdk/Cyclin activity. Inhibition of Chk1 results in the induction of DNA damage and cell death in tumor cells

SAR & Biological Activity of CASC-578 (Ref.1)

- A pyrazole-amino-pyrazine chemical scaffold yielded sub-nanomolar biochemical lead
- Chemical optimization for:
  - Cellular potency
  - Selectivity
  - ADME/PK
  - hERG
- A promising potential development candidate was identified with ideal drug-like properties
  - Balanced PK, selectivity, potency and *in vivo* efficacy
  - Potent single agent efficacy both *in vitro* and *in vivo*
  - Acceptable toxicological profile including cardiovascular safety

Activity	IC_{50} (nM)
Biochemical (Chk1)	~ 0.1
Jeko-1 (MCL)	30
MV411 (AML)	41

Table 1. Chk1 biochemical and cellular IC_{50}. Cells were plated in a 96-well format and CASC-578 was added in serial half-log dilutions after cell attachment/plating. Cellular assays were performed after 72 h and quantitated using Cell-Titer Glo (Promega).

CASC-578 Has a Favorable *In Vitro* Metabolic Profile

Species	Half-life (min)	ER	CL_{int} (mL/min/kg)
Mouse	190	0.17	19.0
Rat	63.3	0.26	19.8
Dog	12.7	0.50	96.1
Monkey	55	0.28	17.6
Human	111	0.21	5.2

Table 2. Microsomal stability *in vitro* assay. CASC-578 (at 1 µg/ml) was incubated, in triplicate, in a 1 mg/mL microsomal reaction at 37°C for 10, 20, 30, or 45 minutes. At each time point, the reaction was stopped, quenched and subjected to LC-MS/MS analysis. Data were used to calculate the half-life, extraction ratio (ER) and intrinsic clearance (CL_{int}) for each of the species tested.

CASCADIAN THERAPEUTICS

CASC-578 is Highly Permeable and is not an Efflux Substrate

Table 3. Caco-2 bidirectional permeability. Clone C2BBe1 cells were grown to confluency (21-28 d) in a Costar transwell plate. CASC-578 was incubated at 5 µM for 120 minutes in triplicate and samples from both apical and basal compartments were extracted and subjected to LC-MS/MS analysis. Results were used to calculate the apparent permeability and the efflux ratio.

	A-to-B ($x\ 10^{-6}$ cm/s)	B-to-A ($x\ 10^{-6}$ cm/s)	Absorption Potential Classification	Efflux Ratio	Significant Efflux
CASC-578	14.0	16.3	High	1.2	No

Absorption Potential Classification: (P_{app} A-B) < 1.0 x 10^{-6} cm/s: **Low**

(P_{app} A-B) ≥ 1.0 x 10^{-6} cm/s: **High**

Significant Efflux: Efflux ratio ≥ 2.0 and (P_{app} B-A) ≥ 1.0 x 10^{-6} cm/s

Transporter Inhibition: CASC-578 is not a Potent Inhibitor

Transporter	% Inhibition (10 µM)
OAT1	None
OAT3	None
OCT1	None
OCT2	None
OATP1B1	None
OATP1B3	None
BSEP	None
BCRP	41.1
P-gp	52.3
BSEP	8.9

Table 4. *In vitro* transporter inhibition. CASC-578 at 10 µM was run in triplicate using polarized monolayer of MDCK-II or MDCK-MDR1 cells or Sf9 membrane vesicles. Incubations were done at 37°C for 60 minutes in triplicate. Samples were analyzed using radiometric detection.

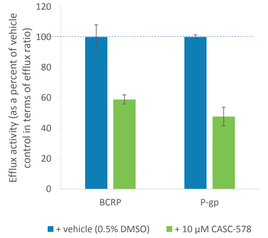


Figure 2. Inhibition of efflux transporter mediated probe substrate transport by CASC-578 (10 µM). The probe substrates for the transporters were 2 µM prazosin (BCRP) and 100 nM quinidine (P-gp). Data represent the mean and standard deviation of triplicate samples.

CASC-578 is Stable in Plasma and has Favorable Plasma Protein Binding

Species	Half-life (min)	Bound (%)	Free (%)
Mouse	>120	96.7	3.3
Rat	> 120	95.3	4.7
Monkey	> 120	98.7	1.3
Human	> 120	95.0	5.0

Table 5. Plasma stability and plasma protein binding in preclinical species. Plasma stability was evaluated in male (pooled) plasma at 37°C for 10, 20, 30, 60, 90, and 120 minutes. Samples were quenched at time points and subjected to LC-MS/MS analysis. Plasma protein binding was determined using ThermoFisher Rapid Equilibrium Dialysis (RED, 8K MWCO) membranes. CASC-578 was spiked in plasma compartment and incubated for 120 min at 37°C. PBS was used in the buffer compartment. Samples were quenched and analyzed via LC-MS/MS. For both assays CASC-578 was tested at 1 µg/mL in triplicate.

Partial Blood to Plasma Partitioning Observed with CASC-578

	Blood to Plasma Partitioning Coefficient ($K_{RBC/PL}$)
CASC-578	2.6

Table 6. *In vitro* whole blood partitioning. CASC-578 (at 1 µg/mL) was incubated in male human whole blood samples in triplicate for 60 minutes at 37°C. Samples were quenched and analyzed by LC-MS/MS. Data from the blood and plasma compartments were used to determine the partitioning coefficient in whole blood.

$$K_{RBC/PL} = \frac{1}{\text{Hematocrit}} * \left(\frac{\text{Reference Plasma}}{\text{Plasma}} * 1 \right) + 1$$

CASC-578 is not a Potent Direct or Time Dependent CYP450 Inhibitor

CYP450	% Inhibition at 20 µM	Fold Shift in IC_{50} for TDI
1A2	7.9	None
2B6	5.2	None
2C8	84 (IC_{50} 5.8 µM)	None
2C9	12.9	None
2C19	16.3	None
2D6	43.9	None
3A4 (midazolam)	16.5	None
3A4 (testosterone)	None	None

Table 7. Direct and time dependent inhibition (TDI) of cytochrome P450 (CYP450). Both *in vitro* assays utilized human liver microsomes (0.3 mg/mL for direct inhibition and 0.1 mg/mL for TDI except for 2C19 TDI which used 1 mg/mL). Samples were prepared in triplicate, incubated at 37°C where the concentration of CASC-578 ranged from 0 to 20 µM. Samples were quenched and analysis was by LC-MS/MS. No IC_{50} was determined with the exception of CYP2C8. The percent inhibition values were taken from the highest concentration tested (20 µM).

CYP3A4 is a Major Metabolizer of CASC-578

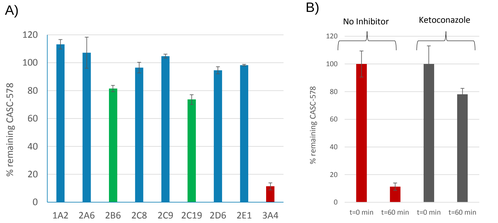


Figure 3. A) Human recombinant CYP (rCYP) *in vitro* profiling. Nine rCYPs (Corning Life Sciences) were tested following recommended assay protocol. CASC-578 was run in triplicate at 100 ng/mL for 60 min at 37°C. Reaction mixtures were quenched and analyzed by LC-MS/MS. B) Human rCYP3A4 *in vitro* profiling. A control with no inhibitor was compared to a control with ketoconazole, a rCYP3A4 inhibitor. Samples were run, quenched and analyzed as above.

CASC-578 is not an Inducer of CYP450s

CYP450	Substrate	Metabolite	% of Positive Control (EA) (n=3)	% of Positive Control (mRNA) (n=5)
1A2	Phenacetin	Acetominophen	nd	6.4
2B6	Buproprion	OH-Buproprion	7.9	7.8
3A4	Testosterone	OH-Testosterone	2.0	5.9

Table 8. *In vitro* CYP induction using human plateable hepatocytes. Primary hepatocytes from single donors were incubated for 3 days with specific inducers (Omeprazole/1A2, Phenobarbitol/2B6, Rifampicin/3A4) or CASC-578 at 5 µM. Cells were processed and CYP mRNAs were quantified utilizing the Quantigene Plex assay from Affymetrix (Luminiex). Replicate wells were treated with specific CYP substrates (100 µM) and incubated for 4 hours at 37°C; after which cell lysates were extracted and subjected to analysis by LC-MS/MS. Enzymatic activity (EA) was determined by quantitating the amount of metabolite of the CYP substrates generated. An inducer is ≥ 40% of the positive control. nd = no data.

Single Dose and Multi-dose PK Parameters

Single Dose PK		CD-1 Mice (n=3, male)		Sprague-Dawley Rats (n=3, male)		Cynomolgus Monkeys (n=3, male)	
Parameters		IV	PO	IV	PO	IV	PO
Dose	(mg/kg)	2	10	2	10	1	5
Half-life	(h)	6	3	4	6	4	
T_{max}	(h)		0.5		3		2
C_{max}	(ng/mL)	228	294	291	725	113	173
C_{max_D}		114	29	146	73	113	35
AUC_{last}	(h*ng/mL)	431	1765	2107	6173	305	1370
AUC_{INF}	(h*ng/mL)	443	1769	2131	6690	335	1438
AUC_D		221	177	1065	669	335	288
MRT_{INF}	(h)	4	4	7	10	5	8
Cl	(mL/min/kg)	75		16		50	
V_{ss}	(L/kg)	16		6		14	
F	(%)		80		63		86

Table 9. Pharmacokinetic parameters in preclinical species after a single dose of CASC-578 given either intravenously (IV) or orally (PO). Plasma samples were collected over a 24 h time period. CASC-578 was extracted from plasma and analyzed by LC-MS/MS. PK parameters were calculated using WinNonLin.

Multi-dose PK		Nude Mice (n=6, female)		Sprague Dawley Rats (n=6, male)		Cynomolgus Monkeys (n=1, male)	
Parameters		Day 1	Day 7	Day 1	Day 7	Day 1	Day 7
Dose	(mg/kg)	25	25	30	30	30	30
Half-life	(h)	3	8	17	7	23	9
T_{max}	(h)	1	4	4	4	4	2
C_{max}	(ng/mL)	1097	790	1428	2247	666	653
C_{max_D}		44	32	48	75	22	22
AUC_{tau}	(h*ng/mL)	4070	3993	19386	24949	10267	8421

Table 10. Pharmacokinetic parameters in preclinical species after multiple oral administrations of CASC-578. Plasma samples were collected over a 24 h period on Day 1 and Day 7. CASC-578 was extracted from plasma and analyzed by LC-MS/MS. PK parameters were calculated using WinNonLin.

Dose Proportionality Across Preclinical Species for CASC-578

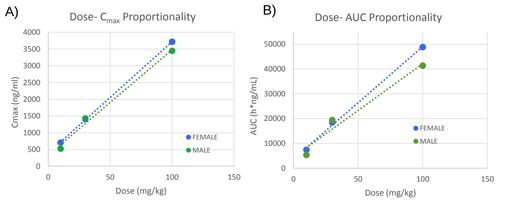
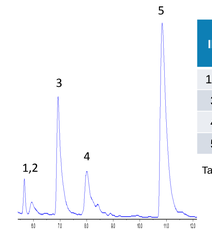


Figure 4. A) Dose-C_{max} proportionality in male and female Sprague-Dawley rats. B) Dose-AUC proportionality in male and female Sprague-Dawley rats. PK values were derived from a multi-dose PK study where CASC-578 was administered orally at 10, 30 & 100 mg/kg. Similar proportionality was also observed in mice and cynomolgus monkeys.

Metabolite Profile/ID of CASC-578



ID	Retention time (min)	Mass Change	Transformation
1,2	5.70/5.90	Unknown	Unknown
3	6.90	+16	Hydroxylation
4	8.00	-14	Demethylation
5	10.84	0	parent

Table 11. CASC-578 Metabolite ID

Figure 5. UV trace of CASC-578 and its metabolites. CASC-578 was incubated in human liver microsome incubation at 40 µM for 120 minutes at 37°C. The sample was quenched, dried down, reconstituted and analyzed using UV-LC-MS/MS.

CASC-578 Has Desirable Drug-like Properties

- CASC-578 has been designated as a development candidate and is well positioned for IND enabling studies
  - Sub-nanomolar Chk1 inhibitor, limited off-target activities
  - Excellent drug-like properties (ADME/PK, oral bioavailability)
  - Completed 7-day repeat dose tolerability studies in mice, rats and cynomolgus monkeys (data not shown)
  - No adverse findings in GLP cardiovascular safety study completed in cynomolgus monkeys, including QTc and LVP/contractility endpoints (data not shown)
  - Active in multiple tumor models as a single agent and in combination with chemotherapy or Wee1 inhibitor Ref. 2,3

References

1. AACR poster # 2721, 2016. April 16-20, New Orleans, LA
2. AACR posters # 295, 2017. April 2-5, Washington, D.C.
3. AACR poster # 297, 2017. April 2-5, Washington, D.C.

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