Supplementary Data for Squires MS et al. Biological characterisation of AT7519, a small molecule inhibitor of Cyclin Dependent Kinases, in human tumour cell lines.

Kinase	AT7519 (IC_{50} nM)	Kinase	AT7519 (IC_{50} nM)
CDK1/Cyclin B	**210**	JAK2*	>1000
CDK2/Cyclin A	**47**	Jnk2*	>10000
CDK3/Cyclin E*	**360**	MAPK 1*	>10000
CDK4/Cyclin D1	**100**	MEK1*	>10000
CDK5/p35	**13**	Met*	>10000
CDK6/Cyclin D3	**170**	SAPK2a (p38)*	>10000
CDK7/Cyclin H/MAT1*	**2400**	p70S6K*	>10000
CDK9/Cyclin T*	**<10**	PDGFR*	>10000
GSK3 beta	89	PDK1*	>10000
Aurora A	>10000	PI3Kβ*	>1000
c-abl*	>10000	PKBβ	>10000
cSrc*	>10000	PLK3*	>1000
Chk 1*	>10000	Ret*	>1000
EGFR*	>10000	SGK*	>1000
FGFR3*	>10000	TrkB*	>1000
IR*	>10000	VEGFR 1*	>10000

Table 1. Activity of AT7519 versus a panel of protein kinases. Enzyme activities were assayed as described below. The IC_{50} values were determined where possible. Data were generated either in house

according to the methods outlined, or at Upstate Ltd., Hampshire, UK. or ProQinase GmbH (Freiburg, Germany)*

Kinase assays for CDK1, CDK2, Aurora, GSK3-β and PKB-B, were all carried out in a radiometric filter binding format. Assays for CDK5 were in DELFIA format and for CDKs 4 and 6 in ELISA format. CDK1/cyclin B, Aurora A GSK3-β and CDK5/p35 were obtained from Upstate, Milton Keynes, UK. Cdk2/cyclinA and PKB-B were produced in-house at Astex. For CDKs 1 and 2 the relevant CDK and 0.12 µg/ml Histone H1 were incubated in 20 mM MOPS, pH 7.2, 25 mM β-glycerophosphate, 5 mM EDTA, 15 mM $MgCl_2$, 1mM sodium orthovanadate, 1 mM DTT, 0.1 mg/ml BSA and 45 µM ATP (0.78 Ci/mmol) for 2 or 4 hours respectively. For PKB-B, the PKB-B enzyme and 25 µM AKTide-2T peptide (HARKRERTYSFGHHA) were incubated in the above buffer, but with 10 µg/ml BSA and 30 µM ATP (1.16 Ci/mmol) for 4 hours. For GSK3-β and Aurora A the relevant enzyme and 5 µM glycogen synthase peptide 2 for GSK3-β and 200 µM Kemptide peptide (LRRASLG) for Aurora A were incubated in 10 mM MOPS pH 7.0, 0.1 mg/ml BSA, 0.001% Brij-35, 0.5% glycerol, 0.2 mM EDTA, 10 mM $MgCl_2$, 0.01% β-mercaptoethanol, 15 µM ATP (2.31 Ci/mmol) for 3 hours and 30 min respectively. Assay reactions were stopped by adding an excess of orthophosphoric acid and filtered using Millipore MAPH filter plates. The plates were then washed, scintillant added and radioactivity measured by scintillation counting on a Packard TopCount.

CDK5/p35 and 1µM of a biotinylated Histone H1 peptide (Biotin-PKTPKKAKKL) (Bachem Ltd, Merseyside, UK) were incubated in 25 mM Tris-HCl, pH 7.5, 2.5 mM $MgCl_2$, 0.025% Brij-35, 0.1 mg/ml BSA, 1 mM DTT, 15 µM ATP for 30min. Assay reactions were stopped using EDTA, transferred to Neutravidin-coated plates and phosphorylated peptide quantified by means of a rabbit phospho-cdk1 substrate polyclonal antibody from Calbiochem (Merck Biosciences, Nottingham, UK) and DELFIA europium-labelled anti-rabbit IgG secondary antibody from Perkin Elmer Ltd.(Beaconsfield, Bucks., UK) using time-resolved fluorescence at λ_{ex}=335nm, λ_{em}=620nm.

For CDK 4 and 6 assays plates were coated with GST- $pRb^{769-921}$ and blocked with Superblock (Perbio Science, Northumberland). CDK4 or 6 were incubated in 15 mM $MgCl_2$, 50 mM HEPES, pH 7.4, 1 mM DTT, 1mM EGTA, pH 8.0, 0.02% Triton X-100 and 2.5% DMSO and the reaction initiated with the

addition of ATP. After 30 min reactions were stopped by the addition of 0.5 M EDTA pH 8.0. Plates were then washed and incubated for one hour with the primary antibody (anti- p-Rb Serine 780, New England Biolab, Hitchin, UK) diluted in Superblock followed by secondary antibody (alkaline phosphatase linked anti-rabbit (New England Biolab, Hitchin, UK) for a further hour. Plates were developed using the Attophos system (Promega, Southampton, UK) and fluorescence read on a Spectramax Gemini plate reader (Molecular Devices) at excitation 450 nm and emission 580 nm. In all cases IC_{50} values were calculated from replicate curves, using GraphPad Prism software. All other enzyme assays were carried out at Upstate Biotechnology (Dundee, UK) or ProQinase GmbH (Freiburg, Germany).

Tissue	t½ (h)	Clp (mL/min/kg)	Vss (L/kg)	Cmax (ng/mL or ng/g)	$AUC_{(0-\infty)}$ (h.ng/mL)
Plasma	1.1	55	1.5	1750	1513
Tumour	3.0	n/a	n/a	684	3015

Table 2. Pharmacokinetic parameters derived from plasma and tumour samples after intravenous administration of AT7519M at 5mg/kg to BALB/c nude mice bearing HCT116 xenografts. Tmax was 0.083h for both plasma and tumour being the first sampling time after dosing. Fraction of AUC extrapolated was 5% or less.