

Structure and inhibitor specificity of the PCTAIRE-family kinase CDK16

Sarah E. Dixon-Clarke, Saifeldin N. Shehata, Tobias Krojer, Timothy D. Sharpe, Frank von Delft, Kei Sakamoto, Alex N. Bullock

SUPPLEMENTARY MATERIAL

SUPPLEMENTARY METHODS

Knockdown of CDK16

The specificity of the anti-CDK16 antibody (HPA001366, Sigma) was tested using siRNA-mediated knockdown of CDK16 in IGR-37 melanoma cells. IGR-37 melanoma cells were plated at 5000 cells per well in 6-well plates. After 24 hours, cells were transfected with different siRNA targeting human CDK16, cyclin Y or scrambled control, at a final concentration of 25 nM using Dharmacon DharmaFECT transfection reagents (Dharmafect reagent at 10 uL per well) according to the manufacturer's instructions (GE Healthcare Dharmacon). Validation of RNAi gene silencing was evaluated 72 hours after transfection by immunoblotting for protein expression. Dharmacon ON-TARGETplus SMARTpool siRNA were used for all knockdown experiments.

SUPPLEMENTAL TABLES

Table S1 DSF screening data for Calbiochem compounds

Compound	Tm Shift (°C)	Compound	Tm Shift (°C)
Cdk1/2 Inhibitor III	9.6	JAK Inhibitor I	1.0
Alsterpaullone, 2-Cyanoethyl	7.9	PIM1 Inhib II	1.0
Indirubin E804	7.6	Compound 52	0.9
Cdk2/9 Inhibitor	7.2	IKK-2 Inhibitor VI	0.9
Aurora/Cdk Inhibitor	6.7	H-89	0.9
(Z,E)-3-(Imidazol-4-ylmethylene)indolin-2-one	6.7	GSK-3beta Inhibitor VIII	0.9
Indirubin-3'-monoxime-5-sulphonic acid	6.7	Bosutinib	0.9
Flt3 Inhib III	6.6	EGFR Inhibitor	0.8
5-iodo-Indirubin-3'-monoxime	6.1	GTP-14564	0.8
SU9516	6.0	Ellipticine	0.8
Cdk/Crk Inhibitor	6.0	IGF-1R Inhibitor II	0.8
Alsterpaullone	5.5	Syk Inhibitor II	0.8
GSK-3 Inhibitor IX	5.3	Roscovitine	0.7
Cdk2 Inhibitor II	5.3	10Z-Hymenialdisine	0.7
Quercetin	5.1	ST638	0.7
Flt3 Inhib III	4.8	Emodin	0.7
Raf1 kinase inhibitor II	4.7	Tpl2 Inhibitor	0.7
Damnacanthal	4.4	GSK-3 inhibitor X	0.6
BIM I	3.9	Lck Inhibitor III	0.6
Indirubin-3monoxime	3.9	Diacylglycerol Kinase Inhibitor I	0.6
SU11652	3.9	PDGF RTKinase Inhibitor IV	0.6
NSC-664704	3.8	VEGF Receptor 2 Kinase Inhibitor	0.5
Oxindole I	3.8	HA 1004	0.5
Cdk1 Inhibitor	3.7	SU6656	0.5
IKK Inhibitor VII	3.5	SB 220025	0.5
Curcumin	3.3	Cdk Inhibitor, p35	0.5
c-Met kinase inhibitor II	3.2	Genistein	0.5
TX-1123	3.2	PD173074	0.5
TX-1918	3.0	piceatannol	0.4
PI3/CK2 Inhib	3.0	ST638	0.4
VEGFR 2/3 Inhibitor	3.0	HA 1077	0.4
GSK inhibitor XIII	2.9	A3	0.4
Wee1/Chk1 inhibitor	2.9	PI3Kg inhib	0.4
K252a	2.7	PIM1 Inhib II	0.4
Apigenin	2.6	Erbstatin Analog	0.4
Aminopurvalanol	2.6	JNK Inhib IX	0.3
Aloisine A	2.5	SB 239063	0.3
JNK inhib V	2.4	LY 364947	0.3
Syk Inhibitor	2.4	IKK-3 Inhibitor IX	0.3
Purvalanol A	2.3	BPDQ	0.3
isogranulatimide	2.3	Cdk1 Inhibitor III	0.3
NU6102; Cdk 1/2 inhibitor II	2.1	Diacylglycerol Kinase Inhibitor II	0.3
PI3/CK2 Inhib	1.9	PI-103	0.3
ATM/ATR inhibitor	1.8	Aurora Kinase Inhibitor II	0.3
Raf1 kinase inhibitor I	1.7	JNK inhib VIII	0.3
JNK Inhibitor II; SAPK Inhibitor II	1.6	IKK Inhibitor X	0.2
BIBX1382	1.6	ML-3163	0.2
Aurora Kinase Inhibitor III	1.4	H-	0.2
Cdk1/5 Inhibitor	1.4	IKK-3 Inhibitor IX	0.2
PKC beta inhibitor	1.3	Cdk2 Inhibitor III	0.2
Cdk4 Inhibitor	1.2	7,8-Dihydroxycoumarin	0.2
Wedelolactone	1.2	Radicicol	0.2
5-Iodotubercidin	1.1	Lavendustin A	0.2
4,5,6,7-Tetrabromobenzotriazole	1.0	PD 169316	0.2

Table S1 cont. DSF screening data for Calbiochem compounds

Compound	Tm Shift (°C)	Compound	Tm Shift (°C)
PI3Kg inhib	0.2	SB 202190	-0.4
Bohemine	0.2	SB 218078	-0.4
VEGF RTKinase Inhibitor III	0.2	GSK-3 Inhibitor II	-0.4
TGFb RI Inhibitor IV	0.2	SB 203580	-0.4
AG 1879	0.1	MEK1/2 Inhibitor	-0.5
GSK inhibitor XII	0.1	Cdk4 inhibitor III	-0.5
Flt3 inhibitor	0.1	AS041164	-0.6
PD 153035	0.1	Diacylglycerol Kinase Inhibitor I	-0.6
PI3Kg inhib II	0.1	FASCAPLYSIN	-0.6
Pfmrk Inhibitor	0.1	ERK Inhibitor II	-0.6
IKK Inhibitor X	0.1	cFMS Receptor Inhibitor III	-0.6
TGFb RI Inhibitor III	0.1	Chk2 inhibitor II	-0.6
SL327	0.1	Scytonemin	-0.7
DNA-PK Inhibitor II	0.1	GSK-3beta inhibitor III	-0.7
6 DMAP	0.1	MEK Inhibitor I	-0.8
BAY 61-3606	0.1	U0126	-0.9
cFMS Receptor Inhibitor IV	0.1	p38 MAP kinase inhibitor V	-0.9
MEK Inhibitor II	0.1	(-)-Arctigenin	-1.0
ML-9	0.1		
PLK inhibitor I	0.1		
1-Na-PP1	0.1		
TYRPHOSTIN B48	0.1		
Debromohymenialdisine	0.1		
Cdk1 inhibitor	0.0		
JNK Inhib IX	0.0		
PI 3-K Inhibitor IV	0.0		
KN-93	0.0		
p38 MAP kinase Inhibitor III	0.0		
cFMS RTKinase inhibitor	0.0		
p38 MAP kinase inhibitor	0.0		
5,6-Dichlorobenzimidazole Riboside	-0.1		
ERK Inhibitor	-0.1		
IC261	-0.1		
Y-27632	-0.1		
Dmbi	-0.1		
PNU 112455A; Cdk2/5 Inhibitor	-0.1		
TG003	-0.1		
Casein Kinase I Inhibitor	-0.1		
PDK/AKT inhibitor	-0.2		
WHI-P180	-0.2		
Olomoucine	-0.2		
Tyrphostin B42	-0.2		
PI3Kg inhib II	-0.2		
LAVENDUSTIN C	-0.2		
VEGF Receptor 2 Kinase Inhibitor I	-0.2		
Tyrphostin A23	-0.2		
PLK inhibitor I	-0.2		
Bcr-abl Inhibitor II	-0.2		
PD 98059	-0.2		
Geldanamycin	-0.3		
TGFb RI Inhibitor IV	-0.3		
ZM 336372	-0.3		
LY 364947	-0.3		
TGFb RI Inhibitor III	-0.3		

Table S2 DSF screening data for PKIS compounds

Compound	T_m Shift (°C)	Compound	T_m Shift (°C)	Compound	T_m Shift (°C)
GW300657X	8.8	GW458787A	1.2	GW643971X	0.5
GW416981X	8.7	SB-390527	1.2	GW278681X	0.5
GW779439X	8.1	GW806776X	1.1	GW441756X	0.5
GW300660X	6.8	GW784752X	1.1	GW794607X	0.5
GW301784X	6.0	SB-242718	1.1	GW445015X	0.5
GSK238583A	5.8	GW275616X	1.1	GW574782A	0.5
SB-732881-H	5.5	GW782612X	1.1	GW693481X	0.5
SB-739245-AC	5.4	GSK317354A	1.0	GW827105X	0.5
SB-732881	5.3	GW830263A	1.0	GSK711701A	0.4
GW780056X	4.8	GW796921X	1.0	GW559768X	0.4
GW276655X	4.7	GW806290X	1.0	SB-390523	0.4
GW580509X	4.5	GW589933X	1.0	SB-711237	0.4
GW784307A	4.5	GW831090X	1.09	GR105659X	0.4
GW290597X	4.5	SB-409514	1.0	GSK2186269A	0.4
GW831091X	4.1	SB-750140	1.0	GW442130X	0.4
SB-278539	3.9	GW700494A	0.9	GW781673X	0.4
SB-686709-A	3.9	SB-739452	0.9	GW813360X	0.4
GW795493X	3.9	GW406108X	0.9	SB-242721	0.4
GW352430A	3.8	GW549390X	0.9	GSK466317A	0.4
SB-278538	3.7	GW810372X	0.9	GSK1007102B	0.4
GW572399X	3.7	GW627512B	0.9	GW759710A	0.4
GW280670X	3.6	SB-376719	0.8	GSK300014A	0.4
SB-675259-M	3.4	GW801372X	0.8	GSK994854A	0.4
GW335962X	3.3	GW305178X	0.8	GW445017X	0.4
GW814408X	3.2	GSK1326255A	0.8	GW575533A	0.4
GW778894X	3.0	GW583373A	0.8	GW833373X	0.4
GW618013A	3.0	GW806742X	0.8	SB-284847-BT	0.4
SB-725317	2.9	GW673715X	0.8	GW827106X	0.4
GW824645A	2.9	GSK182497A	0.8	GW830900A	0.4
SB-742865	2.9	GW809897X	0.7	GSK586581A	0.4
SB-732941	2.8	GW578748X	0.7	GW407323A	0.3
GW708336X	2.6	GW568377A	0.7	GSK625137A	0.3
GW441806A	2.5	GW743024X	0.7	GW827102X	0.3
GW631581B	2.4	GW861893X	0.7	SB-220025-R	0.3
SB-678557-A	2.4	GW615311X	0.7	SB-264865	0.3
SB-698596-AC	2.2	GW811168X	0.7	GSK2220400A	0.3
GW654652C	2.1	GW627834A	0.7	GW282449A	0.3
GW771127A	1.9	GSK2163632A	0.6	GW820759X	0.3
SB-735465	1.9	GW300653X	0.6	SB-251527	0.3
GW680908A	1.8	GW632046X	0.6	GW275944X	0.3
GW770249A	1.7	GW301888X	0.6	GW569530A	0.3
GW581744X	1.7	GW435821X	0.6	GW620972X	0.3
GW284408X	1.6	GW794726X	0.6	SB-741905	0.3
GSK614526A	1.6	SB-742864	0.6	GW439255X	0.3
SB-245392	1.6	GW297361X	0.6	GW440139A	0.3
SB-361058	1.5	SB-738482	0.6	GW607049C	0.3
GW810576X	1.4	SB-743899	0.6	GW612286X	0.3
GW683768X	1.4	GW829906X	0.6	SB-226879	0.3
SB-735467	1.4	GW829877X	0.6	SB-734117	0.3
GW513184X	1.3	GSK980961A	0.5	GW577921A	0.3
GW301789X	1.3	GW680191X	0.5	GW679410X	0.3
GW450241X	1.3	GSK953913A	0.5	GSK978744A	0.3
GW416469X	1.2	GSK2110236A	0.5	GW784684X	0.3
GW566221A	1.2	GW396574X	0.5	GW827396X	0.2

Table S2 cont. DSF screening data for PKIS compounds

Compound	T _m Shift (°C)	Compound	T _m Shift (°C)	Compound	T _m Shift (°C)
SB-737198	0.2	GSK299115A	0.1	GSK635416A	0.0
GSK1173862A	0.2	GW621431X	0.1	GW772405X	0.0
GSK269962B	0.2	GW633459A	0.1	GW775608X	0.0
GW807930X	0.2	GW683003X	0.1	GW837331X	0.0
GW567808A	0.2	GSK200398A	0.1	SKF-86055	0.0
SB-738561	0.2	GW829055X	0.1	GSK2213727A	-0.1
GSK620503A	0.2	SB-253228	0.1	GSK317314A	-0.1
SB-239272	0.2	GR269666A	0.1	GW701032X	-0.1
GW644007X	0.2	GSK326090A	0.1	SB-476429-A	-0.1
GW659893X	0.2	GW769076X	0.1	GSK192082A	-0.1
SB-772077-B	0.2	GW796920X	0.1	GW445012X	-0.1
GSK2219385A	0.2	GW829115X	0.1	GW782912X	-0.1
GW575808A	0.2	SB-264866	0.1	GW805758X	-0.1
GW693917A	0.2	SB-759335-B	0.1	GW829874X	-0.1
GW827099X	0.2	GSK237700A	0.1	GW846105X	-0.1
SB-431542-A	0.2	GW429374A	0.1	GW869810X	-0.1
GI261520A	0.2	GW785404X	0.1	SB-285234-W	-0.1
GSK579289A	0.2	GW832467X	0.1	SB-657836-	-0.1
GW576609A	0.2	GSK180736A	0.1	GW684626B	-0.1
GW711782X	0.2	GSK237701A	0.1	SB-333612	-0.1
GW819230X	0.2	GW641155A	0.1	GSK938890A	-0.1
GW876790X	0.2	GW695874X	0.1	GW284372X	-0.1
SB-242717	0.2	GSK248233A	0.0	GW405841X	-0.1
SB-242719	0.2	GW572738X	0.0	GW461104A	-0.1
GSK238063A	0.2	GW682841X	0.0	SB-236687	-0.1
GW282974X	0.2	GW703087X	0.0	GSK204925A	-0.1
SB-347804	0.2	GW811761X	0.0	GW458344A	-0.1
GW410563A	0.2	SB-409513	0.0	GW621823A	-0.1
GW651576X	0.2	GSK605714A	0.0	GW786460X	-0.1
GW770249X	0.2	GSK718429A	0.0	GW874091X	-0.1
GW828529X	0.2	GW282536X	0.0	GW427984X	-0.1
GW852849X	0.2	GW574783B	0.0	GW434756X	-0.1
SB-220455	0.2	GW785974X	0.0	SB-682330-A	-0.1
SB-250715	0.2	GSK312948A	0.0	GW642125X	-0.1
GSK1511931A	0.2	GW305074X	0.0	GW819077X	-0.1
GW621970X	0.1	GW785804X	0.0	SB-216385	-0.1
GW642138X	0.1	GW817394X	0.0	SB-630812	-0.1
SB-254169	0.1	GW817396X	0.0	GSK1023156A	-0.1
SB-431533	0.1	GW843682X	0.0	GW561436X	-0.2
GW616030X	0.1	GSK1392956A	0.0	GW830365A	-0.2
SB-744941	0.1	GW693881A	0.0	GSK554170A	-0.2
GW770220A	0.1	SB-751399	0.0	GW445014X	-0.2
GW607117X	0.1	GSK561866B	0.0	GW683109X	-0.2
GW809885X	0.1	SB-251505	0.0	SKF-86002-A2	-0.2
GW828525X	0.1	SB-814597	0.0	GW406731X	-0.2
SB-253226	0.1	SKF-62604	0.0	GSK943949A	-0.2
SB-358518	0.1	GSK969786A	0.0	GW549034X	-0.2
SB-736302	0.1	SB-751148	0.0	GSK1030058A	-0.2
GSK1751853A	0.1	GW569293E	0.0	GSK949675A	-0.2
GSK619487A	0.1	GW807982X	0.0	SB-610251-B	-0.2
GW459057A	0.1	SB-736290	0.0	SB-633825	-0.2
GW576924A	0.1	GW680975X	0.0	GW572401X	-0.2
GW701427A	0.1	GSK270822A	0.0	GW678313X	-0.2
GW795486X	0.1	GSK571989A	0.0	SB-223133	-0.2

Table S2 cont. DSF screening data for PKIS compounds

Compound	T_m Shift (°C)
GW580496A	-0.2
GW804482X	-0.2
GW856804X	-0.2
SB-220025-A	-0.2
SB-221466	-0.2
GW683134A	-0.2
GW694234A	-0.2
SB-210313	-0.2
SB-360741	-0.2
SB-400868-A	-0.2
GSK1819799A	-0.3
GW432441X	-0.3
GW708893X	-0.3
GW782907X	-0.3
GSK317315A	-0.3
SB-437013	-0.3
GW568326X	-0.3
GSK1220512A	-0.3
GSK1000163A	-0.3
GW296115X	-0.3
GW799251X	-0.3
GSK1713088A	-0.3
GW576484X	-0.3
GW768505A	-0.3
GSK1030061A	-0.4
SB-614067-R	-0.4
GW279320X	-0.4
GSK466314A	-0.4
GW780159X	-0.4
GW853606X	-0.4
GW659386A	-0.4
GW622055X	-0.4
GW694590A	-0.4
GSK1030062A	-0.4
GW632580X	-0.5
GW853609X	-0.5
GSK259178A	-0.5
GSK319347A	-0.5
GW734508X	-0.5
GSK1030059A	-0.5
GW589961A	-0.6
SB-590885-	-0.6
GW709042A	-0.7

Table S3 DSF screening data for clinical kinase inhibitors

Compound	T_m Shift (°C)	Compound	T_m Shift (°C)	Compound	T_m Shift (°C)
Rebastinib	12.6	Rabusertib	0.2	Nintedanib	-0.3
Dabrafenib	10.4	AP26113	0.2	AZD2014	-0.3
SNS-032	7.0	Seliciclib	0.2	AZ3146	-0.3
Milciclib	6.2	GSK2334470	0.2	KX2-391	-0.3
AZ23	5.8	Doramapimod	0.2	MK-2206	-0.3
AT9283	5.7	SGI-1776	0.2	EMD1214063	-0.3
Ponatinib	5.5	BI 2536	0.2	PF-670462	-0.3
Dinaciclib	4.8	GSK2636771	0.2	Imatinib	-0.3
PIK-75	4.7	BYL719	0.1	ZSTK474	-0.3
Regorafenib	4.1	Apitolisib	0.1	BMS-911543	-0.4
PF 431396	4.1	Lenvatinib	0.1	Alectinib	-0.4
PF-03758309	4.1	Crizotinib	0.1	ASP3026	-0.4
Gandotinib	4.0	Pilralisib	0.1	Galunisertib	-0.4
Lestaurtinib	3.8	Dactolisib	0.1	Vandetanib	-0.4
Momelotinib	3.7	Pimasertib	0.1	Dasatinib	-0.4
TAK-901	3.7	GSK269962	0.1	AZD5363	-0.4
GSK650394	3.3	Dovitinib	0.1	Masitinib	-0.4
Foretinib	3.3	Gefitinib	0.0	Trametinib	-0.4
Fedratinib	3.3	Tivantinib	0.0	Buparlisib	-0.5
Alvociclib	2.9	Tofacitinib	0.0	Ipatasertib	-0.5
Crenolanib	2.7	Erlotinib	0.0	Linsitinib	-0.5
Palbociclib	2.6	Baricitinib	0.0	NVP-BGJ398	-0.5
LY2874455	2.6	Lapatinib	0.0	Bafetinib	-0.5
Linifanib	2.4	OSI-027	0.0	AZD8330	-0.5
MK-1775	2.2	CUDC-101	0.0	Selumetinib	-0.6
Sotrastaurin	2.2	Danuseritib	0.0	PF-04708671	-0.6
Sunitinib	2.1	Volasertib	-0.1	Ralimetinib	-0.6
UCN-01	2.1	Sonolisib	-0.1	Refametinib	-0.6
Sorafenib	2.0	Vatalanib	-0.1	Dacomitinib	-0.6
Pictilisib	1.5	IPI-145	-0.1	Midostaurin	-0.6
AZD1480	1.3	BMS-599626	-0.1	Cediranib	-0.7
Cabozantinib	1.2	Orantinib	-0.1	Alisertib	-0.7
AZD4547	1.1	INK128	-0.1	Brivanib	-0.7
Nilotinib	1.1	Tivozanib	-0.1	VX-11E	-0.8
BX-912	1.0	Axitinib	-0.1	AZD8055	-0.9
(5Z)-7-Oxozeaenol	1.0	GSK1904529A	-0.1	Ruxolitinib	-0.9
Tamatinib	0.9	PF-04691502	-0.1		
Voxtalisib	0.9	Ibrutinib	-0.1		
PF-00477736	0.9	MEK-162	-0.1		
Pazopanib	0.8	AZD7762	-0.1		
Neratinib	0.7	TGX-221	-0.2		
BMS-754807	0.6	TAK-733	-0.2		
SP600125	0.6	GSK1838705A	-0.2		
Ruboxistaurin	0.6	Idelalisib	-0.2		
Enzastaurin	0.6	SCH772984	-0.2		
Fasudil	0.5	GSK2126458	-0.2		
RAF265	0.5	Tideglusib	-0.2		
Bosutinib	0.5	Amuvatinib	-0.2		
Tandutinib	0.4	Motesanib	-0.2		
AZD1152-HQPA	0.4	PF-4800567	-0.2		
Saracatinib	0.4	Rigosertib	-0.3		
Vemurafenib	0.3	Afatinib	-0.3		
Quizartinib	0.3	Icotinib	-0.3		
MGCD-265	0.3	Apatinib	-0.3		

SUPPLEMENTARY FIGURES

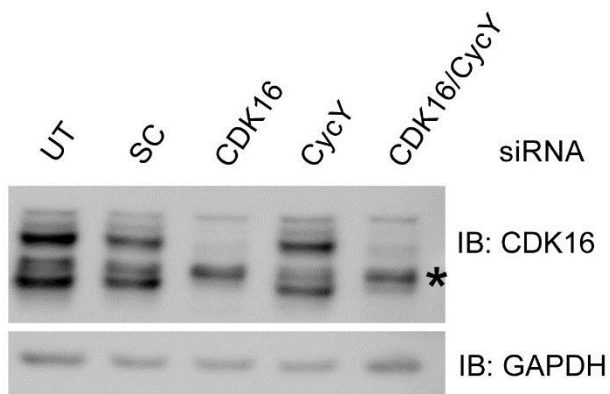


Figure S1. Knockdown of CDK16

Immunoreactive bands specific for CDK16 were confirmed using siRNA-mediated knockdown of CDK16 in IGR-37 melanoma cells. Scrambled siRNA (SC) and cyclin Y siRNA (CycY) were used as additional controls and compared to untreated (UT) cells. GAPDH protein levels were assessed as a loading control. A non-specific band detected by the anti-CDK16 antibody is indicated by an asterisk.