invitrogen™

part of life technologies™

Assessing differences in biochemical and cell-based profiles using SUTENT® as a case study

July 2009

Known Compound Example: SUTENT® (Sunitinib)

invitrogen™ part of life technologies™

SUTENT® (Sunitinib) Background

Usage

- GIST (gastrointestinal stromal tumor)
  - used when Gleevec® does not inhibit cancer growth or when one cannot take Gleevec®
- Advanced kidney cancer (advanced renal cell carcinoma)

Pharmacology

- Inhibits all known PDGF and VEGF receptors

SUTENT® is a registered trademark of Pfizer

Gleevec® is a registered trademark of Novartis

© 2009 Invitrogen Corporation. All Rights Reserved.

Experimental Design

invitrogen™ part of life technologies™

- Step I: Evaluate the compound of interest against a panel of biochemical kinase assays
  - Determine selectivity and potency across a broad kinase panel
  - SelectScreen® Kinase Profiling Service

- Step II: Determine the compound's effect in a cellular environment
  - Analyze selectivity and potency against multiple cellular pathways
  - SelectScreen® Cell-based Pathway Profiling Service

SelectScreen® Services can support you through each step of your screening process

© 2009 Invitrogen Corporation. All Rights Reserved.

Step I: Biochemical Profiling

invitrogen™ part of life technologies™

- Determine selectivity and potency across a broad panel of kinases
  - Profile SUTENT® in single point at 1μM against 260 kinases at $_{Km, app}$ ATP
  - Follow up with IC_{50} determinations

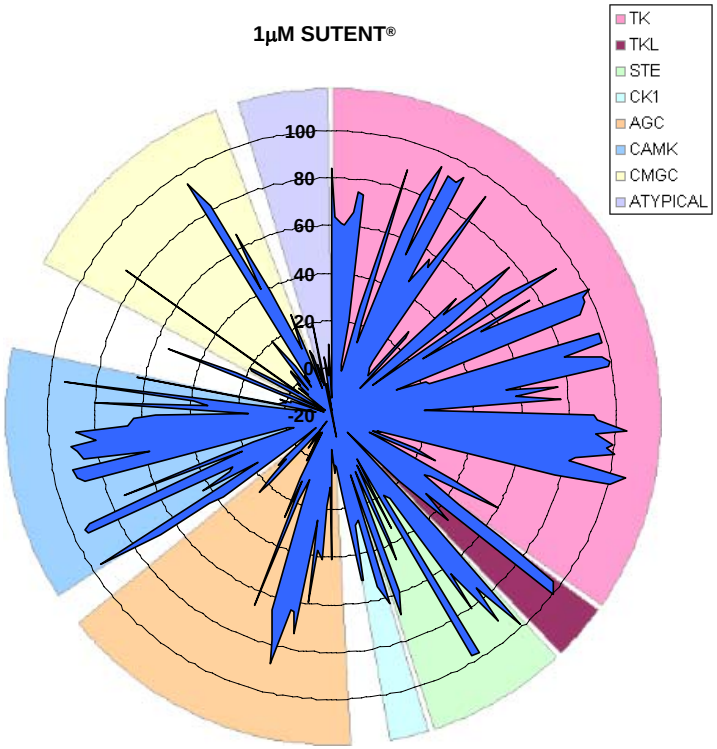
IC_{50} Profiling

Activity Assay- Kinase	Sutent® IC50 at ATP Km,app (nM)	Activity Assay- Kinase	Sutent® IC50 at ATP Km,app (nM)	Activity Assay- Kinase	Sutent® IC50 at ATP Km,app (nM)
ABL 1	362	GSK3B (GSK3 beta)	>5000	PRKCN (PKD3)	108
ABL1 E255K	1250	HCK	660	PRKCQ (PKC theta)	>5000
ABL1 G250E	601	HIPK1 (Myak)	2630	PRKCZ (PKC zeta)	>5000
ABL1 T315I	261	HIPK4	1870	PRKD1 (PKC mu)	65.3
ABL1 Y253F	415	IGF1R	612	PRKD2 (PKD2)	109
ABL 2 (Arg)	3290	IKBKB (IKK beta)	>5000	PRKG1	>5000
ACVR1B (ALK4)	>5000	IKBKE (IKK epsilon)	1660	PRKG2 (PKG2)	>5000
ADRBK1 (GRK2)	>5000	INSR	348	PRKX	>5000
ADRBK2 (GRK3)	>5000	INSRR (IRR)	340	PTK2 (FAK)	233
AKT1 (PKB alpha)	>5000	IRAK4	741	PTK2B (FAK2)	466
AKT2 (PKB beta)	>5000	ITK	49	PTK6 (Brk)	3110
AKT3 (PKB gamma)	>5000	JAK1	2190	RAF1 (cRAF) Y340D Y341D	1310
ALK	764	JAK2	1050	RET	12.7
AMPK A1B1G1	116	JAK2 JH1 JH2	2410	RET V804L	6.29
AMPK A2B1G1	60.3	JAK2 JH1 JH2 V617F	1890	RET Y791F	8.87
AURKA (Aurora A)	2490	JAK3	1840	ROCK1	>5000
AURKB (Aurora B)	3310	KDR (VEGFR2)	65.3	ROCK2	>5000
AURKC (Aurora C)	1050	KIT	12	ROS1	1070
AXL	28.3	KIT T670I	19	RPS6KA1 (RSK1)	267
BLK	188	LCK	138	RPS6KA2 (RSK3)	223
BMX	>5000	LRRK2	33.3	RPS6KA3 (RSK2)	508
BRAF	1270	LRRK2 G2019S	23.1	RPS6KA4 (MSK2)	210
BRAF V600E	3860	LTK (TYK1)	203	RPS6KA5 (MSK1)	>5000
BRSK1 (SAD1)	580	LYN A	168	RPS6KA6 (RSK4)	384
BTK	3340	LYN B	164	RPS6KB1 (p70S6K)	461
CAMK1 (CaMK1)	2260	MAP2K1 (MEK1)	379	SGK (SGK1)	432
CAMK1D (CaMKI delta)	1990	MAP2K2 (MEK2)	168	SGK2	4620
CAMK2A (CaMKII alpha)	311	MAP2K6 (MKK6)	>5000	SGK1 (SGK3)	>5000
CAMK2B (CaMKII beta)	4990	MAP3K8 (COT)	864	SIK1	658
CAMK2D (CaMKII delta)	47.3	MAP3K9 (MLK1)	724	SRC	1330
CAMK4 (CaMKIV)	703	MAP4K2 (GCK)	313	SRC N1	2190
CDC42 BPA (MRCKA)	>5000	MAP4K4 (HGK)	71.4	SRMS (Srm)	>5000
CDC42 BPB (MRCKB)	>5000	MAP4K5 (KHS1)	22.7	SRPK1	>5000
CDK1/cyclin B	>5000	MAPK1 (ERK2)	>5000	SRPK2	>5000
CDK2/cyclin A	>5000	MAPK10 (JNK3)	>5000	STK22B (TSSK2)	>5000
CDK5/p25	4000	MAPK11 (p38 beta)	>5000	STK22D (TSSK1)	206
CDK5/p35	3380	MAPK12 (p38 gamma)	>5000	STK23 (MSSK1)	>5000
CDK7/cyclin H/MNAT1	192	MAPK13 (p38 delta)	>5000	STK24 (MST3)	94.1
CDK9/cyclin T1	4830	MAPK14 (p38 alpha)	>5000	STK25 (YSK1)	2120
CHEK1 (CHK1)	378	MAPK3 (ERK1)	>5000	STK3 (MST2)	367
CHEK2 (CHK2)	76.2	MAPK8 (JNK1)	>5000	STK4 (MST1)	62.8
CHUK (IKK alpha)	>5000	MAPK9 (JNK2)	>5000	SYK	1400
CLK1	301	MAPKAPK2	>5000	TAOK2 (TAO1)	>5000
CLK2	71.5	MAPKAPK3	>5000	TBK1	959
CLK3	>5000	MAPKAPK5 (PRAK)	>5000	TEK (Tie2)	>5000
CSF1R (FMS)	130	MARK1 (MARK)	192	TYK2	609
CSK	>5000	MARK2	401	TYRO3 (RSE)	214
CSNK1A1 (CK1 alpha 1)	>5000	MARK3	302	YES1	44.6
CSNK1D (CK1 delta)	498	MARK4	259	ZAP70	>5000
CSNK1E (CK1 epsilon)	645	MATK (HYL)	>5000		
CSNK1G1 (CK1 gamma 1)	>5000	MEK1 K	125		
CSNK1G2 (CK1 gamma 2)	2440	MERTK (c-MER)	193		
CSNK1G3 (CK1 gamma 3)	1060	MET (c-Met)	>5000		
CSNK2A1 (CK2 alpha 1)	>5000	MET M1250T	3010		
CSNK2A2 (CK2 alpha 2)	>5000	MINK1	121		
DAPK1	1020	MST1R (RON)	>5000		
DAPK3 (ZIPK)	2080	MSK1	1870		
DCAMKL2 (DCK2)	>5000	MUSK	27.6		
DYRK1A	>5000	MYLK2 (skMLCK)	34.4		
DYRK1B	>5000	NEK1	>5000		
DYRK3	>5000	NEK2	>5000		
DYRK4	>5000	NEK4	>5000		
EEF2K	>5000	NEK6	>5000		
EGFR (ErbB1)	>5000	NEK7	>5000		
EGFR (ErbB1) L858R	>5000	Nek9	>5000		
EGFR (ErbB1) 861Q	>5000	NTRK1 (TRKA)	29.2		
EGFR (ErbB1) T790M	4360	NTRK2 (TRKB)	93		
EGFR (ErbB1) T790M L858R	1850	NTRK3 (TRKC)	45.6		
EPHA1	3190	PAK2 (PAK65)	>5000		
EPHA2	>5000	PAK3	>5000		
EPHA3	>5000	PAK4	1500		
EPHA4	>5000	PAK6	>5000		
EPHA5	4340	PAK7 (KIAA1264)	>5000		
EPHA8	>5000	PASK	>5000		
EPHB1	568	PDGFRA (PDGFR alpha)	117		
EPHB2	1400	PDGFRA D842V	555		
EPHB3	>5000	PDGFRA T674I	5.6		
EPHB4	1240	PDGFRA V561D	16.3		
ERBB2 (HER2)	>5000	PDGFRB (PDGFR beta)	249		
ERBB4 (HER4)	>5000	PDK1	3280		
FER	389	PHKG1	47.9		
FES (FES)	347	PHKG2	96.4		
FGFR1	660	PIK3CB	>5000		
FGFR2	209	PIK3CA/PIK3R1 (p110 alpha/p85 alpha)	>5000		
FGFR3	462	PIK3CG (p110 gamma)	>5000		
FGFR3 K650E	310	PIM1	>5000		
FGFR4	>5000	PIM2	>5000		
FGR	102	PKN1 (PRK1)	>5000		
FLT1 (VEGFR1)	2120	PLK1	>5000		
FLT3	1.62	PLK2	>5000		
FLT3 D835Y	3.18	PLK3	>5000		
FLT4 (VEGFR3)	129	PRKACA (PKA)	>5000		
FRAP1 (mTOR)	>5000	PRKCA (PKC alpha)	>5000		
FRK (PTK5)	533	PRKCB1 (PKC beta I)	>5000		
FYN	2360	PRKCB2 (PKC beta II)	>5000		
GRK4	>5000	PRKCD (PKC delta)	>5000		
GRK5	>5000	PRKCE (PKC epsilon)	>5000		
GRK6	837	PRKCG (PKC gamma)	>5000		
GRK7	2660	PRKCH (PKC eta)	>5000		
GSK3A (GSK3 alpha)	>5000	PRKCI (PKC iota)	>5000		

Legend	
low nanomolar	< 100 nM
sub-micromolar	100-1000 nM
low micromolar	1000-10000 nM
above range	> 10000 nM

© 2009 Invitrogen Corporation. All Rights Reserved.

Step I: Biochemical Profiling



SelectScreen® Kinase Profiling Results

- Highly non-selective inhibitor that hits multiple targets across all families
- PDGFR, KDR, KIT, CSF1R. FLT3, and RET are known targets for SUTENT®

Nat. Rev. Drug Discov. 6, 734-745 (2007)

Biochemical profiling of SUTENT® shows a highly non-selective inhibitor profile across all families

© 2009 Invitrogen Corporation. All Rights Reserved.

Step II: Cell-based Profiling

invitrogen™ part of life technologies™

- Evaluate selectivity and potency against multiple pathway specific cellular assays
  - Profile SUTENT® in 10 point titration (½-log dilutions)

IC_{50} Cell-based Profiling

Pathway	Cell Line	Stim	Sutent® IC50 (nM)	% Inhibition at 10 uM	Z´
cAMP/PKA	CRE-bla Jurkat	Forskolin	>10,000	38	0.87
cAMP/PKA	CRE-bla HEK 293T	Forskolin	>10,000	-5	0.91
DNA damage/p53 Response	p53RE-bla HCT-116	NA	>10,000	-5	0.76
Interleukin 4/STAT6	STAT6-bla RA1	IL-4	617	100	0.82
JAK/STAT	ISRE-bla Jurkat	IFN-alpha	650	81	0.74
JAK/STAT	ISRE-bla HEK 293T	IFN-alpha	>10,000	55	0.91
JAK/STAT	SIE-bla ME-180	IL-6	6300	63	0.64
JAK/STAT	SIE-bla ME-180	IFN-gamma	>10,000	38	0.92
JAK2/STAT5	irf1-bla TF1	GM-CSF	5670	60	0.67
JAK2/STAT5	irf1-bla TF1	EPO	4740	74	0.70
MAPK/EGFR/Ras/Raf	AP1-bla ME-180	EGF	> 10,000	58	0.70
NFkB	NFkB-bla ME-180	TNF-alpha	3040	66	0.63
NFkB	NFkB-bla ME-180	IL-1 beta	7160	40	0.76
NFkB	NFkB-bla THP-1	TNF-alpha	> 10,000	42	0.70
PI3K/AKT/FOXO3	TREx FOXO3-DBE-bla HeLa	Insulin	---	-39	0.53
T Cell Receptor	NFAT-bla Jurkat	anti-CD3:CD28	1090	106	0.63
TGF-beta	SBE-bla HEK 293T	TGF-beta	> 10,000	16	0.89
Toll-Like Receptor (TLR4)	NFkB-bla THP-1	LPS	4640	79	0.68

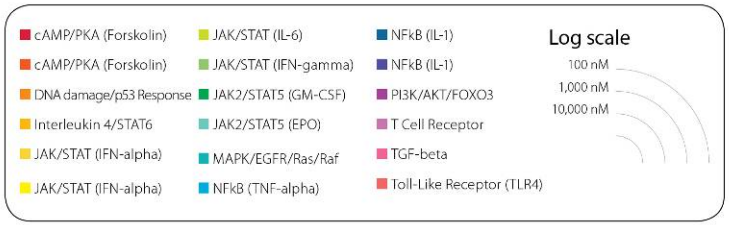
Legend	
low nanomolar	< 100 nM
sub-micromolar	100-1000 nM
low micromolar	1000-10000 nM
above range	> 10000 nM

© 2009 Invitrogen Corporation. All Rights Reserved.

Step II: Cell-based Profiling

invitrogen™ part of life technologies™

Sutent®



SelectScreen® Cell-based Profiling Results

- Sub-micromolar inhibitor of certain JAK/STAT pathways

Cellular profiling shows SUTENT® has a more defined inhibitor profile than that identified in the biochemical profile

© 2009 Invitrogen Corporation. All Rights Reserved.

Results of Case Study

invitrogen™
part of life technologies™

- This case study demonstrates:
  - Determination of a compound's effect on a panel of kinases using biochemical assays.
    - Evaluated the selectivity of the compound of interest by profiling a panel of kinases.
    - *Identified a compound that was highly non-selective across a broad panel of kinases in a biochemical context.*
  - Determination of a compound's effect in a biological context using cellular assays.
    - Identified selectivity by profiling multiple pathway specific cellular assays.
    - *Determined that the compound is more selective in a cellular environment than observed in a biochemical assay.*

Biochemical → *Cellular*

© 2009 Invitrogen Corporation. All Rights Reserved.

Start Your Project Today

invitrogen™
part of life technologies™

- To design your next project and obtain a quote, email us at discoveryservices@invitrogen.com
- To speak with your local Drug Discovery Sales Specialist, contact us at discoveryservices@invitrogen.com
- To learn more about our SelectScreen® Services, please visit our website at www.invitrogen.com/experienceus

© 2009 Invitrogen Corporation. All Rights Reserved.