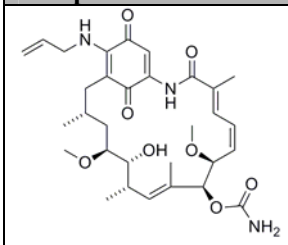
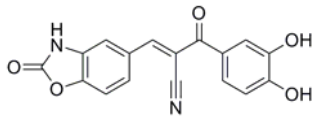
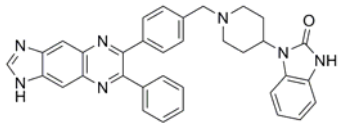
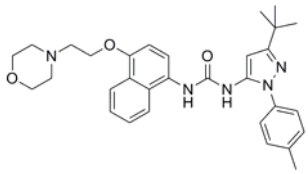
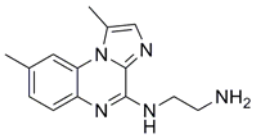
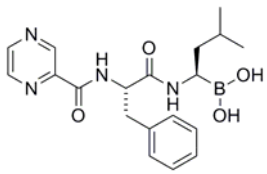
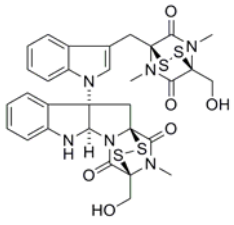
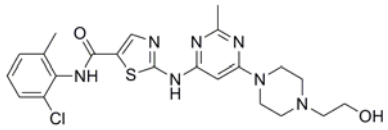
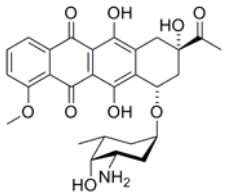
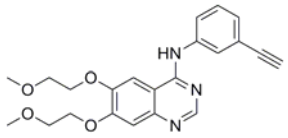
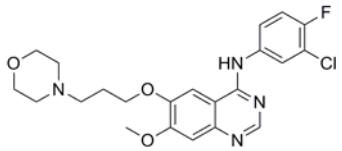
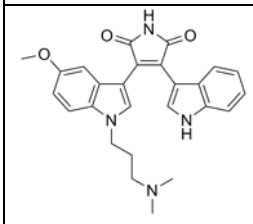
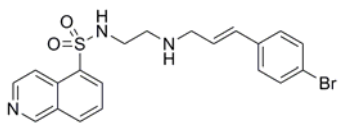
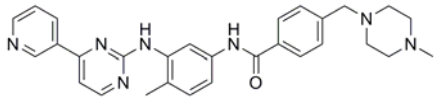
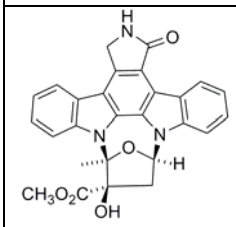
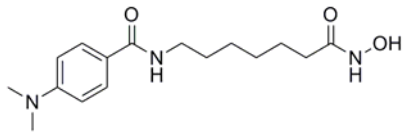
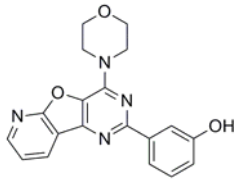
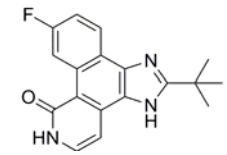
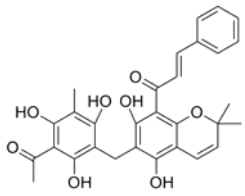
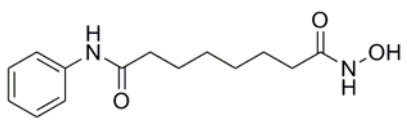
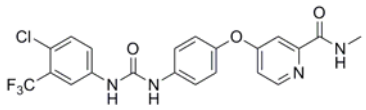
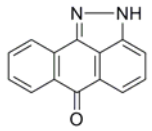
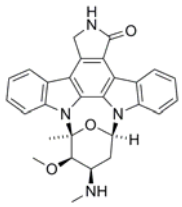
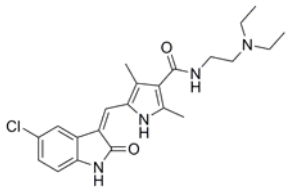
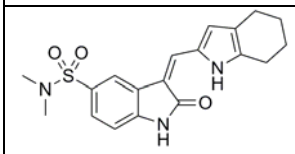
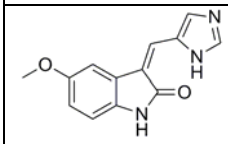
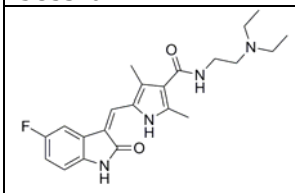
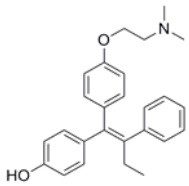
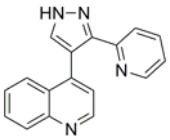
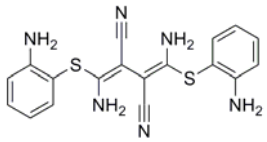
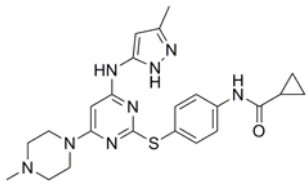
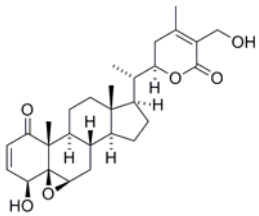
Supplementary Table 1: Compounds used in the profiling study.

Compound	Known target(s)	Reference(s)
 17-AAG	HSP90	Clarke PA, et al., Oncogene 19, 4125-33 (2000)
 AGL 2263	IR, IGF1R	Blum G, et al., J. Bio. Chem. 278, 40442-40454 (2003)
 AKTi-1/2	AKTs	Logie L, et al., Diabetes 56, 2218-27 (2007)
 BIRB-796	p38α, β, γ	Regan J, et al., J. Med. Chem. 46, 4676-86 (2003)
 BMS345541	IKKβ	Burke JR, et al., J. Biol. Chem. 278, 1450-6 (2003)
 Bortezomib (Velcade, MG-341)	Proteasomal inhibitor	Nencioni A, et al., Blood 108, 551-8 (2006)

 Chetomin	HIF1a/p300	Kung AL, et al., Cancer Cell 6, 33-43 (2004)
 Dasatinib (Sprycel, BMS-354825)	ABL1, SRC, KIT	Schittenhelm MM, et al., Cancer Res. 66, 473-481 (2006).
 Daunorubicin	DNA replication and repair	Capranico G, et al., Cancer Res. 55, 312-7 (1995)
 Erlotinib (Tarceva)	EGFR	Moyer JD, et al., Cancer Res. 57, 4838-4848 (1997)
 Gefitinib (Iressa)	EGFR	Ciardiello F, et al., Clin Cancer Res. May;6(5):2053-63 (2002)
 GÖ-6983	PKC	Gschwendt M, et al., FEBS Lett. Aug 26;392(2):77-80 (1996)
 H-89	PKA, ROCK2, PRK2, RSKs	Davies SP, et al., Biochem. J. 351, 95-105 (2000)

 Imatinib (Gleevec)	ABL1, KIT, PDGFRβ	O'Dwyer ME, et al., Lancet Oncol. 1, 207-11 (2000)
 K252a	Trks, Pan-kinase inhibitor	Wang J, et al., Curr. Chem. Gen. 1, 1-7 (2008); Knüsel B, et al., J. Neurochem. 59, 1987-96 (1992).
 M344	HDAC inhibitor	Riessland M, et al., Hum. Genet. 120, 101-10 (2006)
 PI-103	PIK3CA, mTOR	Chaussade C, et al., Biochem. J. 404, 449-458 (2007)
 Pyridone 6	JAKs	Pedranzini L, et al., Cancer Res. 66(19):9714-21 (2006)
 Rottlerin	PKCδ and θ	Gschwendt M, et al., Biochem. Biophys. Res. Commun. 199, 93-8 (1994)
 SAHA (Zolinza, vorinostat)	HDAC inhibitor	Richon VM, et al., Proc. Natl. Acad. Sci. USA 93, 5705-8 (1996)

 Sorafenib (Bay43-9006, Nexavar)	CRAF,BRAF,KIT,FLT3, VEGFR2,VEGFR3,PDGFRB. P38a, SRC	Biochem. J. 408, 297- 315 (2007)
 SP600125	JNK	Bennett BL, et al., Proc. Natl. Acad, Sci. USA 98, 13681-13686 (2001)
 Staurosporine	PKCs, Pan-kinase inhibitor	Rüegg UT, et al., Trends Pharmacol. Sci. 10, 218-20 (1989)
 SU11652	PDGFR,VEGFR, KIT	Liao A, et al., Blood 100, 585-593 (2002)
 SU6656	SRC family	Blake RA, et al., Mol. Cell Biol. 20, 9018-27 (2000)
 SU9516	CDKs	Lane ME, et al., Cancer Res. 61, 6170-6177 (2001)
 Sunitinib (Sutent, SU11248)	PDGFR,VEGFR,KIT, CSF1R,FLT3,RET	Faivre S, et al., Nat. Rev. Drug Discov. 6, 734-745 (2007)

 (Z)-4-hydroxytamoxifen	ER, PKC, CaM	Katzenellenbogen Cancer Res. 44(1):112-9 (1984)
 TGFβR1 Inhibitor	TGFβR1	Sawyer SJ, et al., J. Med. Chem. 46. 3953-3956 (2003)
 U0126	MEK	Favata MF, et al., J. Biol. Chem. 273, 18623-32 (1998)
 VX680 (MK-0457)	AURKA, AURKB, AURKC	Harrington EA, et al., Nat. Med. 10, 262-7 (2004)
 Withaferin A	IKKβ, Proteasome	Kaileh M, et al., J. Biol. Chem. 282, 4253-4264 (2007); Yang H, et al., Mol. Pharmacol. 71, 426-437 (2007)

Stimuli/ Pathway	*CellSensor® line*	*EC_{80} (nM)*	*Response Ratio*	*Z' max*	*Z' EC80*	*Known inhibitor*	*Known target*	*IC_{50} (nM)*	*Pub. IC_{50} (nM)*	*Inhibitor Reference*
EGF/MAPK	AP1-*bla* ME-180	0.008	6	0.7	0.7	PD153035	EGFR	62	100	Lichtner RB, et al., Cancer Res. 61, 5790-5795 (2001)
TNFα/JNK	AP1-*bla* ME-180	0.069	5	0.7	0.5	SP600125	JNKs	2750	3000-5000	Utsugi M, et al., J. Immunol. 171, 628-635 (2003)
TNFα/NFκB	NFκB-*bla* THP1	1.07	6.5	0.9	0.7	Withaferin A	IKKβ, Proteasome	550	250	Kaileh M, et al., J. Biol. Chem. 282, 4253-4264 (2007)
LPS/NFκB	NFκB-*bla* THP1	0.8 ng/ml	5	0.7	0.6	Withaferin A	IKKβ, Proteasome	325	250	Kaileh M, et al., J. Biol. Chem. 282, 4253-4264 (2007)
IL6/JAK/STAT	SIE-*bla* ME-180	0.41	12	0.8	0.7	Pyridone 6	JAKs	13	<250	Pedranzini L, et al., Cancer Res. 66, 9714-9721 (2006)
IFNα/JAK/STAT	ISRE-*bla* Hek293T	0.036	4	0.8	0.7	Pyridone 6	JAKs	20	N/A	N/A
TGFβ/SMAD	SBE-*bla* Hek293T	0.009	4.5	0.8	0.8	TGFβR1 Inhibitor	TGFβR	53	47	Sawyer SJ, et al., J. Med. Chem. 46. 3953-3956 (2003)
Thapsigargin PKC/Ca^{2+}	NFAT-*bla* Jurkat	25	5.2	0.9	0.8	Gö6983	PKCs	5200	5000	Oberg HH, et al., Cell Death and Differ. 11,674-684 (2004)
Forskolin cAMP/PKA	CRE-*bla* Jurkat	5 x 10^4	7	0.9	0.7	H-89	PKA	6000	2000	Reem RH, et al., J. Mol. Endocrinol. 22, 285-292 (1999)
$CoCl_2$/Hypoxia	HRE-*bla* ME-180	10^5	7	0.9	0.8	Chetomin	hypoxia	5	5	Kung AL, et al., Cancer Cell 6, 33-43 (2004)
Serum/Cell Cycle/pRB/E2F	Dhfr(E2F)-*bla* NIH3T3	10%	5	0.7	N/A	SU9516	CDKs	6600	5000-6000	Lane ME, et al., Cancer Res. 61, 6170-6177 (2001)
Insulin/PI3K/ AKT/FOXO3a	T-REx-FOXO3a DBE-bla HeLa	100	3.5	0.9	0.8	PI-103	PI3K/mTOR	90	78	Chaussade C, et al., Biochem. J. 404, 449-458 (2007)
Control	CMV-*bla* Jurkat	N/A	23	0.9	N/A	Clavulanate	Beta-lactamase	1.9 x 10^5	N/A	N/A

Supplementary Table 2: Validation parameters for the 13 cell lines used in the profiling study.

Supplementary Table 3a IC_{50} (nM) heat map summary for 32 compounds against the CMV-*bla* Jurkat control and 9 pathway assays (5 hour stimulation time)

Compound	Constitutive Control	EGF/MAPK	TNFα/JNK	TNFα/NFκB	LPS/NFκB	IL-6 /JAK/STAT	IFNα /JAK/STAT	TGFβ/ SMAD	Thapsigargin /PKC/Ca2+	Forskolin cAMP /PKA
	CMV-*bla* Jurkat	AP1-*bla* ME180	AP1-*bla* ME180	NFκB-*bla* THP-1	NFκB-*bla* THP-1	SIE-*bla* ME180	ISRE-*bla* HEK293T	SBE-*bla* HEK293T	NFAT-*bla* Jurkat	CRE-*bla* Jurkat
Staurosporine	116	1	1	209	62	8	15	85	4	16
Chetomin	2287^	319	140	416	164	1099	494	5	68	95
K252a	1435	142	272	1461	37	16	45	1400	87	51
PI-103	384*	76**	575**	944*	1035*	63**	1026*	183*	457**	>5000
Sorafenib	>10000	709	180	570	354	672	572	526	593	151
Daunorubicin	2089**	886	861	674	469	1370	1790	1007	2894	945
Dasatinib	>10000	9	5	>10000	728**	1799^	7691^	242	6	>10000
Withaferin A	1231^	2763	2727	903	630	835	1182	437	99	3046^
SU6656	>10000	431**	869	>10000	350*	444	1369	167*	126**	396**
Rottlerin	>20000	6160**	3622	389	230	1478	536	1453	744	165
SU11652	7739^	3197^	158	3634^	1945	730	3953^	3732	275	539^
AKTi-1/2	3138*	1045**	2672*	1690*	2502**	6774**	2206**	1757**	614	>10000
Sunitinib	>10000	1039*	571*	>10000	4033^	1477	4179^	>10000	401**	6592^
BMS345541	6267*	2766	2067	2880	1144	5714	2742	5834	5588	2130
SU9516	7299**	4802	8412	3457	2427	1638	3870	8930	817	6582
SP600125	33872^	1667*	3174**	6893**	3254	2330**	5172	6440*	27809^	25924*
Bortezomib	>10000	>10000	>10000	237**	68	>10000	22	189**	87**	>10000
17-AAG	>14230	2170*	3326*	>14230	389*	>14230	>14230	195	151*	>14230
GÖ-6983	>10000	5726^	4625^	8295^	9084^	3867**	>10000	>10000	5287**	>10000
M344	>10000	>10000RA	>10000RA	>10000	>10000	71	210**	596*	1084*	>10000
SAHA	>10000	>10000RA	>10000RA	>10000	>10000	163	219**	973*	3037*	>10000
VX680	>10000	>10000	1810	>10000	>10000	3027^	>10000	9747**	430	>10000
H89	>10000	>10000	>10000	>10000	6151^	>10000	9190^	9110^	7228^	4554^

Compound	Constitutive Control	EGF/MAPK	TNFα/JNK	TNFα/NFκB	LPS/NFκB	IL-6 /JAK/STAT	IFNα /JAK/STAT	TGFβ/ SMAD	Thapsigargin /PKC/Ca2+	Forskolin cAMP /PKA
	CMV-*bla* Jurkat	AP1-*bla* ME180	AP1-*bla* ME180	NFκB-*bla* THP-1	NFκB-*bla* THP-1	SIE-*bla* ME180	ISRE-*bla* HEK293T	SBE-*bla* HEK293T	NFAT-*bla* Jurkat	CRE-*bla* Jurkat
Pyridone 6	>10000	>10000	>10000	>10000	475*	7	42	>10000	304**	>10000
Erlotinib	>10000	132	2177	>10000	>10000	6786^	>10000	>10000	1204**	>10000
Gefitinib	>10000	69	7294^	>10000	6605^	>10000	>10000	>10000	1721**	>10000
UO126	>20000	2194**	>20000	>20000	2312*	>20000	13327^	>20000	2655^	6419
Imatinib	>10000	2316*	>10000	>10000	>10000	>10000	>10000	>10000	2566**	>10000
BIRB-796	>10000	>10000	1753**	>10000	3**	>10000	>10000	>10000	>10000	>10000
TGFβR1 Inh.	>10000	>10000	>10000	>10000	>10000	>10000	>10000	43	>10000	>10000
Tamoxifen	>10000	>10000	>10000	>10000	>10000	>10000	>10000	6171^	>10000	>10000
AGL 2263	>20000	>20000	>20000	>20000	>20000	>20000	>20000	>20000	>20000	>20000

IC_{50} legend: < 100 nM | 100 - 1,000 nM | 1,000 - 10,000 nM | > 10,000 nM

^no clear top to the IC_{50} curve; therefore, IC_{50} was estimated using fixed "top" values rather than actual data points
^{RA}reverse antagonist curve detected
*weak partial antagonist (clear top to IC_{50} curve, but only 25-50% maximal inhibition observed relative to full antagonist controls and/or unstimulated controls)
**strong partial antagonist (clear top to IC_{50} curve, but only 50-75% maximal inhibition observed relative to full antagonist controls and/or unstimulated controls)

To facilitate visualization of the data, a four-color heat map was superimposed onto the table and the data was also sorted top-to-bottom in relative rank order by most-to-least potent multi-pathway inhibition.

The partial effects were marked in order to be distinguished from the complete pathway inhibitory effect of control compounds known to act in a more directed manner towards the pathway of interest. We believe that the partial inhibitory effect on a particular pathway is generally due to the partial involvement (e.g., selectivity for a subset of the target kinase family members present within the given cell background, thereby allowing for a degree of "compensation" by the non-/weakly-targeted family members) and/or

indirect contributions of the primary targets of a given compound to the pathway of interest (i.e., multiple pathways feeding into the final readout). It is also possible that some of the partial effects observed are related to the mechanisms of action and/or cellular half-life of the compounds. For instance, HDAC inhibition by SAHA is known to be rapidly reversible, which could explain the partial effect of SAHA on most of the pathways affected.

Supplementary Table 3b IC_{50} (nM) summary heat map for 32 compounds against the CMV-*bla* Jurkat control and 3 pathway assays (overnight incubation)

Compound	Constitutive Control	Hypoxia	Cell cycle pRB-E2F	Insulin /PI3K/AKT
	CMV-*bla* Jurkat	HRE-*bla* ME180	dhfr(E2F)-*bla* NIH3T3	T-REx FOXO3 DBE-*bla* HeLa
Staurosporine	116	58	2	>1000
Chetomin	104	4	208	>10000
K252a	1878	25	>10000	>10000
PI-103	44*	59	1105**	52**
Sorafenib	>10000	853	4203^	>10000
Daunorubicin	275	91	178	391*
Dasatinib	>10000	9**	466	>10000
Withaferin A	362	139	1136	>10000
SU6656	>10000	8102^	>10000	>10000
Rottlerin	3553*	267**	1414**	>20000
SU11652	4512^	89**	3147	>10000
AKTi-1/2	617*	575	>10000	86*
Sunitinib	5304^	1307	2481	>10000
BMS345541	7450	2316	7747*	>20000
SU9516	11045	2179	7377^	>31600
SP600125	34954^	1773	8155**	>50000
Bortezomib	10	9	14	>10000
17-AAG	>14230	46	258	>14230
GÖ-6983	4009^	7432^	>10000	>10000
M344	509*	1040	305**	>10000
SAHA	724*	1902	322**	>10000
VX680	>10000	7701^	6136^	>10000
H-89	>10000	2612^	>10000	>10000
Pyridone 6	1028*	220	>10000	>10000
Erlotinib	>10000	4245	>10000	>10000
Gefitinib	>10000	9446^	>10000	>10000
UO126	>20000	>20000	>20000	>20000
Imatinib	>10000	4871**	>10000	>10000
BIRB-796	>10000	>10000	>10000	>10000
TGFβR1 Inh.	>10000	>10000	>10000	>10000
Tamoxifen	>10000	>10000	>10000	>10000
AGL 2263	>20000	>20000	>20000	>20000

Compounds such as staurosporine and chetomin exhibited detectable cytotoxic effects at high concentrations. To accurately report the IC_{50} values for the pathway reporter gene assays, those data points were excluded from the IC_{50} calculation. In

addition, the hypoxia, cell cycle and PI3K/AKT pathways assays required an overnight incubation with compounds and respective stimulatory ligands, whereas the rest of the pathway assays utilized only a 5 hour incubation. Therefore, CMV-*bla* controls were run for both 5 and 16 hours. Not surprisingly, the longer 16 hour incubation identified more compound nonspecific effects and more cases of toxicity, especially at higher compound concentrations.

It is also important to point out the unique nature of the insulin/PI3K/AKT pathway reporter line, which was engineered to express the BLA reporter upon induction of FOXO3a expression. Accordingly, when this pathway is activated by insulin, activated AKT negatively regulates FOXO3a, and BLA reporter activity decreases. This is in contrast to the other pathway reporter lines that express increased BLA activity upon pathway stimulation. Therefore, unlike the rest of the pathway read-outs, the PI3K/AKT reporter assay is less prone to false-positive hits due to nonspecific or toxic effects of compounds. As a result only three compounds out of the 32 showed activities in this assay.

Supplementary Table 4 Selectivity scores before (non filtered) and after (filtered) removing IC_{50} values from the calculations that were within a half-log of the corresponding IC_{50} values obtained for the CMV-*bla* Jurkat controls.

Compound	S (< 0.1 μM)		S (< 1 μM)		S (< 10 μM)	
	Non filtered	Filtered	Non filtered	Filtered	Non filtered	Filtered
Staurosporine	0.833	0.583	0.917	0.583	0.917	0.583
Chetomin	0.333	0.333	0.833	0.750	0.917	0.750
K252a	0.500	0.500	0.667	0.667	0.833	0.667
PI-103	0.333	0.250	0.667	0.250	0.917	0.333
Sorafenib	0.000	0.000	0.833	0.833	0.917	0.917
Daunorubicin	0.083	0.000	0.667	0.167	1.0	0.167
Dasatinib	0.333	0.333	0.583	0.583	0.750	0.750
Withaferin A	0.083	0.083	0.417	0.167	0.917	0.167
SU6656	0.000	0.000	0.583	0.583	0.750	0.750
Rottlerin	0.000	0.000	0.583	0.500	0.917	0.833
SU11652	0.083	0.083	0.416	0.416	0.917	0.500
AKTi-1/2	0.083	0.083	0.250	0.167	0.833	0.167
Sunitinib	0.000	0.000	0.167	0.167	0.750	0.667
BMS345541	0.000	0.000	0.000	0.000	0.917	0.167
SU9516	0.000	0.000	0.083	0.083	0.917	0.250
SP600125	0.000	0.000	0.000	0.000	0.750	0.750
Bortezomib	0.417	0.250	0.583	0.417	0.583	0.417
17-AAG	0.083	0.083	0.417	0.417	0.583	0.583
GÖ-6983	0.000	0.000	0.000	0.000	0.583	0.500
M344	0.083	0.083	0.333	0.250	0.500	0.333
SAHA	0.000	0.000	0.333	0.250	0.500	0.333
VX680	0.000	0.000	0.083	0.083	0.500	0.500
H-89	0.000	0.000	0.000	0.000	0.500	0.500
Pyridone 6	0.167	0.167	0.417	0.417	0.417	0.417
Erlotinib	0.000	0.000	0.083	0.083	0.417	0.417
Gefitinib	0.083	0.083	0.083	0.083	0.417	0.417
UO126	0.000	0.000	0.000	0.000	0.333	0.333
Imatinib	0.000	0.000	0.000	0.000	0.250	0.250
BIRB-796	0.083	0.083	0.083	0.083	0.167	0.167
TGFβR1 Inh.	0.083	0.083	0.083	0.083	0.083	0.083
Tamoxifen	0.000	0.000	0.000	0.000	0.083	0.083
AGL 2263	0.000	0.000	0.000	0.000	0.000	0.000