Supplemental Figures:

SF1) C48 can alkylate cysteine using glutathione as a model compound

A) Positive ion electrospray mass spectrum (MS) of the adduct of C48 with glutathione (GSH). A protonated molecular ion was observed at m/z 624.1868 (calculated for $C_{27}H_{34}N_3O_{12}S^+$: 624.1858).

B) Fragment ion spectrum (MS/MS) of the GSH-C48 molecular ion. Collision-induced dissociation fragment ion spectrum (MS/MS) of the ion at m/z 624.1 yielded the C48 carbocation at m/z 317.1021 (calculated for $C_{17}H_{17}O_6^+$: 317.1020), the protonated molecular ion for GSH at m/z 308.0922 (calculated for $C_{10}H_{18}N_3O_3S$+: 308.0911), and the y2 fragment of the protonated molecular ion for GSH at m/z 179.0485 (calculated for $C_5H_{ll}N_2O_3S^+$: 179.0485).

All spectra were acquired by LC-MS/MS on an Agilent 6520 QTOF mass spectrometer equipped with an Agilent 1200 series nanoflow HPLC and Chip-cube source (Agilent Technologies, Santa Clara, CA). The loading and analytical pumps used the same solvent system. Solvent A was 100% aqueous and solvent B was 90% acetonitrile, both with 0.1% formic acid. Sample was loaded onto a standard capacity chip (40 nl trapping column, 43 mm x 75 μm analytical column, packed with Zorbax 5 μm 300 Å C18 silica) at 6 μl/min in 99% solvent A. After loading, the sample was washed with 8 μl of the same solvent to remove salts. After washing, the chip cube valve was switched so the trapping and analytical columns were on-line with the analytical pump. The sample was eluted at 600 nl/min with a linear gradient from 1% to 50% solvent B over 8 min, followed by a 1 min ramp to 98% solvent B, a 1 min hold at 98% B, and a 1 min ramp back to 1% B. The mass spectrometer was operated in a data dependent MS/MS mode. Full mass survey scans of singly charged ions from 300 Th to 3000 Th with specific targeting of GSH-C48 adduct m/z (624.18) were collected for 250 ms. From each survey scan, up to 6 ions with an intensity of at least 1000 counts were selected for MS/MS. MS/MS scans were also 250 ms, but were collected from 50 Th to 3000 Th. After an ion was selected for MS/MS, it was placed on an exclusion list for 15 s. Mass accuracy was improved by monitoring two lock mass ions.

SF2) C48 does not affect Stat3 dimerization

A) Size exclusion chromatography of Stat3. Stat3, residues 127 to 722, is monomeric and elutes at 14.1 mL on a Superdex 200 HR column. Phosphorylation of this construct produces a dimeric species that elutes at 12.4 mL. The addition of C48 (at 200 μM) to the monomeric or phosphorylated dimeric form does not affect their respective elution profiles, indicating that C48 does not affect pY705-mediated dimerization.

B) Sedimentation equilibrium experiments of phospho-Stat3-C468S in the presence of C48 indicate that the protein is dimeric. The bottom panel shows the radial absorbance at 280 nm collected at 20 °C and 5 speeds (light blue = 6000 RPM; black = 8000 RPM; dark blue = 10000 RPM; green = 12000 RPM; red = 14000 RPM). The radial absorbance

for each speed was collected after 10 hrs once at speed and rescanned after 2 hrs to ensure the system was at equilibrium. The data was fit as a single species using Fast Fitter [1]. The calculated molecular weight was 143,000 +/- 1000 g/mol with V_{bar} = 0.718 (calculated from the sequence using SEDNTERP - http://jphilo.mailway.com/download.htm) and the solvent density, ρ = 1.0041, calculated for PBS. The residuals are shown as an autocorrelation function. A flat line (e.g., random error) indicates a good fit.

SF3) Effect of C48 on Jak1 and Jak2 kinase activity *in vitro*

C48 and Staurosporine (positive control compound) were tested in 10-dose IC_{50} mode with 3-fold serial dilution starting at 400 μM (C48) or 20 μM (Staurosporine). Reactions were carried out at 10 μM ATP. A) Summary table with IC_{50}. B) Raw data, % enzyme activity (relative to DMSO control), and curve fits. Assays were performed by Reaction Biology Corporation, Malvern, PA-19355.

Reference:

(1) Arkin, M.; Lear, J. D. *Anal Biochem* **2001**, *299*, 98.

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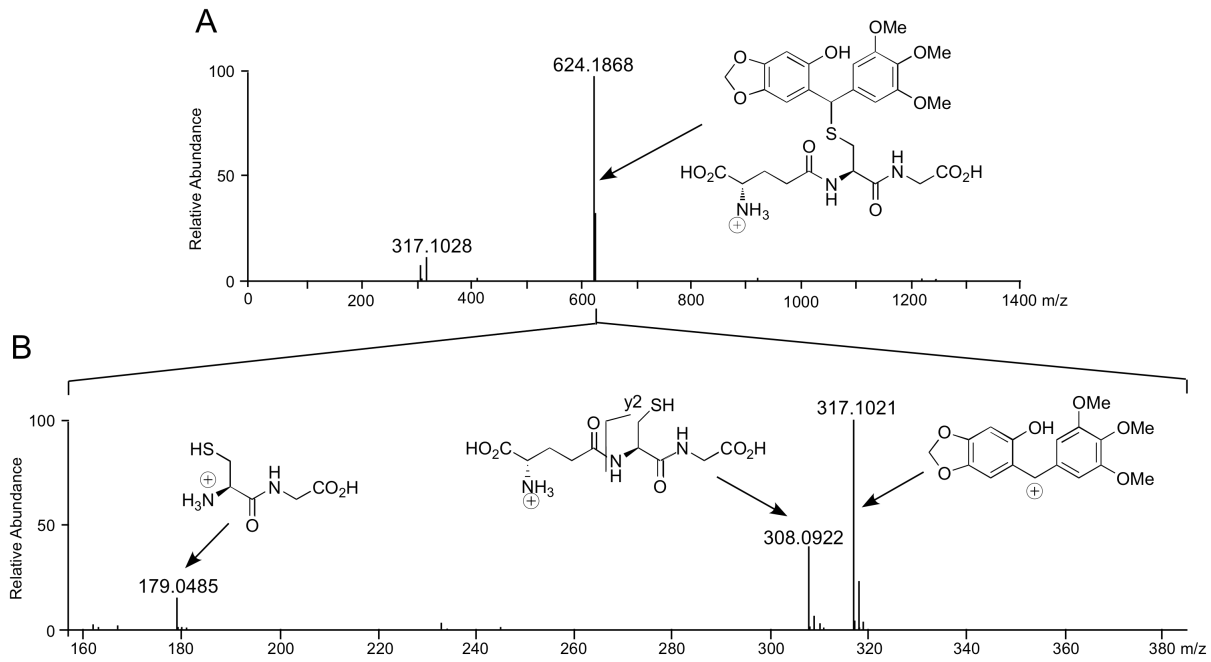
SUPPLEMENTAL
FIGURE 1



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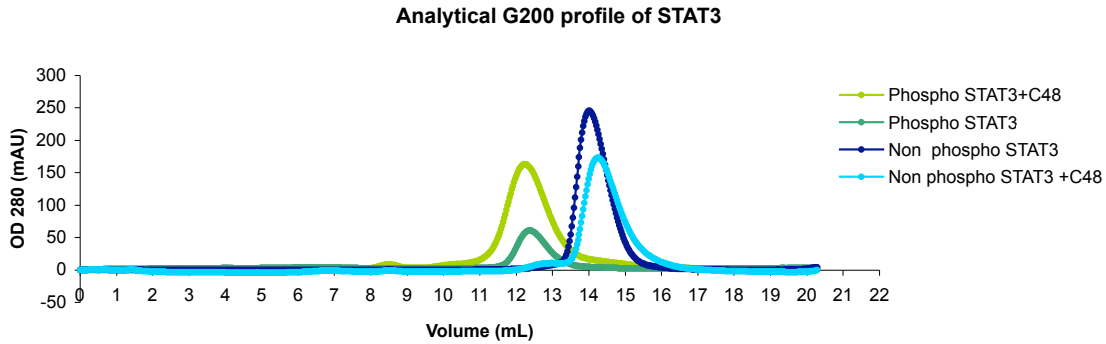
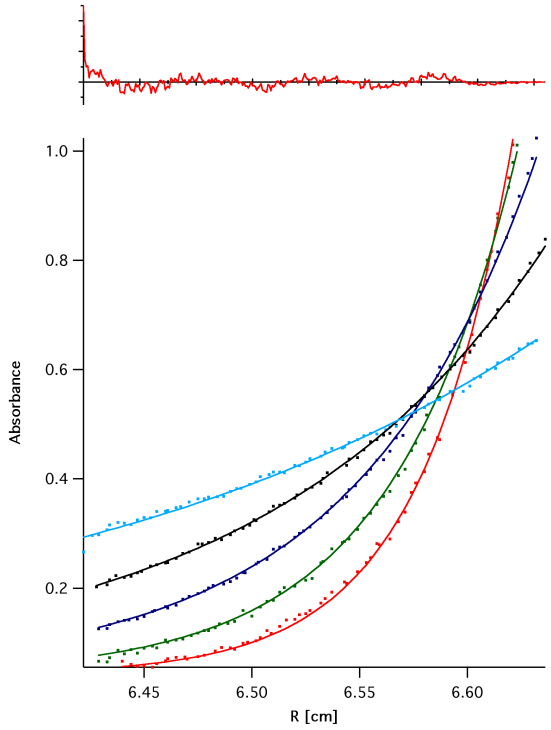
SUPPLEMENTAL
FIGURE 2

A)



B)



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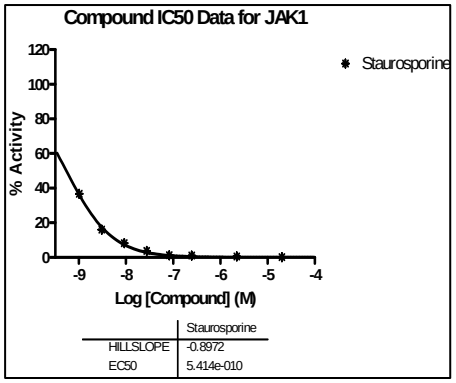
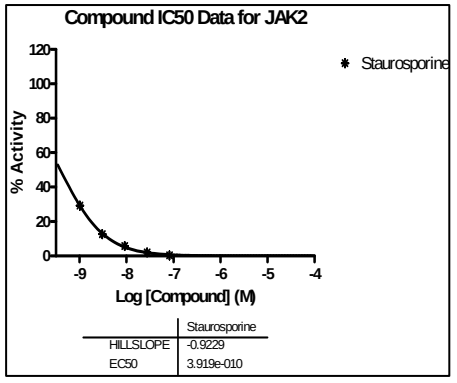
SUPPLEMENTAL
FIGURE 3

A)

	Compound IC_{50}	
Kinase:	**C48**	**Staurosporine**
JAK1	**> 400 μM**	**<1.0 nM**
JAK2	**> 400 μM**	**<1.0 nM**

B)





Kinase Profiling Report for: JAK1

Raw Data

Conc. (M)	C48	Staurosporine	Stauro. Conc. (M)
4.00E-04	447132	921	2.00E-05
1.33E-04	478369	-654	6.67E-06
4.44E-05	513228	3013	2.22E-06
1.48E-05	513611	-1299	7.41E-07
4.94E-06	550720	5878	2.47E-07
1.65E-06	558862	7042	8.23E-08
5.49E-07	565998	22098	2.74E-08
1.83E-07	549340	48381	9.14E-09
6.10E-08	533688	94551	3.05E-09
2.03E-08	551682	216095	1.02E-09
DMSO	584850	586800	DMSO

% Activity

Conc. (M)	C48	Staurosporine	Stauro. Conc. (M)
4.00E-04	76.05	0.16	2.00E-05
1.33E-04	81.37	-0.11	6.67E-06
4.44E-05	87.29	0.51	2.22E-06
1.48E-05	87.36	-0.22	7.41E-07
4.94E-06	93.67	1.00	2.47E-07
1.65E-06	95.06	1.20	8.23E-08
5.49E-07	96.27	3.76	2.74E-08
1.83E-07	93.44	8.23	9.14E-09
6.10E-08	90.77	16.08	3.05E-09
2.03E-08	93.84	36.76	1.02E-09
DMSO	99.48	99.81	DMSO
HILLSLOPE		-0.90	
IC50 (M)		5.41E-10	

Kinase Profiling Report for: JAK2

Raw Data

Conc. (M)	C48	Staurosporine	Stauro. Conc. (M)
4.00E-04	862133	-4829	2.00E-05
1.33E-04	838140	-11367	6.67E-06
4.44E-05	998838	-1782	2.22E-06
1.48E-05	954274	-1898	7.41E-07
4.94E-06	1009545	-1362	2.47E-07
1.65E-06	793471	4335	8.23E-08
5.49E-07	818822	23872	2.74E-08
1.83E-07	1007560	66202	9.14E-09
6.10E-08	963781	145399	3.05E-09
2.03E-08	979128	337185	1.02E-09
DMSO	911982	1143943	DMSO

% Activity

Conc. (M)	C48	Staurosporine	Stauro. Conc. (M)
4.00E-04	94.53	-0.42	2.00E-05
1.33E-04	91.90	-0.99	6.67E-06
4.44E-05	109.52	-0.15	2.22E-06
1.48E-05	104.64	-0.16	7.41E-07
4.94E-06	110.70	-0.12	2.47E-07
1.65E-06	87.01	0.38	8.23E-08
5.49E-07	89.78	2.07	2.74E-08
1.83E-07	110.48	5.75	9.14E-09
6.10E-08	105.68	12.62	3.05E-09
2.03E-08	107.36	29.26	1.02E-09
DMSO	100.00	99.27	DMSO
HILLSLOPE		-0.92	
IC50 (M)		3.92E-10	