

Parthenolide Inhibits STAT3 Signaling by Covalently Targeting Janus Kinases

Supplementary figures

Man Liu ^{1,2}, **Chengqian Xiao** ^{3,4}, **Mingwei Sun** ^{2,5}, **Minjia Tan** ^{2,5}, **Lihong Hu** ³ and **Qiang Yu** ^{1,2,*}

¹ Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China; liuman@simm.ac.cn

² University of Chinese Academy of Sciences, Beijing 100049, China; cmusmw@163.com (M.S.); mjtan@simm.ac.cn (M.T.)

³ Jiangsu Key Laboratory for Functional Substance of Chinese Medicine, Jiangsu Collaborative Innovation Center of Chinese Medicinal Resources Industrialization, State Key Laboratory Cultivation Base for TCM Quality and Efficacy, School of Pharmacy, Nanjing University of Chinese Medicine, Nanjing 210023, China; xchq890503@163.com (C.X.); lhhu@njucm.edu.cn (L.H.)

⁴ State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China

⁵ The Chemical Proteomics Center, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China

* Correspondence: qyu@sibs.ac.cn; Tel.: +86-021-5080-1790

Match to: **G5E852** Score: **28851**

Tyrosine-protein kinase OS=Mus musculus GN=Jak2 PE=1 SV=1

Found in search of 20170112-jak2-PN.mgf

Nominal mass (M_r): **130511**; Calculated pI value: **7.19**

NCBI BLAST search of [G5E852](#) against nr

Unformatted [sequence string](#) for pasting into other applications

Variable modifications: Acetyl (Protein N-term), Oxidation (M), Carbamidomethyl (C), PN (C)

Cleavage by Trypsin/P: cuts C-term side of KR

Sequence Coverage: **85%**

Matched peptides shown in **Bold Red**

1 MGMACLTMTE MEATSTSPVH QNGDIPGSAN SVK**QIEPVLQ VVLYHSLGQA**
51 **EGEYLKFP**SG EYVAEEICVA ASKACGITPV YHNMFALMSE TERIWYPPNH
101 **VFHIDESTRH** DILYRIRFYF PHWYCSGSSR TYRYGVSRGA EAPLLDDFVM
151 **SYLFAQWRHD** FVHWIKVPV THETQEELG MAVLDMRIA KEDQTPHAV
201 **YNSVSYKTFL** PKCVRAKIQD YHILTRKRIR YRFRRIQQF SQCKATARNL
251 **KLKYLINLET** LQSAFYTEQF EVKESARGPS GEEIFATIII TGNGGIQWSR
301 **GKHKESETLT** EQDVQLYCDF PDIIDVSIKQ ANQECNESR ITVHKQDGK
351 **VLEIELSSLK** EALSFVSLID GYYRLTADAH HYLCKEVAPP AVLENIHSNC
401 **HGPISMDFAI** SKLKAGNQT GLYVLRCSKP DFNKYFLTFA VERENVIEYK
451 **HCLITKNENG** EYNLSGTRKN FSNLKDLLNC YQMETVRSDS IIFQFTKCCP
501 **PKPKDKSNLL** VFRTNGISDV QISPTLQRHN NVNQMVFHKI RNEDLIFNES
551 **LGQGTFTKIF** KGVREVGDY GQLHKTEVLL KVLDAHRNY SESFFEAASM
601 **MSQLSHKHLV** LNYGVCVCGE ENILVQEFVK FGSLDTYLKK NKNSINILWK
651 **LGVAQLAWA** MHFLEEKSLI HGNVCAKNIL LIREEDRRTG NPPFIKLSDP
701 **GISITVLPKD** ILQERIPWVP PECIENPKNL NLATDKWSFG TTLWEICSGG
751 **DKPLSALDSQ** RKLQFYEDKH QLPAPKWTEL ANLINNCMDY EPDFRPAFRA
801 **VIRDLNSLFT** PDYELLTEND MLPNMRIGAL GFSGAFEDRD PTQFEERHLK
851 **FLQQLGKGNF** GSVEMCRYDP LQDNTGEVVA VKKLQHSTEE HLRDFEREIE
901 **ILKSLQHDNI** VKYKGVCSYA GRRNLRLIME YLPYGSLRDY LQKHKERIDH
951 **KKLLQYTSQI** CKGMEYLGTK RYIHRDLATR NILVENENRV KIGDFGLTKV
1001 **LPQDKEYYKV** KEPGESPIFW YAPESLTESK FSVASDVWSF GVVLYELFTY
1051 **IEKSKSPPVE** FMRMIGNDKQ GQMIVFHLIE LLKSNGRLPR PEGCPDEIYV
1101 **IMTECWNNNV** SQRPSFRDLS LRVDQIRDSI AA

Figure S1 JAK2 protein was well identified with 85% sequence coverage.

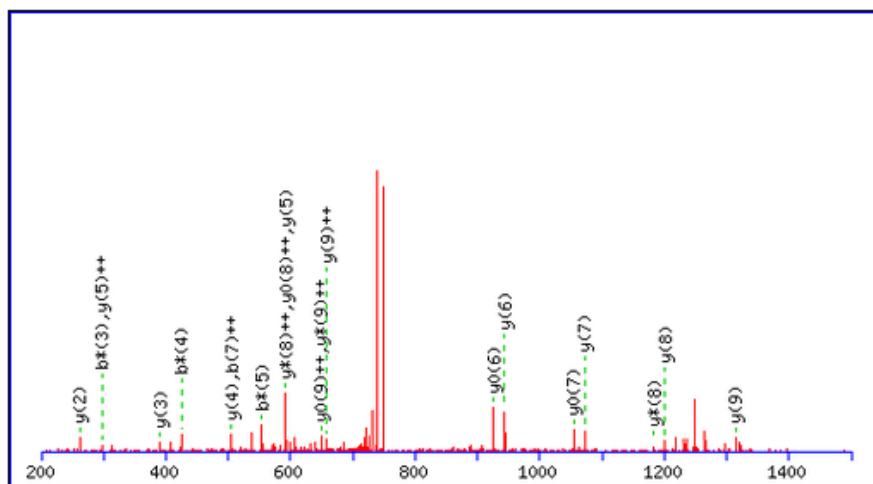
MS/MS Fragmentation of **QANQECSNESR**Found in **G5E852**, Tyrosine-protein kinase OS=Mus musculus GN=Jak2 PE=1 SV=1

Match to Query 7546: 1512.650608 from(757.332580,2+) intensity(633753.4375)

Title: File20882 Spectrum6100 scans: 7377

Data file TEST_151221212047.mgf

Click mouse within plot area to zoom in by factor of two about that point

Or, 200 1500 Da Label all possible matches ☐ Label matches used for scoring ☒**Monoisotopic mass of neutral peptide Mr(calc):** 1512.6514**Variable modifications:****C6** : PN (C)**Ions Score:** 52 **Expect:** 9e-005**Matches** : 21/110 fragment ions using 25 most intense peaks ([help](#))

#	b	b ⁺⁺	b [*]	b ⁺⁺⁺	b ⁰	b ⁰⁺⁺	Seq.	y	y ⁺⁺	y [*]	y ⁺⁺⁺	y ⁰	y ⁰⁺⁺	#
1	129.0659	65.0366	112.0393	56.5233			Q							11
2	200.1030	100.5551	183.0764	92.0418			A	1385.6002	693.3037	1368.5736	684.7904	1367.5896	684.2984	10
3	314.1459	157.5766	297.1193	149.0633			N	1314.5631	657.7852	1297.5365	649.2719	1296.5525	648.7799	9
4	442.2045	221.6059	425.1779	213.0926			Q	1200.5201	600.7637	1183.4936	592.2504	1182.5096	591.7584	8
5	571.2471	286.1272	554.2205	277.6139	553.2365	277.1219	E	1072.4616	536.7344	1055.4350	528.2211	1054.4510	527.7291	7
6	922.3975	461.7024	905.3709	453.1891	904.3869	452.6971	C	943.4190	472.2131	926.3924	463.6998	925.4084	463.2078	6
7	1009.4295	505.2184	992.4030	496.7051	991.4190	496.2131	S	592.2685	296.6379	575.2420	288.1246	574.2580	287.6326	5
8	1123.4725	562.2399	1106.4459	553.7266	1105.4619	553.2346	N	505.2365	253.1219	488.2100	244.6086	487.2259	244.1166	4
9	1252.5150	626.7612	1235.4885	618.2479	1234.5045	617.7559	E	391.1936	196.1004	374.1670	187.5871	373.1830	187.0951	3
10	1339.5471	670.2772	1322.5205	661.7639	1321.5365	661.2719	S	262.1510	131.5791	245.1244	123.0659	244.1404	122.5738	2
11							R	175.1190	88.0631	158.0924	79.5498			1

Figure S3 Cys335 of mouse JAK2 protein was covalently modified by parthenolide.

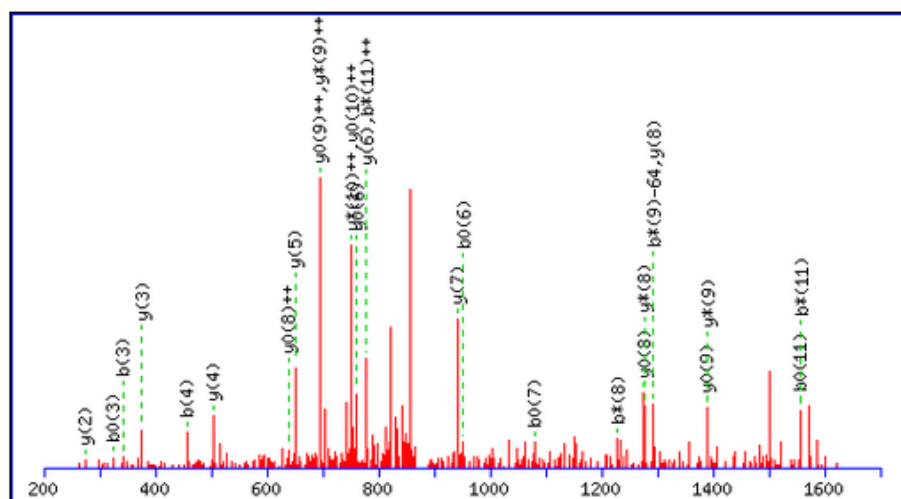
MS/MS Fragmentation of **DLLNCYQMETVR**Found in **G5E852**, Tyrosine-protein kinase OS=Mus musculus GN=Jak2 PE=1 SV=1

Match to Query 9298: 1747.818088 from(874.916320,2+) intensity(46608.8789)

Title: File20882 Spectrum11135 scans: 13419

Data file TEST_151221212047.mgf

Click mouse within plot area to zoom in by factor of two about that point

Or, 200 1700 Da Label all possible matches ☐ Label matches used for scoring ☒Monoisotopic mass of neutral peptide $M_r(\text{calc})$: 1747.8161

Variable modifications:

C5 : PN (C)**M8** : Oxidation (M), with neutral losses 0.0000(shown in table), 63.9983

Ions Score: 34 Expect: 0.022

Matches : 27/188 fragment ions using 42 most intense peaks ([help](#))

#	b	b ⁺⁺	b [*]	b ^{***}	b ⁰	b ⁰⁺⁺	Seq.	y	y ⁺⁺	y [*]	y ^{***}	y ⁰	y ⁰⁺⁺	#
1	116.0342	58.5207			98.0237	49.5155	D							12
2	229.1183	115.0628			211.1077	106.0575	L	1633.7964	817.4019	1616.7699	808.8886	1615.7859	808.3966	11
3	342.2023	171.6048			324.1918	162.5995	L	1520.7124	760.8598	1503.6858	752.3465	1502.7018	751.8545	10
4	456.2453	228.6263	439.2187	220.1130	438.2347	219.6210	N	1407.6283	704.3178	1390.6018	695.8045	1389.6177	695.3125	9
5	807.3957	404.2015	790.3692	395.6882	789.3851	395.1962	C	1293.5854	647.2963	1276.5588	638.7831	1275.5748	638.2910	8
6	970.4590	485.7332	953.4325	477.2199	952.4485	476.7279	Y	942.4349	471.7211	925.4084	463.2078	924.4244	462.7158	7
7	1098.5176	549.7624	1081.4911	541.2492	1080.5070	540.7572	Q	779.3716	390.1894	762.3451	381.6762	761.3611	381.1842	6
8	1245.5530	623.2801	1228.5265	614.7669	1227.5424	614.2749	M	651.3130	326.1602	634.2865	317.6469	633.3025	317.1549	5
9	1374.5956	687.8014	1357.5691	679.2882	1356.5850	678.7962	E	504.2776	252.6425	487.2511	244.1292	486.2671	243.6372	4
10	1475.6433	738.3253	1458.6167	729.8120	1457.6327	729.3200	T	375.2350	188.1212	358.2085	179.6079	357.2245	179.1159	3
11	1574.7117	787.8595	1557.6851	779.3462	1556.7011	778.8542	V	274.1874	137.5973	257.1608	129.0840			2
12							R	175.1190	88.0631	158.0924	79.5498			1

Figure S4 Cys480 of mouse JAK2 protein was covalently modified by parthenolide.