

Supporting Information

***N*-formyl-7-amino-11-cycloamphilectene, a marine sponge metabolite, binds to tubulin and modulates microtubule depolymerization**

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HPLC run of CALe-NH₂ bearing linker.

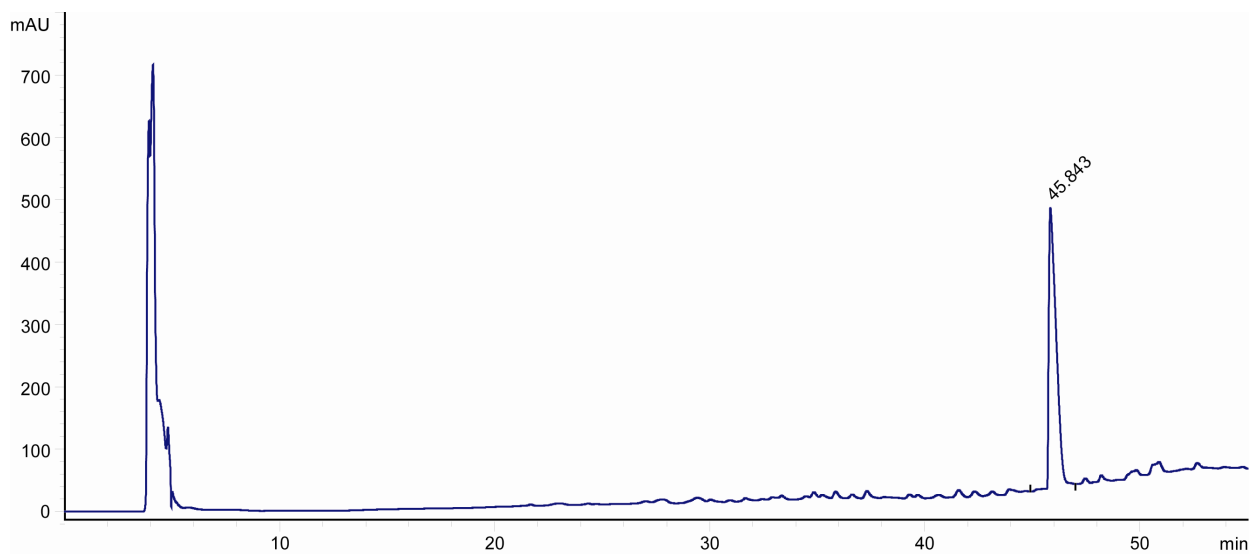


Figure S1. HPLC run of CALe-NH₂ bearing linker shows the purity of the covalent complex (higher than 90%).

MS and MSMS analysis of the covalent adduct between CALe-NH₂ and dithiobis-succinimidylpropionate.

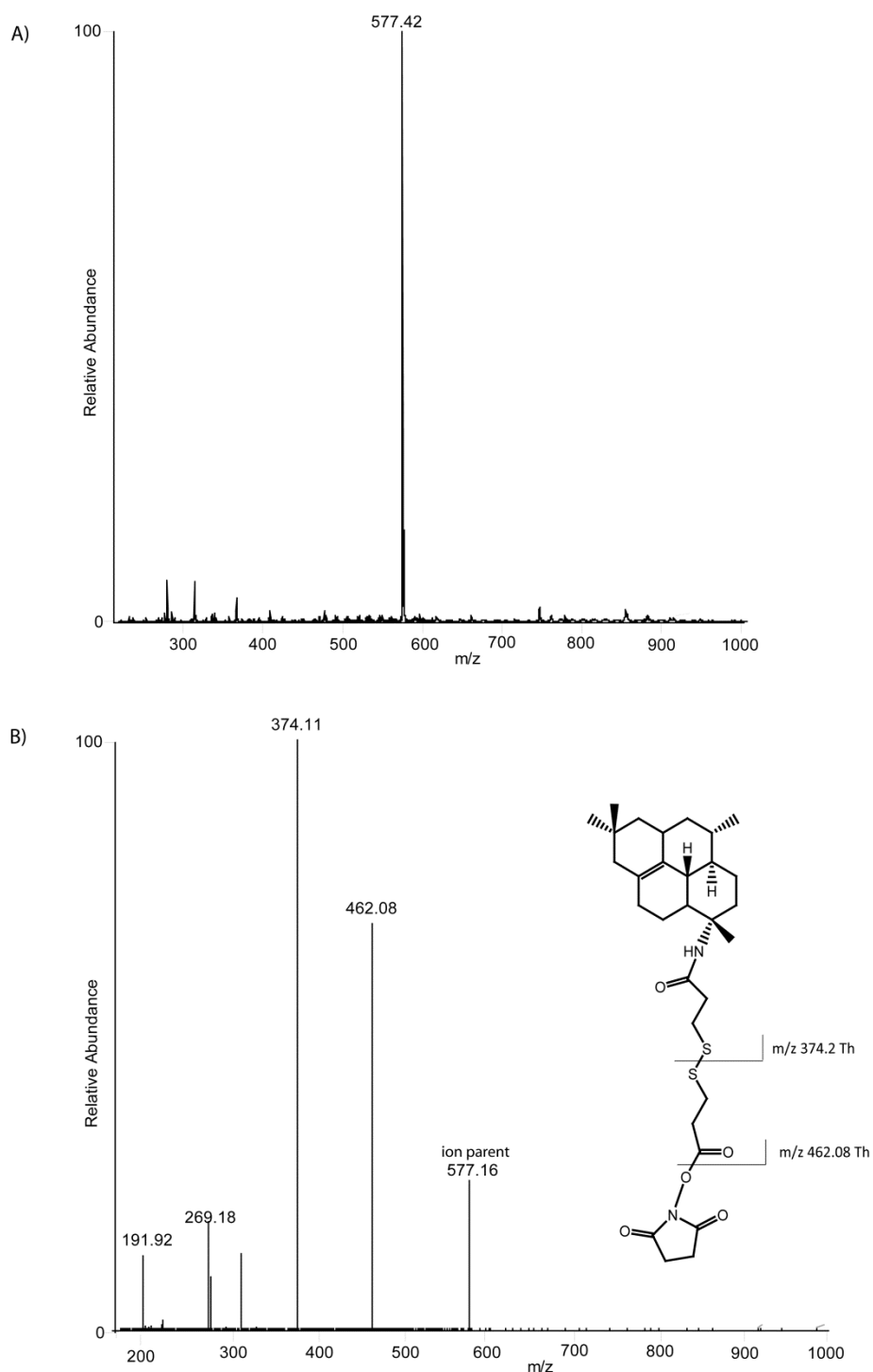


Figure S2. A) MS spectrum of the covalent adduct between CALe-NH₂ and dithiobis succinimidylpropionate, **B)** Ion fragments obtained during tandem MS experiments on the parent ion at m/z of 577.43.

HPLC runs of CALe-NH₂ bearing linker immobilization on amine beads.

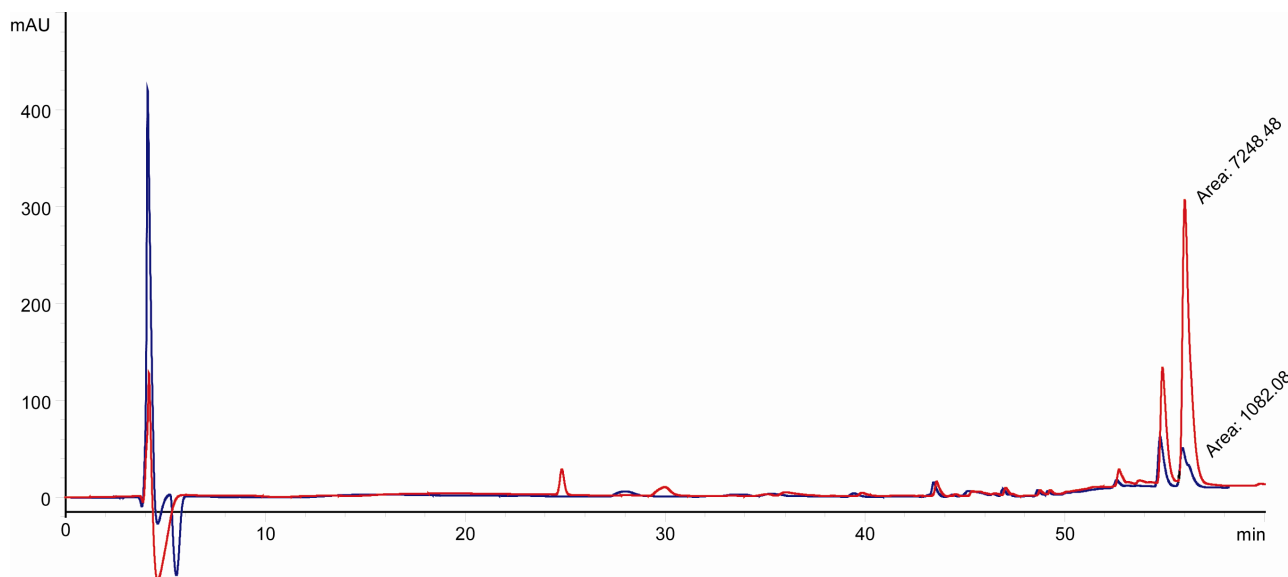


Figure S3. HPLC runs of CALe-NH₂ immobilization reaction on amine beads. Panel shows the overlapping of the chromatographic profiles relative to the peak of free CALe-NH₂ in 20 μ L of reaction buffer, at $t=0$, and 2 h. The strong reduction of the peak area demonstrates that the CALe-NH₂ has been anchored to the amine beads.

Electrophoretic profile of proteins eluted from CALe and control beads.

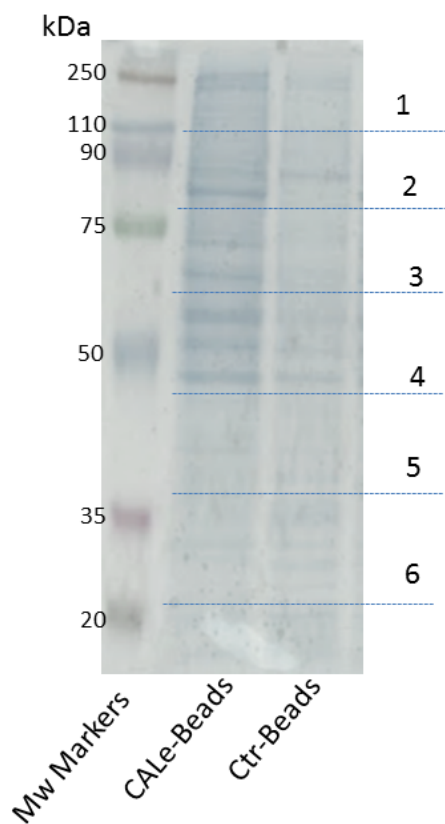


Figure S4. Representative gel of the affinity chromatography elutions reporting number and size of excised bands.

Proteins identified from control experiments.

Accession	Description	Mass	SCR	Matches
GSTP1_HUMAN	Glutathione S-transferase P	23569	276	13
CH60_HUMAN	60 kDa heat shock protein, mitochondrial	61187	260	16
MYH9_HUMAN	Myosin-9 OS=Homo sapiens	227646	93	5
K1C17_HUMAN	Keratin, type I cytoskeletal 17	48361	87	4
RS5_HUMAN	40S ribosomal protein S5	23033	86	1
COF1_HUMAN	Cofilin-1 OS=Homo sapiens	18719	83	1
KPYM_HUMAN	Pyruvate kinase PKM	58470	63	6
ACTB_HUMAN	Actin, cytoplasmic 1	42052	48	5
TBA1A_HUMAN	Tubulin alpha-1A chain	50788	44	1
RAB7A_HUMAN	Ras-related protein Rab-7a	23760	40	1
TPD54_HUMAN	Tumor protein D54	22281	37	1
PLAK_HUMAN	Junction plakoglobin	82434	37	1
GSTM3_HUMAN	Glutathione S-transferase Mu 3	26998	33	3
DCD_HUMAN	Dermcidin	11391	32	4
RAB30_HUMAN	Ras-related protein Rab-30	23386	27	2
RS3_HUMAN	40S ribosomal protein S3	26842	27	1
G3P_HUMAN	Glyceraldehyde-3-phosphate dehydrogenase	36201	22	1
RL19_HUMAN	60S ribosomal protein L19	23565	21	1
RS8_HUMAN	40S ribosomal protein S8	24475	20	1
HSPB1_HUMAN	Heat shock protein beta-1	22826	16	1

Table S1. List of proteins identified in four independent chemical proteomics experiments using control beads. The results are reported in terms of Mascot score (SCR) and number of identified peptides (Matches).

Proteins sequence coverage of the selected putative CALe interactors.

Heat Shock protein beta and tubulin sequence coverage were reported below. In addition, all the peak lists, which were sent to Mascot, have been deposited to the ProteomeXchange Consortium (<http://proteomecentral.proteomexchange.org>) via the PRIDE partner repository with the dataset identifier PXD000106 and DOI 10.6019/PXD000106.

Proteins	Protein sequence coverage			
	Exp1	Exp2	Exp3	Exp4
Heat shock protein HSP 90-beta (HS90B_HUMAN)	46%	33%	26%	25%
Tubulin beta chain (TBB5_HUMAN)	71%	43%	24%	30%
Tubulin alpha-4A chain (TBA4A_HUMAN)	49%	41%	20%	24%
Tubulin alpha-1B chain (TBA1B_HUMAN)	64%	41%	20%	24%

Table S2. Table shows the % of protein sequence coverage for each experiment of the selected putative CALe interactors.

Mascot report and peak lists for Hsp90beta and Tubulin.

MS/MS Fragmentation of ELISNASDALDKIR

Found in HS90B_HUMAN in SwissProt, Heat shock protein HSP 90-beta OS=Homo sapiens GN=HSP90AB1 PE=1 SV=4

Match to Query 92: 1543.853248 from(772.933900,2+) intensity(87.0404) index(37)

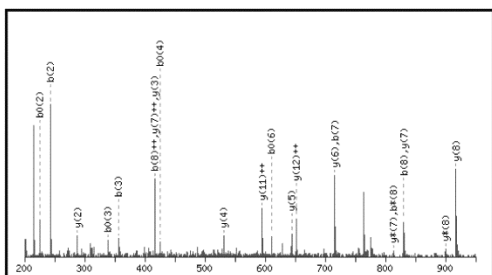
Data file CALExp1_lane3.pkl

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

Or, to Da

Label all possible matches Label matches used for scoring

Show Y-axis



EXP 1

MS/MS Fragmentation of GVVDSEDLPLNISR

Found in HS90B_HUMAN in SwissProt, Heat shock protein HSP 90-beta OS=Homo sapiens GN=HSP90AB1 PE=1 SV=4

Match to Query 53: 1512.781448 from(757.398000,2+) intensity(117.1454) index(124)

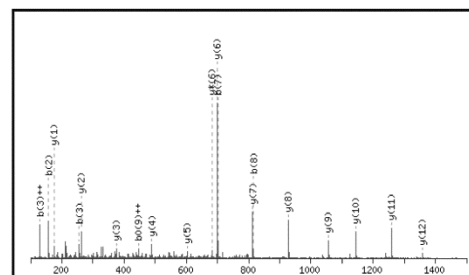
Data file CALExp2_lane4.pkl

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

Or, to Da

Label all possible matches Label matches used for scoring

Show Y-axis



EXP 2

MS/MS Fragmentation of ELISNASDALDKIR

Found in HS90B_HUMAN in SwissProt, Heat shock protein HSP 90-beta OS=Homo sapiens GN=HSP90AB1 PE=1 SV=4

Match to Query 64: 1543.818248 from(772.916400,2+) intensity(5530.0205) index(34)

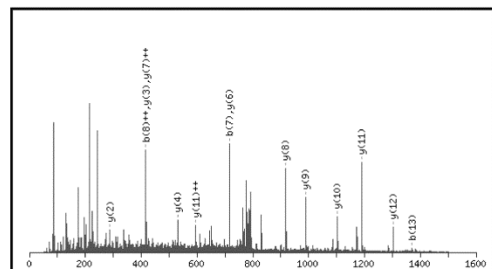
Data file CALExp3_lane3.pkl

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

Or, to Da

Label all possible matches Label matches used for scoring

Show Y-axis



EXP 3

MS/MS Fragmentation of ELISNASDALDKIR

Found in HS90B_HUMAN in SwissProt, Heat shock protein HSP 90-beta OS=Homo sapiens GN=HSP90AB1 PE=1 SV=4

Match to Query 59: 1543.809048 from(772.911800,2+) intensity(2243.3857) index(37)

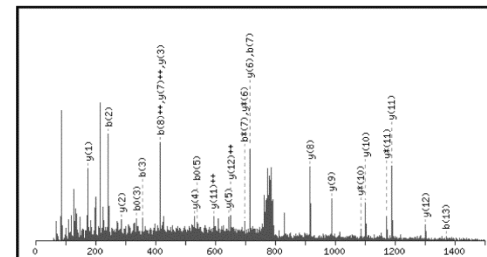
Data file CALExp4_lane3.pkl

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

Or, to Da

Label all possible matches Label matches used for scoring

Show Y-axis



EXP 4

Figure S5. Tandem mass spectra of a Hsp90β (HS90B_HUMAN) peptide for each experiment is shown herein.

Peptide View

MS/MS Fragmentation of EIVHIQAGQCGNQIGAK

Found in TBB5_HUMAN in SwissProt, Tubulin beta chain OS=Homo sapiens GN=TUBB PE=1 SV=2

Match to Query 178: 1822.065248 from(912.039900,2+) intensity(87.3732) index(112)

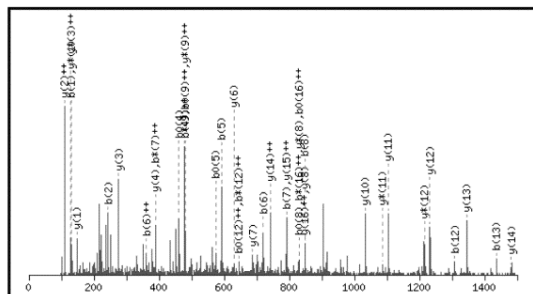
Data file CALExp1_lane5.pkl

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

Or, Plot from Da

Label all possible matches Label matches used for scoring

Show Y-axis



EXP 1

Peptide View

MS/MS Fragmentation of SGPFQIIFRPDNFVFGQSGAGNNWAK

Found in TBB5_HUMAN in SwissProt, Tubulin beta chain OS=Homo sapiens GN=TUBB PE=1 SV=2

Match to Query 194: 2797.341672 from(933.454500,3+) intensity(158.9298) index(97)

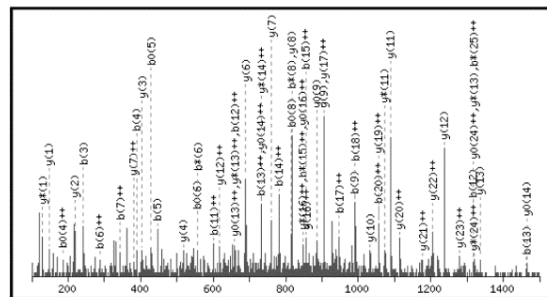
Data file CALExp2_lane5.pkl

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

Or, Plot from Da

Label all possible matches Label matches used for scoring

Show Y-axis



EXP 2

Peptide View

MS/MS Fragmentation of ALTVPELTQQVFDAK

Found in TBB5_HUMAN in SwissProt, Tubulin beta chain OS=Homo sapiens GN=TUBB PE=1 SV=2

Match to Query 135: 1658.882648 from(830.448600,2+) intensity(9950.5332) index(192)

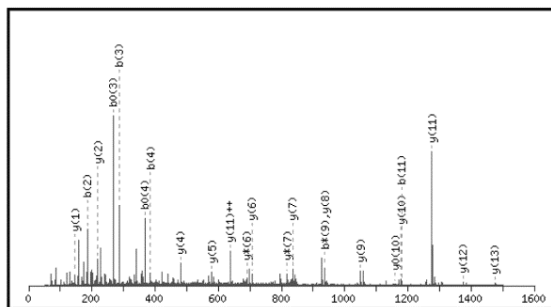
Data file CALExp3_lane5.pkl

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

Or, Plot from Da

Label all possible matches Label matches used for scoring

Show Y-axis



EXP 3

Peptide View

MS/MS Fragmentation of AILVDLEPGTMDSVR

Found in TBB5_HUMAN in SwissProt, Tubulin beta chain OS=Homo sapiens GN=TUBB PE=1 SV=2

Match to Query 143: 1614.825448 from(808.420000,2+) intensity(1412.0271) index(139)

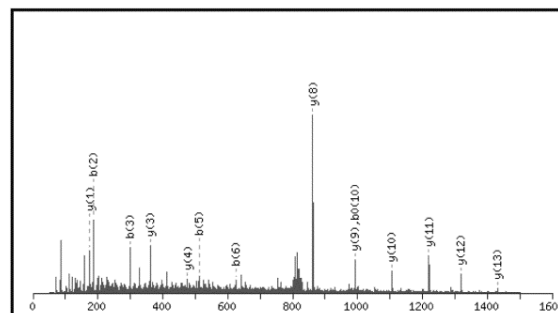
Data file CALExp4_lane5.pkl

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

Or, Plot from Da

Label all possible matches Label matches used for scoring

Show Y-axis



EXP 4

Figure S6. A Tandem mass spectra of a tubulin beta 5 (TBB5_HUMAN) peptide for each experiment is shown herein.

Peptide View

MS/MS Fragmentation of TIGGGDDSFITFFCETGAGK

Found in TBA4A_HUMAN in SwissProt, Tubulin alpha-4A chain OS=Homo sapiens GN=TUBA4A PE=1 SV=1

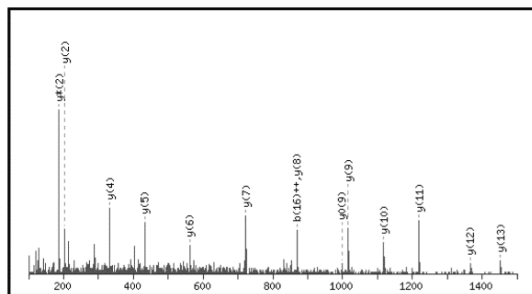
Match to Query 227: 2067.064248 from(1034.539400,2+) intensity(796.1695) index(154)
Data file CALExp1_lane5.pkl

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

Or, to Da

Label all possible matches Label matches used for scoring

Show Y-axis



EXP 1

Peptide View

MS/MS Fragmentation of AVFVDLEPTVIDEIR

Found in TBA4A_HUMAN in SwissProt, Tubulin alpha-4A chain OS=Homo sapiens GN=TUBA4A PE=1 SV=1

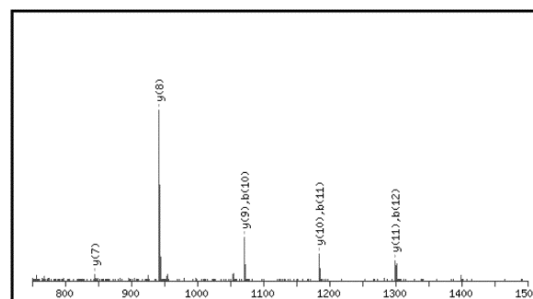
Match to Query 110: 1714.920448 from(858.467500,2+) intensity(250.0330) index(93)
Data file CALExp2_lane5.pkl

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

Or, to Da

Label all possible matches Label matches used for scoring

Show Y-axis



EXP 2

Peptide View

MS/MS Fragmentation of AVFVDLEPTVIDEIR

Found in TBA4A_HUMAN in SwissProt, Tubulin alpha-4A chain OS=Homo sapiens GN=TUBA4A PE=1 SV=1

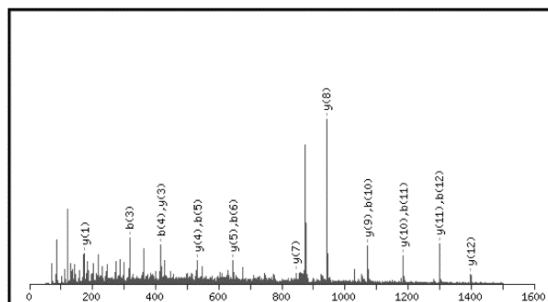
Match to Query 150: 1714.917648 from(858.466100,2+) intensity(3904.6138) index(126)
Data file CALExp3_lane5.pkl

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

Or, to Da

Label all possible matches Label matches used for scoring

Show Y-axis



EXP 3

Peptide View

MS/MS Fragmentation of AVFVDLEPTVIDEIR

Found in TBA4A_HUMAN in SwissProt, Tubulin alpha-4A chain OS=Homo sapiens GN=TUBA4A PE=1 SV=1

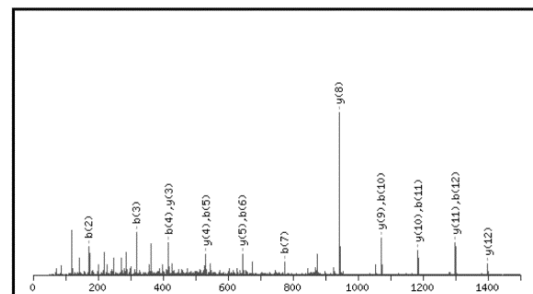
Match to Query 174: 1714.898048 from(858.456300,2+) intensity(1049.9827) index(212)
Data file CALExp4_lane5.pkl

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

Or, to Da

Label all possible matches Label matches used for scoring

Show Y-axis



EXP 4

Figure S7. A Tandem mass spectra of a tubulin alpha 4 (TBA4A_HUMAN) peptide for each experiment is shown herein.

Peptide View

MS/MS Fragmentation of TIGGGDDSFNTFFSETGAGK

Found in TBA1B_HUMAN in SwissProt, Tubulin alpha-1B chain OS=Homo sapiens GN=TUBA1B PE=1 SV=1

Match to Query 220: 2007.056048 from(1004.535300,2+) intensity(2860.0068) index(51)

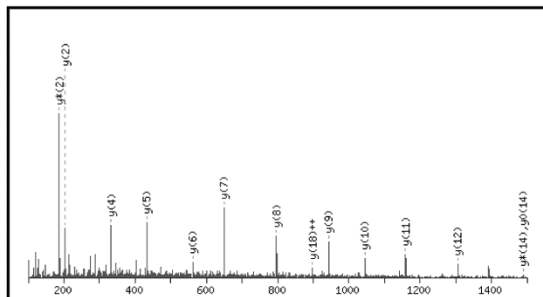
Data file CALExp1_lane5.pkl

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

Or, to Da

Label all possible matches Label matches used for scoring

Show Y-axis



EXP 1

Peptide View

MS/MS Fragmentation of LISQIVSSITASLR

Found in TBA1B_HUMAN in SwissProt, Tubulin alpha-1B chain OS=Homo sapiens GN=TUBA1B PE=1 SV=1

Match to Query 77: 1486.875848 from(744.445200,2+) intensity(107.2513) index(117)

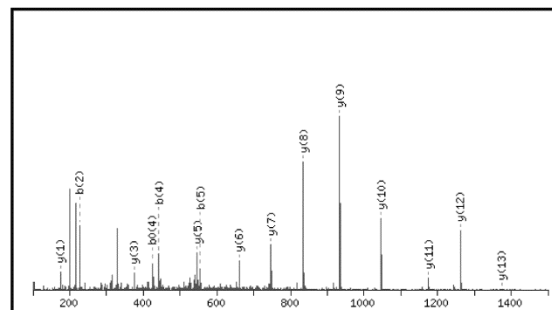
Data file CALExp2_lane5.pkl

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

Or, to Da

Label all possible matches Label matches used for scoring

Show Y-axis



EXP 2

Peptide View

MS/MS Fragmentation of AVFVDLEPTVIDEVR

Found in TBA1B_HUMAN in SwissProt, Tubulin alpha-1B chain OS=Homo sapiens GN=TUBA1B PE=1 SV=1

Match to Query 147: 1700.879648 from(851.447100,2+) intensity(21060.1484) index(123)

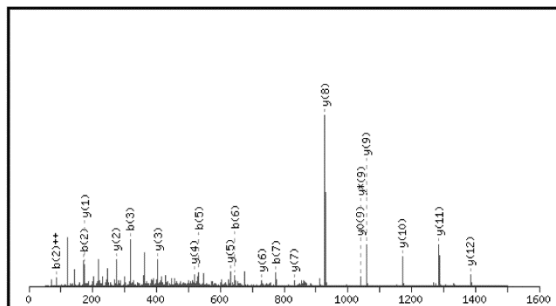
Data file CALExp3_lane5.pkl

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

Or, to Da

Label all possible matches Label matches used for scoring

Show Y-axis



EXP 3

Peptide View

MS/MS Fragmentation of AVFVDLEPTVIDEVR

Found in TBA1B_HUMAN in SwissProt, Tubulin alpha-1B chain OS=Homo sapiens GN=TUBA1B PE=1 SV=1

Match to Query 171: 1700.879048 from(851.446800,2+) intensity(11483.3516) index(155)

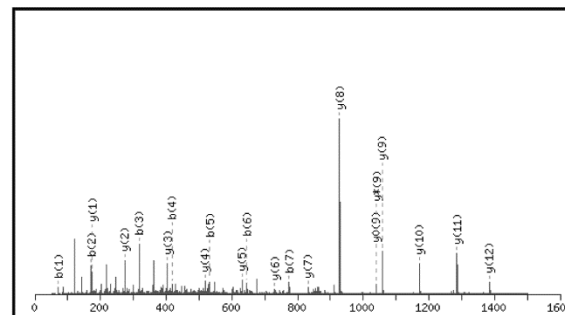
Data file CALExp4_lane5.pkl

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

Or, to Da

Label all possible matches Label matches used for scoring

Show Y-axis



EXP 4

Figure S8. A Tandem mass spectra of a tubulin beta 1 (TBA1B_HUMAN) peptide for each experiment is shown herein.

Analysis of CALe and Hsp90 β affinity by Surface Plasmon Resonance

Sensograms showed in **Figure S9** clearly demonstrate the absence of a direct interaction between CALe and Hsp90 β .

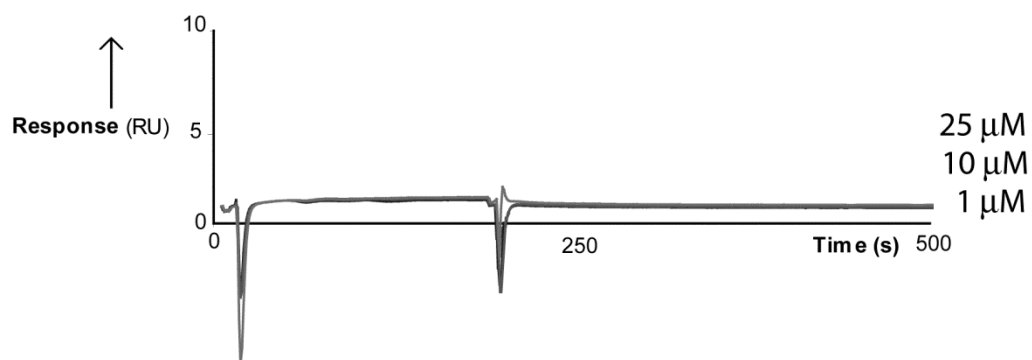


Figure S9. Sensograms obtained from the binding of CALe (0.5-25 μ M) to immobilized Hsp90 beta.

Cell Viability Assay

Cell viability was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cells were seeded in 96-well microtiter plates in 100 μ l of growth medium.

After 16 h incubation at 37 $^{\circ}$ C, cells were exposed to different concentration of CALe, ranging from 5 μ M to 100 μ M, containing 1% DMSO. The same amount of DMSO has been applied as control and the incubation was carried out for 6 and 24 h.

The mitochondrial-dependent reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to formazan was used to assess the possible CALe cytotoxic effect. The experiment was carried out in triplicate and all the values were normalized to control.

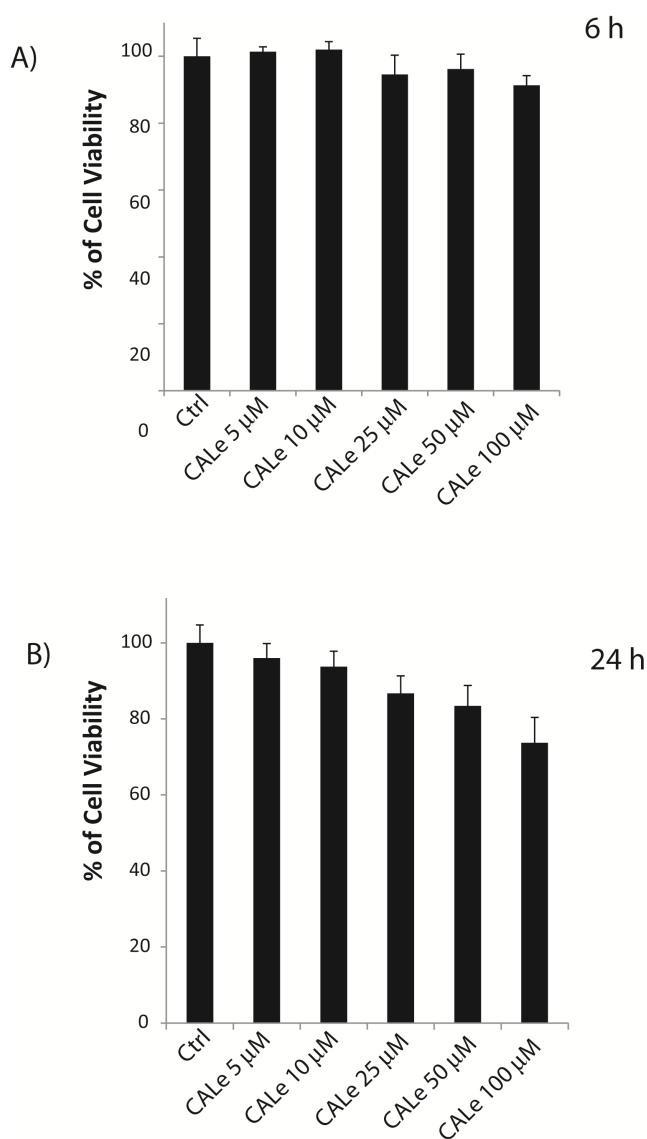


Figure S10. % cell survival of HeLa at different concentration of CALe after 6h (A) and 24 h (B) of incubation. Each value is the mean \pm standard deviation of three different experiments performed in triplicate.