Electronic Supplementary Material (ESI) for Chemical Science.

Supplementary Information

A chemical probe unravels the reactive proteome of health-associated catechols

Angela Weigert Muñoz,[a] Kevin Meighen-Berger,[b] Stephan M. Hacker,[c] Matthias Feige[b] and Stephan A. Sieber*[a]

[a] Center for Functional Protein Assemblies, Department of Bioscience, TUM School of Natural Sciences, Technical University of Munich, Ernst-Otto-Fischer-Straße 8, D-85748 Garching, Germany

[b] Center for Functional Protein Assemblies, Department of Bioscience, TUM School of Natural Sciences, Technical University of Munich, Lichtenbergstraße 4, D-85748 Garching, Germany

[c] Leiden Institute of Chemistry, Leiden University, Einsteinweg 55, 2333 CC Leiden, Netherlands

*E-mail: stephan.sieber@tum.de

Table of contents

Supplementary Figures

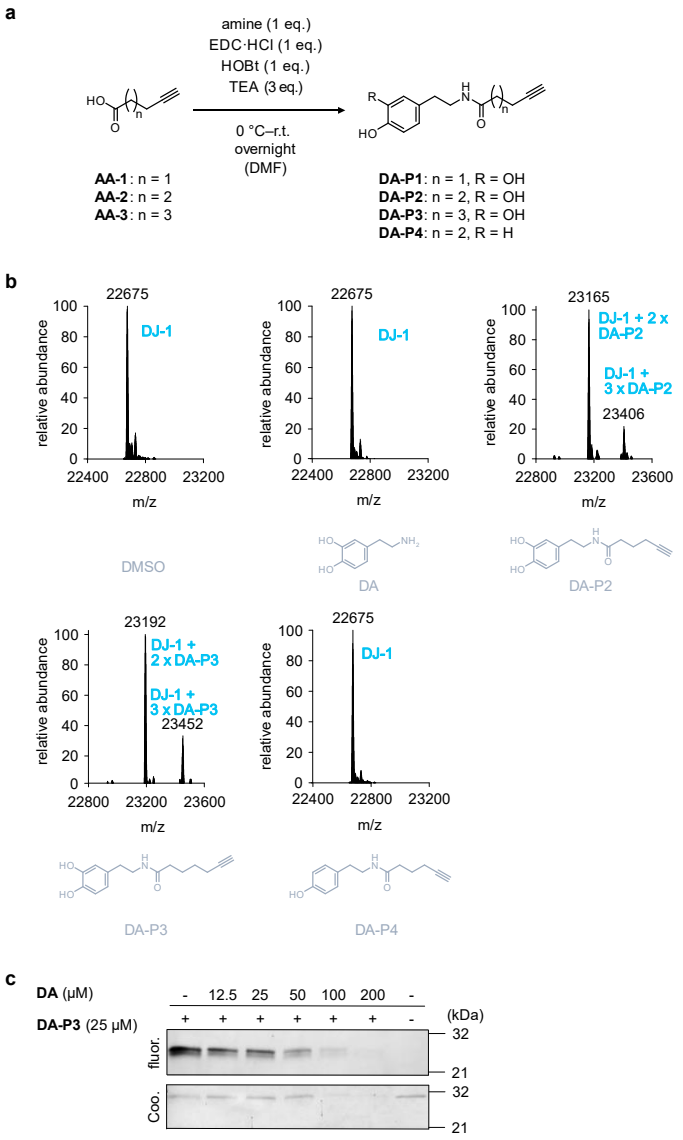


Figure S1. Probe synthesis and analysis of protein binding. (a) Probe synthesis was performed by standard amide coupling of alkyne handles to **DA** or tyramine. TEA = triethylamine; HOBt = hydroxybenzotriazole; DMF = *N*,*N*-dimethylformamide; EDC·HCl = 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride. (b) Analysis of compound binding to purified DJ-1 by intact protein mass spectrometry. DJ-1 (5 μM) was incubated with a 100-fold excess of **DA** derivatives at r.t. in PBS, overnight. (c) Gel-based chemoproteomics analysis of **DA-P3** binding to DJ-1 (1 μM, in PBS) in competition with different concentrations of **DA**. The labelled protein was ligated to a fluorophore, subjected to SDS-PAGE, and imaged. Full protein load was revealed by Coomassie staining; fluor. = fluorescence, Coo. = Coomassie.

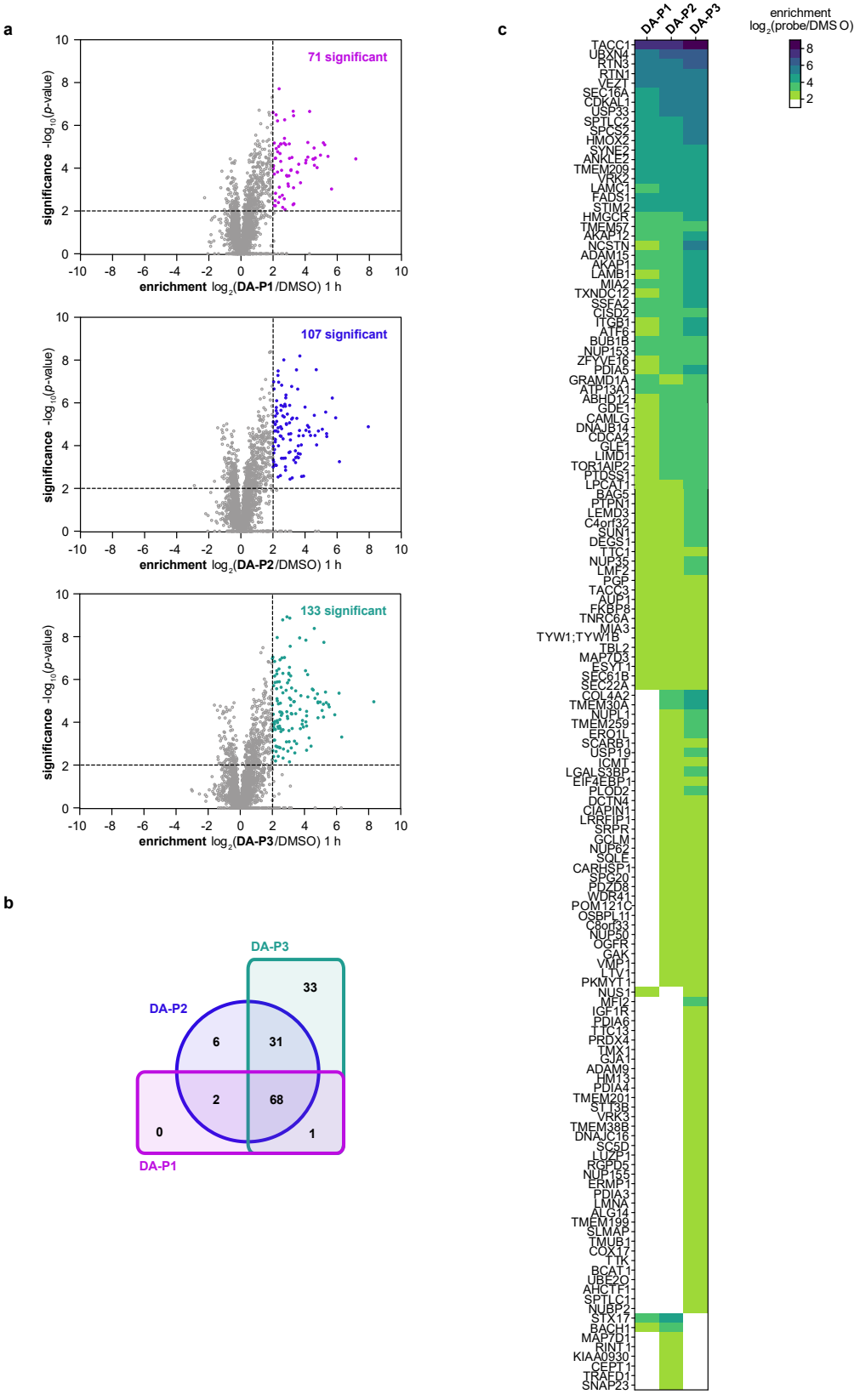


Figure S2. Comparison of target scope of catechol probes in live Hek293 cells labelled for 1 h. (a) Hek293 cells were treated 1 h with 15 µM **DA-P1**, **DA-P2**, **DA-P3**, or DMSO. MS data from four replicates were analysed by MaxLFQ[1] and filtered for proteins identified in three replicates in at least one condition. Samples were compared using a two-sided two-sample *t*-test. Proteins were considered significant hits when they were enriched more than four-fold ($\log_2$(enrichment) ≥ 2) with a *p*-value of less than 0.01 ($-\log_{10}$(*p*-value) ≥ 2); proteins with missing values are shown with $-\log_{10}$(*p*-value) = 0. Significant hits are shown as full circles; non-significantly enriched proteins are shown as open circles. See table S1 for details on identified proteins. (b) Venn diagram showing the overlap of protein hits of the probes. (c) Heat map showing protein enrichment by the different probes compared to DMSO.

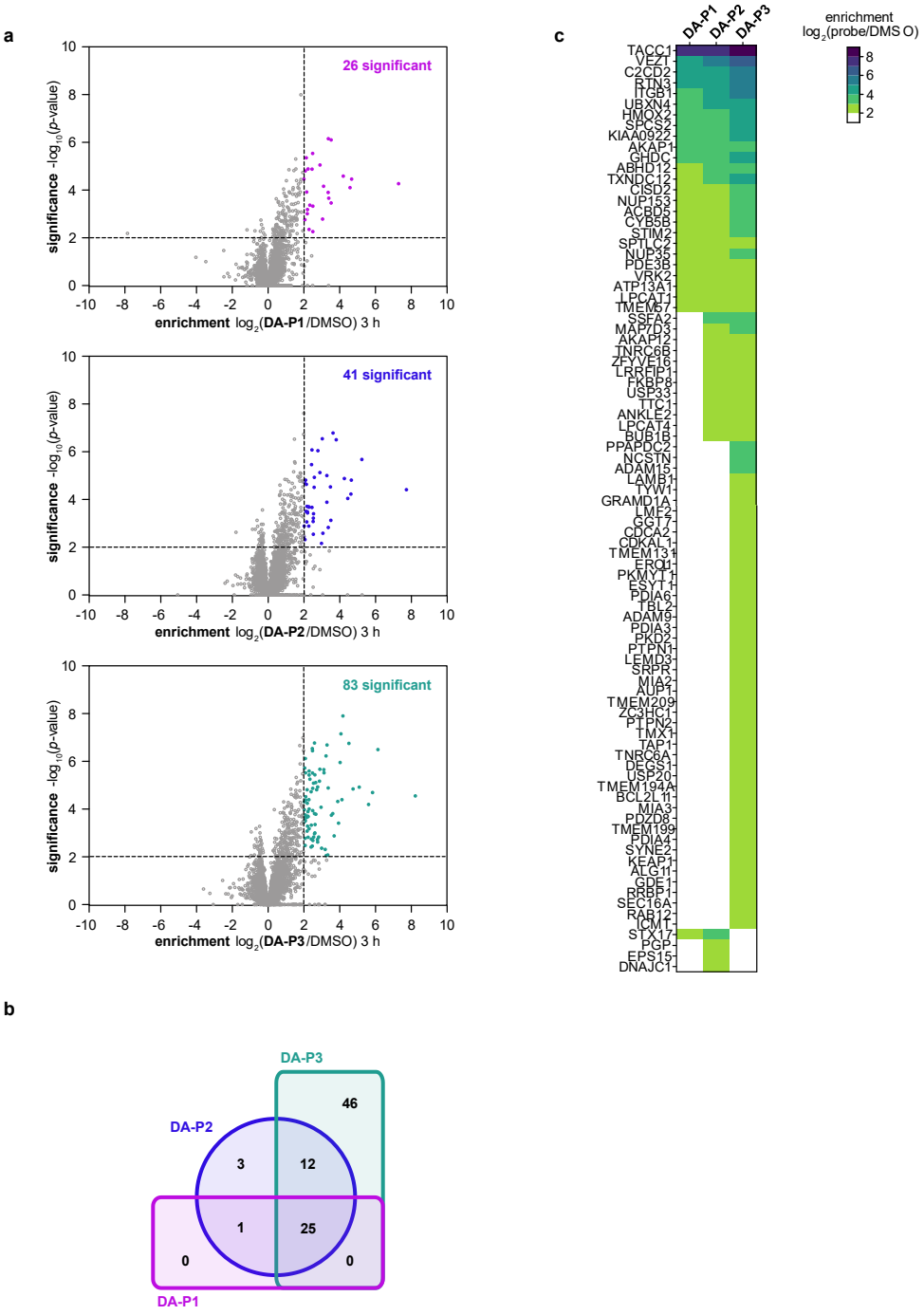


Figure S3. Comparison of target scope of catechol probes in live Hek293 cells labelled for 3 h. (a) Hek293 cells were treated 3 h with 15 µM **DA-P1**, **DA-P2**, **DA-P3**, or DMSO. MS data from four replicates were analysed by MaxLFQ[1] and filtered for proteins identified in three replicates in at least one condition. Samples were compared using a two-sided two-sample *t*-test. Proteins were considered significant hits when they were enriched more than four-fold ($\log_2$(enrichment) ≥ 2) with a *p*-value of less than 0.01 ($-\log_{10}$(*p*-value) ≥ 2); proteins with missing values are shown with $-\log_{10}$(*p*-value) = 0. Significant hits are shown as full circles; non-significantly enriched proteins are shown as open circles. See table S2 for details on identified proteins. (b) Venn diagram showing the overlap of protein hits of the probes. (c) Heat map showing protein enrichment by the different probes compared to DMSO.

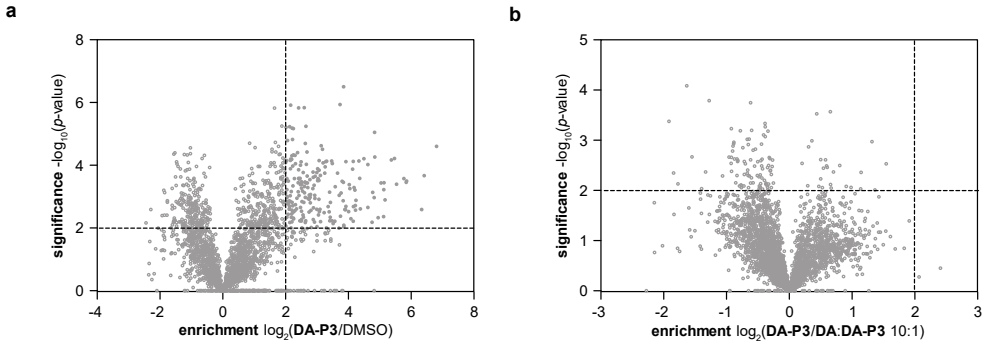


Figure S4. Labelling in SH-SY5Y cells in competition with DA. (a) Volcano plot showing proteins labelled by **DA-P3** (4 µM) in live SH-SY5Y compared to a DMSO-treated control. (b) Volcano plot showing proteins labelled by **DA-P3** (4 µM) in live SH-SY5Y cells compared to samples pre-treated with a 10-fold **DA** excess followed by **DA-P3** (4 µM). (a and b) MS data from three replicates were analysed by MaxLFQ and filtered for proteins identified in three replicates in at least one condition. Samples were compared using a two-sided two-sample *t*-test. Proteins were considered significant when they were enriched more than four-fold ($\log_2$(enrichment) ≥ 2) with a *p*-value of less than 0.01 ($-\log_{10}$(*p*-value) ≥ 2). Proteins with missing values are shown with $-\log_{10}$(*p*-value) = 0. Significant hits are shown as full circles; non-significantly enriched proteins are shown as open circles. See table S4 for details on identified proteins.

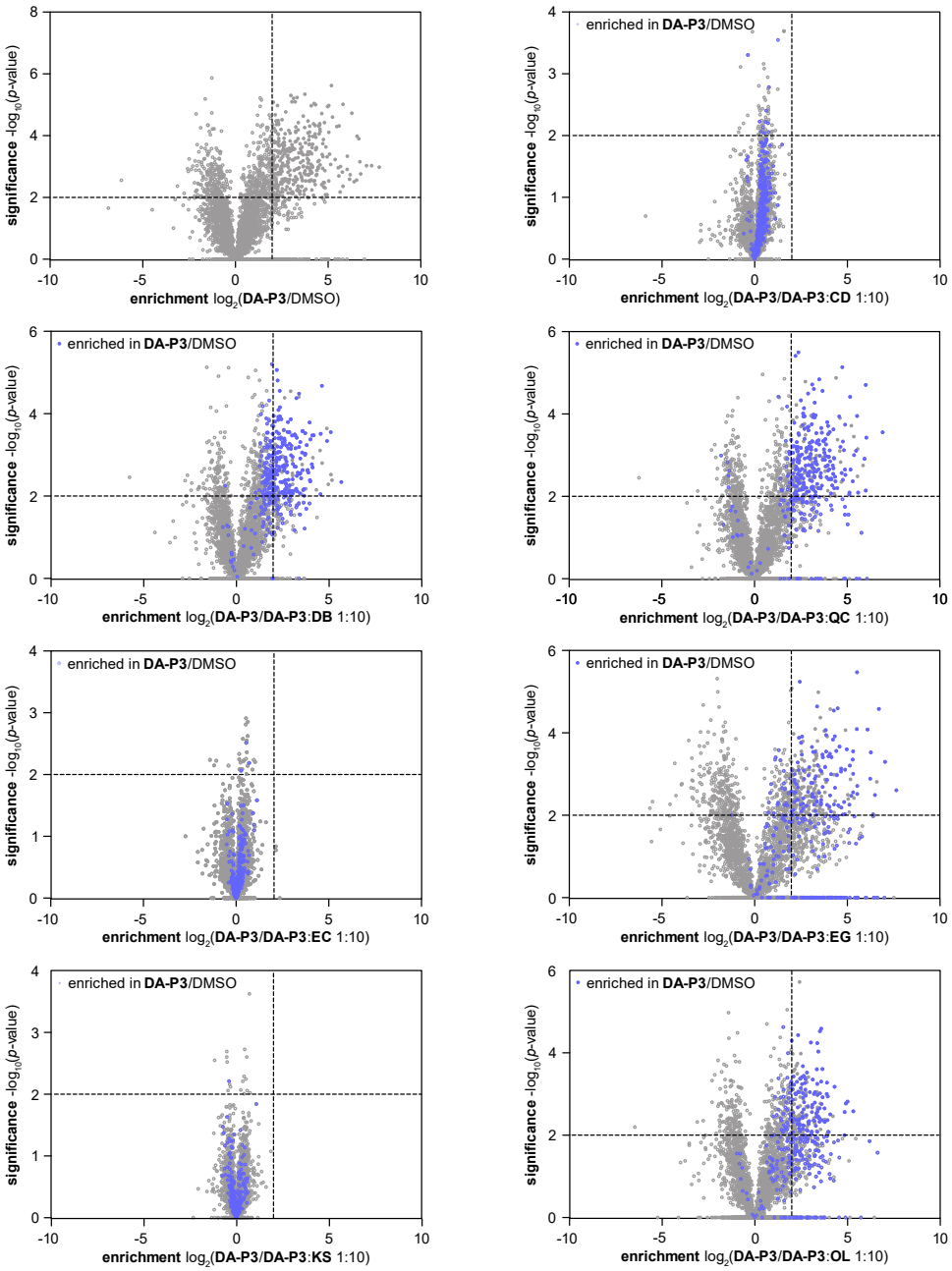


Figure S5: Labelling in competition with a 10-fold excess of different catechol compounds. MS data from three replicates were analysed by MaxLFQ[1] and filtered for proteins identified in three replicates in at least one condition. Samples were compared using a two-sided two-sample *t*-test. Proteins were considered significant hits when they were enriched more than four-fold ($\log_2$(enrichment) ≥ 2) with a *p*-value of less than 0.01 ($-\log_{10}$(*p*-value) ≥ 2); proteins with missing values are shown with $-\log_{10}$(*p*-value) = 0. Proteins significantly enriched against DMSO are highlighted in purple. Significant hits are shown as full circles; non-significantly enriched proteins are shown as open circles. See table S6 for details on identified proteins.

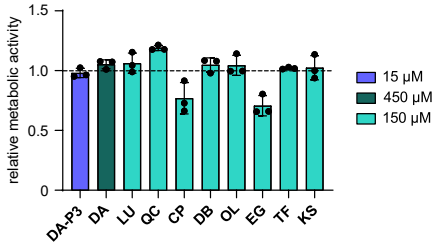


Figure S6. Cytotoxicity assessment by MTT assay. HEK293 were treated as for MS experiments with the compounds as indicated in serum-free medium for 2 h. Metabolic activity was normalised to a DMSO-treated control; error bars denote standard deviation, n = 3.

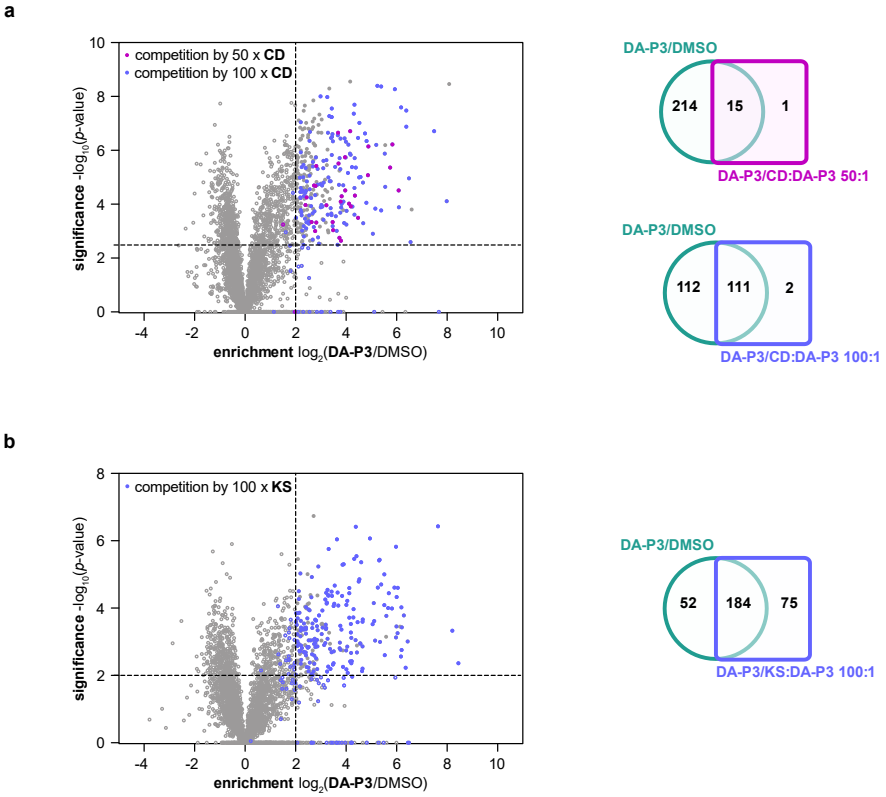


Figure S7. Labelling in HEK293 cells in competition with higher concentrations of CD and KS. (a) HEK293 cells were treated with DMSO, **DA-P3** (15 µM) or with **DA-P3** plus an excess of **CD** (50- or 100-fold). Volcano plot shows proteins labelled by **DA-P3** compared to the DMSO control. Proteins at which **DA-P3** binding was outcompeted by different concentrations of competitor are coloured as indicated. MS data from four replicates were analysed by MaxLFQ and filtered for proteins identified in three replicates in at least one condition. (b) HEK293 cells were treated with DMSO, **DA-P3** (15 µM) or with **DA-P3** plus an excess of **KS** (100-fold). Volcano plot shows proteins labelled by **DA-P3** compared to the DMSO control. Proteins at which **DA-P3** binding was outcompeted by **KS** are highlighted in blue. MS data from three replicates were analysed by MaxLFQ and filtered for proteins identified in three replicates in at least one condition. (a and b) Samples were compared using a two-sided two-sample *t*-test. Proteins were considered significant when they were enriched more than four-fold ($\log_2$(enrichment) ≥ 2) with a *p*-value of less than 0.01 ($-\log_{10}$(*p*-value) ≥ 2). Proteins with missing values are shown with $-\log_{10}$(*p*-value) = 0. Significant hits are shown as full circles; non-significantly enriched proteins are shown as open circles. See table S7–8 for details on identified proteins.

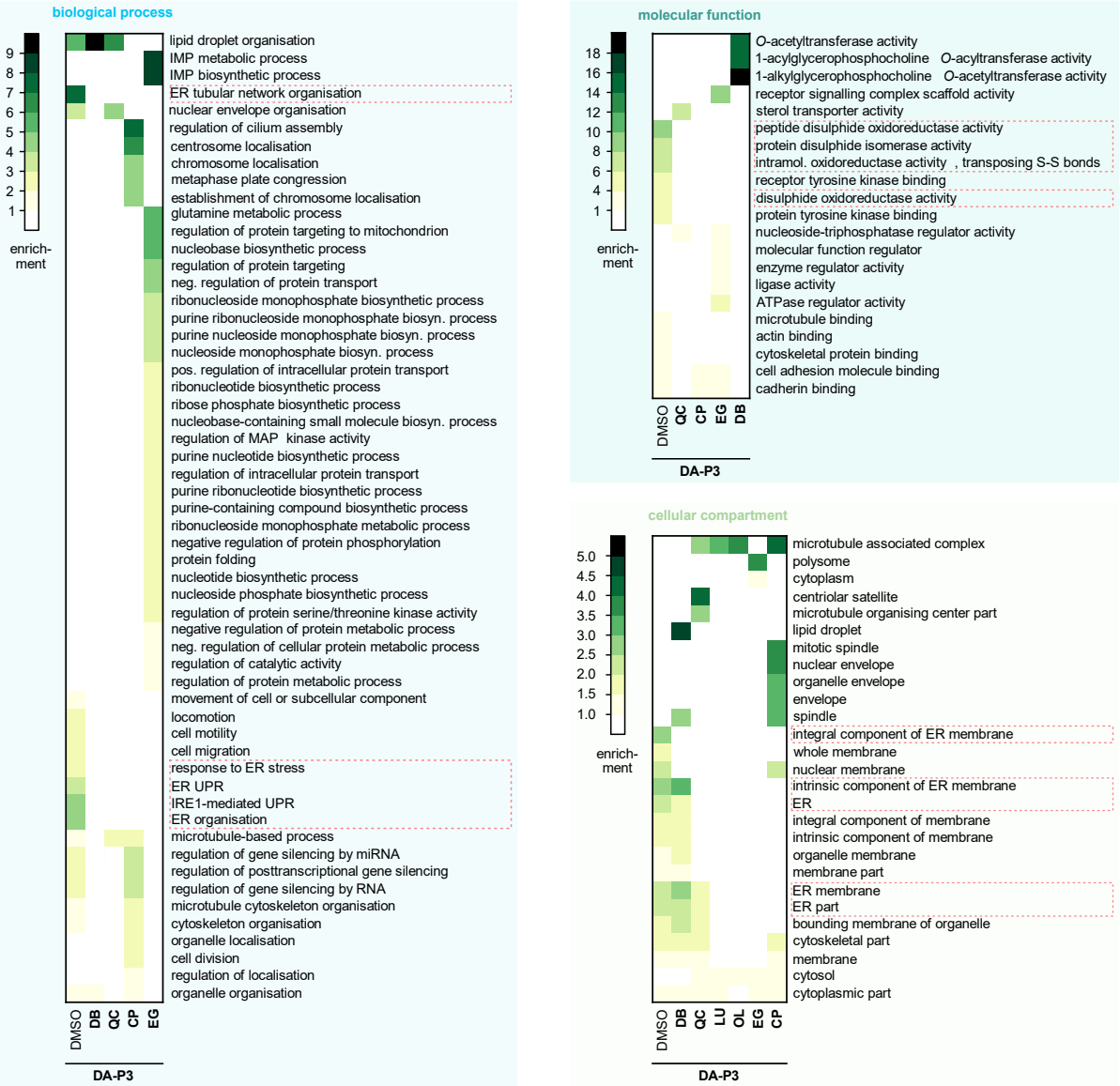


Figure S8. Cellular pathways and structures connected to proteins enriched against reactive catechols (10-fold excess). Heat map shows enrichment of GO term frequency in significantly outcompeted proteins compared to frequency in the corresponding dataset. Compounds for which no GO term was significantly enriched are not shown. ER-associated terms are highlighte by a red, dashed box. Threshold of GO term enrichment analyses: *p*-value ≤ 10^{-3}; IMP = inosine monophosphate; neg. = negative; pos. = positive; biosyn. = biosynthetic; ER = endoplasmic reticulum; UPR = unfolded protein response.

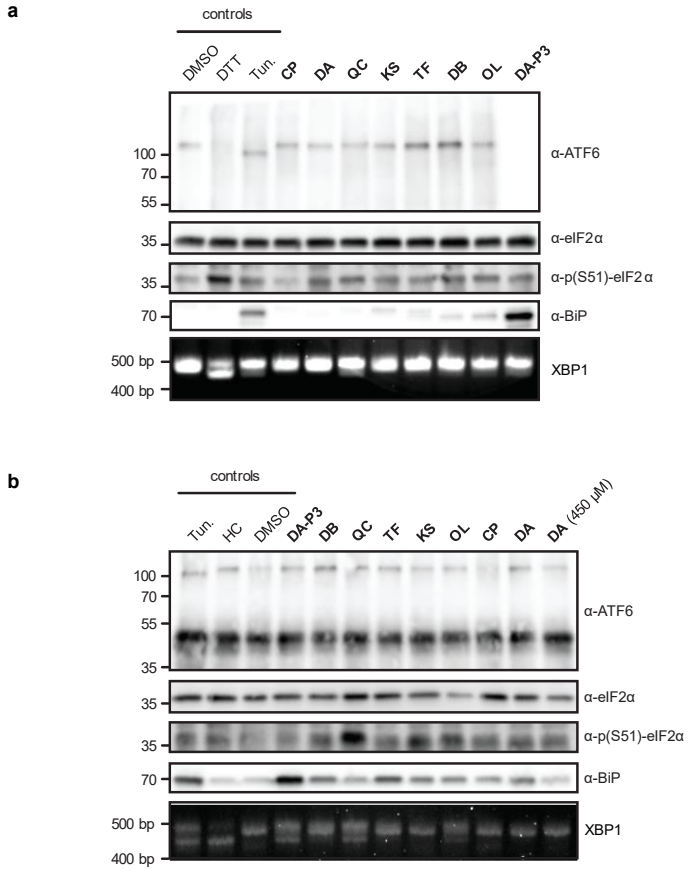


Figure S9 Replicates of UPR assessment. HEK293T cells were treated with the compounds of interest, the positive control tunicamycin (Tun.), DTT, or DMSO (as a vehicle control), or were transfected with IgG heavy chain (HC). Immunoblotting was performed against ATF6, eIF2α, phosphorylated eIF2α, BiP or Hsc70. XBP1 splicing was analysed using agarose gel electrophoresis. Cells were treated with 100 μM compound for 16 h unless otherwise indicated. (a) Replicate 1 and (b) replicate 2. Full gel images are shown in the appendix.

Supplementary Methods

Biological Methods

Chemical compounds

Code	name	CAS	supplier
DA	dopamine HCl	62-31-7	Alfa Aesar
DB	dobutamine HCl	49745-95-1	TCI
CD	carbidopa monohydrate	38821-49-7	Acros Organics
CP	capsaicin	404-86-4	Sigma Aldrich
EC	(-)-epicatechin	490-46-0	TCI
EG	epigallocatechin gallate	989-51-5	Sigma Aldrich
KS	caffeic acid	331-39-5	Sigma Aldrich
LU	luteolin	491-70-3	TCI
TF	(+)-taxifolin	480-18-2	TCI
OL	oleacein	149183-75-5	Phytolab
QC	quercetin	849061-97-8	Cayman Chemical

Catechol compounds were stored as indicated by the manufacturer and chemical probes as 100 mM dimethyl sulphoxide (DMSO) stocks at -20 °C.

Intact protein mass spectrometry

DJ-1 was kindly provided by Dr. Jonas Drechsel.[2] Chemical probes (500 µM) were added to DJ-1 (5 µM) in phosphate-buffered saline (PBS) from DMSO stocks (1% final concentration), and incubated at r.t. with mild shaking and protection from light for 15 h. LC-MS analysis of 1 µL protein solution was carried out on a LTQ FT Ultra™ mass spectrometer (Thermo Scientific) equipped with an electro spray ionisation (ESI) source operated in positive ionisation mode coupled to a Dionex Ultimate 3000 HPLC system (Thermo Scientific). Samples were loaded on a desalting column, eluted with an acetonitrile gradient, and transferred to the MS unit. Protein spectra were deconvoluted using the Thermo Xcalibur software (Thermo Scientific).

DJ-1 gel-based labelling

DJ-1 (1 µM) in PBS was incubated with **DA** at the indicated concentrations (added from 100-fold concentrated DMSO stocks) for 30 min in a shaker at 37 °C, 500 rpm, protected from light. **DA-P3** was added to 25 µM (from a 100-fold concentrated DMSO stock) and incubated for 2.5 h at 37 °C, 500 rpm, protected from light. The protein was precipitated in 600 µL acetone at -20 °C overnight, pelleted by centrifugation (15 min, 21,100 x g, 4 °C), and washed with 300 µL methanol in a sonication bath. Proteins were pelleted, air-dried, and resuspended in 150 µL 0.4% (w/v) SDS/PBS with sonication. Labelled proteins were ligated to rhodamine-azide by CuAAC and separated by SDS-PAGE as described below. The experiment was performed twice on two different days.

Cell culture for proteomics

All cells were grown in a humidified incubator at 37 °C in a 5% CO_2 atmosphere. For subculturing, cells were washed with PBS, detached with accutase® solution (Sigma), and split in a ratio of 1:9 (Hek293) or 1:19 (SH-SY5Y).

HEK293 (preparative scale labelling)

HEK293 cells were cultured in Dulbecco´s Modified Eagle´s Medium (DMEM high glucose, Sigma) supplemented with 2 mM L-glutamine and 10% (v/v) heat-inactivated fetal calf serum (FCS) and 10 x 10^6 cells were seeded the day before labelling to achieve 60–80% confluency. For competition experiments, competitors were added to the concentrations as indicated and 0.5% DMSO (from 200-fold concentrated DMSO stocks) in 10 mL FCS-free growth medium, swirled gently, and incubated 1 h, 37 °C, 5% CO_2. The probe was added to 15 µM and 0.1% DMSO (from a 15 mM stock), swirled, and incubated 1 h, 37 °C, 5% CO_2. For probe comparison experiments, the probes were directly added and incubated for 1 h or 3 h as indicated. After cell treatment, the medium was aspirated off and cells were scraped off in 10 mL PBS (on ice), transferred to tubes, washed 1–2 x with 1 mL cold PBS (500 x g, 5 min, 4 °C), flash-frozen, and stored at -80 °C until lysis. Frozen cell pellets were resuspended in 1 mL lysis buffer (1% [v/v] Nonidet P-40 [NP-40], 1% [w/v] sodium deoxycholate in PBS + EDTA-free protease inhibitor cocktail, Roche) and sodium dodecyl sulphate (SDS) was added to 0.2% (20 µL 10% SDS/PBS). Cells were sonicated (2 x 15 s, 60% intensity) to shear nucleic acids, insoluble debris was pelleted (21,000 x g, 15 min, 4 °C), and the supernatant transferred to a new tube. For competition experiments (except **DA**), proteins were precipitated in acetone (≥ 4 x volumes) at -20 °C, washed twice with ice-cold methanol, air-dried, and resuspended in 0.8% SDS/PBS as some catechols interfered with the protein concentration determination. Protein concentration was adjusted to 450 µg in 1 mL lysis buffer + 0.2% (w/v) SDS or to 500 µg in 500 µL 0.8% (w/v) SDS/PBS). Labelling was performed in three or four independent replicates and samples were processed together after lysis.

SH-SY5Y (preparative scale labelling)

SH-SY5Y cells were grown in Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F-12, Gibco™) supplemented with 2 mM L-glutamine (Sigma), non-essential amino acids (Gibco™), and 10% (v/v) heat-inactivated FCS (Sigma). For labelling, SH-SY5Y cells were grown in full growth medium in 150 mm cell culture dishes to 70% confluency. The medium was aspirated off, replaced by 10 mL FCS-free growth medium, and **DA** was added to 40 µM and 0.1% DMSO (10 µL of a 40 mM stock) and incubated 1 h, 37 °C, 5% CO_2. **DA-P3** was added to 4 µM and 0.1% DMSO (10 µL of a 4 mM stock) and incubated 1 h, 37 °C, 5% CO_2. Labelled cells were washed twice with 5 mL PBS, scraped off the plates in 1 mL lysis buffer, and transferred to microcentrifuge tubes. To shear nucleic acids, samples were sonicated with an ultrasound lance (2 x 15 s, 60% intensity) with constant cooling on ice. Insoluble debris was pelleted by centrifugation (21,000 x g, 15 min, 4 °C) and the supernatant transferred to a new tube. Protein concentration was adjusted to 500 µg in 1000 µL lysis buffer and SDS was added to 0.8% (w/v). Labelling was performed in three independent replicates and samples were processed together after lysis.

HEK293 (analytical scale labelling)

Analytical gel-based labelling was performed as MS-based labelling, seeding 0.5–0.75 x 10^6 cells in 3 mL medium in 6-well dishes. Competitors and probes were added from 200 x stocks as indicated and labelling was performed in 1 mL medium. Cells were harvested in 1 mL PBS and lysed in 100 µL lysis buffer. The experiment was performed in two biological replicates.

Click reaction preparative scale

Protein concentration was determined using the Roti®-Quant universal kit (Carl Roth) following the manufacturer's instructions. Click reagents were added to the lysate from a premix to the following concentrations: 100 µM rhodamine-biotin-azide tag (10 mM stock in DMSO)[3] or biotin-PEG_3-azide (10 mM stock in DMSO, *Jena Bioscience*), 1 mM $CuSO_4$ (50 mM stock in water), 1 mM tris(2-carboxyethyl)phosphine hydrochloride (52 mM stock in water), and 100 µM tris((1-benzyl-4-triazolyl)methyl)amine (1.667 mM stock in 20% (v/v) DMSO/*t*-BuOH) and incubated 1 h, 25 °C, 400 rpm. Proteins were precipitated in 4 mL acetone at -20 °C overnight, pelleted (20,450 × g, 15 min, 4° C), and washed twice with 1 mL ice-cold methanol with sonication (1 × 10 s, 10% intensity). Pellets were air-dried, and proteins were resolubilised in 500 µL 0.4% (w/v) SDS/PBS by sonication and transferred to LoBind microcentrifuge tubes.

CuAAC, analytical scale labelling

CuAAC for analytical labeling experiments was performed as for preparative scale experiments but with rhodamine-azide (tetramethylrhodamine 5-carboxamido-(6-azidohexanyl), 5-isomer, 5 mM stock in DMSO, Base Click). Click reaction was quenched by addition of the same volume of 2 x sample loading buffer (63 mM Tris/HCl, 10% (v/v) glycerol, 2% (w/v) SDS, 0.0025% (w/v) bromophenol blue, 5% (v/v) 2-mercaptoethanol).

SDS-PAGE for proteomics

Stacking gels consisted of 4% (w/v) acrylamide (in 50 mM Tris, pH 6.8) and resolving gels of 12.5% (w/v) acrylamide (in 300 mM Tris, pH 8.8) and were run in a Tris-glycine buffer (25 mM Tris, 192 mM glycine, 0.1% (w/v) SDS, pH 8.3). Samples were boiled (90 °C, 3 min, 400 rpm) and centrifuged (16,249 x g, 3 min) before SDS-PAGE. Typically, 30 µL sample (~15 µg protein), 8 µL fluorescent marker (BenchMark™ Fluorescent Protein Standard, Thermo Fisher), and 12 µL protein marker (Roti®-Mark Standard, Carl Roth) were loaded and gels were run at 150–300 V (depending on gel size) on a EV265 Consort power supply (*Hoefer*). Fluorescence was scanned in a LAS-4000 imaging system equipped with a Fujinon VRF43LMD3 lens and a 575DF20 filter (Fujifilm). Gels were stained in Coomassie staining solution (0.25% (w/v) Coomassie Brilliant Blue R-250, 9.2% (v/v) concentrated acetic acid, 45.4% (v/v) ethanol) overnight and destained in 10% (v/v) acetic acid, 40% (v/v) ethanol).

MS sample preparation

Protein LoBind microcentrifuge tubes and MS-grade reagents were used throughout MS sample preparation.

Enrichment for target identification

Protein samples were centrifuged to remove particulates, added to 50 µL avidin slurry (Sigma) (pre-washed 3 x with 0.4% (w/v) SDS/PBS [3 min, 400 x g]), and incubated 1 h at r.t. under constant rotation. Samples were centrifuged, the supernatant discarded, and the beads were washed 3 x with 1 mL 0.4% (w/v) SDS/PBS, 2 x with 1 mL 6 M urea/ddH_2O, and 3 x with 1 mL PBS. Beads were resuspended in 200 µL MS denaturation buffer (7 M urea, 2 M thiourea, 20 mM Hepes, pH 7.5) and reduced with 5 mM tris(2-carboxyethyl)phosphine hydrochloride for 1 h at 37 °C with shaking. Next, thiols were alkylated with 10 mM iodoacetamide for 30 min at 25 °C with shaking, and alkylation was quenched with 10 mM dithiothreitol (DTT) for 30 min at 25 °C with shaking. Proteins were digested with 0.5 µg Lys-C (0.5 µg/µL, MS-grade, Wako) for 2 h at 25 °C with shaking. Samples were diluted by addition of 800 µL 50 mM triethylammonium bicarbonate buffer (TEAB) and proteins were digested with 0.75 µg trypsin (from 0.5 µg/µL in 50 mM acetic acid, Promega) for 16 h at 37 °C with vigorous shaking. The digest was stopped by addition of formic acid to 3% (pH 2–3), the beads pelleted (16,249 x g, 3 min, r.t.), and the supernatant desalted.

Desalting (target identification experiments)

For desalting, 50 mg SepPak C18 columns (Waters) were washed with 1 x 1 mL acetonitrile and 1 x 1 mL elution buffer (80% [v/v] acetonitrile, 0.5% [v/v] formic acid/water) and then equilibrated with 3 x 1 mL 0.1% (v/v) trifluoroacetic acid (TFA)/water. Samples were loaded onto the columns and desalted with 3 x 1 mL 0.1% (v/v) TFA and 1 x 0.5 mL 0.5% formic acid. Peptides were eluted into 2 mL LoBind tubes with 3 x 250 µL elution buffer and dried in a speed vac. Dried peptides were stored at -80 °C until LC-MS/MS analysis.

Chemoproteomic experiments with isoDTB tags

HEK293 cells were treated with **DA-P3** (15 µM) *in situ* and lysed as for target identification experiments. Protein concentration was adjusted to 1 µg/µL in 1400 µL lysis buffer (1% NP-40, 1% deoxycholic acid/PBS) containing 0.8% SDS. Click reaction with a light and heavy isoDTB tag,[4] MS sample preparation, and measurement were performed as previously reported.[5] The experiment was performed in two independent replicates.

Peptide reconstitution (all proteomics experiments)

Peptides were reconstituted in 30 µL 1% (v/v) formic acid with vortexing and in a sonication bath (10 min) and filtered through centrifugal filters (0.22 µm, Durapore, PVDF, Merck KGaA) previously equilibrated with 300 µL 1% (v/v) formic acid (16,249 x g, 2 min, r.t.).

LC-MS/MS measurements

Target identification peptide samples were analyzed on an UltiMate 3000 nano HPLC system (Dionex) equipped with an Acclaim C18 PepMap100 (75 µm ID × 2 cm) trap column and a 25 cm Aurora Series emitter column (25 cm × 75 µm ID, 1.6 µm FSC C18) (*Ionopticks*) separation column (column oven heated to 40 °C) coupled to a Q Exactive Plus (Thermo Fisher) in EASY-spray setting. Peptides were loaded on the trap column and washed with 0.1% (v/v) TFA before being transferred to the analytical

column and separated using a 152 min gradient (buffer A: 0.1% (v/v) formic acid in water, buffer B: 0.1% (v/v) formic acid in acetonitrile. Gradient of buffer B: 5% (v/v) for 7 min, increase to 22% (v/v) in 105 min, then to 32% (v/v) in 10 min, then to 90% (v/v) in 10 min, hold at 90% (v/v) for 10 min, decrease to 5% (v/v) in 0.1 min and hold at 5% (v/v) for 9.9 min) with a flow rate of 400 nL/min. The Q Exactive Plus was operated in a TOP10 data dependent mode full scan acquisition in the orbitrap was performed with a resolution of 140 000 and an AGC target of 3e6 (maximum injection time of 80 ms) in a scan range of 300–1,500 m/z. Most intense precursors with charge states of > 1, a minimum AGC target of 1e3, and intensities greater than 1e4 were selected for fragmentation. Peptide fragments were generated using higher-energy collisional dissociation (HCD, normalized collision energy: 27%) and detected in the orbitrap with a resolution of 17 500 m/z. The AGC target was set to 1e5 (maximum injection time 100 ms) and the dynamic exclusion duration to 60 s. Isolation in the quadrupole was performed with a window of 1.6 m/z.

Differential isotopic labeling samples were measured on a Q Exactive Plus spectrometer with a different gradient (buffer B: 5% (v/v) for 7 min, increase to 40% (v/v) in 105 min, then to 60% (v/v) B in 10 min, and to 90% (v/v) B in 10 min, hold at 90% (v/v) for 10 min, then decrease to 5% (v/v) in 0.1 min and hold at 5% (v/v) for another 9.9 min) at the same flow rate. All parameters were the same as for photoaffinity labeling experiments except full MS scans were collected at a resolution of 70 000.

Analysis of MS data from target identification experiments

MS data were analysed using MaxQuant[6, 7] version 1.6.5.0 and peptides were searched against the UniProt database for *Homo sapiens* (taxon identifier 9606, downloaded on 29.01.2019) where Titin and all proteins containing U, O, or X were removed. Cysteine carbamidomethylation was set as fixed modification and methionine oxidation and N-terminal acetylation as variable modifications. Trypsin/P was set as proteolytic enzyme with a maximum of two allowed missed cleavages. Label-free quantification (LFQ) mode[1] was performed with a minimum ratio count of 1. Protein quantification was performed with the minimal ratio count set to 1 and the match between runs (0.7 min match and 20 min alignment time window) and second peptide identification options were activated. All other parameters were used as pre-set in the software. LFQ intensities were further processed with Perseus[8] version 1.6.1.1. Peptides of the categories “only identified by site”, “reverse”, or “potential contaminant” were removed and LFQ intensities were $\log_2$-transformed. Data were filtered to retain only protein groups identified with at least three valid values in at least one group. A two-sided two-sample Student´s *t*-test with permutation-based FDR (FDR 0.05) was performed and the significance cut-off was set at *p*-value = 0.01 ($-\log_{10}$(*p*-value) = 2) and an enrichment factor of 4 ($\log_2(x) = 2$) as indicated in the plots.

Analysis of isoDTB data

IsoDTB data were analysed as previously described,[5, 9] using the MSconvert tool (version: 3.0.19172-57d620127) of the ProteoWizard software (version: 3.0.19172 64bit),[10] the FragPipe interface (version: 14.0),[11, 12] MSFragger (version: 3.1.1),[11,12] Philosopher (version: 3.3.10),[13] IonQuant (version 1.4.6),[14] and Python (version: 3.7.3). The FASTA file (*Homo sapiens*, taxon identifier 9606, downloaded on 29.01.2019), where Titin and all proteins containing U, O, or X were removed, was further modified by adding the reverse sequences manually. Modifications were analysed as previously described.[9]

Amino acid selectivity was analysed and data were evaluated and filtered as previously published[9] performing an Offset Search in MSFragger[11, 12] with mass offsets set as 754.4120 or 760.4206. Run MS1 quant was enabled with Labelling based quant with masses set as 754.4120 or 760.4206. Specific amino acids were quantified and data were evaluated and filtered as previously published[9] performing a Closed Search in MSFragger with variable modifications set to 754.4120 or 760.4206 on Cys. Run MS1 quant was enabled with Labelling based quant with masses set as 754.4120 or 760.4206.

Gene ontology (GO) term enrichment analyses

GO enrichment analysis was performed using the GOrilla tool.[15, 16] Gene names of proteins enriched above the cut offs ($-\log_{10}(p\text{-value}) \geq 2$; $\log_2(\text{enrichment}) \geq 2$) were used as target set (unranked) and compared against all proteins detected (after filtering). The *p*-value threshold was set to 10^{-3}–10^{-5} as indicated.

Heat maps

Heat maps with hierarchical clustering were generated with Origin 2021 using group average as cluster method and Euclidian distance. Heat maps of probe comparison experiments were created without clustering.

Data availability

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository[17] with the dataset identifier PXD043348.

Cytotoxicity assays (MTT)

HEK293 cells were seeded in a clear flat-bottom 96-well plate (Thermo Fisher Scientific) previously coated with poly-L-Lys (Sigma Aldrich) at a density of 50 000 cells in 150 µL full growth medium per well and allowed to adhere overnight. The next day, the medium was removed and replaced with 100 µL serum-free medium containing the compounds or DMSO at the indicated concentrations (added from 100-fold concentrated DMSO stocks). Following incubation with the compounds for 2 h at 37 °C, 5% CO_2 the medium was aspirated off, replaced by 80 µL full growth medium plus 20 µL MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide) solution (5 mg/mL in PBS) and incubated for 4 h, 37 °C, 5% CO_2. The medium was removed by suction and the resulting formazan was dissolved in 200 µL and the optical density was determined at 570 nm with a background control at 630 nm using a TECAN Infinite® M200 Pro plate reader. Percent inhibition of metabolic activity was calculated as a fraction of DMSO controls. The experiment was performed in technical triplicates.

Cell culture for UPR assays

HEK293T cells were cultivated in Dulbecco's Modified Eagle's Medium (DMEM), high glucose (Sigma-Aldrich) supplemented with 10% (v/v) foetal bovine serum (Biochrom) and 1% (v/v) antibiobotic-antimycotic solution (25 µg/mL amphotericin B, 10 mg/mL streptomycin, 10,000 units of penicillin [Sigma-Aldrich]) at 37 °C and 5% CO_2. 24 h before treatment, 700,000 HEK293T cells were seeded per

p35 plate (uncoated Nunclon 6-well plates, #140685, Thermo Scientific). ER stress assays were performed in three biological replicates.

Induction of ER stress

To assess the effect of the compounds of interest, medium of the cells was exchanged with medium supplemented with 100 µM of the compound from 100 mM stock solutions in DMSO 16 h prior to lysis, unless otherwise stated. As a control, the unfolded protein response was induced by addition of 5 µg/ml tunicamycin (Sigma-Aldrich) in medium and subsequent incubation for 6 h prior to lysis, or by 24-hour transient transfection of antibody heavy chain under a CMV promoter. XBP1 splicing was assessed by RNA extraction using an RNAeazy Mini Kit (Qiagen) in an RNAse free environment. RT-PCR of the purified RNA was then performed using an OligodT20 Primer (18418020, Thermo Fisher) and SuperScript III Reverse Transcriptase (18080044, Thermo Fisher). The resulting cDNA was amplified using a Jumpstart Taq DNA Polymerase (D4184, Sigma Aldrich) with 1.5 mM $MgCl_2$ added and the following primers: forward $_5$'AAACAGAGTAGCAGCTCAGACTGC$_3$' and reverse $_5$'TCCTTCTGGGTAGACCTCTGGGA$_3$'. The following product was analysed on 3% agarose gels.

Transient transfection

24 h before transfection, 700.000 HEK293T cells were seeded per per p35 plate (uncoated Nunclon 6-well plates, #140685, Thermo Scientific). Transient transfection was performed using Metafectene Pro (T040, Biontex) according to the manufacturer's protocol with maximum recommended amounts of DNA.

Cell lysis

Cells were harvested 24 h after transfection on ice using ice-cold solutions. Cells were washed twice using PBS and then lysed for 20 min by addition of 500 µL NP-40 lysis buffer (1% [v/v] NP-40, 1 mM $MgCl_2$, 150 mM NaCl, 5% [v/v] glycerol) supplemented with cOmplete protease inhibitor cocktail (Roche). For phosphorylated eIF2α, the lysis buffer was additionally supplemented with 50-fold phosphatase inhibitor (Phosphatase Inhibitor Mix II solution, Serva). The resulting lysate was centrifuged 20 min at 15,000 x g and the supernatant was supplemented with 5x Laemmli buffer containing 2% (v/v) β-mercaptoethanol and incubated at 95 °C for 10 min or used for immunoprecipitation for ATF6 samples. Here, the supernatant was agitated at 4 °C for 2 h after being supplemented with 25 µL of magnetic protein A/G beads (PureProteome Protein A/G Mix, Millipore) pre-coupled to ATF6 antibody using the manufacturer's instructions and washed three times with NP-40 wash buffer (1% [v/v] NP-40, 1 mM $MgCl_2$, 400 mM NaCl, 5% [v/v] glycerol). The sample was then eluted from the beads using 25 µL of 2x Laemmli buffer containing β-mercaptoethanol and boiling at 95 °C for 10 min. The resulting solutions were used for subsequent SDS-PAGE.

SDS-PAGE and immunoblotting

Proteins were separated by SDS-polyacrylamide gel electrophoresis (PAGE) in 12% SDS-PAGE gels. The gels were then blotted overnight at 4 °C onto polyvinylidene difluoride (PVDF) membranes (Biorad) and then blocked at room temperature for 6 h with Tris-buffered saline supplemented with skim milk powder and Tween-20 (M-TBST; 25 mM Tris/HCl, pH 7.5, 150 mM NaCl, 5% [w/v] skim milk powder,

0.05% [v/v] Tween-20) or with 5% (w/v) BSA (A3294, Sigma-Aldrich) solution in TBST for eIF2α and p-eIF2α. Primary anti-BiP antibody (polyclonal rabbit, C50B12, Cell Signaling) at 1:500 in M-TBST, anti-ATF6 antibody (mouse monoclonal, ab122897, Abcam) at 1:500 in M-TBST, anti-Hsc70 antibody (mouse monoclonal, sc-7298, Santa Cruz) at 1:1000 in M-TBST, p-eIF2α antibody (rabbit polyclonal, #9721, Cell Signaling Technology) at 1:500 in 5% (w/v) BSA in TBST, or anti-eIF2A antibody (rabbit polyclonal, #9722, Cell Signaling Technology) at 1:500 in 5% (w/v) BSA in TBST was applied at 4 °C overnight. After washing (1 x 5 min TBS, 2 x 5 min TBST, 3 x 5 min TBS), the blots were then incubated at room temperature for 1 h in M-TBST or 5% (w/v) BSA in TBST supplemented with HRP-conjugated anti-rabbit antibodies (mouse monoclonal, IgG-HRP (sc-2357, Santa Cruz)) or mouse IgG kappa binding protein (sc-516102, Santa Cruz) at 1:5000. Following another round of washing, the blots were then analysed using Amersham ECL prime solution (GE Life Sciences) and a Fusion Pulse 6 imager (Vilber Lourmat).

Chemical Synthesis

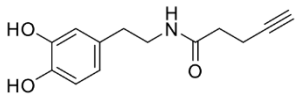
General remarks

Chemicals with reagent or higher grade and anhydrous solvents were purchased from Sigma Aldrich, Acros Organics, or Alfa Aesar. Analytical thin layer chromatography was performed on aluminium-coated TLC silica gel plates (silica gel 60, F254, Merck KGaA) using UV light (λ = 254 nm) and $KMnO_4$-stain (3.0 g $KMnO_4$, 20.0 g K_2CO_3 and 5 mL 5% (w/v) NaOH in 300 mL ddH_2O) for visualisation. Column chromatography was performed with silica gel (40–63 µm (Si 60), Merck KGaA). High-resolution mass spectrometry (HRMS) measurements were performed on a LTQ-FT Ultra (Thermo Fisher) equipped with an ESI ion source. NMR spectra were measured at room temperature on Avance-III HD NMR systems with 400 or 500 MHz (Bruker Co.). Chemical shifts are reported in parts per million (ppm) and residual proton signals of deuterated solvents were used as internal reference (^{1}H-NMR: DMSO-d_6 δ = 2.50 ppm. ^{13}C-NMR: DMSO-d_6 δ = 39.52 ppm). Coupling constants (*J*) are reported in Hertz (Hz). Signal multiplicities are denoted with the following abbreviations: s – singlet, dt – doublet of triplets, t – triplet, td – triplet of doublets, p – pentet, and m – multiplet. NMR data were analysed using MestReNova (Mestrelab Research).

Amide coupling general protocol[18]

To a solution of carboxylic acid (1.0 eq.) in DMF (24 mL), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl, 1.0 eq.) was added followed by hydroxybenzotriazole (HOBt, 1.0 eq.). The solution was stirred at 0 °C for 30 min and then at r.t. for 1.5 h and anhydrous triethylamine (TEA, 3.0 eq.) was added dropwise. The amine (1.0 eq.) was added, and the mixture was stirred at r.t. overnight protected from light. EtOAc (100 mL) was added, washed three times with water (30 mL), and the combined aqueous phases were reextracted with EtOAc (20 mL). The combined organic phases were washed with brine (50 mL) and dried over Na_2SO_4. Solvents were removed under reduced pressure and the crude mixture was purified by SiO_2 chromatography (0.5% AcOH, 1–3% MeOH/CH_2Cl_2).

N-(3,4-dihydroxyphenethyl)pent-4-ynamide (**DA-P1**)



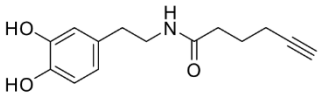
DA-P1
$C_{13}H_{15}NO_3$
233.27 g/mol

DA-P1 was obtained from dopamine·HCl (569 mg, 3.00 mmol, 1.0 eq.) and 4-pentynoic acid (294 mg, 3.00 mmol, 1.0 eq.) as a grey solid (186 mg, 0.798 mmol, 27%).

TLC: R_f = 0.26 (0.5% AcOH, 3% MeOH/CH_2Cl_2) [UV/$KMnO_4$]. **^{1}H-NMR:** (500 MHz, DMSO-d_6) δ [ppm]: 8.83–8.53 (m, 2H), 7.90 (t, *J* = 5.3 Hz, 1H), 6.65–6.60 (m, 1H), 6.58–6.54 (m, 1H), 6.45–6.40 (m, 1H), 3.20–3.13 (m, 2H), 2.75 (t, *J* = 2.6 Hz, 1H), 2.53–2.47 (m, 2H + DMSO), 2.36–2.30 (m, 2H), 2.27–2.21 (m, 2H). **^{13}C-NMR:** (75 MHz, DMSO-d_6) δ [ppm]: 170.05, 145.04, 143.52, 130.20, 119.21, 115.98,

115.49, 83.81, 71.26, 40.63, 34.70, 34.24, 14.26. **HRMS:** (ESI) $C_{13}H_{16}NO_3^+$ $[M+H]^+$ calculated: 234.1125; found: 234.1124.

N-(3,4-dihydroxyphenethyl)hex-5-ynamide (**DA-P2**)

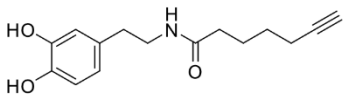


DA-P2
$C_{14}H_{17}NO_3$
247.29 g/mol

DA-P2 was obtained from dopamine·HCl (284 mg,1.50 mmol, 1.0 eq.) and 5-hexynoic acid (168 mg, 166 µL, 1.50 mmol, 1.0 eq.) as a brown oil (217 mg, 0.876 mmol, 58%).

TLC: R_f = 0.34 (0.5% AcOH, 3% MeOH/CH_2Cl_2) [UV/$KMnO_4$]. **^{1}H-NMR:** (400 MHz, DMSO-d_6) δ [ppm]: 8.65 (s, 2H), 7.81 (t, J = 5.6 Hz, 1H), 6.62–6.56 (m, 1H), 6.56–6.51 (m, 1H), 6.43–6.35 (m, 1H), 3.13 (dt, J = 8.1, 6.0 Hz, 2H), 2.73 (t, J = 2.7 Hz, 1H), 2.47 (t, J = 7.5 Hz, 2H), 2.10 (td, J = 7.2, 1.9 Hz, 4H), 1.62 (p, J = 7.3 Hz, 2H). **^{13}C-NMR:** (101 MHz, DMSO-d_6) δ [ppm]: 171.25, 145.03, 143.48, 130.27, 119.19, 115.94, 115.45, 84.14, 71.42, 40.53, 34.69, 34.20, 24.29, 17.41. **HRMS:** (ESI) $C_{14}H_{18}NO_3^+$ $[M+H]^+$ calculated: 248.1281; found: 248.1281.

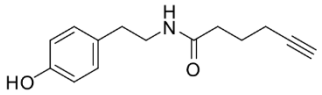
N-(3,4-dihydroxyphenethyl)hept-6-ynamide (**DA-P3**)



DA-P3
$C_{15}H_{19}NO_3$
261.32 g/mol

DA-P3 was obtained from dopamine·HCl (398 mg, 2.10 mmol, 1.0 eq.) and 6-heptynoic acid (265 mg, 280 µL, 2.10 mmol, 1.0 eq.) as a brown oil (352 mg, 1.35 mmol, 64%). **TLC:** R_f = 0.46 (5% MeOH/CH_2Cl_2) [UV/$KMnO_4$]. **^{1}H-NMR:** (400 MHz, DMSO-d_6) δ [ppm]: 8.67 (s, 2H), 7.79 (t, J = 5.5 Hz, 1H), 6.64–6.58 (m, 1H), 6.58–6.53 (m, 1H), 6.44–6.37 (m, 1H), 3.20–3.10 (m, 2H), 2.74 (t, J = 2.6 Hz, 1H), 2.53–2.46 (m, 2H + DMSO-d_6), 2.13 (td, J = 7.0, 2.6 Hz, 2H), 2.03 (t, J = 7.4 Hz, 2H), 1.61–1.49 (m, 2H), 1.44–1.32 (m, 2H). **^{13}C-NMR:** (101 MHz, DMSO-d_6) δ [ppm]: 171.64, 145.02, 143.47, 130.25, 119.17, 115.93, 115.43, 84.36, 71.20, 48.59, 40.47, 34.78/34.73 (rotamers), 27.50, 24.39, 17.43. **HRMS:** (ESI) $C_{15}H_{20}NO_3^+$ $[M+H]^+$ calculated: 262.1438; found: 262.1438.

N-(4-hydroxyphenethyl)hex-5-ynamide **(DA-P4)**



DA-P4
$C_{14}H_{17}NO_2$
231.30 g/mol

DA-P4 was obtained from 4-(2-aminoethyl)phenol (412 mg, 3.00 mmol, 1.0 eq.) and 5-hexanoic acid (336 mg, 331 µL, 3.00 mmol, 1.0 eq.) as an off-white amorphous solid (565 mg, 2.44 mmol, 81%). **TLC:** R_f = 0.42 (3% MeOH, 0.5% AcOH/CH_2Cl_2) [UV/$KMnO_4$]. **^{1}H-NMR:** (400 MHz, DMSO-d_6) δ [ppm]: 9.20 (s, 1H), 7.86 (t, J = 5.4 Hz, 1H), 6.97 (d, J = 8.4 Hz, 2H), 6.66 (d, J = 8.4 Hz, 2H), 3.18 (q, J = 6.5 Hz, 2H), 2.77 (t, J = 2.6 Hz, 1H), 2.57 (t, J = 7.4 Hz, 2H), 2.17–2.08 (m, 4H), 1.64 (p, J = 7.2 Hz, 2H). **^{13}C-NMR:** (101 MHz, DMSO-d_6) δ [ppm]: 171.25, 155.61, 129.52, 129.43, 115.06, 84.12, 71.40, 40.48, 34.39, 34.17, 24.28, 17.38. **HRMS:** (ESI) $C_{14}H_{18}NO_2^+$ $[M+H]^+$ calculated: 232.1332; found: 232.1332.

Supplementary tables

see separate Excel files

Table S1: Chemical proteomics data of labelling with **DA-P1**, **DA-P2**, or **DA-P3** for 1 h in HEK293 cells. Includes protein annotation, MS data, statistics, and LFQ intensities.

Table S2: Chemical proteomics data of labelling with **DA-P1**, **DA-P2**, or **DA-P3** for 3 h in HEK293 cells. Includes protein annotation, MS data, statistics, and LFQ intensities.

Table S3: Chemical proteomics data of **DA-P3** labelling in competition with **DA** in HEK293 cells. Includes protein annotation, MS data, statistics, and LFQ intensities.

Table S4: Chemical proteomics data of **DA-P3** labelling in competition with **DA** in SH-SY5Y cells. Includes protein annotation, MS data, statistics, and LFQ intensities.

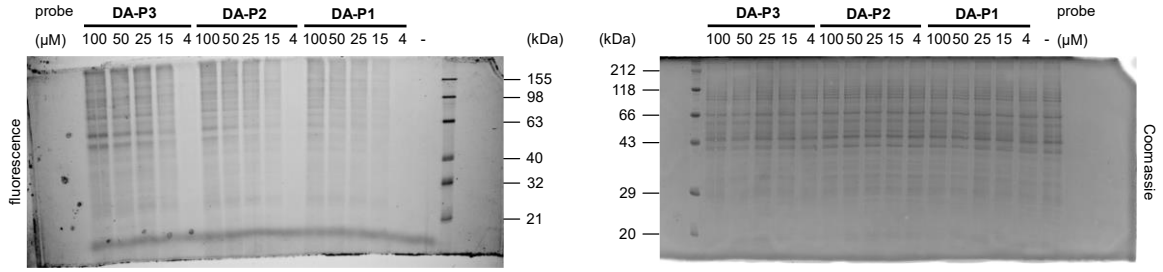
Table S5: Proteomics data of isoDTB labelling experiment. Includes amino acid modification, selectivity, and quantification results.

Table S6: Chemical proteomics of **DA-P3** labelling in competition with **CD**, **DB**, **QC**, **TF**, **EC**, **LU**, **EG**, **KS**, **OL**, or **CP**. Includes protein annotation, MS data, statistics, and LFQ intensities.

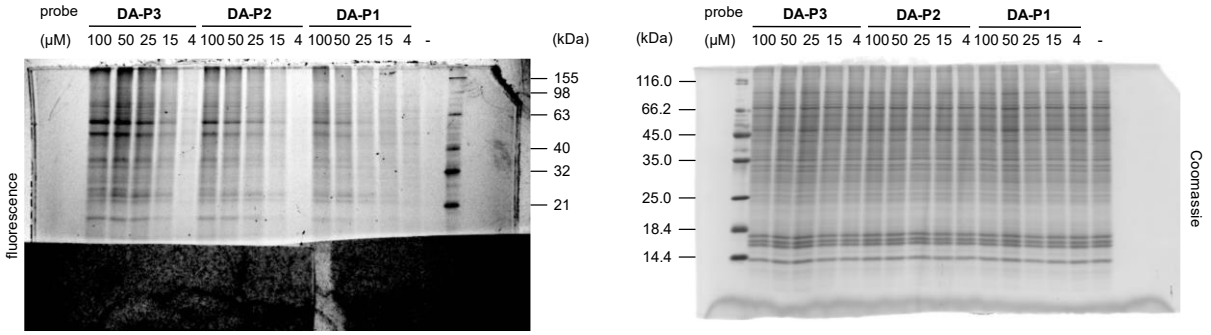
Table S7: Chemical proteomics data of **DA-P3** labelling in competition with **CD** (50–100-fold excess) in HEK293 cells. Includes protein annotation, MS data, statistics, and LFQ intensities.

Table S8: Chemical proteomics data of **DA-P3** labelling in competition with **KS** (100-fold excess) in HEK293 cells. Includes protein annotation, MS data, statistics, and LFQ intensities.

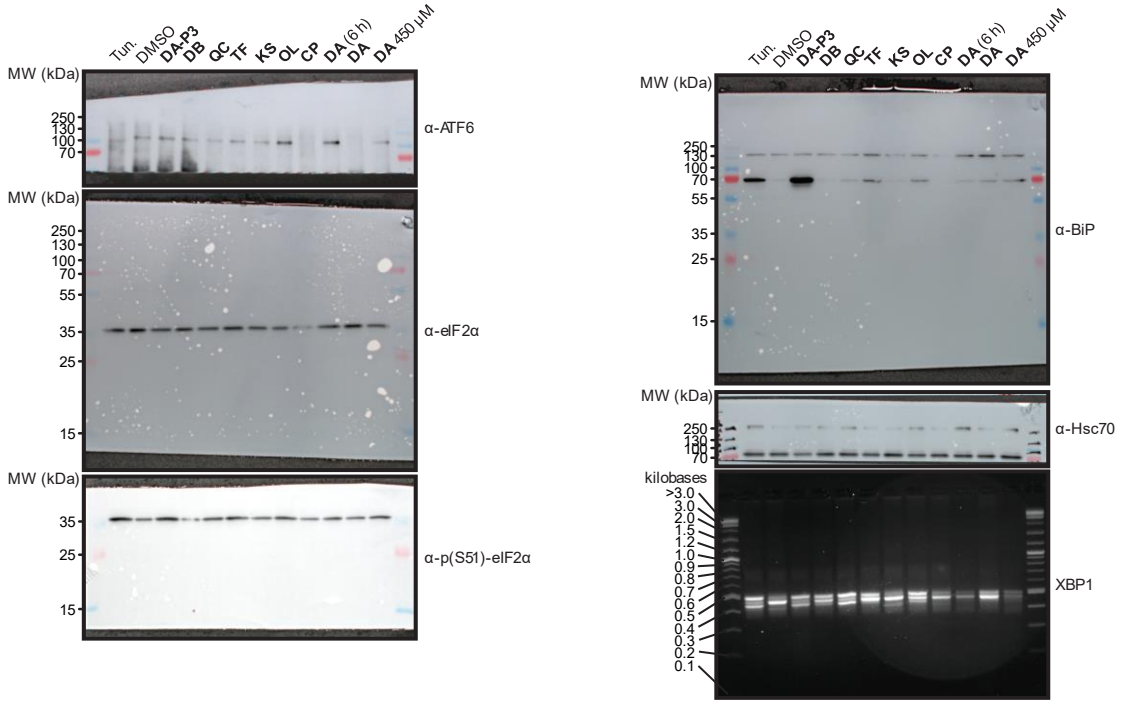
Full images and replicates of electrophoretic data



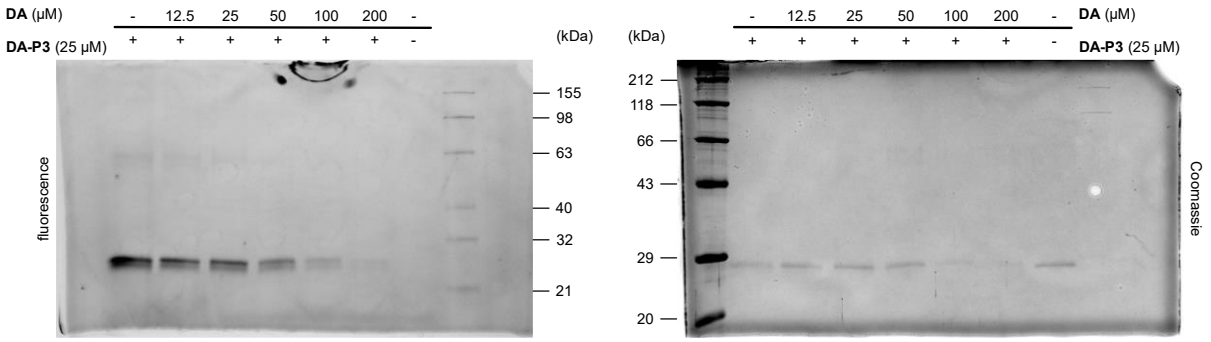
Full image of fluorescence visualisation and Coomassie staining of HEK293 proteome separated by SDS-PAGE after labelling *in situ* with different concentrations of **DA-P1**, **DA-P2**, and **DA-P3**, lysis, and ligation to rhodamine azide. Associated with figure 2 b.



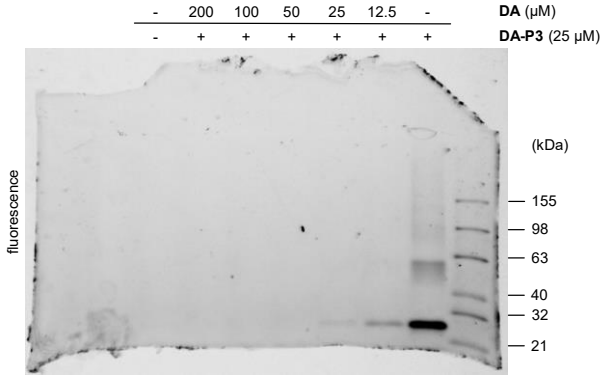
Full image of replicate experiment of fluorescence visualisation and Coomassie staining of HEK293 proteome separated by SDS-PAGE after labelling *in situ* with different concentrations of **DA-P1**, **DA-P2**, and **DA-P3**, lysis, and ligation to rhodamine azide.



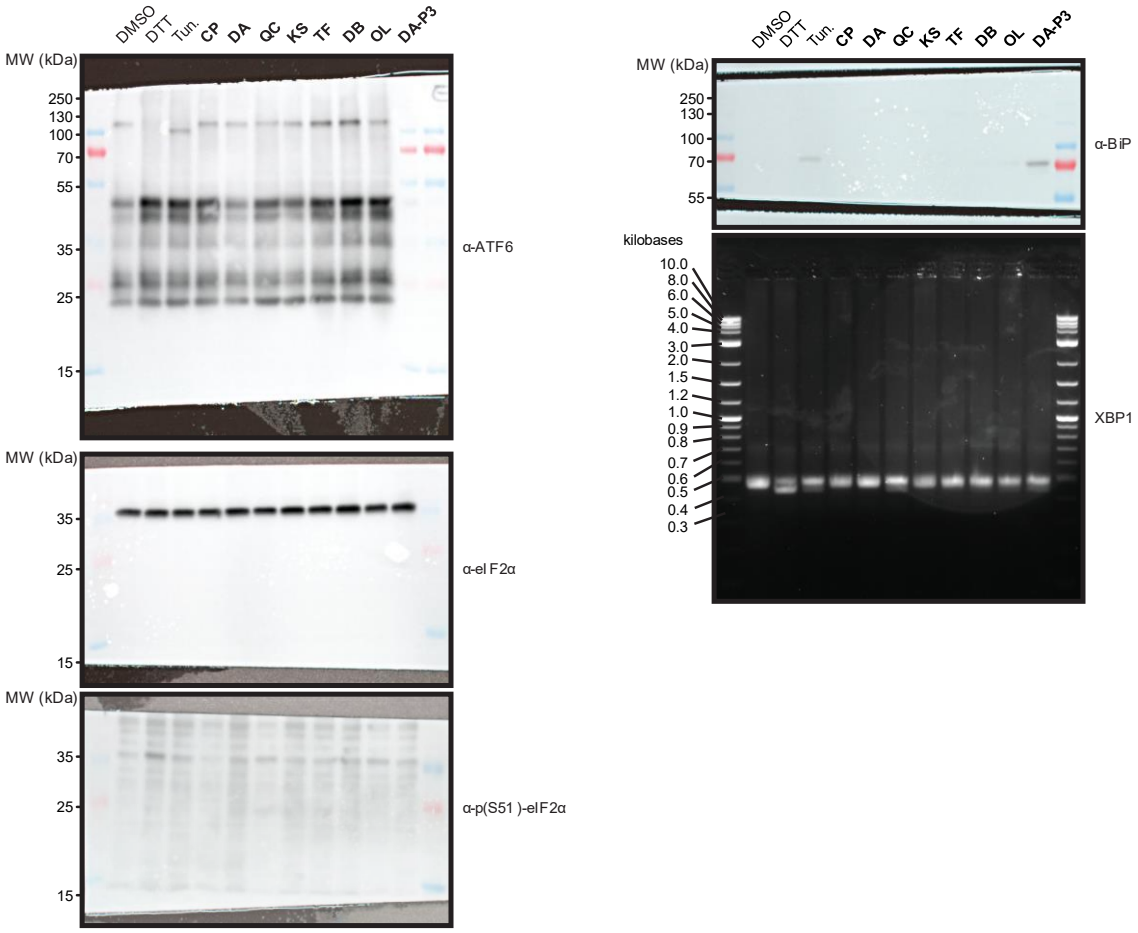
Full image of UPR assessment in HEK293T cells treated with the compounds of interest, the positive control tunicamycin (Tun.), or DMSO (vehicle control). Associated with figure 5.



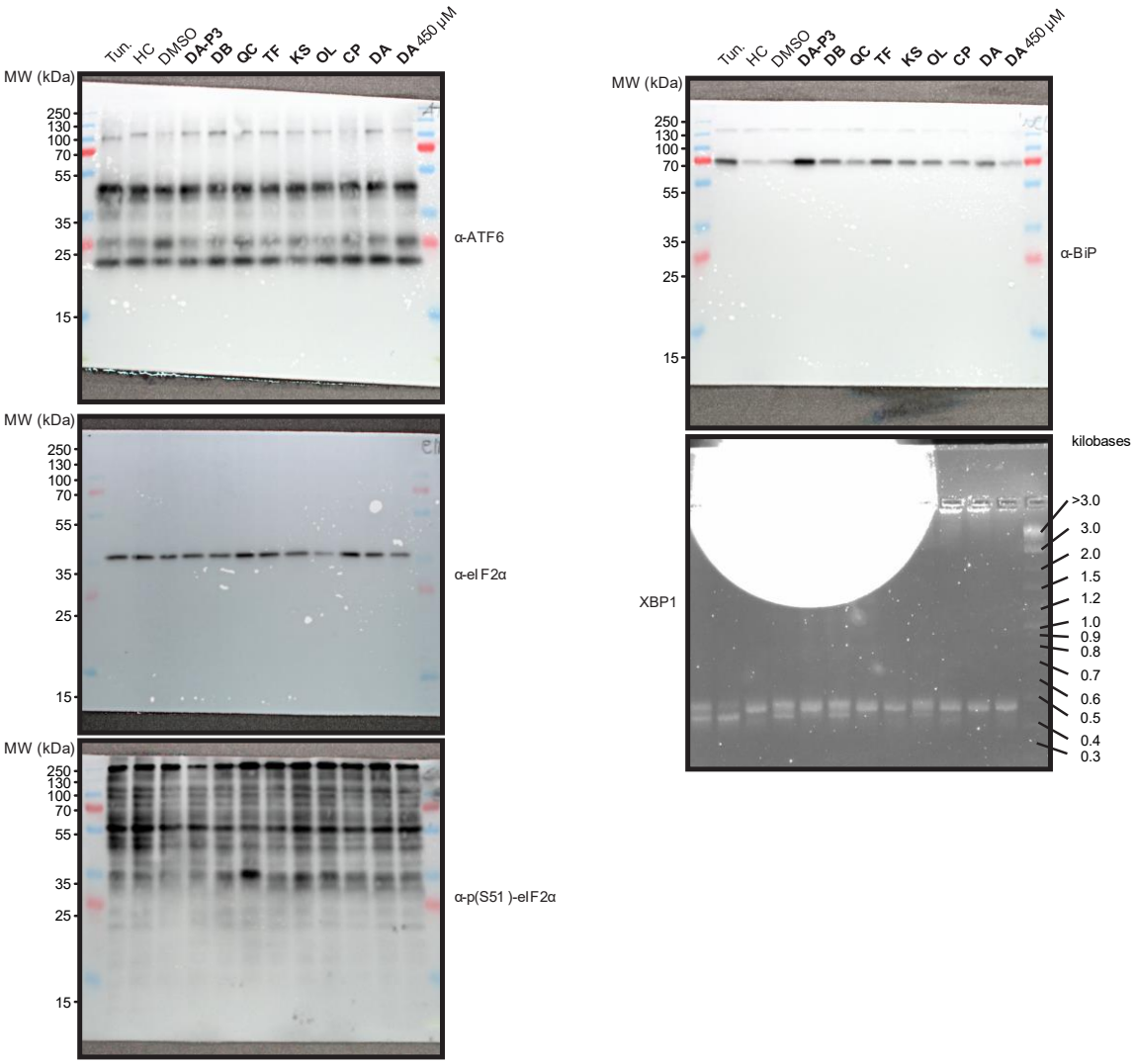
Full image of gel-based chemoproteomics analysis of **DA-P3** binding to DJ-1 (1 μM, in PBS) in competition with different concentrations of **DA**. The labelled protein was ligated to a fluorophore, subjected to SDS-PAGE, and imaged. Full protein load was revealed by Coomassie staining. Associated with figure S1 c.



Full image of replicate of gel-based chemoproteomics analysis of **DA-P3** binding to DJ-1 (1 μM, in PBS) in competition with different concentrations of **DA**. The labelled protein was ligated to a fluorophore, subjected to SDS-PAGE, and imaged.



Full image of third independent replicate of UPR assessment in HEK293T cells treated with the compounds of interest, the positive control tunicamycin (Tun.), DTT, or DMSO (vehicle control). Associated with figure S9 a.

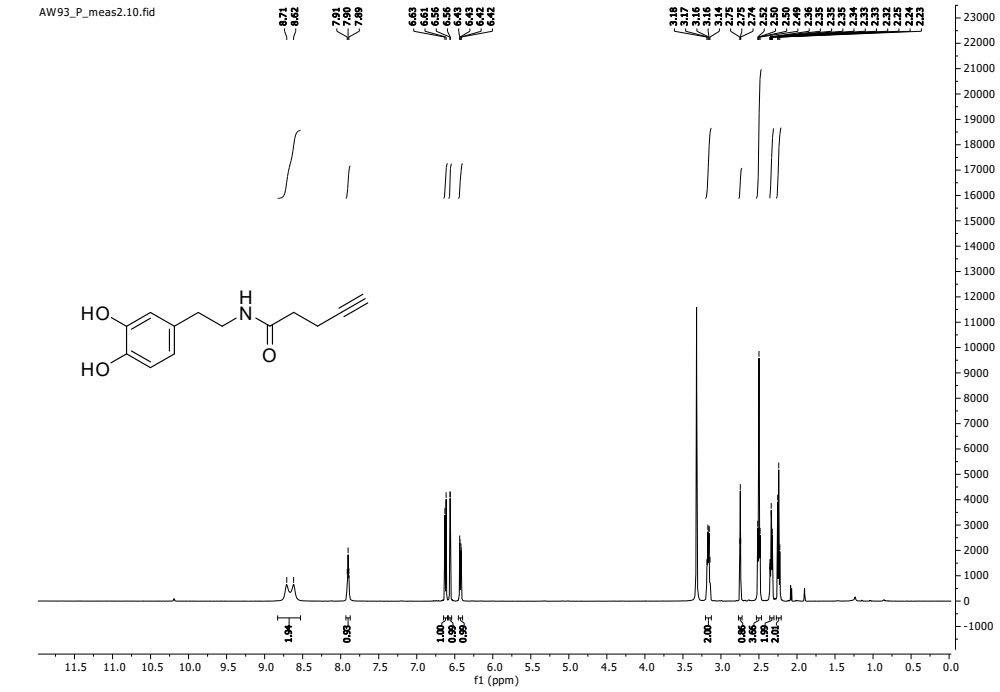


Full image of second independent replicate of UPR assessment in HEK293T cells treated with the compounds of interest, the positive control tunicamycin (Tun.), or DMSO (vehicle control), or transfected with IgG heavy chain (HC). Associated with figure S9 b.

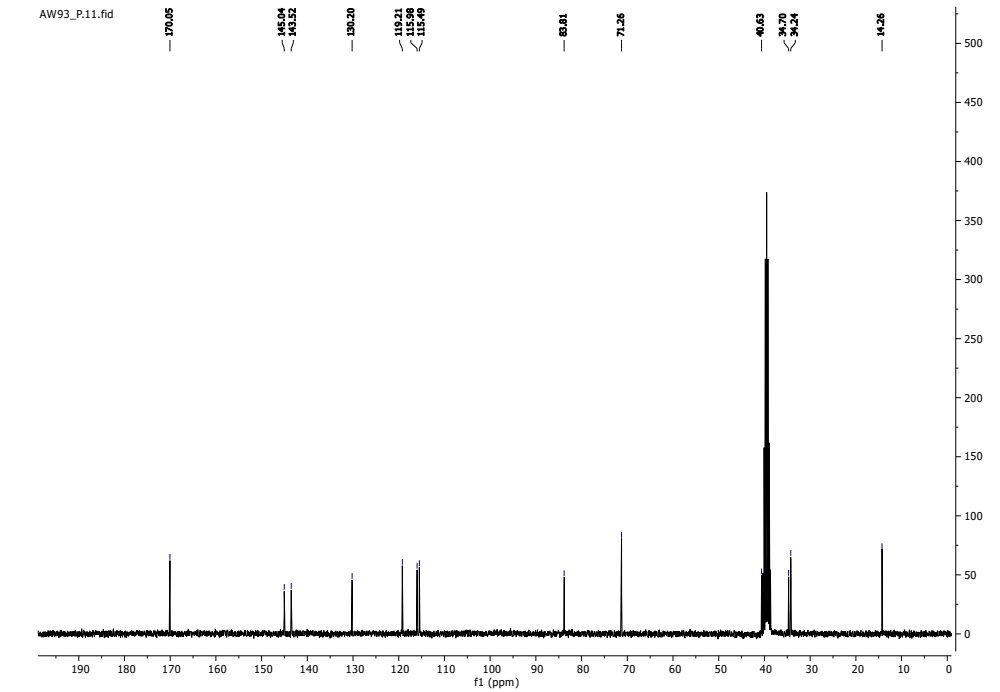
NMR spectra

DA-P1

a



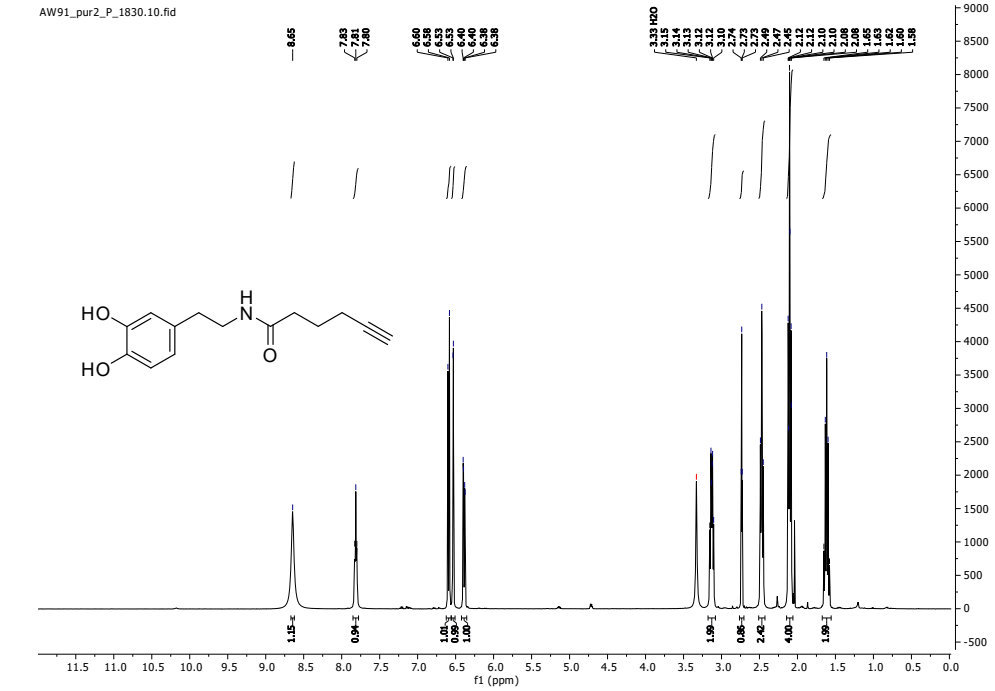
b



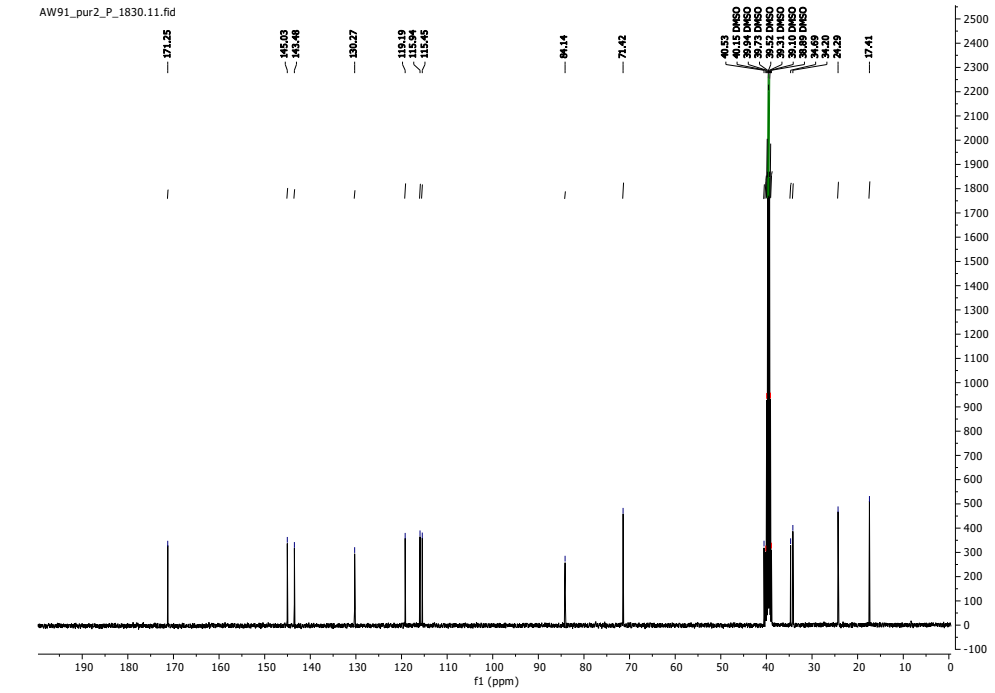
NMR spectra of *N*-(3,4-dihydroxyphenethyl)pent-4-ynamide (**DA-P1**) in DMSO-d_6. (a) ^{1}H-NMR (500 MHz). (b) ^{13}C-NMR (75 MHz).

DA-P2

a



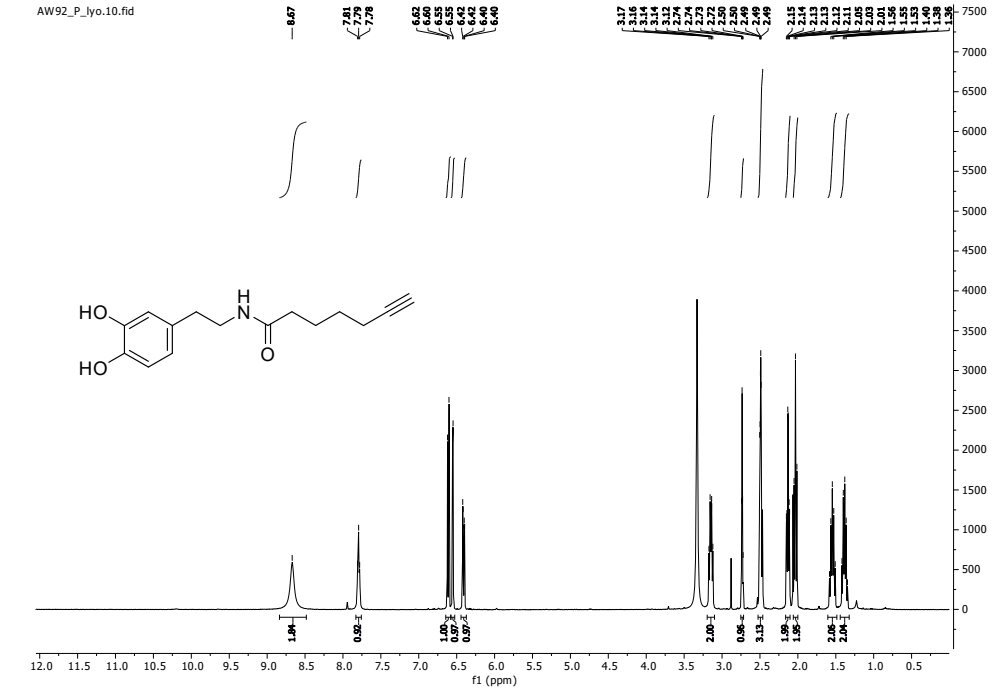
b



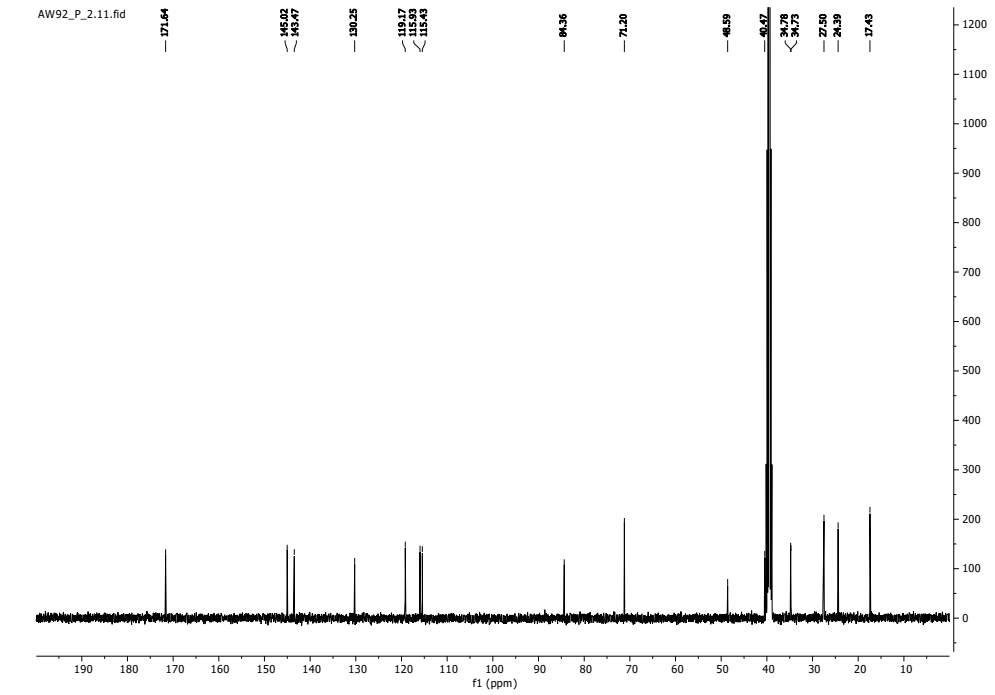
NMR spectra of *N*-(3,4-dihydroxyphenethyl)hex-5-ynamide (**DA-P2**) in DMSO-d_6. (a) ^{1}H-NMR (400 MHz). (b) ^{13}C-NMR (101 MHz).

DA-P3

a



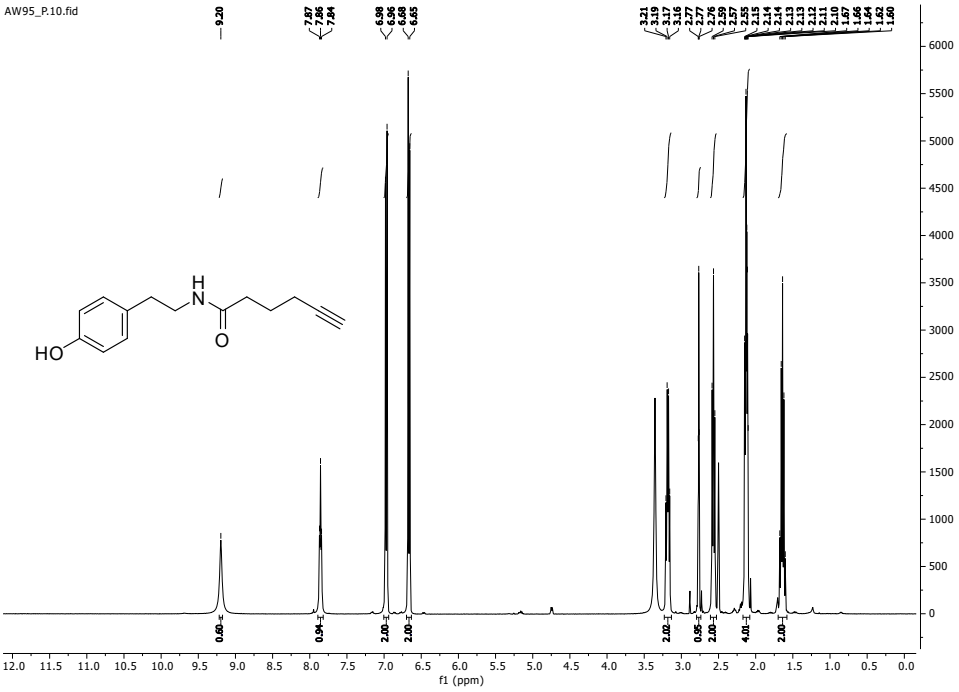
b



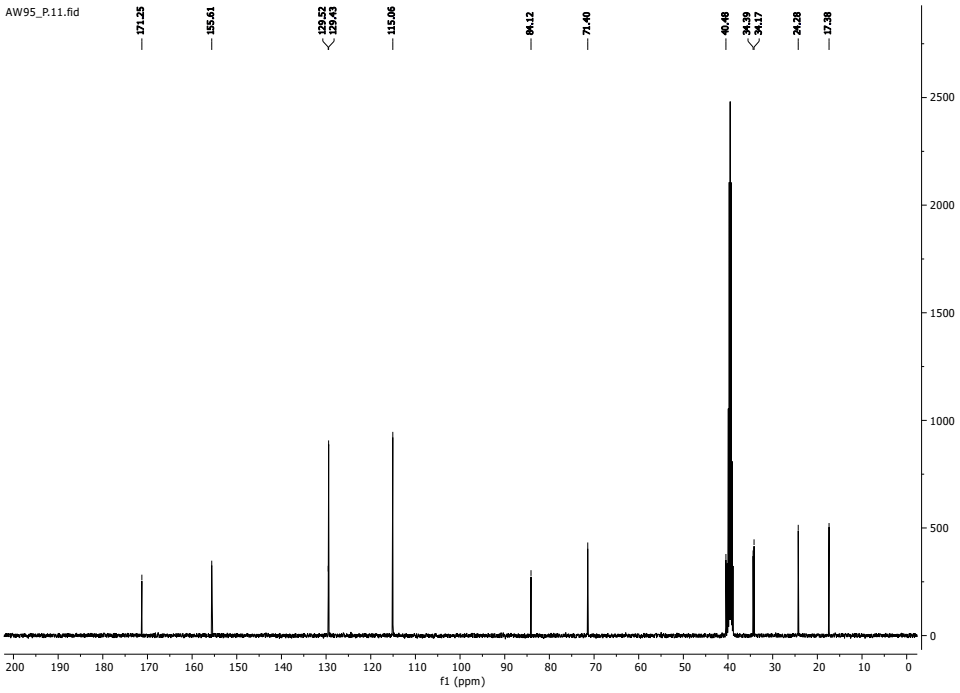
NMR spectra of *N*-(3,4-dihydroxyphenethyl)hept-6-ynamide (**DA-P3**) in DMSO-d_6. (a) ^{1}H-NMR (400 MHz). (b) ^{13}C-NMR (101 MHz).

DA-P4

a



b



NMR spectra of *N*-(4-hydroxyphenethyl)hex-5-ynamide (**DA-P4**) in DMSO-d_6. (a) ^{1}H-NMR (400 MHz). (b) ^{13}C-NMR (101 MHz).

References

1. J. Cox, M. Y. Hein, C. A. Luber, I. Paron, N. Nagaraj and M. Mann, *Molecular & cellular proteomics : MCP*, 2014, **13**, 2513-2526.
2. J. Drechsel, F. A. Mandl and S. A. Sieber, *ACS Chem Biol*, 2018, **13**, 2016-2019.
3. J. Eirich, J. L. Burkhart, A. Ullrich, G. C. Rudolf, A. Vollmar, S. Zahler, U. Kazmaier and S. A. Sieber, *Molecular bioSystems*, 2012, **8**, 2067-2075.
4. P. R. A. Zanon, L. Lewald and S. M. Hacker, *Angewandte Chemie (International ed. in English)*, 2020, **59**, 2829-2836.
5. A. Weigert Muñoz, E. Hoyer, K. Schumacher, M. Grognot, K. M. Taute, S. M. Hacker, S. A. Sieber and K. Jung, *Proceedings of the National Academy of Sciences of the United States of America*, 2022, **119**, e2118227119.
6. J. Cox and M. Mann, *Nature biotechnology*, 2008, **26**, 1367-1372.
7. S. Tyanova, T. Temu and J. Cox, *Nature protocols*, 2016, **11**, 2301-2319.
8. S. Tyanova, T. Temu, P. Sinitcyn, A. Carlson, M. Y. Hein, T. Geiger, M. Mann and J. Cox, *Nature methods*, 2016, **13**, 731-740.
9. P. R. A. Zanon, F. Yu, P. Z. Musacchio, L. Lewald, M. Zollo, K. Krauskopf, D. Mrdović, P. Raunft, T. E. Maher, M. Cigler, C. J. Chang, K. Lang, F. D. Toste, A. I. Nesvizhskii and S. M. Hacker, *ChemRxiv*, 2021, DOI: https://doi.org/10.26434/chemrxiv.14186561.v1.
10. D. Kessner, M. Chambers, R. Burke, D. Agus and P. Mallick, *Bioinformatics (Oxford, England)*, 2008, **24**, 2534-2536.
11. A. T. Kong, F. V. Leprevost, D. M. Avtonomov, D. Mellacheruvu and A. I. Nesvizhskii, *Nature methods*, 2017, **14**, 513-520.
12. F. Yu, G. C. Teo, A. T. Kong, S. E. Haynes, D. M. Avtonomov, D. J. Geiszler and A. I. Nesvizhskii, *Nature communications*, 2020, **11**, 4065.
13. F. da Veiga Leprevost, S. E. Haynes, D. M. Avtonomov, H. Y. Chang, A. K. Shanmugam, D. Mellacheruvu, A. T. Kong and A. I. Nesvizhskii, *Nature methods*, 2020, **17**, 869-870.
14. F. Yu, S. E. Haynes, G. C. Teo, D. M. Avtonomov, D. A. Polasky and A. I. Nesvizhskii, *Molecular & cellular proteomics : MCP*, 2020, **19**, 1575-1585.
15. E. Eden, R. Navon, I. Steinfeld, D. Lipson and Z. Yakhini, *BMC Bioinformatics*, 2009, **10**, 48.
16. E. Eden, D. Lipson, S. Yogev and Z. Yakhini, *PLOS Computational Biology*, 2007, **3**, e39.
17. Y. Perez-Riverol, A. Csordas, J. Bai, M. Bernal-Llinares, S. Hewapathirana, D. J. Kundu, A. Inuganti, J. Griss, G. Mayer, M. Eisenacher, E. Pérez, J. Uszkoreit, J. Pfeuffer, T. Sachsenberg, S. Yilmaz, S. Tiwary, J. Cox, E. Audain, M. Walzer, A. F. Jarnuczak, T. Ternent, A. Brazma and J. A. Vizcaíno, *Nucleic acids research*, 2019, **47**, 442-450.
18. N. Elumalai, A. Berg, K. Natarajan, A. Scharow and T. Berg, *Angewandte Chemie (International ed. in English)*, 2015, **54**, 4758-4763.