Supporting Information

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SI Materials and Methods

Cell Culture. PC12 mHTT^{Q103} cells were a gift from Erik S. Schweitzer (David Geffen School of Medicine School of Medicine, University of California, Los Angeles). These cells are stably transfected with the first exon of the human *HTT* gene containing the pathogenic 103 CAG/CAA repeat expansion, under the control of the ecdysteroid promoter (1). The plasmid also contains a *Bombyx mori* ecdysone receptor gene fused at the N terminus with VP16 transactivation domain (2, 3). Addition of the ecdysone analog, tebufenozide, to the cell culture medium is used to initiate the transcription of mutant *HTT* (1).

PC12 mHTT^{Q103} cells were cultured in DMEM containing 4.5 g/L glucose, 25 mM Hepes, sodium pyruvate, and no L-glutamine (cat. no. 15-018-CV; Mediatech), supplemented with 10% (vol/vol) Cosmic calf serum, 2 mM L-glutamine, 100 U/mL of penicillin-streptomycin, and 0.5 mg/mL active geneticin. Cells were grown at 37 °C, 9.5% CO_2, and medium was replaced with fresh medium every 2–3 d. To induce mHTT^{Q103} expression for experiments, tebufenozide, a gift from Lynne Moore and Fred H. Gage (The Salk Institute for Biological Studies, La Jolla, CA), was added to the medium at 200 nM final concentration from 1 mM stock in 85% (vol/vol) ethanol.

High-Throughput Screen of LOC Library. LOC mother plates with compounds at 4 mg/mL were thawed and spun down (215 × *g*, 20 °C, 1 min) before use. Biomek FX (Beckman Coulter) robotic liquid dispenser was used to handle all liquid transferring and mixing. Replica daughter plates (D1) were prepared by transferring 2 μL of compound from the mother plate into 384-deep-well clear, round bottom, polypropylene plates (Grenier cat. no. 781270) containing 98 μL of PC12 medium without selective agent geneticin to obtain compound concentration at 80 μL/mL in 2% (vol/vol) DMSO. Twofold serial dilution was performed across five daughter plates by transferring 50 μL of compounds (at 80 μL/mL) from the D1 plate into 50 μL of PC12 medium in daughter plate D2, mixing, and then repeating the process for the remaining three plates. Daughter plate D1 with compounds at 80 μL/mL, daughter plate D3 with compounds at 20 μL/mL, and daughter plate D5 with compounds at 5 μL/mL were then used for the screen. Assay plates were set up by seeding tebufenozide-induced PC12 mHTT^{Q103} cells into 384-well black, clear-bottom plates (cat. no. 3712; Corning) at a density of 7,500 cells per well in 57 μL PC12 medium without geneticin. Three microliters of compound from the daughter plates (D1, D3, and D5) were added to the assay plates for a final compound concentration of 4, 1, and 0.25 μL/mL. Four wells containing uninduced PC12 mHTT^{Q103} cells and four wells containing medium only were also included on each plate as controls. The assay plates were incubated at 37 °C, 9.5% CO_2 for 48 h. Twenty microliters of 40% (vol/vol) Alamar blue (cat. no. DAL1100; Life Technologies) solution in PC12 medium was added to each well (1:10 final dilution), and the plates were incubated for an additional 12–24 h at 37 °C, 9.5% CO_2. Alamar blue fluorescence was read on a fluorescence plate reader (PerkinElmer Victor3) with a 530-nm excitation filter and 590-nm emission filter. Each compound concentration was tested in triplicate.

The follow-up testing of primary screen hits was performed in a similar way. Fresh powder stocks of primary hits were reordered from vendors, dissolved in DMSO, and tested in a twofold serial dilution across 10 wells in both tebufenozide-induced and uninduced PC12 mHTT^{Q103} cells. Dose–response curves are plotted as mean ± SD and fit to either four-parameter sigmoidal (for uninduced cells) or bell-shaped (induced) function using Prism (GraphPad Software).

Molecular Cloning, Protein Expression, and Purification of PDIa. Sequence for the human catalytic PDI A1 *a* domain was generated by PCR from Ultimate ORF Clone IOH9865 (Life Technologies) as an NdeI-BamHI fragment. The amplified a domain (amino acids 18–134 in the full-length PDI sequence) was then subcloned into NdeI-BamHI sites of pET-15b vector (Novagen) containing the N-terminal His_6 tag and confirmed by DNA sequencing (GeneWiz). The catalytic PDI A1 *a* domain was referred as PDIa throughout the paper.

The PDIa construct was transformed into *Escherichia coli* BL21-Gold (DE3) competent cells (Agilent Technologies) and grown at 37 °C in Luria broth medium with 100 μg/mL ampicillin until OD_{600nm} reached 0.5. Expression was induced with 0.5 mM IPTG (isopropyl β-D-1-thiogalactopyranoside) at 37 °C for overnight (usually 12–15 h). Cells were pelleted (4,000 × *g*, 20 min, 4 °C) and lysed by sonication in buffer containing 50 mM Tris·HCl, pH 8.0, 150 mM NaCl, 1 mM TCEP [Tris(2-carboxyethyl)phosphine], and 5 mM $MgCl_2$. Cell lysate was then centrifuged at 19,784 × *g* for 30 min at 4 °C. The supernatant was loaded onto a chromatography column containing Ni Sepharose 6 Fast Flow beads (GE Life Sciences) equilibrated with PDI Suspension Buffer (50 mM Tris·HCl, pH 8.0, 150 mM NaCl, and 1 mM TCEP). The bound PDIa was eluted with 250 mM imidazole in the same buffer. Recombinant PDIa was further purified using gel filtration Superdex 100 column (GE Life Sciences) in a buffer containing 20 mM Tris·HCl, pH 8.0, 150 mM NaCl, and 1 mM TCEP. The fractions containing PDIa were concentrated, flash frozen, and stored at −80 °C. Protein concentration was determined using absorbance at 280 nm with molar extinction coefficient (ε) 19,940 $M^{-1}\cdot cm^{-1}$ (for reduced PDIa with N-terminal His_6 tag as calculated from amino acid sequence by ExPASy ProtParam). PDIa purity was verified by SDS/PAGE as more than 98% pure.

For NMR studies, uniformly ^{15}N-labeled PDIa protein with N-terminal His_6 tag was prepared. PDIa construct was transformed into *E. coli* BL21-Gold (DE3) competent cells (Agilent Technologies). Cells were grown at 37 °C in 1 L of M9 minimal medium supplemented with 2 mM $MgSO_4$, 0.1 mM $CaCl_2$, 100 μg/mL ampicillin, 22.2 mM glucose, metals 44 solution, 30 mg nicotinic acid, 3 mg *p*-aminobenzoic acid, 0.3 mg biotin, 0.5 mg thiamine hydrochloride, and 0.6 g $^{15}NH_4Cl$ as the sole nitrogen source. When OD_{600nm} reached 0.9, temperature was reduced to 20 °C, and protein expression was induced with 0.5 mM IPTG for overnight. Protein was purified as described above except the histidine tag was removed after size exclusion chromatography. Thrombin was added at 5 U/mg protein to cleave the N-terminal His_6 tag. Reaction was allowed to proceed overnight at 4 °C. Next day, protein solution was passed over Ni Sepharose 6 Fast Flow beads (GE Life Sciences) equilibrated with PDI Suspension Buffer, and flow-through containing the ^{15}N-labled PDIa protein without histidine tag was concentrated and incubated with 5 mM TCEP overnight with gentle shaking at 4 °C. Four residues, GSHM, which are part of the backbone vector, were present after removal of the His_6 tag. The next day, the protein was dialyzed into MilliQ water, flash frozen, and stored at −80 °C. ^{15}N-labeled PDIa purity was verified by SDS/PAGE as more than 98% pure.

ITC. All ITC experiments were carried out at 25 °C on MicoCal Auto-ITC_{200} system (GE Healthcare). Reduced PDIa was

dialyzed into ITC buffer (20 mM sodium phosphate buffer, pH 7.8) and loaded into sample cell at either 30 or 40 μM concentration. The compound solution was loaded into syringe at 400 μM in the same ITC buffer with a final DMSO concentration at 0.4% (vol/vol). ITC titration experiments were carried out at 25 °C with 19 injections, 2 μL per injection, and 180 s between each injection. Reference cell power was set to 5 μcal/s. The control experiment was performed for each compound, where each compound was titrated into buffer to account for heat released due to dilution. This background was subtracted from test data before the final dissociation constant was obtained. Data were analyzed using a one-site binding model in Origin 7.1 software. The dissociation constant, K_d, was calculated according to equation $K_d = 1/K_a$. Gibbs free energy, ΔG, was calculated from the equation $\Delta G = \Delta H - T\Delta S$. All other parameters, K_a, n, ΔH, and ΔS, were determined directly from the titration data.

For ITC experiments with 16F16 pretreatment, 40 μM PDIa (after dialysis into ITC buffer) was treated with 200 μM 16F16 for 12–15 h at 4 °C. The next day, the whole solution was loaded into the sample cell. LOC14 at 400 μM in ITC buffer was loaded into syringe and titrated into PDIa + 16F16 loaded cell. For control experiments, ITC buffer with 200 μM 16F16 was used in the cell, whereas 400 μM LOC14 in the ITC buffer was used in the syringe.

Size Exclusion Buffer Dialysis Experiments for Reversibility of Binding. Before dialysis, fluorescence emission spectra were recorded from 315- to 550-nm wavelength with excitation at 280 nm on a Tecan Infinite 200 microplate reader. Fluorescence readings were carried out in a 384-well low volume, black bottom plate. Each well contained 40 μL of either LOC14 (300 μM), PDIa (20 μM), or PDIa (20 μM) treated with LOC14 (300 μM) overnight. All samples were dissolved in buffer B (20 mM sodium phosphate buffer, pH 7.8). After recording the initial fluorescence spectra, samples were then transferred to Amicon Ultra 10-kDa size exclusion filter spin columns for dialysis; 400 μL of buffer B was added to the spin column, the samples were centrifuged for 8 min on a table top microcentrifuge (10,616 × *g*, 4 °C), and afterward, the flow-through was transferred to a new tube. This step was repeated three more times with fresh buffer B. Forty microliters of the collected samples from the flow-through and the spin-column chamber were transferred to a new 384-well plate, and the emission spectra were recorded as described above.

Reversibility of LOC14 Binding Using in Vitro Insulin Reduction Assay. The assay was carried out in a 384-well black, clear bottom plate. For samples containing diluted protein-compound complexes, 500 μM PDIa was incubated with either 750 μM LOC14 or 750 μM irreversible inhibitor 16F16 for 3 h at room temperature. The mixture was then diluted 100-fold into buffer A (10 mM Tris·HCl, pH 8, 150 mM NaCl, and 2 mM EDTA) containing 100 μM bovine insulin. DTT was added last to a 350 μM final concentration. For control samples, the same stock of concentrated PDIa at 500 μM was diluted 100-fold into the same buffer A (containing 100 μM bovine insulin) and then treated with either 7.5 or 750 μM LOC14 or 16F16 test compound. DTT was added last to all wells to a 350 μM final concentration. The final reaction volume in all wells was 80 μL. All experiments were done in triplicate. The assay plate was incubated at 25° C for 1 h, and then the absorbance at 650 nm was read on a Tecan Infinite 200 microplate reader for each sample consecutively, at 5-min intervals for 1 h. Increase in absorbance is indicative of insulin's β-chain aggregation and precipitation out of solution.

NMR Spectroscopy. The residue numbering in all HSQC spectra are based on the sequence of the mature PDI protein, i.e., residue 1 of the mature PDI corresponds to residue 18 in the full-length PDI. The first 17 amino acids in full-length PDI are the signal sequence that is proteolytically removed out to generate the mature PDI.

The ^{1}H-^{15}N HSQC spectra were performed on Bruker Avance III 500 Ascend (500 MHz) spectrometers (Columbia University) at 300 K. Uniformly ^{15}N-labeled PDIa was dissolved at 50 or 100 μM in 90% H_2O/10% D_2O (vol/vol), pH 5.1. The ^{1}H carrier frequency was positioned at the water resonance. The ^{15}N carrier frequency was positioned at 115 ppm. The spectral width in the ^{1}H dimension was 7,500 Hz, and the width in ω_1 (^{15}N) dimension was 1,824.6 Hz. Suppression of the water signal was accomplished using the WATERGATE sequence. Heteronuclear decoupling was accomplished using the GARP decoupling scheme.

The 3D NMR experiments were performed on a Bruker Avance 500 MHz spectrometer equipped with a 5-mm TXI cryogenic probe (New York Structural Biology Center). The ^{15}N-NOESY-HSQC (^{15}N-nuclear Overhauser effect spectroscopy-HSQC) and ^{15}N-TOCSY-HSQC (^{15}N-total correlated spectroscopy-HSQC) spectra were recorded at 300 K on the uniformly ^{15}N-labeled PDIa that was dissolved at 500 μM in 90% H_2O/10% D_2O (vol/vol), pH 5.1. The ^{1}H carrier frequency was positioned at the water resonance. The ^{15}N carrier frequency was positioned at 118 ppm. The spectral width in the ^{1}H dimension was 7,501.9 Hz, and the width in ω_2 (^{15}N) dimension was 2,027.3 Hz. The ^{15}N-NOESY-HSQC was recorded with a mixing time of 150 ms. The ^{15}N-TOCSY-HSQC was recorded using an *rf* field strength of 8.3 kHz during the DIPSI-2 mixing time of 60 ms (4). The TOCSY-HSQC spectrum was recorded with gradient enhancement. Data were processed and analyzed using TopSpin 3.1 (Bruker) and Sparky (T. D. Goddard and D. G. Kneller, University of California, San Francisco). The mean chemical shift difference for ^{1}H and ^{15}N ($\Delta\ \delta_{NH}$) was calculated using (5)

$$\Delta\delta_{NH} = \frac{\sqrt{(\Delta\ \delta H_N)^2 + \left[\left(\frac{\Delta\ \delta N}{10}\right)\right]^2}}{2}.$$

In Vitro Drug Metabolism Studies: Mouse Microsome Stability Assay. The test compound (0.5 μM) was incubated at 37 °C for up to 45 min in 100 mM of potassium phosphate buffer (pH 7.4) containing microsomal protein (0.5 mg/mL) and an NADPH generating system [0.34 mg/mL β-nicotinamide adenine dinucleotide phosphate (NADP), 1.56 mg/mL glucose-6-phosphate, 1.2 U/mL glucose-6-phosphate dehydrogenase]. At 0-, 5-, 15-, 30-, and 45-min intervals, an aliquot was taken and quenched with acetonitrile (ACN) containing internal standard. No-cofactor controls at 45 min were prepared. Following completion of the experimentation, the samples were analyzed by LC-MS/MS. The half-life ($t_{1/2}$) is calculated using the following equation: $t_{1/2} = 0.693/k$, where k is the elimination rate constant of test compounds obtained by fitting the data to the following equation: C = initial × exp(–k × t). Intrinsic clearance (CL_{int}) is calculated as liver clearance from the half-life using the following equation: CL_{int} = k × (mL incubation/0.5 mg protein) × (52.5 mg protein/g liver). Results were reported as peak area ratios of analyte to internal standard. The intrinsic clearance (CL_{int}) was determined from the first-order elimination constant by nonlinear regression.

In Vitro Drug Metabolism Studies: Mouse Plasma Stability Assay. The test compound (1 μM) was incubated at 37 °C for up to 120 min in mouse plasma. At 0-, 15-, 30-, 60-, and 120-min intervals, a 100-μL aliquot was taken and quenched with 200 μL acetonitrile (ACN) containing internal standard. Following completion of the experimentation, the samples were analyzed by LC-MS/MS. The half-life ($t_{1/2}$) is calculated using the following equation: $t_{1/2} = 0.693/k$, where k is the elimination rate constant of test compounds obtained by fitting the data to the following equation: C = initial × exp(–k × t). Results were reported as peak area ratios of analyte to internal standard.

In Vitro Drug Metabolism Studies: Mouse Plasma Protein Binding Assay. A test compound at the concentration of 2,000 ng/mL in plasma was added into the sample chamber, a dialysis buffer (PBS) was added into the buffer chamber, and the unit was covered with sealing tape and incubated for 4 h at 37 °C on an orbital shaker. Samples were taken from both plasma and the buffer chamber at the end of the incubation, and then analyzed by LC-MS/MS. Protein binding and free fraction percentage were determined using the peak area ratio of analyte to the internal standard (IS). The fraction bound percent was calculated as % Bound $=100 \times (C_{plasma} - C_{PBS})/C_{plasma}$.The fraction recovered percent was calculated as % Recovery $= (V_{PBS} \times C_{PBS} + V_{plasma} \times C_{plasma})/(V_{plasma} \times C_{spike})$, where V_{PBS} is volume of PBS, V_{plasma} is volume of plasma, C_{PBS} is drug concentration in PBS (analyte/IS peak area ratio), C_{plasma} is drug concentration in plasma (Analyte/IS peak area ratio), and C_{spike} is drug concentration in spiked plasma (analyte/IS peak area ratio).

In Vitro Drug Metabolism Studies and in Vivo PK Study. The microsome stability assay, plasma stability assay, plasma protein binding assay, and in vivo PK study were performed by Alliance Pharma.

LC/MS. Proteins were separated by one-dimensional SDS/PAGE electrophoresis and digested with trypsin as described previously (6). Peptides were separated with a NanoAcquity UPLC as described previously (7) except that Solvent B was increased in a 30-min linear gradient between 5% and 40% and postgradient cycled to 95% B for 7 min, followed by a postrun equilibration at 5% B.

Spectra were recorded in sensitivity positive ion mode with a Synapt G2 quadrupole-time-of-flight HDMS mass spectrometer (Waters Corp). Spectra were acquired for the first 59 min of the chromatographic run. Source settings were capillary voltage (3.2 kV), extraction cone (4 V), sampling cone (30 V), and source temperature of 80 °C. The cone gas N_2 flow was 30 L/h. Analyzer settings included quadrupole profile set at manual with mass 1 as 400 (dwell time, 25%; ramp time, 25%), mass 2 as 500 (dwell time, 25%; ramp time, 25%), and mass 3 as 600. A reference sprayer was operated at 500 nL/min to produce a lockmass spectrum with Glu-1-fibrinopeptide B (EGVNDNEEGFFSAR; *m/z* 785.8426) and leucine enkephalin (YGGFL) at *m/z* 556.2771 every 30 s.

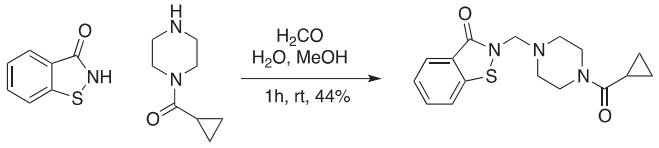
Data were collected by data-dependent acquisition with a scan time of 0.25 s. A survey scan was conducted over the range of 300–2,000 Da. Acquisition was performed in sensitivity mode and switched when individual ion counts exceeded 1,000 count/s. MS/MS spectra were acquired over the range of 50–2,000 *m/z*. A maximum of the five most intense ions were selected from a single MS survey scan. Return to MS survey scan was triggered when the intensity exceeded 60,000 counts/s or 3 s had elapsed. Charge state peak detection was enabled for +2, +3, and +4. Collision energy in the trap was ramped from 12 to 20 V for low mass (300 Da) and 40–60 V for high mass (2,000 Da).

Raw spectrum processing was performed with the PLGS software (Vers. 2.5, RC9). The electrospray survey was calibrated to a lock mass of Glu-1-fibrinopeptide B at 785.8426 *m/z*, averaging three scans with a tolerance of 0.1 Da, adaptive background subtraction with slow algorithm deisotoping function with 30 iterations, and a 3% threshold. MS/MS spectra were calibrated to singly charged leucine enkephalin at *m/z* 556.2771 with the same settings as for the survey scan. Spectra were processed and exported as .pkl files that were then imported into the Mascot database search program (Vers. 2.3.02; Matrix Science.). Observed masses were searched with Mascot against the full National Center for Biotechnology Information nr protein database of Jan. 4, 2012 (16,826,875 sequences; 5,780,204,515 residues). In addition, a custom database was used with sequence of the protein disulfide isomerase construct used for the reaction. Decoy search was enabled and no taxonomic restriction and up to one missed trypsin cleavage with no fixed modifications. Charge states of +1, +2, or +3 were considered. Monoisotopic mass tolerance for precursor peptides was 10 ppm, and for products, 0.02 Da. Variable modifications included carbamidomethyl (C), oxidation (M), and a custom modification of C representing inhibitor 16F16 with a monoisotopic mass shift of 284.1160 Da and an elemental composition of C(16) H(16) N(2) O(3).

All raw data files and DDA .pkl files are being deposited in a public repository at https://chorusproject.org.

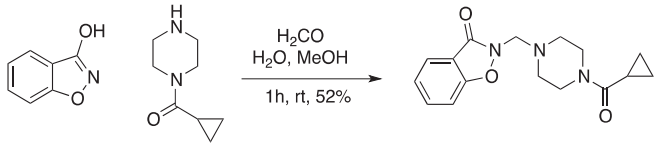
HD Brain Slice Assay. Brain slice explants were prepared and transfected as previously described (8). Briefly, brains were taken from postnatal day 10 Sprague–Dawley rat pups (Charles River) and cut into 250-μm coronal slices on a vibratome (Vibratome). Brain slices containing striatum and cortex were then placed in individual wells of 12-well plates atop culture medium set in 0.5% agarose and maintained at 32 °C under 5% CO_2. Co-transfection with YFP and *HTT* exon 1 containing a 73 polyglutamine repeat was done using a biolistic device (Bio-Rad Helios Gene Gun). Positive controls were transfected with YFP only or treated with a combination of 50 μM KW-6002 (istradefylline) and 30 μM SP600125. Negative control brain slices were treated with 0.1% DMSO carrier only. Striatal MSNs expressing YFP were visualized under fluorescence microscopy and identified based on their location within brain slices and their characteristic morphology. MSNs exhibiting normal-sized cell bodies, and even and continuous expression of YFP in at least two discernible primary dendrites at least two cell body diameters long were scored as healthy.

Compound Synthesis. All commercial reagents were used without further purification. All solvents used were reagent or HPLC grade. All reactions were carried out in flame-dried glassware under a nitrogen atmosphere. Chemical yields refer to isolated, spectroscopically pure compounds. Proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded on a Bruker Avance III 400- or 500-MHz spectrometer at ambient temperature. Chemical shifts are recorded in parts per million relative to residual solvent $CDCl_3$ (^{1}H, 7.26 ppm; ^{13}C, 77.16 ppm). Multiplicities are reported as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, comp m = complex multiplet, td = triplet of doublets.



2-((4-(cyclopropanecarbonyl)piperazin-1-yl)methyl)benzo[d]isothiazol-3(2H)-one (LOC14). Methanol (1 mL) and a 38% solution of formaldehyde in water (75 μL 1.2 eq.) were combined and stirred at room temperature. 1,2-Benzisothiazol-3*(2H)*-one (98 mg, 1 eq.) and 1-(cyclopropylcarbonyl)piperazine (92 μL, 1 eq.) were then added, and the solution was allowed to stir at room temperature until white solids precipitated in about 1 h. These solids were collected via vacuum filtration and recrystallized from ethyl acetate and hexanes providing LOC14 (91 mg, 44%) as a white powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.037 (d, J = 8 Hz, 1H), 7.63 (t, J = 7.2 Hz, 1H), 7.55 (d, J = 8 Hz, 1H), 7.397 (t, J = 7.2 Hz, 1H), 4.73 (s, 1H), 3.67 (bs, 4H), 2.73 (bs, 4H), 1.67 (m, 1H), 0.96 (m, 2H), 0.74 (m, 2H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 172.0, 166.3, 141.1, 132.2, 126.9, 125.6, 124.7, 120.4, 65.7, 50.7, 50.2, 45.4, 42.1, 11.0, 7.5. LRMS (APCI+): calculated for ($C_{16}H_{19}N_3O_2S$) 317.4 g/mol, found *m/z* (relative intensity %): 317.07 (M^+, 1%), 309.10 (1%), 302.90 (1%), 269.16 (4%), 184.15 (10%), 167.19 (4%), 155.18 (100%), 152.08 (100%), 122.12 (6%). This mass fragmentation pattern is consistent with the properties reported for Mannich bases (9–11). The identity of LOC14 as a single compound

under nonionizing conditions was verified by TLC in 10% MeOH/90% dichloromethane, showing a single spot with an *Rf* of 0.5 on iodine visualization and by NMR (Fig. S2).



2-((4-(cyclopropanecarbonyl)piperazin-1-yl)methyl)benzo[d]isoxazol-3(2H)-one (Oxy-LOC14). Methanol (1 mL) and a 38% solution of formaldehyde in water (75 μL, 1.2 eq.) were combined and stirred at room temperature. 3-Hydroxybenzisoxazole (87 mg, 1 eq.) and 1-(cyclopropylcarbonyl)piperazine (92 μL, 1 eq.) were then added, and the solution was allowed to stir at room temperature for 1 h. Precipitation of a white solid was induced by sonication. These solids were collected via vacuum filtration and recrystallized from ethyl acetate and hexanes providing Oxy-LOC14 (101 mg, 52%) as a white powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (d, J = 8 Hz, 1H), 7.64 (t, J = 6 Hz, 1H), 7.30 (t, J = 8 Hz, 1H), 7.23 (d, J = 8.4 Hz, 1H), 4.93 (s, 2H), 3.68 (bs, 4H), 2.78 (bs, 4H), 1.67 (m, 1H), 0.95 (m, 2H), 0.74 (m, 2H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 171.9, 163.4, 160.3, 133.69, 124.5, 123.6, 115.94, 110.0, 67.136, 50.5, 50.0, 45.3, 42.0, 10.8, 7.42.

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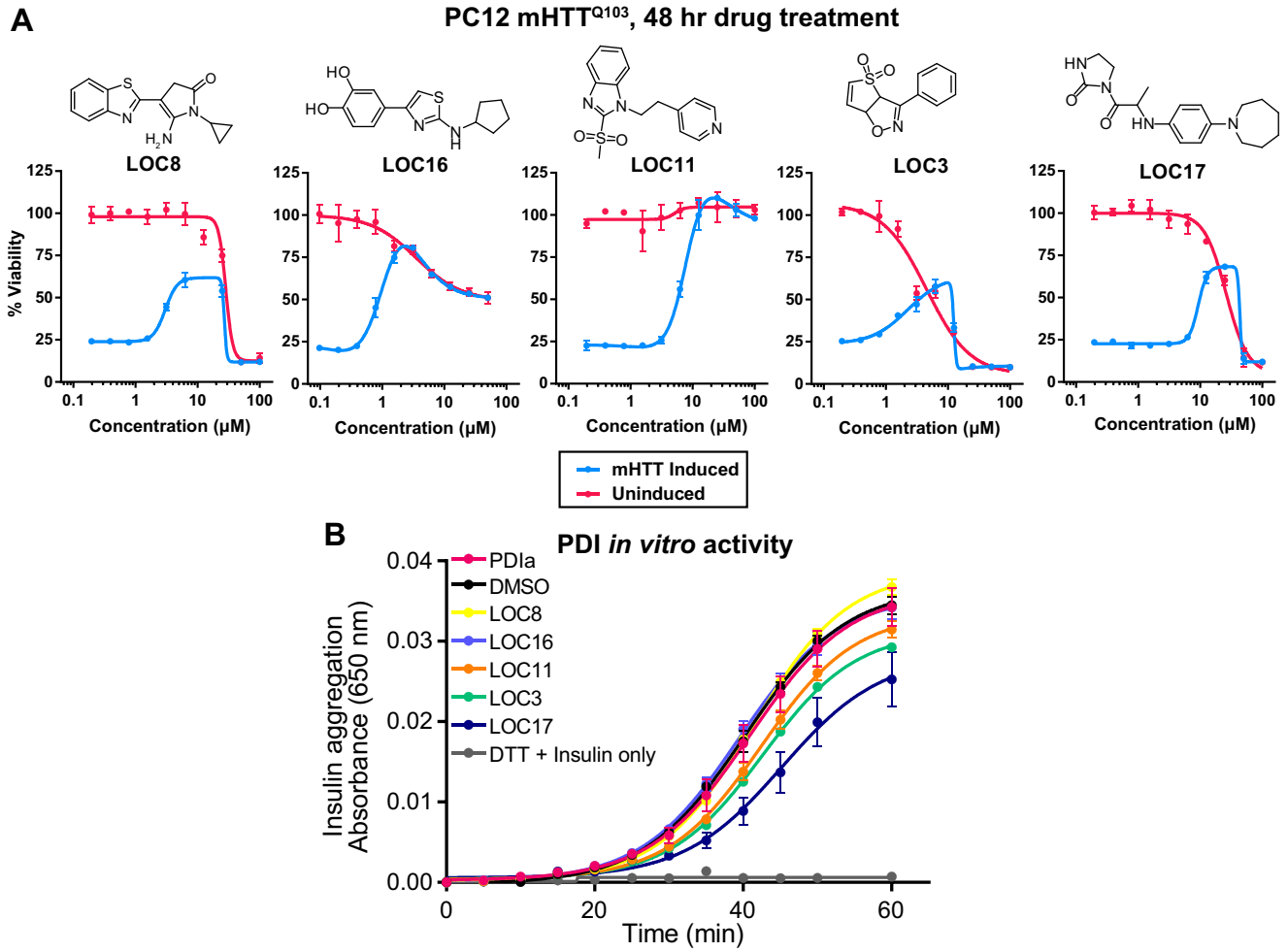

Fig. S1. Evaluation of hits from high-throughput screen. (*A*) Dose–response curves of five additional HTS hits that rescued PC12 cells from $mHTT^{Q103}$-induced cell death as measured by Alamar blue fluorescence after 48-h treatment. Data from cells induced to express $mHTT^{Q103}$ (blue) and cells not expressing $mHTT^{Q103}$ (red) are plotted as mean percent of DMSO-treated uninduced cells ± SD. Experiments were performed in triplicate. (*B*) The five hits at 75 μM are evaluated in counter screen for their ability to inhibit the enzymatic activity of PDIa (5 μM) in the insulin aggregation assay. Experiments were performed in duplicate with data plotted as mean ± SEM.

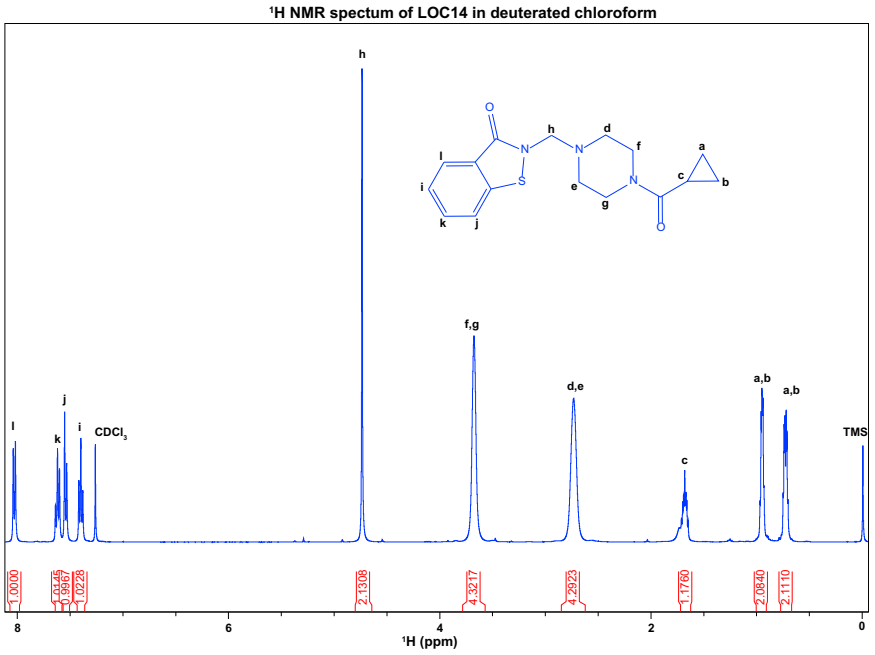

Fig. S2. [1]H NMR spectrum of LOC14. Resynthesized LOC14 structure validated by NMR.

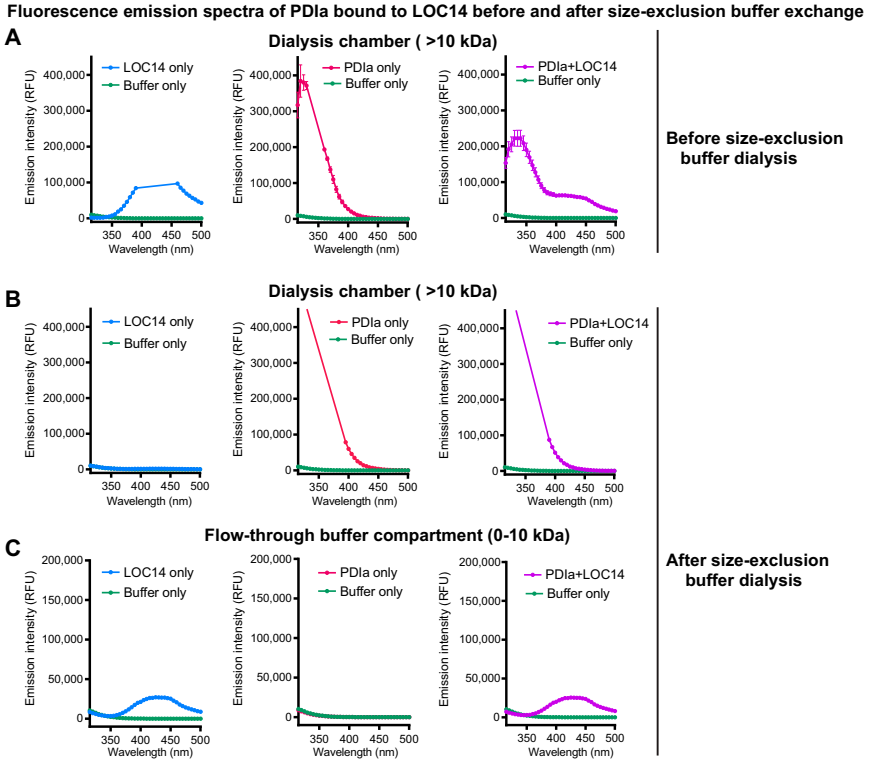

Fig. S3. LOC14 binds reversibly to PDIa. Fluorescence emission spectra of LOC14, PDIa, or PDIa-LOC14 complex (*A*) before or (*B* and *C*) after size-exclusion buffer dialysis. For the dialysis 10-kDa size exclusion filter spin columns were used. Fluorescence emission spectra of (*B*) sample fractions larger than 10 kDa that were retained in the dialysis chamber or (*C*) those collected from the flow-through of dialysis. All samples were excited at 280 nm, and emission spectra recorded from 315 to 550 nm.

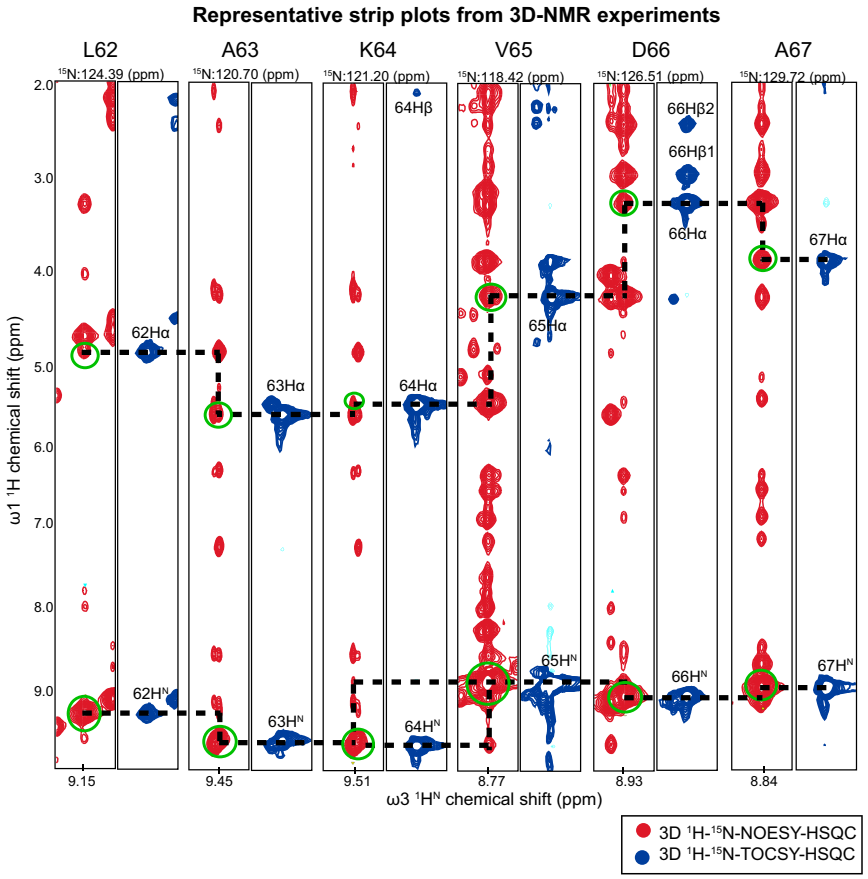

Fig. S4. 3D-NMR experiments to assign reduced PDIa. Representative strip plots of 3D ^{1}H-^{15}N-NOESY-HSQC (red) and 3D ^{1}H-^{15}N-TOCSY-HSQC(blue) for residues L62-A67 in the reduced *a* domain of PDI A1. The peaks represent NOE signal between the amide hydrogen to any hydrogen within its own spin system (for ^{15}N-TOCSY-HSQC) or to any hydrogen within 5 Å proximity in space (^{15}N-NOESY-HSQC). ^{15}N-TOCSY-HSQC spectrum helped identify the spin system and the NOEs corresponding to the amide, alpha, or beta protons within that spin system (labeled). Those assigned NOEs were then transferred to ^{15}*N*-NOESY-HSQC (green circles) to be used for sequential assignments (dotted black path).

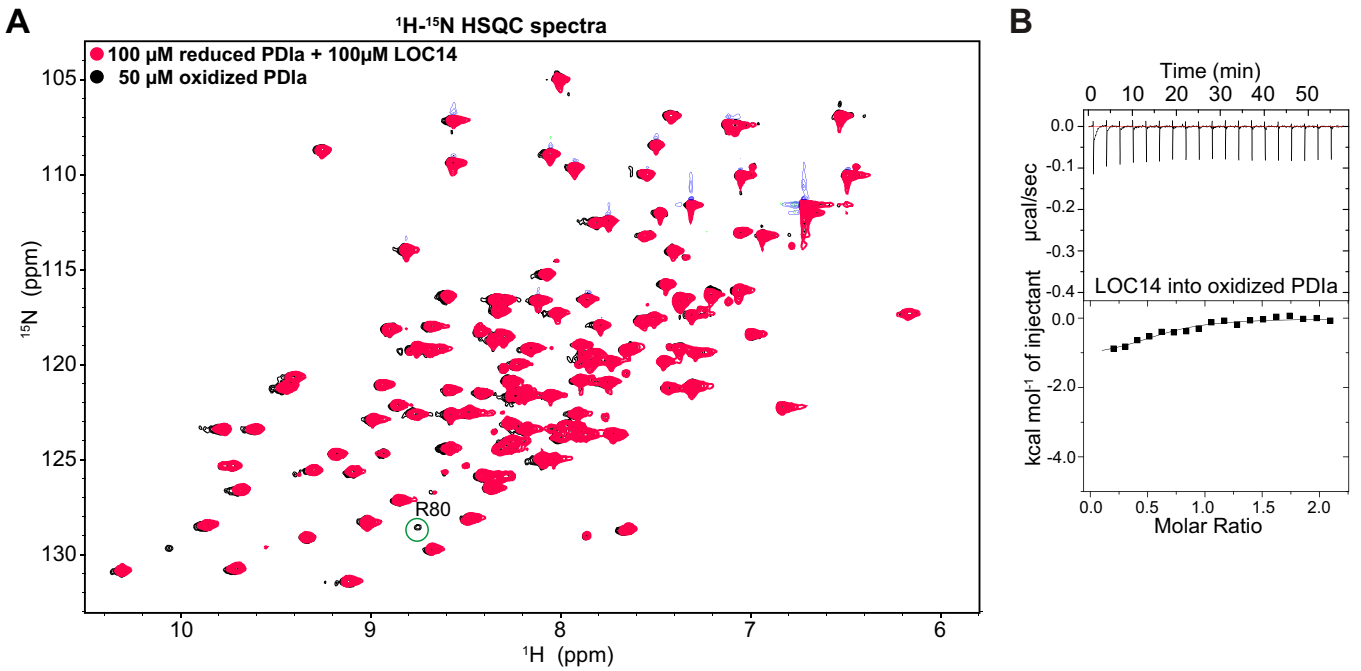

Fig. S5. LOC14 binding to PDIa induces an oxidized conformation in the protein. (*A*) Superimposed ^{1}H-^{15}N HSQC spectra of 50 μM oxidized PDIa alone (black) and 100 μM reduced PDIa treated with 100 μM LOC14 (red). Residue R80 (green circle) is the only peak that is different between the two spectra. (*B*) Calorimetric titration of 400 μM LOC14 into 40 μM oxidized PDIa. (*Upper*) Raw data of the heat released. (*Lower*) Binding isotherm of the reaction. Data are fit to one-site binding model after subtracting the heat released from titrating the compound alone into buffer.

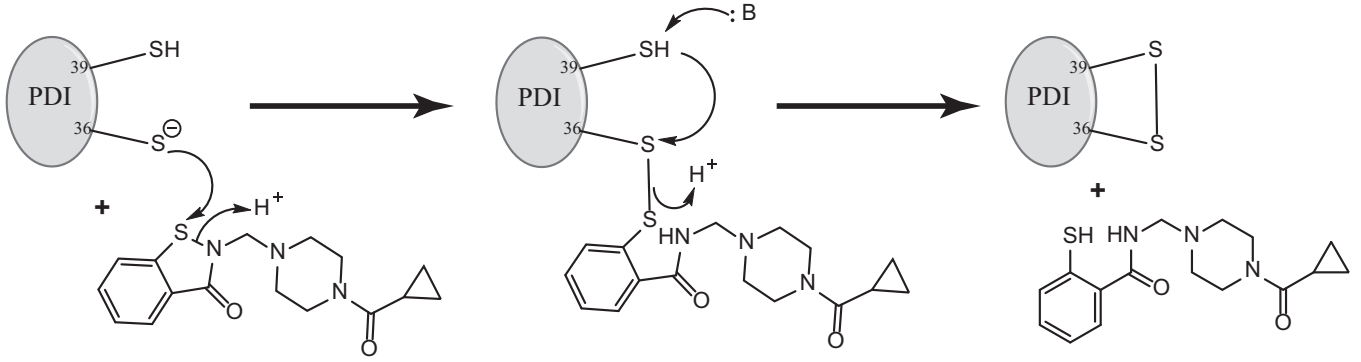

Fig. S6. Possible mechanism for LOC14 modulation of PDI activity; 36 and 39 correspond to the residue number of the two cysteines in the active site. The residue numbering are based on the sequence of the mature PDI protein.

Table S1. ^{1}H and ^{15}N chemical shifts of reduced PDIa at 300 K

Residue	N, ppm	H^N, ppm	Side chain, ppm
E4	122.606	8.592	
E5	120.830	8.278	
E6	122.476	8.770	
V9	123.758	7.881	
L10	128.210	9.010	
V11	124.223	8.285	
L12	126.230	8.438	
R13	118.214	8.923	
K14	122.697	8.888	
S15	108.871	8.077	
N16	117.748	7.567	$N^{\delta 2}$= 106.808, $H^{\delta 21/\ \delta 22}$= 7.424/6.529
F17	123.466	7.890	
A18	119.035	8.760	
E19	119.095	8.164	
A20	123.469	7.710	
L21	117.734	7.482	
A22	116.058	7.069	
A23	118.910	7.356	
H24	116.088	7.157	
K25	123.330	8.247	
Y26	115.457	8.195	
L27	126.080	8.386	
L28	131.530	9.127	
V29	126.747	9.687	
E30	128.172	8.455	
F31	129.058	9.879	
Y32	121.531	8.973	
A33	119.954	7.087	
W35	109.824	5.927	$N^{\varepsilon 1}$ = 130.763, $H^{\varepsilon 1}$ = 10.265
C36	126.466	6.658	
G37	118.879	9.039	
H38	127.681	9.495	
C39	128.106	9.655	
K40	123.673	8.755	
A41	119.935	7.481	
L42	116.669	7.386	
A43	121.064	7.298	
E45	118.420	7.006	
Y46	124.312	8.615	
A47	117.847	7.785	
K48	120.818	7.882	
A49	123.428	8.177	
A50	119.508	7.810	
G51	107.237	8.569	
K52	124.986	8.111	
K54	119.796	7.720	
A55	122.995	7.943	
E56	113.965	7.404	
G57	109.639	7.934	
S58	112.370	7.751	
E59	125.733	9.108	
I60	122.727	8.517	
R61	122.631	7.928	
L62	124.677	9.170	
A63	120.708	9.426	
K64	121.355	9.496	
V65	119.453	8.757	
D66	127.272	8.925	
A67	130.461	8.824	
T68	109.454	8.563	
E69	122.351	6.819	
E70	120.370	8.359	
S71	115.507	7.457	

Table S1. Cont.

Residue	N, ppm	H^N, ppm	Side chain, ppm
D72	121.609	8.363	
L73	121.094	7.787	
A74	119.028	8.054	
Q75	116.627	8.116	$N^{\varepsilon 2}$ = 111.587, $H^{\varepsilon 21/\varepsilon 22}$ = 7.315/6.725
Q76	121.674	8.059	$N^{\varepsilon 2}$ = 110.057, $H^{\varepsilon 21/\varepsilon 22}$ = 7.071/6.492
Y77	113.025	7.102	
G78	108.218	7.454	
V79	119.717	7.869	
R80	128.940	8.751	
G81	107.673	7.120	
Y82	117.429	8.215	
T84	117.726	8.725	
I85	130.612	9.677	
K86	125.608	9.317	
F87	123.446	9.632	
F88	129.165	9.316	
R89	122.288	8.888	
N90	123.221	9.885	$N^{\delta 2}$ = 111.981, $H^{\delta 21/\ \delta 22}$ = 7.490/6.714
G91	108.367	9.284	
D92	119.065	7.617	
T93	116.728	8.284	
A94	123.961	8.267	
K97	121.314	8.613	
E98	124.993	8.968	
Y99	126.503	8.354	
G102	104.770	8.033	
R103	118.531	8.460	
E104	116.493	7.840	
A105	125.291	9.731	
D106	113.952	8.841	
D107	119.391	7.297	
I108	121.087	7.404	
V109	117.320	8.005	
N110	116.555	8.376	$N^{\delta 2}$ = 113.21, $H^{\delta 21/\ \delta 22}$ = 7.547/6.950
W111	121.654	8.194	$N^{\varepsilon 1}$ = 129.61, $H^{\varepsilon 1}$ = 10.057
L112	118.570	8.303	
K113	118.715	8.345	
K114	117.413	7.326	
R115	116.669	7.386	
T116	107.365	7.075	
G117	110.022	7.539	
A119	123.961	8.267	
A120	128.636	7.643	

No HSQC peak detected for D1, A2, D7, H8, L53, S95, T100, and A101. Proline residues (P3, P34, P44, P83, P96, and P118) that lack amide protons are also not shown in Table S1. A94 and A119 peaks overlap and L42 and R115 peaks overlap.

Table S2. Intrinsic clearance of LOC14 in mouse liver microsomes

Compound	Elimination rate constant, k	Half-life ($t_{1/2}$), min	Intrinsic clearance (CL_{int}), mL/min/g liver
LOC14	0.0016	438.7	0.17
7-Ethoxycoumarin	0.2341	3.0	24.58

Compound concentration was 0.5 μM. 7-Ethoxycoumarin, a substrate of cytochrome P450 enzymes, was used as a control.

Table S3. Stability of LOC14 in mouse plasma

Compound	Elimination rate constant, k	Half-life ($t_{1/2}$), h	% Remaining after 2-h incubation
LOC14	0.0049	2.4	49.9
Enalapril	0.0201	0.6	4.3

Compound concentration was 1.0 μM. Enalapril, which undergoes degradation in plasma, was used as a control compound.

Table S4. LOC14 plasma protein binding

Compound	% Bound	% Recovery
LOC14	−18.76 ± 4.09	113.81 ± 0.76
Warfarin	94.21 ± 0.10	109.50 ± 3.54

Compound concentration was 2000 ng/mL. Warfarin, an anticoagulant, was used as a control compound. Data are shown as mean ± SD (n = 3).