Ferrostatins inhibit oxidative lipid damage and cell death in diverse disease models

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Supplementary figures, bioassays and protocols for the synthesis of Ferrostatins and their ^{1}H, ^{13}C NMR and HRMS spectra

Contents

III.4. General procedure C (reductive amination reaction)

III.5. General procedure D (alkylation reaction)

III.6. General procedure E (Addition of Fer-1 to an acyl chloride, alkyl- or benzyl-chloroformates)

III.7. ^{1}H, ^{13}C and mass spectrometry characterization of ferrostatin analogs (Tables 2-8 and Schemes 1-5)

I. Supplementary Figures

Evaluation of selected set of Fer-1 analogs in an in vitro model of periventricular leukomalacia (PVL) (Figure S1)

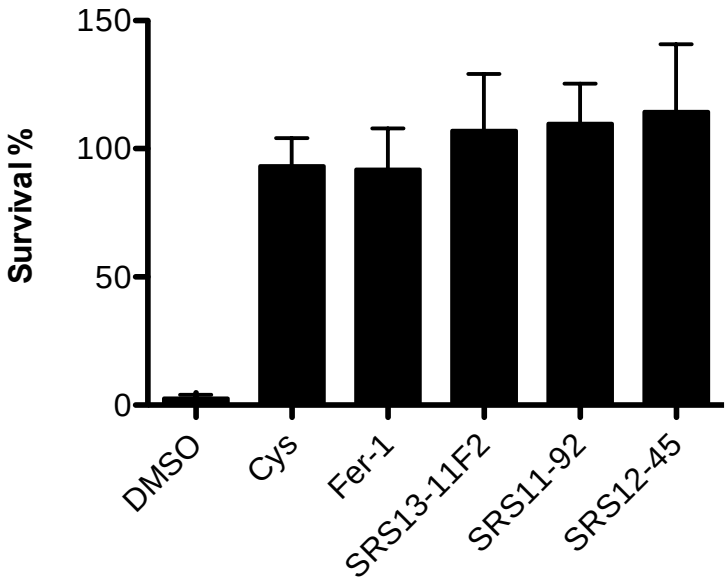


Figure S1. Evaluation of selected set of Fer-1 analogs (100 nM) in a model of periventricular leukomalacia (PVL) in vitro.

Evaluation of selected set of Fer-1 analogs in isolated mouse kidney proximal tubules (Figure S2)

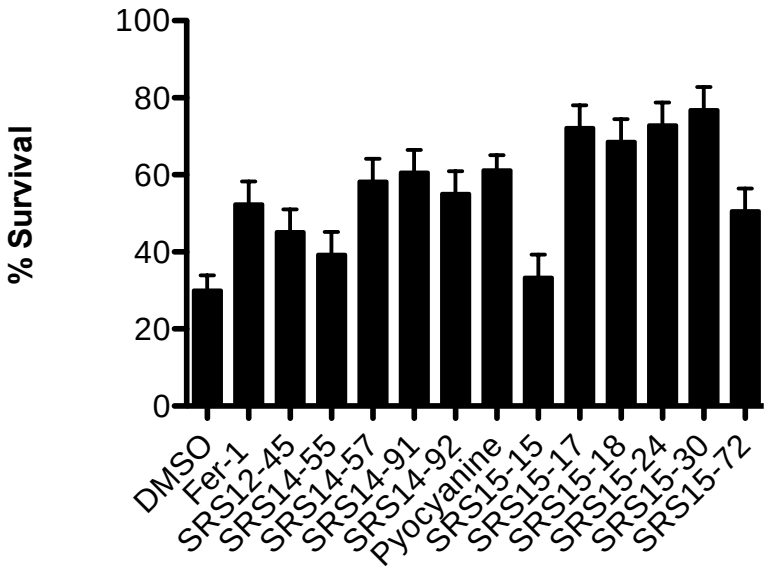
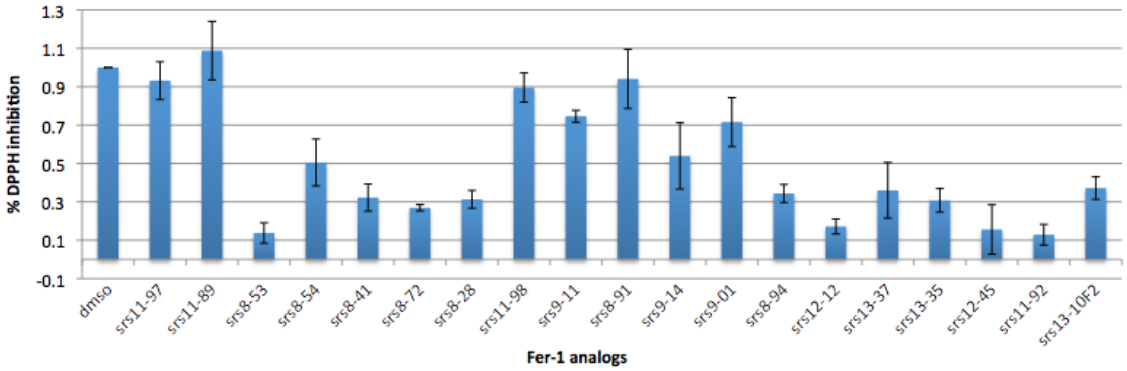


Figure S2. Evaluation of selected set of ferrostatins in a model of iron-induced cell death in freshly isolated mouse kidney proximal tubules that simulate major elements of rhabdomyolysis-induced acute kidney injury. Cell death was quantified by monitoring LDH release after 60 min at 0.5 μM.

% DPPH inhibition of selected Fer-1 analogs (Figure S3)

A)



B)

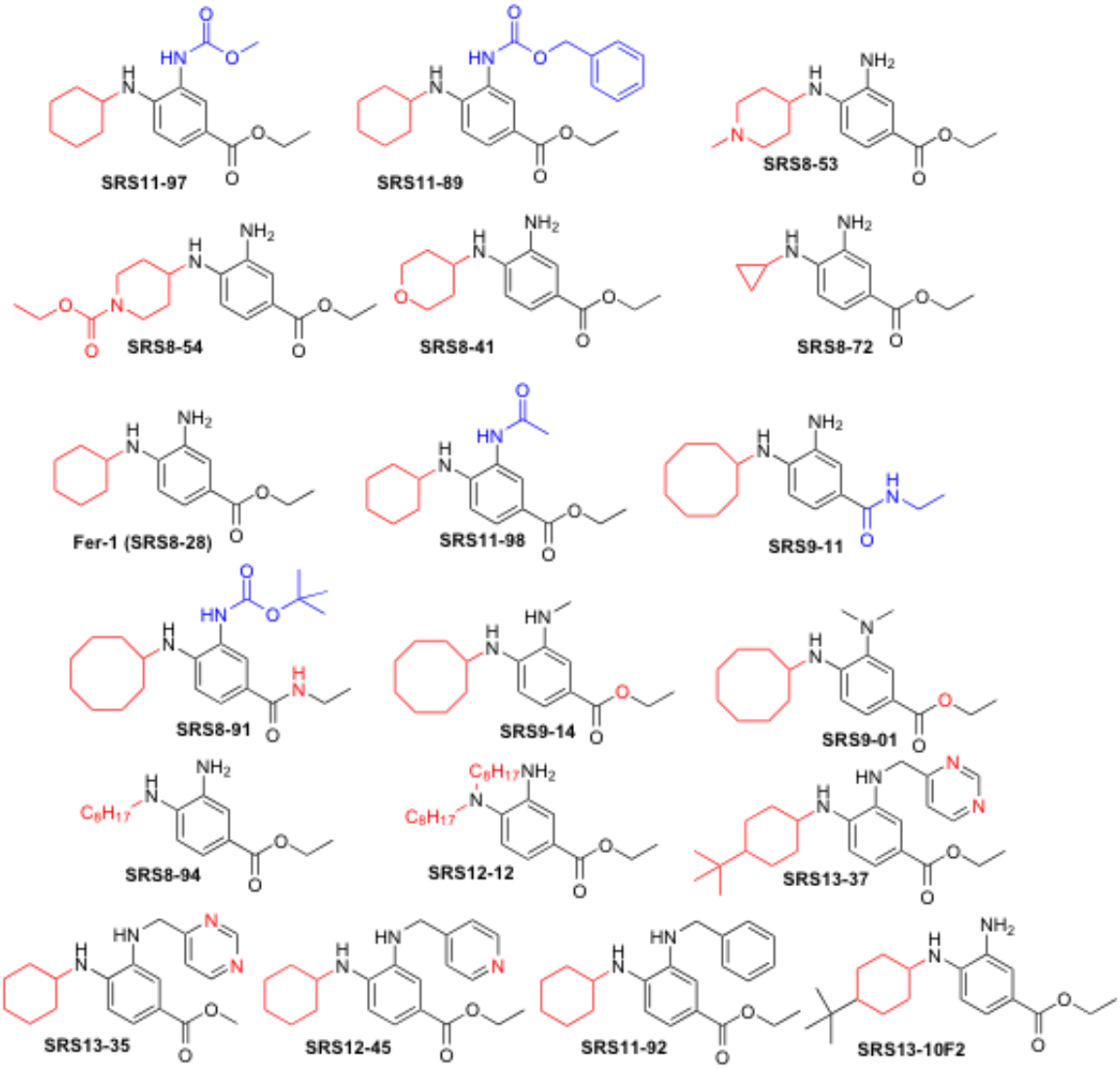
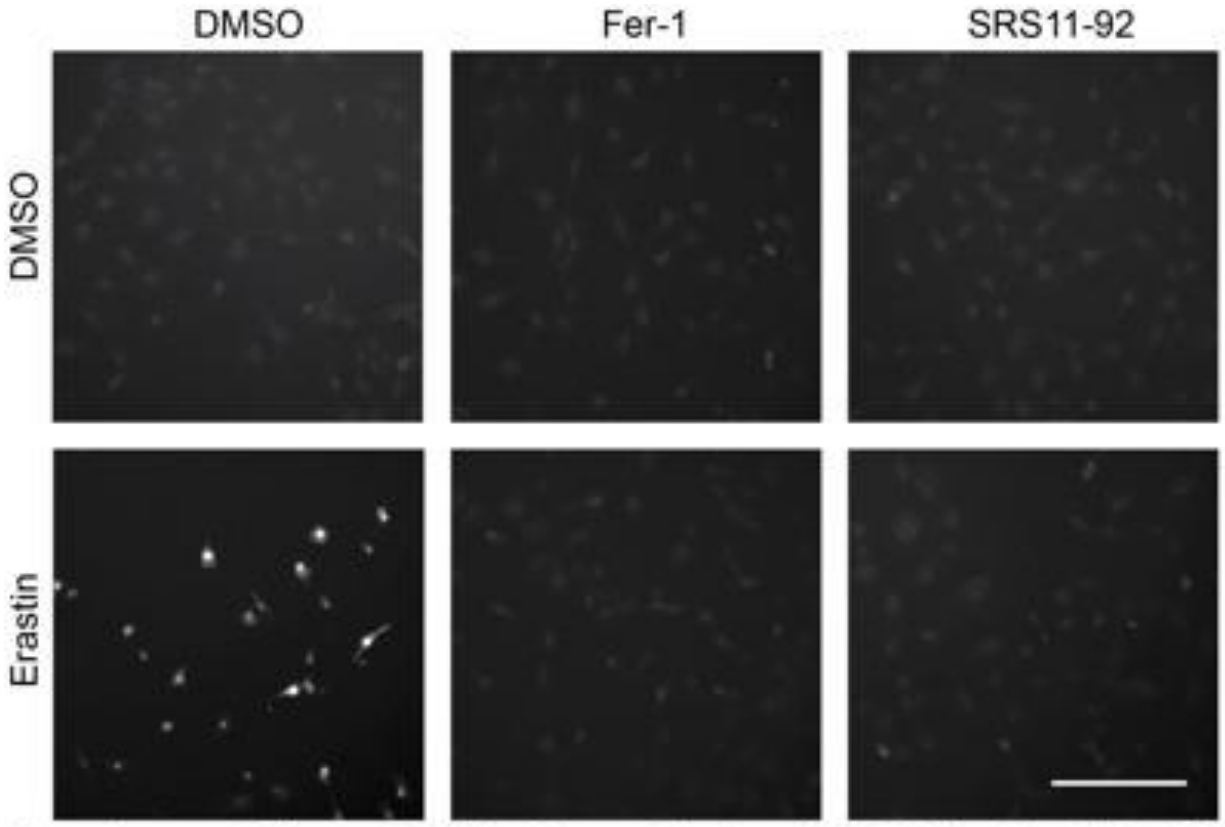


Figure S3. A) % DPPH inhibition of selected Fer-1 analogs. DPPH: 2,2-diphenyl-1-picrylhydrazyl radical. B) Chemical structure of selected Fer-1 analogs

Lipid peroxidation assay (Figure S4)



Supplemental Figure S4. Lipid peroxidation in HT-180 cells. HT-1080 cells were incubated with 50 μM linoleamide alkyne (LAA) and treated for 7.5 hours with the indicated compounds Lipid ROS formation was assessed using a click-chemistry approach involving conjugation of Alexa fluor-488 azide to the oxidative breakdown product of LAA and imaged using an AMG EVOS fl fluorescent microscope. The experiment was repeated three times with similar results. Scale bar = 200 μm.

II. Supplementary Experimental Procedures

II.1. Bioassays, material and methods

II.1.1. 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

The stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) [1] was dissolved in methanol to a final working concentration of 0.05 mM. This was prepared as follows. First, a 100x stock concentration (5 mM) was prepared by dissolving 3.9 mg DPPH in 2 mL methanol. Then, for 25 mL of 0.05 mM final working solution,

250 µL of the 5 mM solution was added to 24.75 mL of methanol. 1 mL of DPPH solution was added to a small volume (< 5 µL) each test compound dissolved in DMSO. The final concentration of each test compound was 0.05 mM. Samples were inverted several times and allowed to incubate at room temperature for 30 minutes. Samples were then aliquoted to white 96-well solid-bottom dishes (Corning) and absorbance at 517 nm was recorded using a TECAN M200 plate reader. All values were normalized to background (methanol only). The experiment was repeated three times and the data was averaged.

II.1.2. Flow cytometry experiments

200,000 HT-1080 cells were seeded into 6-well dishes (Corning). The next day, cells were treated with +/- rotenone +/- ferrostatin-1 for 3 hours (for MitoSOX experiments) or +/- STS +/- ferrostatin-1 for 4 hours (for 10-N-nonylacridine orange [NAO] experiments), harvested by trypsinization and analyzed as follows. For the analysis of mitochondrial ROS, cells were incubated with 5 µM MitoSOX (Molecular Probes/Invitrogen) in HBSS for 10 minutes in the dark at 37^{o}C. For the analysis of mitochondrial cardiolipin peroxidation, cells were incubated with NAO dissolved to a final concentration of 25 ng/mL in HBSS for 10 minutes in the dark at 37^{o}C. Cells were then washed once in HBSS and measurements were subsequently acquired using an flow cytometer (BD/Accuri C6) equipped with a 488 nm laser for excitation of fluorophores. MitoSOX data were collected in the FL2 channel and NAO data was collected in the FL1 channel. Data are from

three independent biological replicates of each experiment. Data were analyzed by one-way ANOVA with Bonferroni's post-hoc test.

II.1.3. Analysis of acridine orange (AO) relocalization

200,000 HT-1080 cells were seeded into 6-well dishes (Corning). The next day, cells were incubated with 25 ng/mL of AO for 15 minutes, then medium was removed and cells were incubated in growth medium for 15 minutes. The medium was then removed again and replaced with medium containing DMSO or erastin +/- ciclipirox olamine (CPX, an iron chelator) or ferrostatin-1 (Fer-1). Relocalization of AO from lysosomes (discrete red puncta) to the cytosol (diffuse green color) was determined using an EVOS fl digital inverted fluorescent microscope (Advanced Microscopy Group) equipped with GFP (ex/em 470/525) and RFP (ex/em 531/593) light cubes.

II.1.4. Metabolomic analysis

HT-1080 cells were seeded overnight in 15 cm dishes in HT-1080 media (DMEM, 10% FBS, 1% Pen-Strep supplemented with 1x nonessential amino acids). The next day, cells were treated for 6 hours in media containing DMSO, erastin (10 μM), Fer-1 (1 μM) or erastin+Fer-1. Cells were then harvested by trypsinization and snap frozen at -80°C. Four independent biological replicate experiments were performed on separate days and approximately 10 million cells were collected per samples. Frozen samples were shipped to Metabolon Inc (Durham, NC) for further processing. At Metabolon, the sample preparation process was

carried out using the automated MicroLab STAR system (Hamilton Company). Sample preparation was conducted using a series of organic and aqueous extractions to remove the protein fraction, while allowing maximum recovery of small molecules. The resulting extract was divided into two fractions: one for analysis by liquid chromatography (LC) and one for analysis by gas chromatography (GC). Samples were placed briefly on a TurboVap (Zymark) to remove the organic solvent, then frozen and dried under vacuum and prepared for analysis by either LC/MS or GC/MS.

II.1.5. Liquid chromatography/Mass Spectrometry (LC/MS, LC/MS^2, (for Table 1, see Excel sheet)

LC/MS analysis was performed using a Waters ACQUITY UPLC and a Thermo-Finnigan LTQ mass spectrometer, which consisted of an electrospray ionization (ESI) source and linear ion-trap (LIT) mass analyzer. The dried sample extract was split into two aliquots then reconstituted in acidic or basic LC-compatible solvents, each of which contained 11 or more injection standards at fixed concentrations. One aliquot was analyzed using acidic positive ion optimized conditions and the other using basic negative ion optimized conditions in two independent injections using separate dedicated columns. Extracts reconstituted in acidic conditions were gradient eluted using water and methanol both containing 0.1% formic acid, while the basic extracts, which also used water/methanol, contained 6.5 mM ammonium bicarbonate. The MS analysis alternated between MS and data-dependent MS^2 scans using dynamic exclusion.

For ions with counts greater than 2 million, an accurate mass measurement could be performed. Accurate mass measurements could be made on the parent ion as well as fragments. The typical mass error was less than 5 ppm.

Gas chromatography/Mass Spectrometry (GC/MS, for Table 1, see Excel sheet)

The samples destined for GC/MS analysis were dried under vacuum desiccation for a minimum of 24 hours then derivatized under dried nitrogen using bistrimethylsilyltriflouroacetamide (BSTFA). The GC column was 5% phenyl and the temperature ramp is from 40° to 300° C in a 16 minute period. Samples were analyzed on a Thermo-Finnigan Trace DSQ fast-scanning single-quadrupole mass spectrometer using electron impact ionization.

II.1.6. Compound identification and Statistical Analysis (for Table 1, see Excel sheet)

Compounds were identified by comparison to library entries of 1,000 purified standards or recurrent unknown entities. The combination of chromatographic properties and mass spectra gave an indication of a match to the specific compound or an isobaric entity. ANOVA procedures implemented in R (http://cran.r-project.org/) were employed to compare the levels of different metabolites between the 4 different treatment conditions (4 replicates per condition). In addition, the experiments data for the Htt, in the *brain slice assay for HD,* were analyzed ‘using a one-way ANOVA in Prism’.

II.1.7. Cancer cell lines and cell line screening.

HT-1080 cells were obtained from ATTC, cultured in DMEM containing 10% fetal bovine serum, 1% supplemented non-essential amino acids and 1% pen/strep mixture (all from Gibco) and maintained in a humidified environment at 37 ^{o}C with 5% CO_2 in a tissue culture incubator. HT-1080 cells were used to test the ability of ferrostatin-1 and analogs to prevent erastin-induced death. 1,000 HT-1080 cells were seeded per well of duplicate 384-well plates (Corning) using a BioMek FX liquid handling robot (Beckman Coulter). The next day, the medium was replaced with 36 µL of medium containing 10 µM erastin with 4 µL of medium containing a dilution series (previously prepared) of DMSO, ferrostatin-1 or ferrostatin-1 analogs. 24 hours later, 10 µL Alamar Blue (Invitrogen) cell viability solution was added to the growth media to a final concentration of 10%. Cells were incubated a further 6 hours and then the Alamar Blue fluorescence intensity was recorded using a Victor 3 platereader (PerkinElmer)(ex/em 530/590). All experiments were performed at least twice and the background (no cells)-subtracted Alamar Blue values for each combination was averaged between biological replicates. From these data, sigmoidal dose-response viability curves and EC_{50} values were computed using Prism 5.0 (GraphPad).

II.1.8. S. cerevisiae viability assays

A yeast strain harboring a deletion of the gene *COQ3* (*coq3Δ*), a kind gift of Dr. Elizabeth Miller (Columbia University), was used for all experiments. For spot

dilution assays, cells harboring the *coq3Δ* mutation were picked from single colonies and grown overnight in YPED media (1% Bacto yeast extract, 2% Bacto peptone, 2% glucose) + G418. The next morning, cells were diluted in YPED + G418 to an OD_{600} = 0.1-0.5 and allowed to grow for 2 hours to log phase. Cells were then washed 2x with sterile water and diluted to an OD_{600} = 0.2 in 100 mM phosphate buffer (pH 6.2) +0.2% dextrose. 0.5 mL aliquots were incubated for 6 hours +/- linolenic acid (500 μM) and +/- DMSO, trolox, ciclopirox olamine or ferrostatin-1. After six hours, cultures were normalized to an OD of 0.2, and 1:5 spot dilutions were performed on YPED+agar plates. Plates were grown for 72 hours and imaged using a G:Box imaging station (Syngene). This experiment was performed three times with similar results and representative data from one experiment is shown.

II.1.9. Brain slice assay for HD

250 μm corticostriatal brain slices were prepared from postnatal day 10 CD Sprague-Dawley rat pups (Charles River) as previously described[22]. Brain slice explants were placed in interface culture in 6-well plates using culture medium containing 15% heat-inactivated horse serum, 10 mM KCl, 10 mM HEPES, 100 U/ml penicillin/streptomycin, 1 mM MEM sodium pyruvate, and 1 mM L-glutamine in Neurobasal A (Invitrogen) and maintained in humidified incubators under 5% CO_2 at 32 deg. C. A custom-modified biolistic device (Helios Gene Gun; Bio-Rad) was used to transfect the brain slices with a human htt exon-1 expression construct containing a 73 CAG repeat ("HttN90Q73") in the gWiz backbone

(Genlantis) together with a YFP expression construct to visualize transfected neurons. Control brain slices were transfected with gWiz blank vector and YFP at the equivalent DNA amounts. After 4 days of incubation, MSNs were identified by their location within the striatum and by their characteristic dendritic morphology and scored as healthy if expressing bright and continuous YFP labeling throughout, normal-sized cell bodies, and >2 primary dendrites >2 cell bodies long, as previously described[21]. Data were expressed as mean numbers of healthy MSNs per striatal region in each brain slice, with statistical significance tested by ANOVA followed by Dunnett's *post hoc* comparison test at the 0.05 confidence level. Fer-1 was added to the culture medium at the time of brain slice preparation; positive control brain slices were treated with a combination of the adenosine receptor 2A modulator KW-6002 (50 μM) and the JNK inhibitor SP600125 (30 μM). Final DMSO concentration of 0.1% for all conditions.

II.1.10. Brain slice assay for HD Fer-1 analogs protected developing oligodendrocytes from cystine deprivation induced cell death

Primary pre-oligodendrocytes cultures were prepared from the forebrains of P2 Sprague Dawley (Charles River Laboratory) rat pups using a differential detachment method. Forebrains free of meninges were dissociated with Hanks' Balanced Salt Solution containing 0.01% trypsin and 10μg/ml DNase, and triturated with DMEM containing 10% heat-inactivated fetal bovine serum and 100 U/ml penicillin and 100 μg/ml streptomycin. Dissociated cells were plated onto poly-D-lysine-coated 75 cm2 flasks and fed cells every other day for 10 – 17

days. On day 10 or 17, following 1 hour pre-shake at 200 rpm 37^{o}C to remove microglia, the flasks were shaken overnight to separate pre-oligodendrocytes from astrocyte layer. The cell suspension was passed through a 20 µm filter and plated onto uncoated (bacteriological) petri dishes for 1 hour in incubator to remove residual microglia/astrocytes. Cell suspension was plated onto poly-D,L-ornithine-coated plates with DMEM, 1x ITS (Life Technologies), 2 mM L-glutamine, 1mM sodium pyruvate, 0.5% FBS and 0.05% gentamicin (Sigma), 10 ng/ml PDGF and 10 ng/ml FGF (Peprotech), with full medium change the next day and half medium change every other day. At day 8, cells were washed twice with cystine deprivation medium, treated with Fer-1 and analogs (stock 1 mM in DMSO) in cystine deprivation medium plus PDGF and FGF (treatment medium) for 24 hrs. Cells were treated with treatment medium plus 100 µM cystine as positive control; and cells were treated with treatment medium as negative control. Cells in each well, received same amount of DMSO as a vehicle. After 24 hrs, cells were assayed with Alamar Blue (Treck Diagnostics) by full medium change with 1x AlamarBlue in Earle's Balance Salt Solution for 2 hours at 37^{o}C and 5% CO_2. Fluorescence was assayed in each well using FluoroCount Plate Reader (Packard), with Packard Plate Reader Version 3.0, and 530 nm excitation, and 590 nm emission filters.

II.1.11. Studies of isolated mouse proximal tubules

Tubule preparation[17, 21]**:** 8-12 week old C57/BL6 female mice were euthanized with isoflurane. Kidneys were removed and immediately injected

intraparenchymally with a cold 95% O_2/5% CO_2-gassed solution consisting of 115 mM NaCl, 2.1 mM KCl, 25 mM $NaHCO_3$, 1.2 mM KH_2PO_4, 2.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, 1.2 mM $MgS0_4$, 25 mM mannitol, 2.5 mg/ml fatty acid free bovine serum albumin, 5 mM glucose, 4 mM sodium lactate, I mM alanine, and 1 mM sodium butyrate (Solution A) with the addition of 1 mg/ml collagenase (Type I, Worthington Biochemical Corp., Freehold). The cortices were then dissected and minced on an ice cold tile, then resuspended in additional Solution A for 8-10 min. of digestion at 37^{o}C followed by enrichment of proximal tubules using centrifugation on self-forming Percoll gradients as previously described for rabbit tubules.

Experimental procedures for isolated tubules [17, 21]**:** Tubules were suspended at 1.5-2.0 mg tubule protein/ml in a 95% air/5% CO_2-gassed medium containing (in mM) 110 NaCl, 2.6 KCl, 25 $NaHCO_3$, 2.4 KH_2PO_4, 1.25 $CaCl_2$, 1.2 $MgCl_2$, 1.2 $MgSO_4$, 5 glucose, 4 sodium lactate, 0.3 alanine, 5 sodium butyrate, 2 glycine, and 1.0 mg/ml bovine gelatin (75 bloom) (Solution B). After precincubation for 15 min. at 37^{o}C, then were then resuspended in fresh Solution B containing 2 mM heptanoic acid instead of sodium butyrate along with experimental compounds.

Measurement of lethal plasma membrane damage by lactate dehydrogenase (LDH) release: LDH activity was measured before and after the addition of 0.1% Triton X-100 by following the production NAD from NADH fluorometrically in the presence of pyruvate [17]. Survival was calculated as the

percentage of Triton-releasable LDH retained intracellularly, which is 100% at the start of incubation of control non-injured tubules.

II.1.11. Lipid peroxidation assay

The formation of lipid peroxides was detected using a Click-iT lipid peroxidation detection kit (Molecular Probes/Life Technologies) as follows. The day before the experiment, HT-1080 cells were seeded at a density of 200,000 cells per well into 6-well dishes (Corning). The day of the experiment cells were incubated with 50 μM linoleamide alkyne and either DMSO or erastin (10 μM) +/- ferrostatin-1 (1 μM) for 7.5 hours. At this point cells were washed three times in PBS and fixed for 15 minutes at room temperature in a 3.7% paraformaldehyde solution prepared fresh in PBS from a 32% stock solution (Electron Microscopy Sciences). The fixative was removed by three washes with PBS and cells were then permeabilized for 10 minutes at room temperature using 0.5% Triton X-100 (Sigma) in PBS. The permeabilization solution was removed and cells were blocked for 30 minutes at room temperature in a 1% (w/v) BSA solution in PBS. Next the blocking solution was removed, cells were washed twice with PBS, and 450 μL of click chemistry reaction mix containing Alexa Fluor 488 azide was added to each well and incubated for 30 minutes at room temperature in the dark. The reaction mix was prepared exactly according to the manufacturer's protocol. Following the reaction cells were washed twice in PBS+BSA and once in regular PBS before the addition of a final 1 mL of PBS to each well. Cells were then imaged on an EVOS fl microscope (Life Technologies) equipped with

a 10x objective lens and GFP light cube appropriate for the detection of Alexa Fluor-488.

III. General procedures for the synthesis of Ferrostatin-1 and derivatives (Supplementary Synthesis Section)

III.1. General Information

Chemicals:

Solvents, inorganic salts, and organic reagents were purchased from commercial sources such as Sigma and Fisher and used without further purification unless otherwise mentioned. Erastin was dissolved in DMSO to a final concentration of 73.1 mM and stored in aliquots at -20^{o}C.

Chromatography: Merck pre-coated 0.25 mm silica plates containing a 254 nm fluorescence indicator were used for analytical thin-layer chromatography. Flash chromatography was performed on 230-400 mesh silica (SiliaFlash® P60) from Silicycle.

Spectroscopy: ^{1}H, ^{13}C and ^{19}F NMR spectra were obtained on a Bruker DPX 400 MHz spectrometer. HRMS spectra were taken on double focusing sector type mass spectrometer HX-110A. Maker JEOL Ltd. Tokyo Japan (resolution of 10,000 and 10 KV accel. Volt. Ionization method; FAB (Fast Atom Bombardment) used Xe 3Kv energy. Used Matrix, NBA (m-Nitro benzyl alcohol).

III. 2. General procedure A (ArS_N2 reaction)[2]

To the ethyl 4-chloro-3-nitrobenzoate (1 equiv., 200 mg, 0.871 mmol) in dry DMSO (2 mL) was added K_2CO_3 (2 equiv., 240.8 mg, 1.742 mmol) and various amines (1.2 equiv., 119.5 μL, 1.045 mmol). The mixture was stirred for 17 h at 60°C. The solution was poured into water and the organic layer was extracted three times with ethyl acetate. After drying with anhydrous magnesium sulfate the solvents were removed under vacuum. The residue was purified by flash column chromatography on silica gel to provide the desired ethyl 4-(substituted-amino)-3-nitrobenzoate derivatives (*SI*, Tables 2-4).

III.3. General procedure B (hydrogenolysis)[1]

The ethyl 4-(substituted-amino)-3-nitrobenzoates (130 mg, 0.445 mmol) were dissolved in MeOH (10 mL) and hydrogenated (H_2 gas) over 10% $Pd(OH)_2$ on charcoal (90 mg) for 17 h at room temperature. The solution was filtered through a pad of celite and volatiles were removed under vacuum. The residue was purified by flash-column chromatography on silica gel to provide the desired Ferrostatin-1 derivatives.

III.4. General procedure C (reductive amination reaction)[3]

A representative example is the reductive amination of Fer-1 with benzaldehyde (Table 7, entry 1):

Method I: ethyl 3-amino-4-(cyclohexylamino)benzoate (Fer-1) (100 mg, 0.382 mmol, 1 equiv) and benzaldehyde (39 μL, 0.382 mmol, 1 equiv) were heated in DCE for 1h at 80 °C in the presence of molecular sieves (4 Å), then the mixture

was cooled down to room temperature before addition of the $NaBH(OAc)_3$ in small portions over 3h. The reaction mixture was stirred at room temperature under a nitrogen atmosphere for 17h. The reaction mixture was quenched with aqueous saturated $NaHCO_3$, and the product was extracted with EtOAc. The EtOAc extract was dried ($MgSO_4$), and the solvent was evaporated. The residue was purified by flash-column chromatography on silica gel to provide the desired ethyl 3-(benzylamino)-4-(cyclohexylamino)benzoate (SRS11-92).

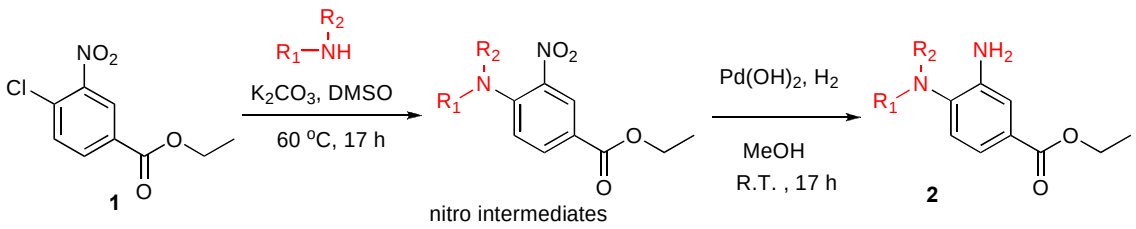
Method II: To the ethyl 3-amino-4-(cyclohexylamino)benzoate (Fer-1) (100 mg, 0.382 mmol, 1 equiv) and benzaldehyde (39 μL, 0.382 mmol, 1 equiv) in DCE was added $NaBH(OAc)_3$ (129.5 mg, 0.611 mmol, 1.6 equiv). The reaction mixture was treated in the same way as in method I.

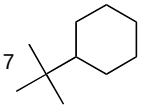
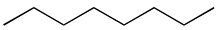
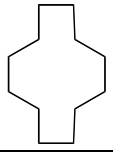
III.5. General procedure D (alkylation reaction)

A representative example is the methylation of the SRS8-70 using methyl iodide (*SI*, Table 5, entry 8). To the ethyl 3-amino-4-(cyclooctylamino)benzoate (SRS8-70; 58 mg, 0.199 mmol) in DMF (1 mL), MeI (28 μL, 0.398 mmol) and K_2CO_3 (82mg, 0.508 mmol) were added. The mixture was stirred at 40 ^oC during 6 h then poured into water. The organic layer was extracted with EtOAc then dried under $MgSO_4$, and the solvent was evaporated. The residue was purified by flash-column chromatography on silica gel to provide the desired ethyl 4-(cyclooctylamino)-3-(dimethylamino)benzoate (SRS9-01).

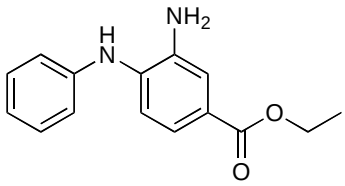
III.6. General procedure E (Addition of the Fer-1 to an acylchloride, alkyl- or benzyl-chloroformates)

A representative example is the addition of aniline of Fer-1 to the benzylchloroformate (*SI*, Table 5, entry 2). To the ethyl 3-amino-4-(cyclohexylamino)benzoate (Fer-1; SRS8-28; 22 mg, 0.084 mmol) in THF (1 mL), benzylchloroformate (24 μL, 0.168 mmol) and DIPEA (44 μL, 0.252 mmol) were added at 0 ^{o}C. The mixture was stirred at room temperature during 17h then poured into water. The organic layer was extracted with EtOAc then dried under $MgSO_4$, and the solvent was evaporated. The residue was purified by flash-column chromatography on silica gel to provide the desired ethyl 3-(benzyloxycarbonylamino)-4-(cyclohexylamino)benzoate (SRS11-89).



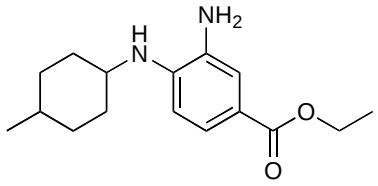
Entry	R_1	R_2	Name (Yield)	EC50(nM)	Log P
1		H	Fer-1 (SRS8-28)[1] (85%)	95	3.2
2		H	SRS8-72[1] (74%)	880	1.9
3		H	SRS8-71[1] (80%)	265	2.3
4		H	SRS8-73[1] (85%)	120	2.7
5		H	SRS8-42[1] (64%)	200	2.9
6		H	SRS8-48[1] (89%)	90	3.5
7		H	(80% as mixture of isomers) SRS13-10_F1_single isomer SRS13-10_F2_single isomer SRS13-10[1]_F1+F2_mixture of isomers	44 13 62	4.7
8		H	SRS8-90[1] (86%)	130	3.6
9		H	SRS8-70[1] (89%)	80	4.1
10		H	SRS8-94[1] (90%)	80	4.3
11		H	SRS9-06[1] (80%)	70	5.8

***SI*, Table 2**: Synthetic scheme of Ferrostatin-1 analogs with various hydrophobic moieties. [1]These analogs were breifly reported in our recent paper (Dixon et al., *Cell* **2012**, *149*, 1060-1072).



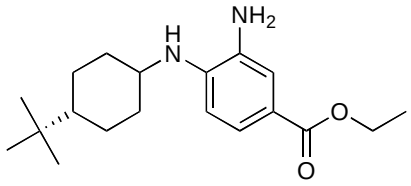
Synthesis of ethyl 3-amino-4-(phenylamino)benzoate (SRS8-42. SI, Table2)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (d, J = 7.9 Hz, 2H), 7.30 (t, J = 7.9 Hz, 2H), 7.19 (d, J = 8.1 Hz, 1H), 6.97 (d, J = 8.3 Hz, 3H), 5.74 (s, NH), 4.37 (q, J = 7.1 Hz, 2H), 3.70 (s, NH_2), 1.41 (t, J = 7.1 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.8, 142.9, 137.9, 135.6, 129.4, 124.6, 121.8, 121.3, 118.4, 117.9, 60.7, 14.4.



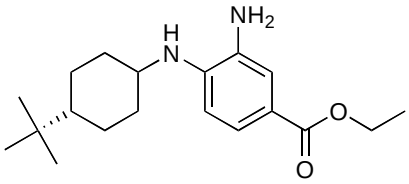
Synthesis of ethyl 3-amino-4-(4-methylcyclohexylamino)benzoate (SRS8-48. SI, Table2)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.58 (d, *J* = 8.3 Hz, 1H), 7.41 (s, 1H), 6.59 (d, J = 8.4 Hz, 1H), 4.30 (d, *J* = 7.1 Hz, 2H), 3.26 (s, 1H), 2.13 (d, *J* = 11.2 Hz, 2H), 1.78 (d, *J* = 12.3 Hz, 2H), 1.35 (t, *J* = 7.0 Hz, 4H), 1.23–1.07 (m, 3H), 0.93 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.98, 141.15, 132.14, 124.13, 119.19, 118.69, 110.50, 60.25, 52.21, 33.91, 33.11, 32.23, 22.20, 14.46; HRMS (FAB) calculated for $C_{16}H_{24}N_2O_2$: 276.37; found: 276.2.



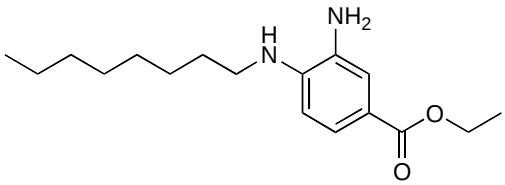
Synthesis of single isomer of ethyl 3-amino-4-(4-*tert*-butylcyclohexyl-amino)benzoate (SRS13-10_F1. SI, Table2)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.49 (d, J = 8.4 Hz, 1H), 7.43 (s, 1H) .6.61 (d, J = 8.4 Hz, 1H), 4.32 (q, J = 7.1 Hz, 2H), 3.86 (b, 1H), 3.25 (b, 1H + NH_2), 2.26-2.13 (m, 2H), 1.95-1.81 (m, 2H), 1.43-1.33 (m, 3H), 1.22-1.12 (m, 4H), 1.10-1.04 (m, 1H), 0.89 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.0, 142.3, 131.67, 124.3, 118.6, 118.4, 109.52, 60.2, 52.0, 47.7, 33.82, 32.4, 27.6, 26.2, 14.5; HRMS (FAB) calculated for $C_{19}H_{30}N_2O_2$: 318.45; found: 318.24.



Synthesis of single isomer of ethyl 3-amino-4-(4-*tert*-butylcyclohexyl-amino)benzoate (SRS13-10_F2. SI, Table2)

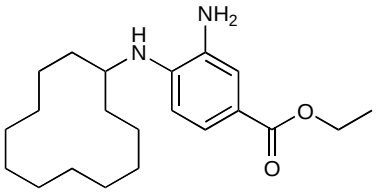
^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.66-7.56 (m, 1H), 7.44 (s, 1H). 6.65-6.56 (m, 1H), 4.39-4.26 (m, 2H), 3.75 (s, 1H), 3.22 (B, NH_2), 2.10-2.96 (m, 2H), 1.70-1.52 (m, 4H), 1.43-1.34 (m, 3H), 1.30-1.20 (m, 2H), 1.13-1.05 (m, 1H), 0.88 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.1, 142.4, 131.9, 124.5, 118.7, 118.4, 109.6, 60.1, 47.8, 46.5, 32.5, 30.3, 27.4, 21.6, 14.45; HRMS (FAB) calculated for $C_{19}H_{30}N_2O_2$: 318.45; found: 318.24.



Synthesis of ethyl 3-amino-4-(octylamino)benzoate (SRS8-94. SI, Table2)

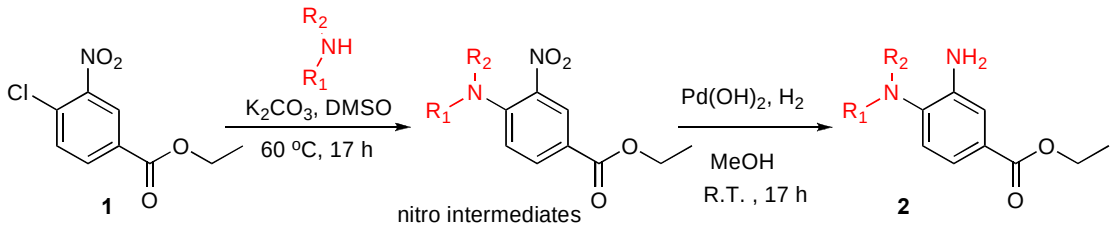
^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.61 (d, *J* = 8.3 Hz, 1H), 7.43 (d, *J* = 2.0 Hz, 1H), 6.60 (d, *J* = 8.3 Hz, 1H), 4.33 (q, *J* = 7.1 Hz, 2H), 3.17 (t, *J* = 7.1 Hz, 2H), 1.69 (q, *J* = 7.3 Hz, 2H), 1.52–1.23 (m, 13 H), 0.91 (t, *J* = 6.6 Hz, 3H); ^{13}C NMR (100

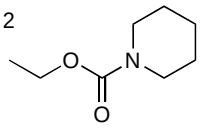
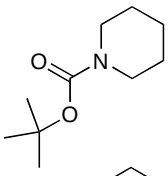
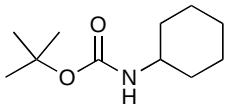
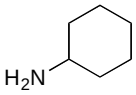
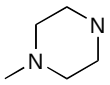
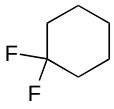
MHz, $CDCl_3$) δ 167.1, 143.2, 131.9, 124.3, 118.9, 118.1, 109.2, 60.2, 43.7, 31.8, 29.4, 29.3, 27.2, 22.7, 14.5, 14.1; HRMS (FAB) calculated for $C_{17}H_{28}N_2O_2$: 292.42; found: 292.22.



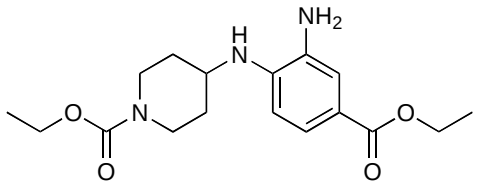
Synthesis of ethyl 3-amino-4-(cyclododecylamino)benzoate (SRS9-06. SI, Table2)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (d, *J* = 8.4 Hz, 1H), 7.43 (s, 1H), 6.60 (d, *J* = 9.6 Hz, 1H), 4.32 (d, *J* = 9.6 Hz, 2H), 3.19 (s, 1H), 1.43 (d, *J* = 26.7 Hz, 25H), 1.28 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.1, 142.6, 131.7, 124.5, 118.6, 118.16, 109.2, 60.2, 49.2, 29.6, 24.33, 24.0 23.3, 23.2, 21.22, 14.5; HRMS (FAB) calculated for $C_{21}H_{34}N_2O_2$: 346.51; found: 346.29.



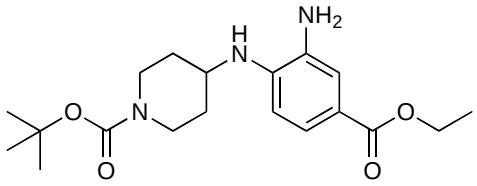
Entry	R_1	R_2	Name (Yield)	EC50 (nM)	Log P
1		H	SRS8-41 (85%)	1450	1.8
2		H	SRS8-54 (50%)	2500	1.9
3		H	SRS8-47 (65%)	710	2.8
4		H	SRS8-81 (86%)	350	1.5
5		H	SRS8-46 (81%0	515	3.6
6		H	SRS8-80 (81%)	380	1.9
7		H	SRS8-53 (70%)	3600	1.7
8		H	SRS8-52 (87%)	650	3.8

***SI*, Table 3**: EC50 and Log P of Ferrostatin-1 analogs with various cyclic amines bearing heteroatoms.



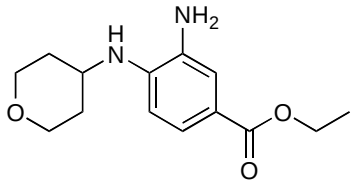
Synthesis of ethyl 4-(2-amino-4-(ethoxycarbonyl)phenylamino)piperidine-1-carboxylate (SRS8-54. SI, Table3)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 (d, J = 8.0 Hz, 1H), 7.36 (s, 1H), 6.55 (d, J = 7.8 Hz, 1H), 4.28–4.21 (m, NH2), 4.12– 4.00 (m, 4H), 3.47 (s, 1H), 2.96 (s, 2H), 1.98 (s, 2H), 1.44–1.11 (m, 10H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.0, 155.5, 141.1, 132.5, 123.8, 119.0, 118.3, 109.5, 61.4, 60.2, 49.5, 42.6, 32.0, 14.7, 14.4; HRMS (FAB) calculated for $C_{17}H_{25}N_3O_4$: 335.40; found: 335.18.



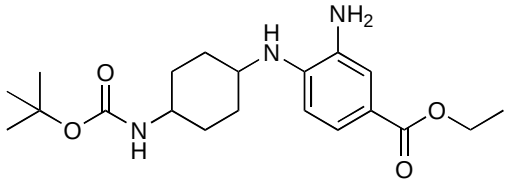
Synthesis of *tert*-butyl 4-(2-amino-4-(ethoxycarbonyl)phenylamino)-piperidine-1-carboxylate (SRS8-47. SI, Table3)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 (d, *J* = 10.1 Hz, 1H), 7.42 (s, 1H), 6.60 (d, *J* = 8.4 Hz, 1H), 4.31 (q, *J* = 7.1 Hz, 2H), 3.26 (s, 2H), 2.97 (t, *J* = 11.7 Hz, 2H), 2.05 (d, *J* = 12.6 Hz, 2H), 1.41 (d, *J* = 45.5 Hz, 16H); ^{13}C NMR (100MHz, $CDCl_3$) δ 167.0, 154.7, 141.3, 132.3, 124.0, 119.0, 118.5, 109.6, 77.0, 60.2, 53.5, 49.6, 32.1, 28.4, 14.4; HRMS (FAB) calculated for $C_{19}H_{29}N_3O_4$: 363.45; found: 363.21.



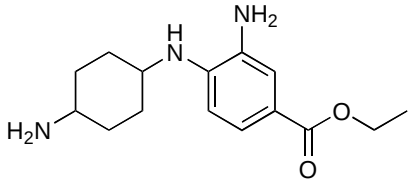
Synthesis of ethyl 3-amino-4-(cyclohexylamino)benzoate (SRS8-81. SI, Table3)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55 (d, J = 8.2 Hz, 1H), 7.41 (s, 1H), 6.57 (d, J = 8.2 Hz, 1H), 4.30 (d, J = 6.8 Hz, 2H), 3.49 (s, 1H + NH_2), 3.24 (b, NH), 2.83 (s, 2H), 2.11 (s, 2H), 1.55 (d, J = 9.2 Hz, 2H), 1.35 (s, 3H), 1.26 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.0, 141.4, 132.1, 124.1, 119.0, 118.6, 109.6, 60.3, 49.5, 44.9, 32.9, 29.7, 14.5; LC/MS (APCI+, M+1) 264.67



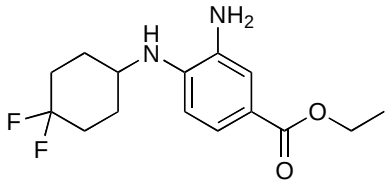
Synthesis of ethyl 3-amino-4-(4-(tert-butoxycarbonylamino)cyclohexyl-amino)benzoate (SRS8-46. SI, Table3)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55 (d, J = 8.1 Hz, 1H), 7.39 (s, 1H), 6.56–6.51 (m, 1H), 4.55 (s, 1H), 4.29 (d, J = 9.9 Hz, 2H), 3.45 (s, 2H), 3.25 (s, 1H), 2.09 (d, J = 26.0 Hz, 4H), 1.44 (s, 9H), 1.29 (d, J = 30.4 Hz, 7H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.0, 155.3, 141.8, 132.0, 124.1, 118.7, 118.5, 109.4, 79.2, 60.2, 50.9, 49.2, 32.0, 31.8, 28.4, 14.5; HRMS (FAB) calculated for $C_{20}H_{31}N_3O_4$: 377.48; found: 377.23.



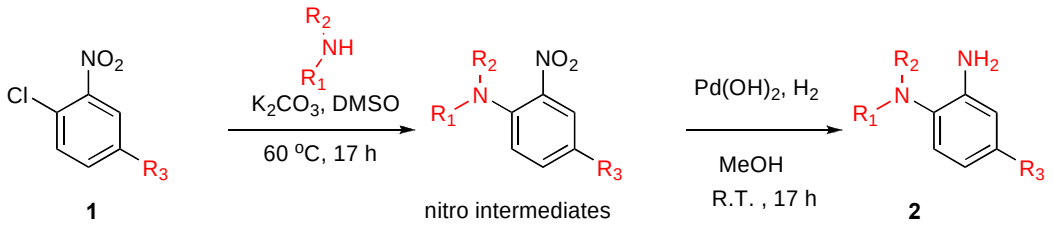
Synthesis of ethyl 3-amino-4-(4-aminocyclohexylamino)benzoate (SRS8-80. SI, Table3)

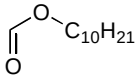
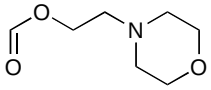
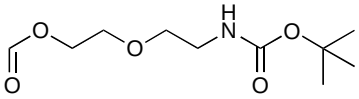
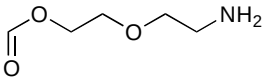
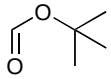
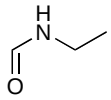
^{1}H NMR (400 MHz, MeOD) 7.45 (d, *J* = 8.3 Hz, 1H), 7.38 (s, 1H), 6.62 (d, *J* = 8.4 Hz, 1H), 4.27 (d, *J* = 7.1 Hz, 2H), 3.41 (s, 1H), 2.18 (s, 2H), 2.04 (s, 2H), 1.37 (t, *J* = 7.1 Hz, 4H), 1.31 (s, 3H); ^{13}C NMR (100 MHz, MeOD) δ 167.8, 141.0, 132.7, 122.8, 117.5, 116.79, 108.8, 59.9, 50.4, 49.7, 31.78, 30.82, 13.33; HRMS (FAB) calculated for $C_{15}H_{23}N_3O_2$: 277.36; found: 277.18.



Synthesis of ethyl 3-amino-4-(4,4-difluorocyclohexylamino)benzoate (SRS8-52. SI, Table3)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (s, 1H), 7.46 (s, 1H), 6.63 (s, 1H), 4.37 (s, 2H), 3.95 (s, 1H) 3.28 (s, 1H), 2.16 (s, 4H), 1.96 (d, *J* = 13.7 Hz, 2H), 1.68 (s, 2H), 1.37 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.8, 141.5, 132.1, 124.3, 119.6, 119.0, 109.9, 60.3, 49.2, 31.9, 30.9, 28.7, 14.4; ^{19}F ($CDCl_3$) -94.9, -98.5; HRMS (FAB) calculated for $C_{15}H_{20}F_2N_2O_2$: 298.33; found: 298.15.

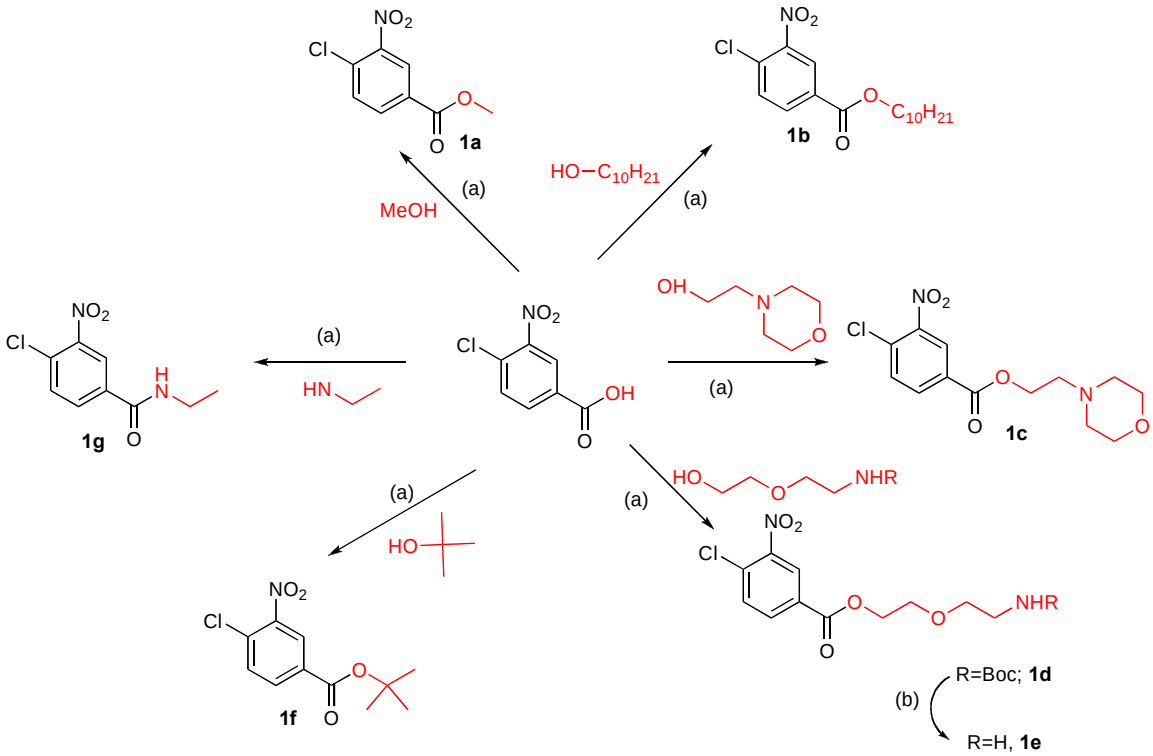


Entry	R_1	R_2	R_3	Name (Yield)	EC50 (nM)	Log P
1		H	H	SRS8-75 (86%)	330	2.9
2		H		SRS8-61 (90%)	150	2.8
3		H		SRS12-29 (92%)	76	6.9
4		H		SRS12-47 (81%)	660	2.3
5		H		SRS8-37 (98%)	160	3.2
6		H		SRS8-43 (89%)	420	1.6
7		H		SRS8-87 (87%)	90	4.9
8		H		SRS9-11 (66%)	950	3.4

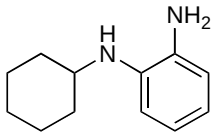
***SI*, Table 4**: Synthetic scheme of Ferrostatin-1 analogs with various R_3 substitutions.

Preparation of the nitrobenzene bearing various esters (Table 4, entries 2-7) and amide (Table 4, entries 8)

A representative example is the coupling reaction between the 4-chloro-3-nitrobenzoic acid and the 4-(2-hydroxyethyl)morpholine (**SI, Scheme 1, 1c**). To the 4-chloro-3-nitrobenzoic acid (500 mg, 2.487 mmol) in DCM, under nitrogen, was added the 4-(2-hydroxyethyl)morpholine (332 μL, 2.736 mmol) and DMAP (91 mg, 0.467 mmol). The mixture was cooled to 0 ^{o}C before the addition of the *N,N'*-Dicyclohexylcarbodiimide (DCC) (615 mg, 2.985 mmol). Then the reaction mixture was left at room temperature for 17h. The precipitate was filtered over celite and the organic solvent was evaporated. The residue was purified by flash-column chromatography on silica gel to provide the desired 2-morpholinoethyl 4-chloro-3-nitrobenzoate (**SI, Scheme 1, 1c**).

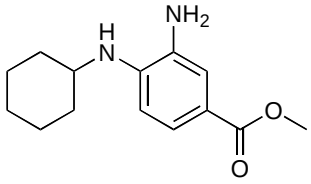


***SI*, Scheme 1**: Synthesis of 4-chloro-3-nitrobenzene analogs bearing various esters and amide (**1a-g**). (a) DCC, DMAP, DCM, r.t., 17h. (b) HCl in Dioxane 4.0 M, r.t. 17h.



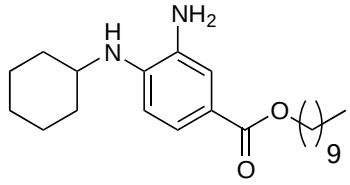
Synthesis of N1-cyclohexylbenzene-1,2-diamine (SRS8-75. SI, Table4)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.91 – 6.81 (m, 1H), 6.74 (dt, *J* = 14.4, 6.6 Hz, 3H), 3.30 (s, 1H), 2.12 (d, *J* = 10.5 Hz, 2H), 1.83 (d, *J* = 12.7 Hz, 2H), 1.72 (d, *J* = 9.0 Hz, 1H), 1.53-1.37 (m, 2H), 1.36-1.19 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 136.9, 134.5, 120.7, 118.3, 117.0, 112.8, 51.9, 33.8, 26.2, 25.2; HRMS (FAB) calculated for $C_{12}H_{18}N_2$: 190.28; found 190.15.



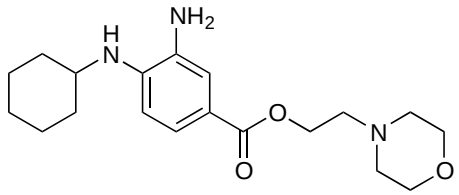
Synthesis of methyl 3-amino-4-(cyclohexylamino)benzoate (SRS8-61. SI, Table4)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.64–6.57 (m, 1H), 7.42 (s, 1H), 6.64–6.57 (m, 1H), 3.35 (s, 1H), 2.09 (d, *J* = 11.4 Hz, 2H), 1.80 (d, *J* = 12.6 Hz, 2H), 1.69 (dt, *J* = 12.9, 4.2 Hz, 1H), 1.42 (d, *J* = 11.5 Hz, 2H), 1.31–1.20 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.5, 142.2, 131.7, 124.4, 118.6, 118.0, 109.5, 51.6, 51.3, 33.3, 25.8, 24.9; HRMS (FAB) calculated for $C_{14}H_{20}N_2O_2$: 248.32; found: 248.15.



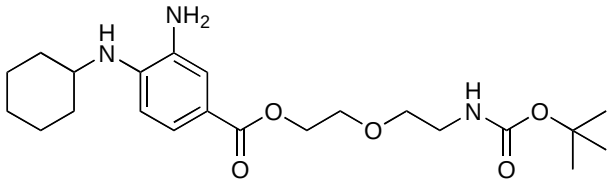
Synthesis of decyl 3-amino-4-(cyclohexylamino)benzoate (SRS12-29. SI, Table4)

^{1}H NMR ($CDCl_3$, 400MHz) δ 7.59 (d, *J* = 8.4 Hz, 1H), 7.42 (s, 1H), 6.61 (d, *J* = 8.4 Hz, 1H), 4.27-4.24 (m, 2H), 3.35 (b, 1H), 2.10-2.07 (m, 2H), 2.78-1.72 (m, 4H), 1.43-1.29 (m, 22H), 0.90-0.88 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.0, 141.2, 132.2, 124.2, 119.2, 118.7, 110.4, 64.5, 51.8, 33.1, 31.9, 29.55, 29.3, 28.9, 26.1, 25.8, 24.9, 22.7, 14.1; HRMS (FAB) calculated for $C_{23}H_{38}N_2O_2$: 374.56; found: 374.47.



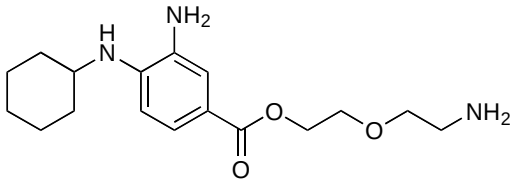
Synthesis of 2-morpholinoethyl 3-amino-4-(cyclohexylamino)benzoate (SRS12-47. SI, Table4)

^{1}H NMR ($CDCl_3$, 400MHz) δ 7.56 (d, *J* = 5.0 Hz, 1H), 7.39 (d, *J* = 2.0 Hz, 1H), 6.59 (d, *J* = 8.4 Hz, 1H), 4.40 (t, *J* = 6.0 Hz, 2H), 3.90 (b, NH), 3.73-3.71 (m, 4H), 3.33 (b, 1H), 3.19 (b, NH_2), 2.76 (t, *J* = 6.0 Hz, 2H), 2.59-2.56 (m, 4H), 2.09-2.04 (m, 2H), 1.82-1.17 (m, 8H); 13C NMR (100 MHz, $CDCl_3$) δ 166.8, 142.3, 131.8, 124.5, 118.6, 117.9, 109.4, 67.0, 61.8, 57.3, 53.9, 51.3, 49.1, 34.0, 33.3, 25.8, 24.9; HRMS (FAB) calculated for $C_{19}H_{29}N_3O_3$: 347.45; found: 348.31.



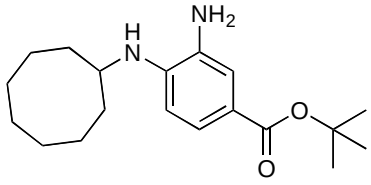
Synthesis of 2-(2-(*tert*-butoxycarbonylamino)ethoxy)ethyl 3-amino-4-(cyclohexylamino)benzoate (SRS8-37. SI, Table4)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (d, J = 10.2 Hz, 1H), 7.42 (s, 1H), 6.59 (d, J = 8.4 Hz, 1H), 4.41 (s, 2H), 3.77 (s, 2H), 3.58 (d, J = 4.7 Hz, 2H), 3.33 (s, 3H), 2.07 (d, J = 11.6 Hz, 2H), 1.78 (d, J = 12.6 Hz, 2H), 1.73–1.64 (m, 1H), 1.44 (s, 12H), 1.24 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.9, 156.0, 142.4, 131.7, 124.6, 118.7, 117.8, 109.5, 79.3, 70.2, 69.2, 63.3, 51.3, 40.4, 33.3, 28.4, 25.9, 24.9; LC/MS (APCI+, M+1) 421.69



Synthesis of 2-(2-aminoethoxy)ethyl 3-amino-4-(cyclohexylamino)benzoate (SRS8-43. SI, Table4)

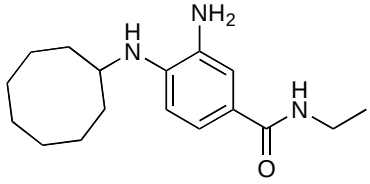
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 (d, J = 8.0 Hz, 1H), 7.39 (s, 1H), 6.70 (d, J = 8.0 Hz, 1H), 4.48–4.41 (m, 2H), 3.95 (s, 4H), 3.84–3.80 (m, 2H), 3.75–3.68 (m, 2H), 3.28 (s, 1H), 3.10–3.00 (m, 2H), 2.07 (d, J = 11.1 Hz, 2H), 1.79 (d, J = 9.5 Hz, 2H), 1.69 (d, J = 8.6 Hz, 1H), 1.41 (d, J = 11.7 Hz, 2H), 1.29–1.18 (m, 3H); LC/MS (APCI+, M+1) 321.69



Synthesis of *tert*-butyl 3-amino-4-(cyclooctylamino)benzoate (SRS8-87. SI, Table4)

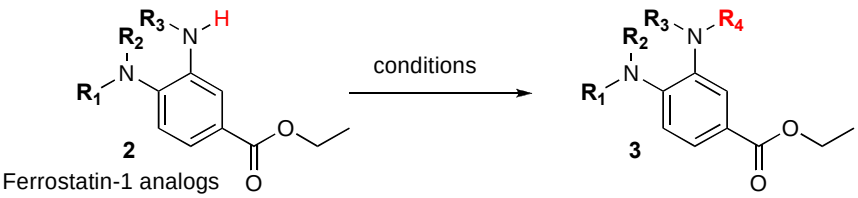
^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.53 (dd, *J* = 8.3, 1.9 Hz, 1H), 7.35 (d, *J* = 1.9 Hz, 1H), 6.51 (d, *J* = 8.4 Hz, 1H), 3.20 (s, 1H), 1.91 (s, 2H), 1.75 (d, *J* = 7.7 Hz, 2H), 1.56 (s, 19H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.4, 141.6, 132.0, 124.0, 120.0,

118.3, 109.7, 79.7, 52.3, 32.7, 28.4, 27.0, 26.0, 24.1; HRMS (FAB) calculated for $C_{19}H_{30}N_2O_2$: 318.45; found: 318.23.

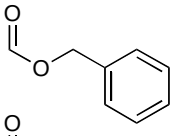
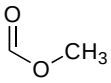
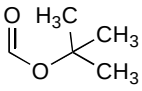
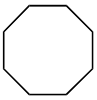


Synthesis of 3-amino-4-(cyclooctylamino)-N-ethylbenzamide (SRS9-11. SI, Table4)

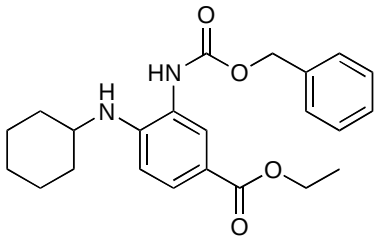
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 (d, *J* = 2.1 Hz, 1H), 7.20 (dd, *J* = 8.3, 2.1 Hz, 1H), 6.52 (d, *J* = 8.3 Hz, 1H), 5.93 (s, 1H), 3.54 (s, 1H), 3.48--3.44 (m, 2H), 1.90 (s, 2H), 1.76 (d, *J* = 8.1 Hz, 1H), 1.60 (s, 11H), 1.23 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.5, 140.3, 132.9, 123.2, 119.9, 116.4, 110.2, 52.5, 34.7, 32.8, 27.0, 26.0, 24.1, 15.1; LC/MS (APCI+, M+1) 389.83



Ferrostatin-1 analogs

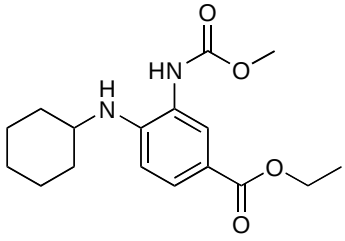
Entry / Name (Yield)	R1	R_2	R_3	R_4	EC50 (nM)	Log P
1 Ferrostatin-1 (SRS8-28; 85%)		H	H	H	**88**	3.2
2 SRS11-89 (88%)[1]		H	H		**> 10,000**	5.2
3 SRS11-97 (86%)[1]		H	H		**> 10,000**	3.4
4 SRS11-98 (70%)[1]		H	H		**> 10,000**	3.1
5 SRS8-91(95%)[2]		H	H		**> 10,000**	5.5
6 SRS8-70 (92%)		H	H	H	**69**	4.1
7 SRS9-14 (70%)[3]		H	H	CH_3	**70**	4.4
8 SRS9-01 (92%)[4]		H	CH_3	CH_3	**3000**	4.6
9 SRS8-94 (95%)	C_8H_{17}	H	H	H	**80**	4.4
10 SRS12-12 (89%)	C_8H_{17}	C_8H_{17}	H	H	**15267**	7.8

***SI*, Table 5.** EC50 and Log P of Ferrostatin-1 analogs. Conditions. [1] Addition of Fer-1 to acylchloride, alkyl- or benzyl-chloroformates (1 equiv.), DIPEA, DCM, R.T., 17 h. [2] $(Boc)_2O$ (1 equiv.), DMAP (cat.), THF, R.T., 17 h. [3] using reductive amination conditions (H_2CO (1 equiv.), $NaBH(OAc)_3$ (1.2-1.6 equiv.), DCE, R.T., 17 h). [4] MeI (2.2 equiv), K_2CO_3, DMF, 40 ^oC, 17 h.



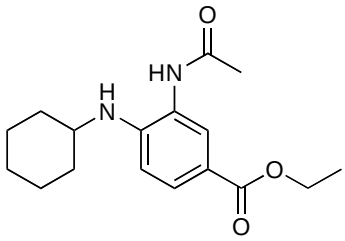
Synthesis of ethyl 3-(benzyloxycarbonylamino)-4-(cyclohexylamino)-benzoate (SRS11-89. SI, Table5)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (d, *J* = 10.3 Hz, 2H), 7.38 (s, 5H), 6.66 (d, *J* = 8.5 Hz, 1H), 4.34-4.11 (m, 4H), 3.32 (s, 1H), 1.99 (s, 2H), 1.74 (s, 2H), 1.64 (s, 1H), 1.36 (t, *J* = 7.1 Hz, 5H), 1.23-1.11 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.4, 154.6, 146.7, 136.0, 130.2, 128.6, 128.4, 121.0, 117.9, 110.6, 67.5, 60.3, 51.3, 33.0, 25.7, 24.8, 14.5; HRMS (FAB) calculated for $C_{23}H_{28}N_2O_4$: 396.48; found: 396.20.



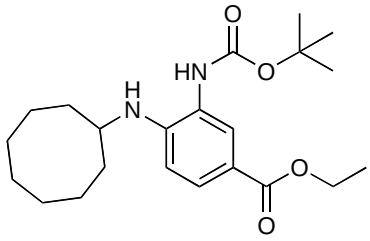
Synthesis of ethyl 4-(cyclohexylamino)-3-(methoxycarbonylamino)benzoate (SRS11-97. SI, Table5)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (s, 2H), 6.67 (d, J = 9.0 Hz, 1H), 6.04 (s, 1H), 4.32 (q, J = 7.1 Hz, 2H), 3.78 (s, 2H), 3.36 (s, 1H), 2.06 (d, J = 12.4 Hz, 2H), 1.79 (d, J = 13.4 Hz, 2H), 1.68 (d, J = 12.6 Hz, 1H), 1.37 (t, J = 7.1 Hz, 5H), 1.29–1.18 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.4, 155.9, 146.7, 130.2, 129.1, 121.1, 117.9, 110.5, 60.3, 52.8, 51.3, 33.1, 25.7, 24.8, 14.4; HRMS (FAB) calculated for $C_{17}H_{24}N_2O_4$: 320.38; found: 320.17.



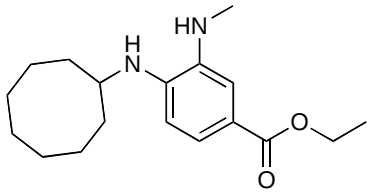
Synthesis of ethyl 4-(cyclohexylamino)-3-ethanamidobenzoate (SRS11-98. SI, Table5)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (d, J = 8.6 Hz, 1H), 7.75 (s, 1H), 6.68 (d, J = 8.6 Hz, 1H), 4.57–4.46 (m, 1H), 4.33 (d, J = 9.8 Hz, 2H), 3.35 (s, 1H), 2.22 (s, 3H), 2.04 (d, J = 12.1 Hz, 2H), 1.88 (s, 1H), 1.79 (s, 3H), 1.38 (s, 4H), 1.24 (d, J = 8.6 Hz, 3H); HRMS (FAB) calculated for $C_{17}H_{24}N_2O_3$: 304.38; found: 304.18.



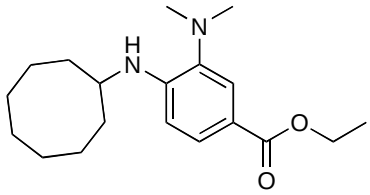
Synthesis of ethyl 3-(*tert*-butoxycarbonylamino)-4-(cyclooctylamino)-benzoate (SRS8-91. SI, Table5)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.54 (d, J = 1.5 Hz, 1H), 7.93 (dd, J = 8.4, 1.7 Hz, 1H), 7.08 (d, J = 8.4 Hz, 1H), 4.44–4.35 (m, 2H), 2.23 (q, J = 10.7 Hz, 2H), 1.86 (d, J = 9.0 Hz, 4H), 1.70 (s, 8H), 1.64–1.56 (m, 6H), 1.42–1.38 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.4, 150.5, 148.6, 132.0, 125.9, 123.9, 115.8, 108.6, 85.0, 61.0, 31.9, 28.1, 27.8, 27.0, 26.2, 26.0, 25.3, 14.4; HRMS (FAB) calculated for C22H34N2O4: 390.52;



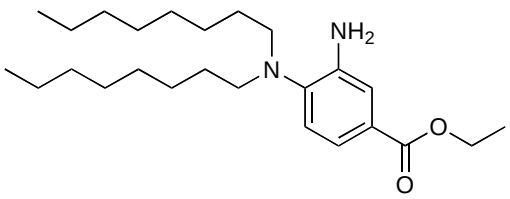
Synthesis of ethyl 4-(cyclooctylamino)-3-(methylamino)benzoate (SRS9-14. SI, Table5)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (d, J = 8.0 Hz, 1H), 7.37 (s, 1H), 6.54 (d, J = 8.3 Hz, 1H), 4.34 (q, J = 7.1 Hz, 2H), 3.56 (d, J = 9.7 Hz, 1H), 2.90 (s, 3H), 1.94–1.86 (m, 2H), 1.80 1.72 (m, 2H), 1.67–1.53 (m, 10H), 1.38 (t, J = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.5, 141.4, 136.3, 123.0, 119.0, 112.6, 109.2, 60.2, 52.3, 32.8, 31.4, 27.0, 26.0, 24.1, 14.5; HRMS (FAB) calculated for $C_{18}H_{28}N_2O_2$: 304.43; found: 304.21.



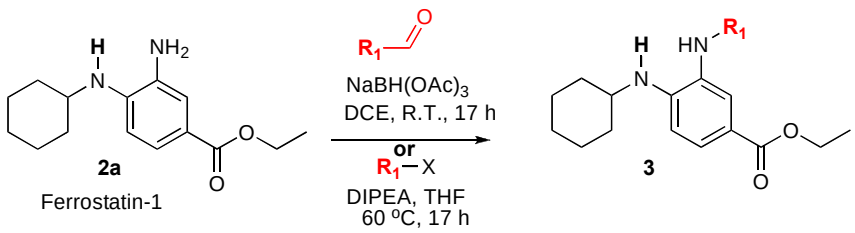
Synthesis of ethyl 4-(cyclooctylamino)-3-(dimethylamino)benzoate (SRS9-01. SI, Table5)

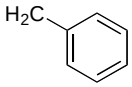
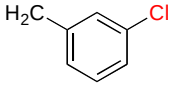
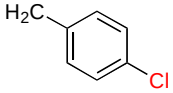
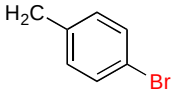
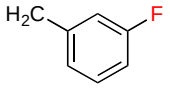
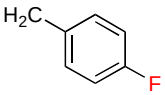
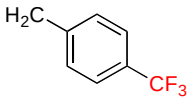
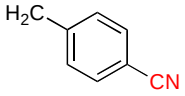
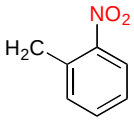
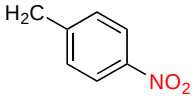
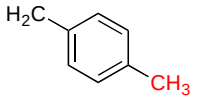
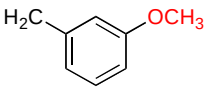
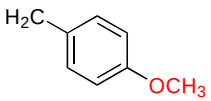
^{1}H NMR (100 MHz, $CDCl_3$) δ 7.78-7.70 (m, 2H), 6.50 (d, *J* = 8.5 Hz, 1H), 4.33 (q, *J* = 7.1 Hz, 2H), 3.58 (s, 1H), 2.64 (s, 6H), 1.92 (s, 2H), 1.63 (d, *J* = 23.7 Hz, 12H), 1.38 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.2, 146.0, 139.3, 128.0, 121.0, 117.0, 109.0, 60.1, 52.0, 44.0, 32.5, 27.2, 25.8, 24.0, 14.5; HRMS (FAB) calculated for $C_{19}H_{30}N_2O_2$: 318.45; found 318.23.



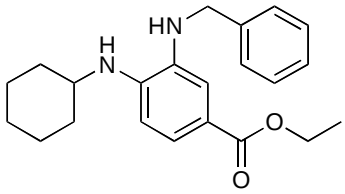
Synthesis of ethyl 3-amino-4-(dioctylamino)benzoate (SRS12-12. SI, Table5)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 (s, 2H), 7.03 (d, J = 8.7 Hz, 1H), 4.34 (d, J = 7.1 Hz, 2H), 2.95 (s, 3H), 1.38 (d, J = 7.1 Hz, 6H), 1.24 (s, 22H), 0.87 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.9, 142.8, 142.3, 126.0, 121.8, 119.7, 116.1, 60.6, 52.8, 31.8, 29.4, 29.3, 27.2, 22.6, 14.4, 14.1; LC/MS (APCI+, M+1) 403.96



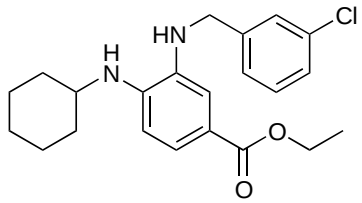
Entry	R1	Name (Yield)	EC50 (nM)	Log P
1		SRS11-92 (98%)	6	5.3
2		SRS12-58 (90%)	41	5.9
3		SRS12-49 (91%)	371	5.9
4		SRS12-35 (89%)	44	6.1
5		SRS12-57 (85%)	100	5.5
6		SRS12-33 (85%)	50	5.4
7		SRS12-48 (86%)	46	6.2
8		SRS12-50 (92%)	100	4.9
9		SRS12-71 (94%)	126	5.2
10		SRS12-36 (90%)	36	5.2
11		SRS12-34 (96%)	40	5.6
12		SRS12-69 (85%)	58	5.3
13		SRS12-43 (86%)	69	5.3

***SI*, Table 6.** EC50 and Log P of Ferrostatin-1 analogs.



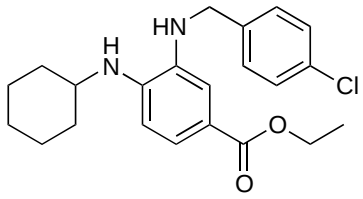
Synthesis of ethyl 3-(benzylamino)-4-(cyclohexylamino)benzoate (SRS11-92. SI, Table6)

^{1}H NMR ($CDCl_3$, 400MHz) δ 7.69-7.61 (m, 1H), 7.53-7.30 m, 6H), 6.71-6.62 (m, 1H), 4.40-4.28 (m, 4H), 3.97 (b, NH), 3.36 (b, 1H), 3.23 (m, NH), 2.17-2.03 (m, 2H), 1.87-1.76 (m, 2H), 1.75-1.66 (m, 1H), 1.47-1.34 (m, 5H), 1.32-1.19 (m, 3H); ^{13}C NMR (100 MHz, CDCl3) δ 167.3, 142.0, 139.2, 134.8, 128.2, 127.44, 123.7, 118.8, 114.7, 109.3, 60.2, 51.4, 49.5, 33.3, 25.9, 25.0 14.5; LC/MS (APCI+, M+1) 353.06



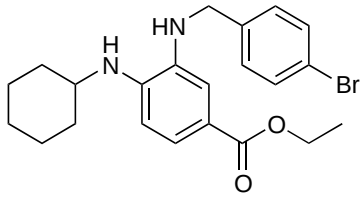
Synthesis of ethyl 3-(3-chlorobenzylamino)-4-(cyclohexylamino)benzoate (SRS12-58. SI, Table6)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.63 (d, *J* = 4.2 Hz, 1H), 7.43 (s, 2H), 7.30-7.28 (m, 3H), 6.65 (d, *J* = 8.4 Hz, 1H), 4.35-4.30 (m, 4H), 3.37 (b, 1H), 2.14-2.05 (m, 2H), 1.87-1.76 (m, 2H), 1.75-1.67 (m, 1H), 1.47-1.36 (m, 5H), 1.29-1.24 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.2, 142.1, 141.3, 134.5, 134.4, 129.9, 128.1, 127.6, 126.2, 123.9, 118.9, 115.0, 110.0, 60.2, 51.5, 48.9, 33.3, 25.9, 24.9, 14.5; HRMS (FAB) calculated for $C_{22}H_{27}ClN_2O_2$: 386.91; found: 386.18.



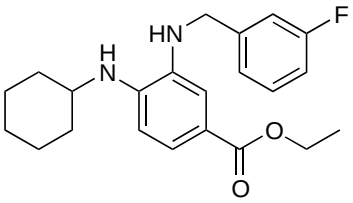
Synthesis of ethyl 3-(4-chlorobenzylamino)-4-(cyclohexylamino)benzoate (SRS12-49. SI, Table6)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.60 (d, *J* = 8.0 Hz, 1H), 7.41 (s, 1H), 7.34 (s, 4H), 6.64 (d, *J* = 8.3 Hz, 1H), 4.36-4.25 (m, 4H), 3.32 (b, 1H), 2.07-2.04 (m, 2H), 1.79-1.76 (m, 2H), 1.69-1.33 (5H), 1.26-1.20 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.2, 142.0, 137.7, 134.4, 133.2, 129.4, 128.8, 123.9, 118.9, 114.9, 109.5, 60.2, 51.4, 48.7, 33.3, 25.9, 24.9 14.5; HRMS (FAB) calculated for $C_{22}H2_7ClN_2O_2$: 386.91; found: 386.17.



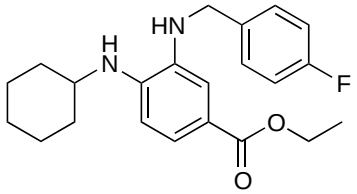
Synthesis of ethyl 3-((4-bromobenzyl)amino)-4-(cyclohexylamino)benzoate (SRS12-35. SI, Table6)

^{1}H NMR ($CDCl_3$, 400MHz,) δ 7.62 (d, *J* = 8.0 Hz, 1H), 7.50 (d, *J* = 8.4 Hz, 2H), 7.42 (s, 1H), 7.31-7.28 (m, 2H), 6.64 (d, *J* = 8.4 Hz, 1 H), 4.35-4.28 (m, 4H), 3.35 (b, 1H), 2.10-2.07 (m, 2H), 1.81-1.59 (m, 4H), 1.44-1.35 (m, 4H), 1.28-1.22 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.2, 142.0, 138.2, 134.4, 131.7, 129.74, 123.9, 121.2, 118. 9, 114.9, 109.5, 60.2, 51.4, 48.8, 33.3, 25.9, 24.9, 14.5; HRMS (FAB) calculated for $C_{22}H_{27}BrN_2O_2$: 431.37; found: 430.13.



Synthesis of ethyl 4-(cyclohexylamino)-3-(3-fluorobenzylamino)benzoate (SRS12-57. SI, Table6)

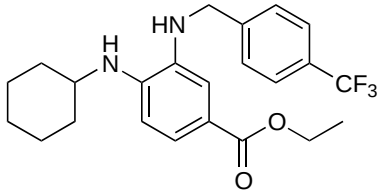
^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.62 (d, *J* = 8.4 Hz, 1H), 7.43 (s, 1H), 7.34-7.31 (m, 1H), 7.21-7.20 (m, 1H), 7.16-7.13 (m, 1H), 7.03-6.99 (m, 1H), 6.65 (d, *J* = 8.4 Hz, 1H), 4.35-4.30 (m, 4H), 3.94 (b, NH), 3.56 (b, 1H), 3.24 (b, NH), 2.15-2.03 (m, 2H). 1.88-1.75 (m, 2H), 1.76-1.66 (m, 1H), 1.49-1.33 (m, 5H). 1.32-1.19 (3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.2, 164.3, 161.9, 142.1, 134.4, 130.1, 123.9, 123.5, 123.49, 118.9, 115.0, 114.9, 114.7, 114.4, 114.2, 109.5, 60.2, 51.4, 48.9, 33.3, 25.86, 24.9, 14.5; ^{19}F ($CDCl_3$) *δ* -112.0; HRMS (FAB) calculated for $C_{22}H_{27}FN_2O_2$: 370.48; found: 370.20.



Synthesis of ethyl 4-(cyclohexylamino)-3-((4-fluorobenzyl)amino)benzoate (SRS12-33. SI, Table6)

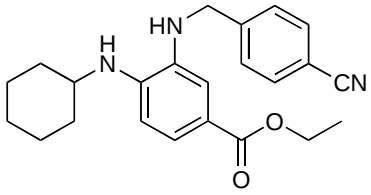
^{1}H NMR ($CDCl_3$, 400MHz) δ 7.62 (d, *J* = 8.4 Hz, 1H), 7.45 (s, 1H), 7.41-7.38 (m, 2H), 7.07 (d, *J* = 8.8 Hz, 2H), 6.65 (d, *J* = 8.0 Hz, 1H), 4.36-4.288 (m, *J* = 4H), 3.35 (b, 1H), 2.10-2.07 (m, 2H), 1.81-1.61 (m, 4H), 1.46-1.44 (m, 4H), 1.39 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, CDCl3) δ 167.3, 163.4, 161.0, 142.0, 134.5, 129.7, 123.8, 118.8, 115.4, 114.8, 109.4, 60.2, 51.4, 48.7, 33.3, 25.9, 25.0, 14.5;

^{19}F ($CDCl_3$) δ -114.3; HRMS (FAB) calculated for $C_{22}H_{27}FN_2O_2$: 370.46; found: 371.13.



Synthesis of ethyl 4-(cyclohexylamino)-3-(4-(trifluoromethyl)benzylamino)-benzoate (SRS12-48. SI, Table6)

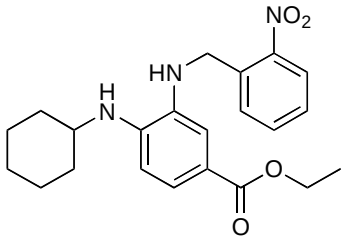
^1H NMR ($CDCl_3$, 400 MHz) δ 7.61 (td, *J* = 6.2, 2.9 Hz, 3H), 7.52 (d, *J* = 8.0 Hz, 2H), 7.40 (d, *J* = 1.9 Hz, 1H), 6.64 (d, *J* = 8.4 Hz, 1H), 4.38 (s, 2H), 4.30 (q, *J* = 7.1 Hz, 2H), 3.34 (b, 1H2.11-2.03 (m, 2H), 1.84-1.74 (m, 2H). 1.70-1.66 (m, 1H), 1.43-1.32 (m, 5H). 1.2-1.17 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.2, 143.3, 142.1, 134.2, 128.2, 125.6, 124.0, 118.9, 115.0, 109.6, 60.2, 51.4, 50.8, 48.9, 33.3, 25.8, 24.9, 14.4; ^{19}F ($CDCl_3$) δ -61.3; HRMS (FAB) calculated for $C_{23}H_{27}F_3N_2O_2$: 420.47; found: 420.22.



Synthesis of ethyl 3-(4-cyanobenzylamino)-4-(cyclohexylamino)benzoate (SRS12-50. SI, Table6)

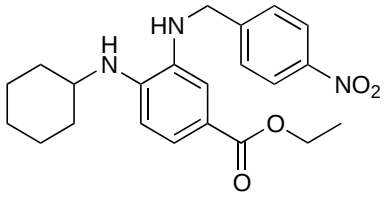
^1H NMR ($CDCl_3$, 400 MHz) δ 7.61 (d, *J* = 2.0 Hz, 2H), 7.59 (d, *J* = 1.6 Hz, 1H), 7.50 (d, *J* = 8.4 Hz, 2H), 7.33 (d, J = 1.9 Hz, 1H), 6.64 (d, *J* = 8.4 Hz, 1H), 4.40 (s, 2H), 4.29 (q, J = 7.1 Hz, 2H), 3.34 (b, 1H), 2.09-2.04 (m, 2H), 1.77-1.32 (m, 8H), 1.27-1.21 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.1, 144.8, 142.1, 133.9,

132.4, 128.4, 124.1, 119.0, 118.8, 115.0, 111.2, 110.0, 60.2, 51.4, 48.8, 33.3, 25.9, 24.9, 14.4: HRMS (FAB) calculated for C23H27N3O2: 377.48; found: 377.39.



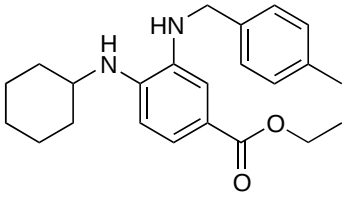
Synthesis of ethyl 4-(cyclohexylamino)-3-(2-nitrobenzylamino)benzoate (SRS12-71. SI, Table6)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.99 (dd, J = 8.1, 1.3 Hz, 1H), 7.62–7.58 (m, 1H), 7.57–7.54 (m, 1H), 7.52 (d, J = 1.5 Hz, 1H), 7.44 (ddd, J = 8.1, 7.1, 1.7 Hz, 1H), 7.32 (d, J = 1.9 Hz, 1H), 6.65–6.58 (m, 1H), 4.58 (s, 2H), 4.28 (q, J = 7.1 Hz, 2H), 4.08 (s, NH), 3.53 (s, NH), 3.39-3.27 (m, 1H), 2.06 (dd, J = 12.5, 4.0 Hz, 2H), 2.11-2.02 (m, 2H), 1.82-1.73 (m, 2H), 1.71-1.62 (m, 2H), 1.39-1.31 (m, 4H), 1.28-1.22 (m, 3H), 1.27–1.16 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.1, 149.1, 142.9, 134.5, 133.6, 133.3, 131.1, 128.4, 124.9, 124.6, 118.6, 116.6, 109.7, 60.17, 51.42, 47.0, 33.2, 25.9, 24.9, 14.4; HRMS (FAB) calculated for $C_{22}H_{27}N_3O_4$: 397.47; found: 397.20.



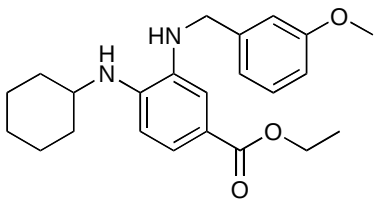
Synthesis of ethyl 4-(cyclohexylamino)-3-((4-nitrobenzyl)amino)benzoate (SRS12-36. SI, Table6)

^{1}H NMR ($CDCl_3$, 400MHz) δ 8.24 (d, *J* = 8.4 Hz, 2H), 7.62-7.7.55 (m, 3H), 7.34-7.2 (m, 1H), 6.66 (d, 8.4 Hz, 1H), 4.45 (s, 2H), 4.32-4.27 (m, 2H), 3.37 (b, 1H), 2.10-2.03 (m, 2H), 1.82-1.67 (m, 4H), 1.44-1.33 (m, 4H), 1.29-1.24 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.1, 146.8, 142.0, 133.8, 129.5, 128.4, 124.1, 123.9, 118.9, 114.9, 109.7, 60.3, 51.5, 48.5, 33.3, 25.9, 24.9, 14.5; LC/MS (APCI+, M+1) 397.96



Synthesis of ethyl 4-(cyclohexylamino)-3-((4-methylbenzyl)amino)benzoate (SRS12-34. SI, Table6)

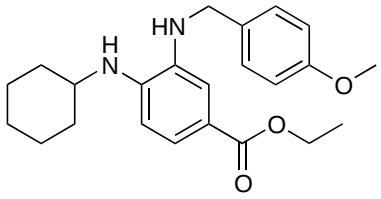
^{1}H NMR ($CDCl_3$, 400MHz) δ 7.62 (d, *J* = 8.0 Hz, 1H), 7.49 (s, 1H), 7.33 (d, *J* = 8.0 Hz, 2H), 7.20 (d, *J* = 7.6 Hz, 2H), 6.66 (d, *J* = 8.4 Hz, 1 H), 4.33 (q, *J* = 6.8, 2H), 4.27 (s, 2H), 3.35 (b, 1H), 2.39 (s, 3H), 2.13-2.0 (m, 2H), 1.84-1.74 (m, 2H), 1.73-1.64 (m, 1H), 1.41-1.36 (m, 4H), 1.28-1.21 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.3, 141.8, 137.1, 136.1, 134.8, 129.3, 128.2, 123.6, 118.9, 114. 7, 109.3, 60.2, 51.5, 49.25, 33.3, 25.9, 25.0, 21.1, 14.5; HRMS (FAB) calculated for $C_{23}H_{30}N_2O_2$: 366.50; found: 366.23.



Synthesis of ethyl 4-(cyclohexylamino)-3-(4-(methoxycarbonyl)-

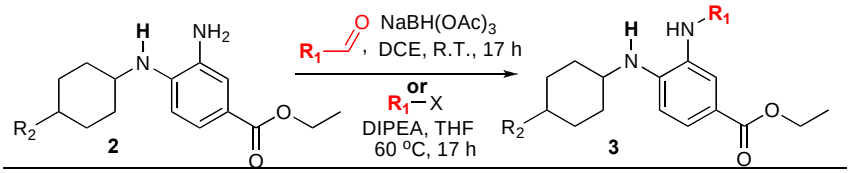
benzylamino)benzoate (SRS12-69. SI, Table6)

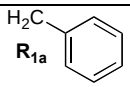
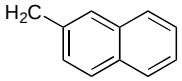
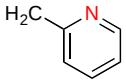
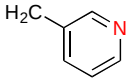
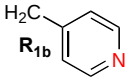
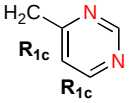
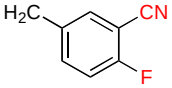
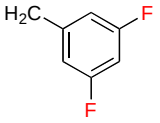
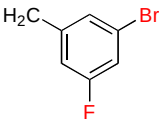
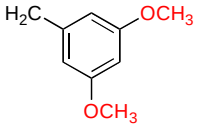
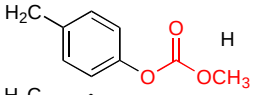
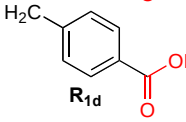
^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.60 (ddd, J = 8.4, 1.9, 1.0 Hz, 1H), 7.45 (d, J = 1.8 Hz, 1H), 7.32–7.27 (m, 1H), 7.04–6.95 (m, 2H), 6.89–6.81 (m, 1H), 6.62 (dd, J = 8.7, 1.3 Hz, 1H), 4.31 (tdd, J = 7.2, 6.7, 1.2 Hz, 2H), 4.27 (s, 2H), 3.90 (s, NH), 3.82 (d, J = 1.1 Hz, 3H), 3.33 (s, 1H), 2.09-2.02 (m, 2H), 1.82–1.73 (m, 2H), 1.71–1.63 (m, 1H), 1.45-1.32 (m, 5H), 1.26-1.17 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.3, 159.9, 141.9, 140.8, 134.7, 129.7, 123.7, 120.5, 118.9, 114.8, 113.8, 112.8, 109.4, 60.2, 55.2, 49.5, 33.3, 25.9, 25.0, 14.5; HRMS (FAB) calculated for $C_{23}H_{30}N_2O_3$: 382.50; found: 382.37.



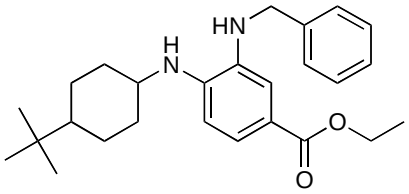
Synthesis of ethyl 4-(cyclohexylamino)-3-((4-methoxybenzyl)amino)-benzoate (SRS12-43. SI, Table6)

^{1}H NMR ($CDCl_3$, 400MHz) δ 7.63 (d, *J* = 8.4 Hz, 1H), 7.49 (s, 1H), 7.37 (d, *J* = 8.4 Hz, 2H), 6.94 (d, *J* = 8.8 Hz, 2H), 6.65 (d, *J* = 8.4 Hz, 1 H), 4.5 (q, *J* = 7.2, 2H), 4.26 (s, 2H), 3.85 (s, 3H), 3.35 (b, 1H), 2.10-2.07 (m, 2H), 1.82-1.60 (m, 4H), 1.47-1.33 (m, 4H), 1.30-1.27 (m, 3H); LC/MS (APCI+, *M+1*) 282.19.



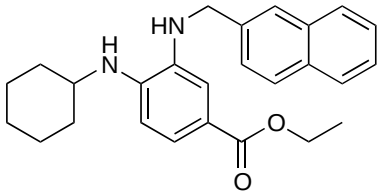
Entry	R_1	R_2	Name (Yield)	EC50 (nM)	Log P
1		H	SRS11-92 (98%)	6	5.3
2	R_{1a}	*tert*-butyl	SRS13-29 (95%)	83	6.8
3		H	SRS12-51 (91%)	58	6.5
4		H	SRS12-46 (85%)	33	4.0
5		H	SRS13-12 (87%)	48	4.0
6		H	SRS12-45 (90%)	25	4.0
7	R_{1b}	*tert*-butyl	SRS13-30 (89%)	114	5.6
8		H	SRS13-35 (92%)	27	2.9
9	R_{1c}	*tert*-butyl	SRS13-37 (88%)	15	4.4
10		H	SRS12-54 (85%)	159	5.1
11		H	SRS12-59 (86%)	96	5.7
12		H	SRS12-52 (89%)	105	6.3
13		H	SRS12-53 (85%)	41	5.3
14		H	4MO43 (85%)	47	5.2
15		H	SRS12-80; R_2= CH_3 (86%)	33	5.2
16	R_{1d}	H	SRS12-84; R_2= CH_2CH_3 (88%)	53	5.6

***SI*, Table 7.** EC50 of Ferrostatin-1 analogs.



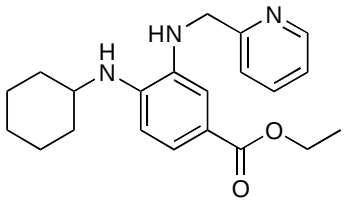
Synthesis of single isomer of ethyl 3-(benzylamino)-4-(4-*tert*-butylcyclohexylamino)benzoate (SRS13-29. SI, Table7)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.62 (d, J = 8.3, 1H), 7.48-7.32 (m, 6H), 6.65 (d, J = 8.3 Hz, 1H), 4.35-4.29 (m, 4H), 3.36 (b, 1H), 2.19 (s, 2H), 1.86 (s, 2H), 1.38 (t, J = 7.1, 0.8 Hz, 3H), 1.22-1.15 (m, 4H), 1.09-1.03 (m, 1H), 0.90 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.3, 141.9, 139.2, 134.8, 128.7, 128.3, 127.5, 123.6, 118.8, 114.5, 109.3, 60.2, 52.1, 49.5, 47.6, 33.8, 32.4, 27.6, 26.2, 14.5; HRMS (FAB) calculated for $C_{26}H_{36}N_2O_2$: 408.58; found: 408.28.



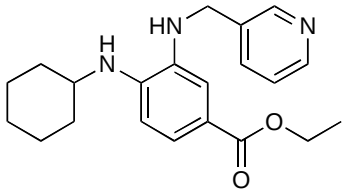
Synthesis of ethyl 4-(cyclohexylamino)-3-(naphthalen-2-ylmethylamino)-benzoate (SRS12-51. SI, Table7)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.87-7.82 (m, 4H), 7.63-7.60 (m, 1H), 7.55 (d, *J* = 5.0 Hz, 1H), 7.51 (s, 1H), 7.50-7.48 (m, 2H), 6.64 (d, *J* = 8.4 Hz, 1H), 4.46 (s, 2H), 4.31 (q, *J* = 7.1 Hz, 2H), 3.93 (s, NH,) 3.34 (b, 1H), 3.24 (b, NH), 2.09-2.03 (m, 2H), 1.79-1.63 (m, 3H), 1.45-1.33 (m, 5H), 1.27-1.19 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.3, 142.0, 136.7, 134.8, 133.5, 132.86, 128.4, 127.8, 127.7, 126.7, 126.4, 126.2, 125.9, 123.8, 118.9, 114.8, 109.4, 60.2, 51.4, 49.7, 33.3, 25.9, 25.0, 14.5; HRMS (FAB) calculated for $C_{26}H_{30}N_2O_2$: 402.53; found: 402.23.



Synthesis of ethyl 4-(cyclohexylamino)-3-(pyridin-2-ylmethylamino)-benzoate (SRS12-46. SI, Table7)

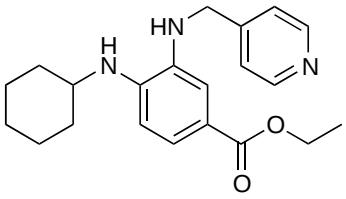
^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.62 (d, *J* = 4.4 Hz, 1H), 7.71-7.67 (m, 1H), 7.61-7.59 (m, 1H), 7.42 (s, 1H), 7.34 (d, *J* = 8.0 Hz, 1H), 7.28 (s, 1H), 7.24-7.21 (m, 1H), 6.64 (d, *J* = 8.4 Hz, 1H), 4.45 (s, 2H), 4.32 (q, J = 7.2, 2H), 3.36 (b, 1H), 2.12-2.09 (m, 2H), 1.85-1.66 (m, 3H), 1.46-1.34 (m, 5H), 1.32-1.24 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.3, 158.1, 149.2, 142.1, 136.7, 134.6, 123.6, 122.3, 122.2, 118.6, 114.7, 109.2, 51.4, 50.1, 33.3, 26.1, 25.9, 25.0, 14.5; HRMS (FAB) calculated for $C_{21}H_{27}N_3O_2$: 353.46; found: 353.21.



Synthesis of ethyl 4-(cyclohexylamino)-3-(pyridin-3-ylmethylamino)-benzoate (SRS13-12. SI, Table7)

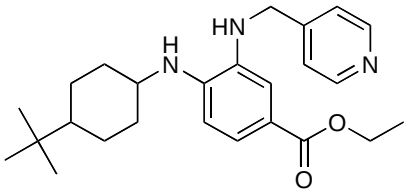
^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.70 (d, J = 2.3Hz, 1H), 8.59-8.56 (m, 1H), 7.75 (d, J = 7.6 Hz, 1H), 7.64-7.53 (m, 1H), 7.44 (s, 1H), 7.37-7.26 (m, 1 H), 6.66 (d, J = 8.8 Hz, 1H), 4.36-4.31 (m, 4H) 3.39-3.33 (b, 1H), 2.13-2.01 (m, 2H), 1.87-1.75 (m, 2H), 1.71-1.66 (m, 1H), 1.43-1.33 (m, 5H), 1.30-1.20 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.2, 149.3, 148.6, 136.1, 134.0, 124.18, 123.7, 115.1, 112.3,

109.6, 60.3, 51.5, 46.9, 33.29, 31.3, 25.83, 24.9, 14.5; HRMS (FAB) calculated for $C_{21}H_{27}N_3O_2$: 353.46; found: 354.12.



Synthesis of ethyl 4-(cyclohexylamino)-3-((pyridin-4-ylmethyl)amino)-benzoate (SRS12-45. SI, Table7)

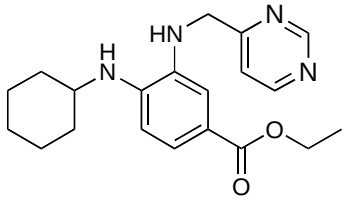
^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.50 (d, *J* = 1.2 Hz, 1H), 7.62 (d, *J* = 7.6 Hz, 1H), 7.35 (s, 1H), 6.66 (d, *J* = 8.4 Hz, 1H), 4.37-4.28 (m, 4H), 3.36 (b, 1H), 2.11-2.08 (m, 2H), 1.84-1.76 (m, 2H), 1.76-1.70 (m, 1H), 1.44-1.33 (m, 4H), 1.29-1.22 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.08, 150.04, 148.31, 142.06, 133.98, 124.05, 122.63, 118.98, 115.01, 109.73, 60.22, 51.44, 48.06, 33.34, 25.85, 24.91, 14.44: HRMS (FAB) calculated for $C_{21}H_{27}N_3O_2$: 353.46; found: 354.21.



Synthesis of ethyl 4-(4-*tert*-butylcyclohexylamino)-3-(pyridin-4-ylmethylamino)benzoate (SRS13-30. SI, Table7)

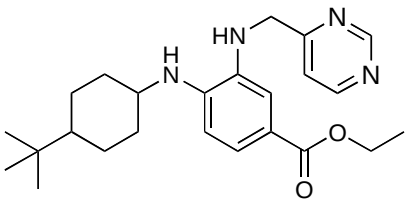
^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.63 – 8.55 (m, 2H), 7.62 (dd, J = 8.4, 1.9 Hz, 1H). 7.38 – 7.30 (m, 3H), 6.67 (d, J = 8.4 Hz, 1H), 4.37 (s, 2H), 4.31 (q, *J* = 7.1 Hz, 2H), 3.30 –3.24 (m, 1H), 2.28–2.16 (m, 2H), 1.93–1.82 (m, 2H), 1.36 (t, *J* = 7.1 Hz, 3H), 1.24-1.16 (m, 4H,), 1.11-1.05 (m, 1H), 0.91 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.1, 150.0, 148.3, 142.2, 134.0, 124.0, 122.6, 119.0, 114.9, 109.8,

60.2, 52.1, 48.1, 47.7, 33.9, 32.4, 27.6, 26.2, 14.4; HRMS (FAB) calculated for $C_{25}H_{35}N_3O_2$: 409.56; found: 409.27.



Synthesis of ethyl 4-(cyclohexylamino)-3-(pyrimidin-5-ylmethylamino)-benzoate (SRS13-35. SI, Table7)

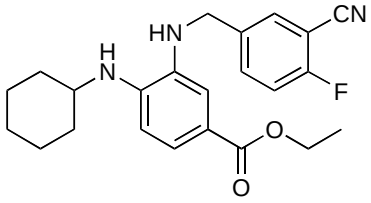
^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.17 (s, 1H), 8.63 (d, *J* = 5.1 Hz, 1H), 7.57 (d, *J* = 8.3 Hz, 1H), 7.34–7.27 (m, 2H), 6.62 (d, *J* = 8.4 Hz, 1H), 4.41 (s, 2H), 4.26 (q, *J* = 7.1 Hz, 2H), 4.15 (s, 2H), 3.34 (b, 1H), 2.07 (d, *J* = 11.8 Hz, 2H), 1.77 (d, *J* = 13.1 Hz, 2H), 1.66 (d, *J* = 12.1 Hz, 1H), 1.39–1.21 (m, 8H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.1, 158.6, 157.0, 141.9, 133.8, 123.8, 119.3, 118.7, 114.4, 109.5, 60.2, 51.4, 49.3, 33.2, 25.8, 24.9, 14.5; LC/MS (APCI+, *M+1*) 354.69



Synthesis of ethyl 4-(4-tert-butylcyclohexylamino)-3-(pyrimidin-5-ylmethylamino)benzoate (SRS13-37. SI, Table7)

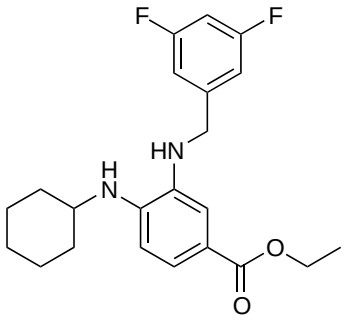
^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.23 (s, 1H), 8.74–8.64 (m, 1H), 7.62 (s, 1H), 7.41–7.31 (m, 2H), 6.66 (d, *J* = 8.4 Hz, 1H), 4.47 (s, 2H), 4.31 (d, *J* = 10.0 Hz, 2H), 4.04 (d, *J* = 16.2 Hz, 2H), 2.23 (s, 2H), 1.89 (s, 2H), 1.35 (t, *J* = 7.1 Hz, 3H), 1.21 (s, 4H), 1.09 (d, *J* = 9.0 Hz, 1H), 0.92-0.088 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$)

δ 166.9, 158.7, 157.1, 142.1, 133.8, 123.9, 119.3, 118.8, 114.6, 109.6, 60.2, 52.1, 49.3, 47.7, 33.8, 32.4, 27.6, 26.2, 14.5; LC/MS (APCI+, *M+1*) 410.19



Synthesis of ethyl 3-(3-cyano-4-fluorobenzylamino)-4-(cyclohexylamino)-benzoate (SRS12-54. SI, Table7)

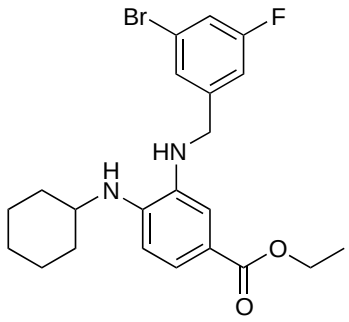
^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.70-7.65 (m, 1H), 7.62-7.60 (m, 2H), 7.31 (s, 1H), 7.20 (t, *J* = 8.8 Hz, 1H), 6.65 (d, *J* = 8.4 Hz, 1 H), 4.34-4.27 (m, 4H), 3.35 (b, 1H), 2.10-2.04 (m, 2H), 1.83-1.75 (m, 2H), 1.72-1.65 (m, 1H), 1.45-1.31 (m, 5H), 1.29-1.19 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.1, 163.7, 161.1, 142.1, 136.4, 134.4, 133.6, 132.5, 124.2, 118.9, 116.5, 115.0, 113.9, 109.8, 60.3, 51.5, 47.8, 33.4, 25.84, 24.9, 14.4; ^{19}F ($CDCl_3$) δ -107.7; HRMS (FAB) calculated for $C_{23}H_{26}FN_3O_2$: 395.47; found: 395.08.



Synthesis of ethyl 4-(cyclohexylamino)-3-(3,5-difluorobenzylamino)-benzoate (SRS12-59. SI, Table7)

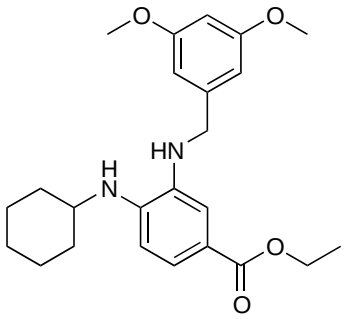
^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.62 (d, *J* = 8.0 Hz, 1H), 7.37 (s, 1H), 7.30-6.95 (d, *J* = 6.8 Hz, 2H), 6.76-6.72 (m, 1H), 6.65 (d, *J* = 8.4 Hz, 1H), 4.32-4.30 (m, 4H),

3.93 (b, NH), 3.36 (b, 1H), 3.28 (b, NH), 2.11-2.08 (m, 2H), 1.82-1.79 (m, 2H), 1.71-1.69 (m, 1H), 1.47-1.33 (m, 5H), 1.31-1.20 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.1, 164.5, 162.0, 143.4, 142.1, 134.0, 124.1, 118.9, 115.0, 110.5, 109.7, 102.7, 60.2, 51.4, 48.6, 33.3, 25.86, 24.9, 14.4; ^{19}F ($CDCl_3$) δ -108.7; HRMS (FAB) calculated for $C_{22}H_{26}F_2N_2O_2$: 388.45; found: 388.00.



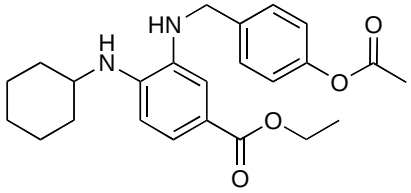
Synthesis of ethyl 3-(3-bromo-5-fluorobenzylamino)-4-(cyclohexylamino)-benzoate (SRS12-52. SI, Table7)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.60 7.61 (dd, *J* = 8.4, 1.9 Hz, 1H), 7.35 (d, *J* = 1.8 Hz, 2H), 7.17 (dt, J = 8.1, 2.1 Hz, 1H), 7.06 (d, *J* = 9.2 Hz, 1H), 6.64 (d, *J* = 8.4 Hz, 1H), 4.33-4.27 (m, 4H), 3.91 (s, NH), 3.34 (b, 1H), 3.24 (s, NH), 2.12-2.03 (m, 2H), 1.84-1.75 (m, 2H), 1.72-1.65 (m, 1H), 1.45-1.32 (m, 5H), 1.29-1.21 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.1, 164.1, 161.6, 143.5, 142.1, 134.0, 126.7, 124.1, 122.7, 118.2, 115.1, 113.8, 109.7, 60.2, 51.5, 48.4, 33.3, 25.9, 24.9, 14.4; ^{19}F ($CDCl_3$) -109.6; HRMS (FAB) calculated for $C22H_{26}BrFN_2O_2$: 449.36; found: 450.00.



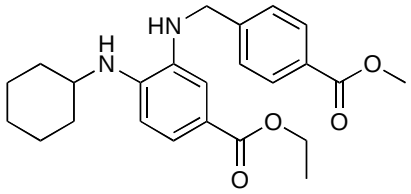
Synthesis of ethyl 4-(cyclohexylamino)-3-(3,5-dimethoxybenzylamino)-benzoate (SRS12-53. SI, Table7)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.60 (dd, *J* = 8.3, 1.9 Hz, 1H), 7.44 (d, *J* = 2.0 Hz, 1H), 6.62 (d, *J* = 8.4 Hz, 1H), 6.58 (d, *J* = 2.3 Hz, 2H), 6.42-6.4 (m, 1H), 4.31 (q, *J* = 7.1 Hz, 2H), 4.23 (s, 2H), 3.80 (s, 6H), 3.33 (b, 1H), 2.07-2.04 (m, 2H), 1.79-1.34 (m, 8H), 1.26-1.20 (m, 3H); LC/MS (APCI+, *M+1*) 412.67



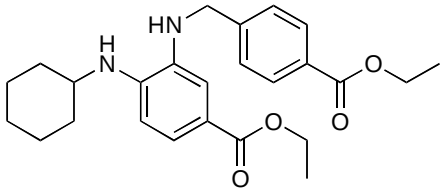
Synthesis of ethyl 4-(cyclohexylamino)-3-(4-(ethanoyloxy)benzylamino)-benzoate (4MO43. SI, Table7)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (dd, J = 8.3, 1.9 Hz, 1H), 7.46–7.40 (m, 3H), 7.09 (d, J = 8.5 Hz, 2H), 6.63 (d, J = 8.5 Hz, 1H), 4.37–4.29 (m, 2H), 4.28 (s, 2H), 3.33 (s, 1H), 2.31 (s, 3H), 2.10–2.03 (m, 2H), 1.78 (d, J = 13.4 Hz, 2H), 1.67 (d, J = 12.8 Hz, 1H), 1.41 (s, 1H), 1.36 (t, J = 7.1 Hz, 4H), 1.27–1.19 (m, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.6, 167.3, 150.0, 142.0, 136.8, 134.61, 129.4, 123.8, 121.8, 118.8, 114.6, 109.3, 60.2, 51.5, 48.9, 33.4, 25.9, 25.0, 21.1, 14.5; LC/MS (APCI+, M+1) 411.01



Synthesis of ethyl 4-(cyclohexylamino)-3-(4-(methoxycarbonyl)-benzylamino)benzoate (SRS12-80. SI, Table7)

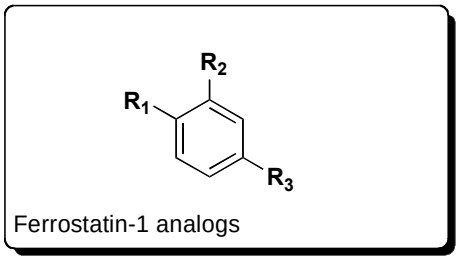
^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.07–7.99 (m, 2H), 7.60 (dd, J = 8.4, 1.9 Hz, 1H), 7.51–7.44 (m, 2H), 7.40 (d, J = 1.9 Hz, 1H), 6.64 (d, J = 8.4 Hz, 1H), 4.37 (s, 2H), 4.30 (q, J = 7.1 Hz, 2H), 3.92 (d, J = 0.6 Hz, 3H), 3.89 (d, J = 0.7 Hz, 1H), 3.39–3.29 (m, 1H), 2.11–2.02 (m, 2H), 1.83–1.73 (m, 2H), 1.72–1.62 (m, 1H), 1.48–1.30 (m, 5H), 1.30–1.16 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.2, 166.9, 144.5, 142.0, 134.3, 130.0, 128.7, 127.8, 123.9, 118.9, 114.9, 109.5, 60.2, 52.1, 51.4, 49.0, 33.3, 25.9, 24.9, 14.5; HRMS (FAB) calculated for $C_{24}H_{30}N_2O_4$: 410.51; found: 410.10.



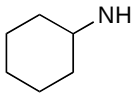
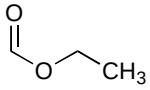
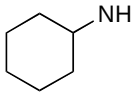
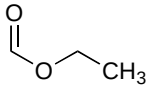
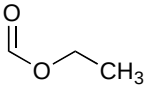
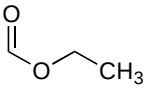
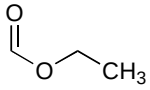
Synthesis of ethyl 4-(cyclohexylamino)-3-(4-(ethoxycarbonyl)benzylamino)-benzoate (SRS12-84. SI, Table7)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.05 (d, *J* = 8.3 Hz, 2H), 7.61 (dd, *J* = 8.3, 1.9 Hz, 1H), 7.48 (d, *J* = 8.3 Hz, 2H), 7.41 (d, *J* = 1.8 Hz, 1H), 6.66 - 6.62 (m, 1H), 4.41-4.36 (m, 4H), 4.30 (t, *J* = 7.1 Hz, 2H), 3.35 (s, 1H), 3.11 (d, *J* = 7.5 Hz, 1H), 2.07 (d, *J* = 12.8 Hz, 2H), 1.79 (d, *J* = 13.4 Hz, 2H), 1.68 (d, *J* = 13.0 Hz, 1H), 1.44-

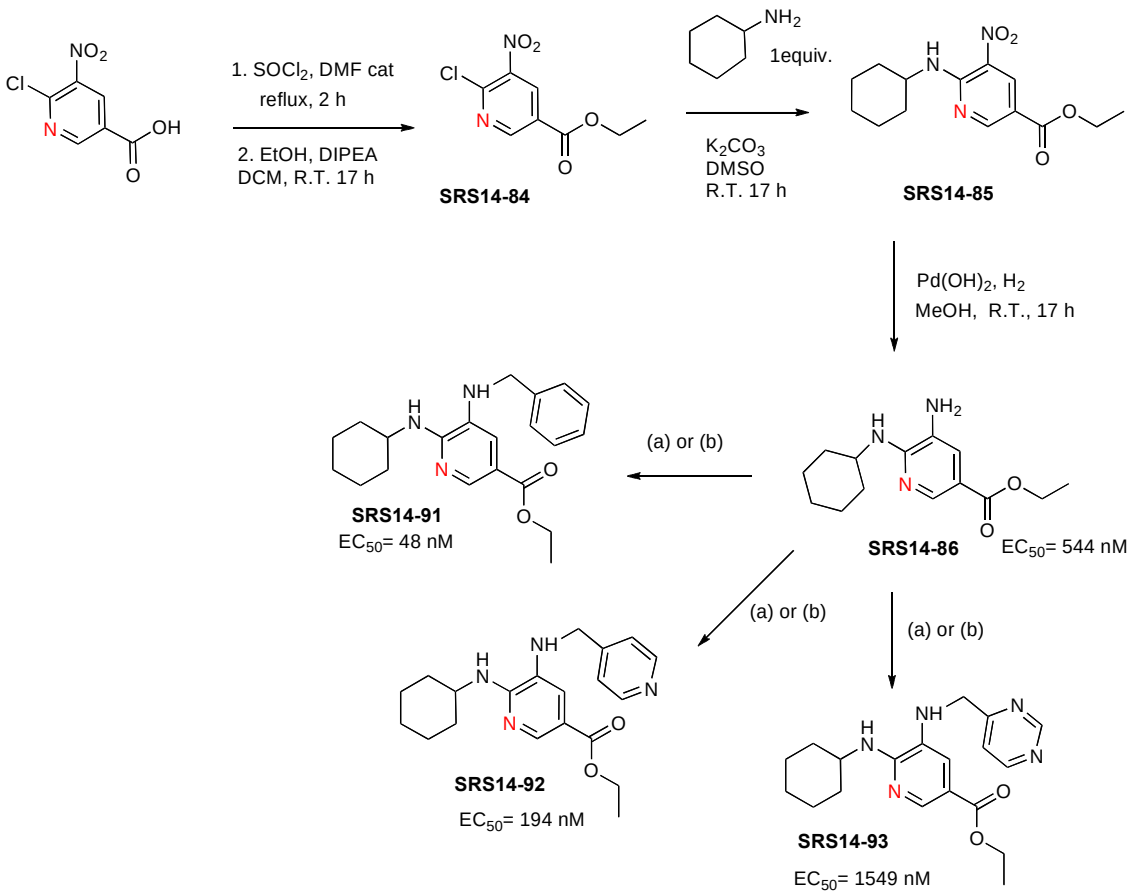
1.36 (m, 8H), 1.26 (d, *J* = 7.9 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.2, 166.4, 144.3, 142.0, 134.3, 129.9, 129.6, 128.7, 127.8, 123.9, 118.9, 114.9, 109.5, 60.9, 60.2, 57.8, 51.4, 49.0, 33.3, 25.9, 24.9, 14.5; HRMS (FAB) calculated for $C_{25}H_{32}N_2O_4$: 424.53; found: 424.12.



Ferrostatin-1 analogs

Entry	**R1**	**R_2**	**R_3**	Name (Yield)	EC50(nM)	Log P
1 Ferrostatin-1		NH_2		SRS8-28 (85%)	**88**	3.2
2		NO_2		SRS8-24 (77%)	**> 10,000**	3.8
3	Cl	NH_2		SRS8-62 (89%)	**> 10,000**	2.1
4	H	NH_2		CA_1	**> 10,000**	1.6
5	NH_2	NH_2		CA_2	**6900**	0.5

SI, Table 8. EC50 of Ferrostatin-1 analogs.



SI, Scheme 2. Synthesis of Ferrostation analogs SRS14-86, SRS14-91, SRS14-92, SRS14-93. (a) alkylation reaction: arylhalide, DIPEA, THF, 60 ^{o}C, 17 h. (b) reductive amination reaction: arylhaldehyde, $NaBH(OAc)_3$, molecular sieve (4 Å), DCE , R.T. to 80 ^{o}C, 17 h.

Synthesis of ethyl 5-amino-6-(cyclohexylamino)pyridine-3-carboxylate (SRS14-86. SI, Scheme 2)

Following the above general **procedure A** and **B** and starting from the ethyl ester (SRS14-84, SI, Scheme 2), which was prepared from the corresponding acid, the crude reaction mixture was purified by column chromatography (dichloromethane: methanol = 20:1) to provide the desired ethyl 5-amino-6-(cyclohexylamino)pyridine-3-carboxylate (SRS14-86, SI, Scheme 2) (195 mg, 0.739 mmol, 85% (2 steps)). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.42 (d, *J* = 2.0 Hz,

1H), 7.36 (d, *J* = 2.0 Hz, 1H), 4.78 (d, *J* = 7.6 Hz, 1H), 4.30 – 4.21 (m, 2H), 4.08 – 3.92 (m, 1H), 3.36 (s, 1H), 2.07 – 1.56 (m, 10H), 1.42 – 1.24 (m, 3H); LC/MS (APCI+, *M+1*) 264.26.

Synthesis of ethyl 5-(benzylamino)-6-(cyclohexylamino)pyridine-3-carboxylate (SRS14-91. SI, Scheme 2)

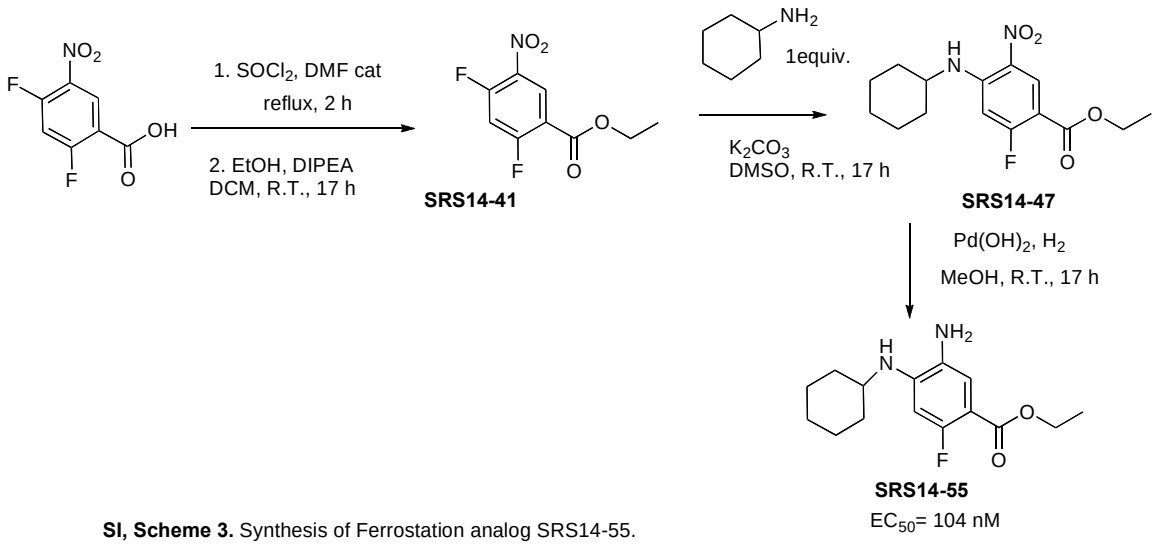
Following the above general **procedure C or D**, the crude reaction mixture was purified by column chromatography (dichloromethane: methanol = 40:1) to provide the desired ethyl 5-(benzylamino)-6 (cyclohexylamino)-pyridine-3-carboxylate (SRS14-91, SI, Scheme 2) (14.8 mg, 0.04 mmol, 53%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.49 (d, *J* = 1.9 Hz, 1H), 7.40 (dd, *J* = 34.8, 27.7 Hz, 5H), 4.59 (s, 1H), 4.47 – 4.25 (m, 4H), 4.08 (s, 1H), 2.11 - 1.51 (m, 10H), 1.38 (t, *J* = 11.5, 4.4 Hz, 3H); LC/MS (APCI+, *M+1*) 354.66.

Synthesis of ethyl 6-(cyclohexylamino)-5-(pyridin-4-ylmethylamino)pyridine-3-carboxylate (SRS14-92. SI, Scheme 2)

Following the above general **procedure C or D**, the crude reaction mixture was purified by column chromatography (dichloromethane: methanol = 20:1) to provide the desired ethyl 6-(cyclohexylamino)-5-(pyridin-4-ylmethylamino)-pyridine-3-carboxylate (SRS14-92, SI, Scheme 2) (16 mg, 0.045 mmol, 54%), ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.55 (d, *J* = 50.9 Hz, 3H), 7.46 – 7.15 (m, 3H), 4.67 (s, 1H), 4.40 – 4.25 (m, 2H), 4.07 (s, 2H), 2.10 – 1.62 (m, 10H), 1.40 – 1.19 (m, 3H); LC/MS (APCI+, *M+1*) 355.36.

Synthesis of ethyl 6-(cyclohexylamino)-5-(pyrimidin-4 ylmethylamino)-pyridine-3-carboxylate (SRS14-93. SI, Scheme 2)

Following the above general **procedure C or D**, the crude reaction mixture was purified by column chromatography (dichloromethane: methanol = 15:1) to provide the desired ethyl 6-(cyclohexylamino)-5-(pyrimidin-4-yl-methylamino)-pyridine-3-carboxylate (SRS14-93, SI, Scheme 2), (18 mg, 0.051 mmol, 58%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.26 (s, 1H), 8.74 (d, *J* = 5.1 Hz, 1H), 8.49 (d, *J* = 1.8 Hz, 1H), 7.37 (d, *J* = 5.1 Hz, 1H), 7.32 (d, *J* = 1.8 Hz, 1H), 4.40 (d, *J* = 40.5 Hz, 2H), 4.34 (dd, *J* = 14.2, 7.1 Hz, 2H), 4.11 (s, 1H), 2.11–1.48 (m, 10H), 1.42–1.31 (m, 3H); LC/MS (APCI+, *M+1*) 356.26.

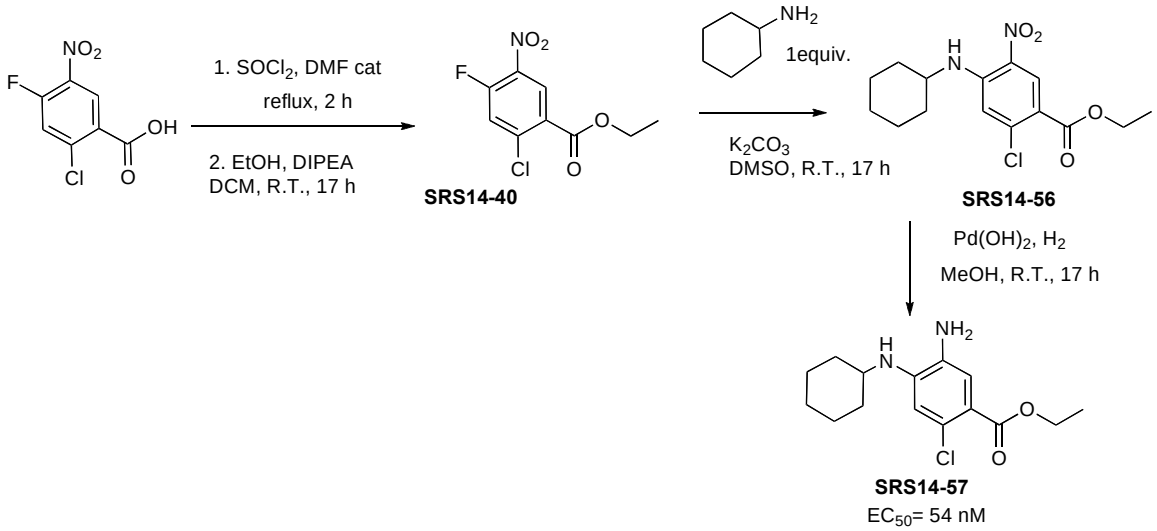


SI, Scheme 3. Synthesis of Ferrostation analog SRS14-55.

Synthesis of ethyl 5-amino-4-(cyclohexylamino)-2-fluorobenzoate (SRS14-55. SI, Scheme 3)

Following the above general procedure A and B and starting from the ethyl ester (SRS14-41, SI, Scheme 3), which was prepared from the corresponding acid, the

crude reaction mixture was purified by column chromatography (dichloromethane: methanol = 20:1) to provide the desired ethyl 5-amino-4-(cyclohexylamino)-2-fluorobenzoate (SRS14-55, SI, Scheme 3) (109 mg, 0.389 mmol, 90% (2 steps)). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 – 7.26 (m, 1H), 6.36 – 6.23 (m, 1H), 4.34 (dd, *J* = 8.9, 6.2, 1.8 Hz, 2H), 3.27 (s, 1H), 3.01 (s, 1H), 2.07 (d, *J* = 8.6 Hz, 2H), 1.81 (d, *J* = 8.5 Hz, 2H), 1.69 (s, 1H), 1.38 (ddd, *J* = 8.9, 6.3, 3.1 Hz, 5H), 1.27 (s, 3H); LC/MS (APCI+, *M+1*) 281.36.

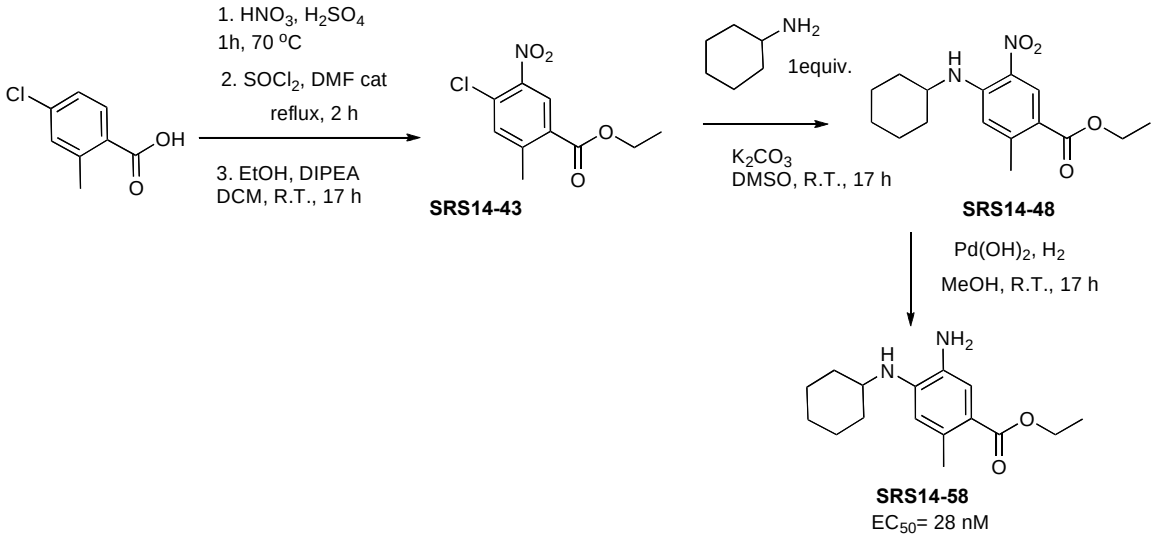


SI, Scheme 4. Synthesis of Ferrostation analog SRS14-57.

Synthesis of ethyl 5-amino-2-chloro-4-(cyclohexylamino)benzoate (SRS14-57. SI, Scheme 4)

Following the above general **procedure A and B** and starting from the ethyl ester (SRS14-40, SI, Scheme 4), which was prepared from the corresponding acid, the crude reaction mixture was purified by column chromatography (dichloromethane: methanol = 20:1) to provide the desired ethyl 5-amino-2-chloro-4-(cyclohexylamino)benzoate (SRS14-57, SI, Scheme 4) (136 mg, 0.459 mmol, 75% (2 steps)).^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 (d, *J* = 8.4 Hz, 1H), 7.42

(s, 1H), 6.59 (d, *J* = 8.4 Hz, 1H), 4.17 – 4.06 (m, 2H), 3.47 (s, 1H), 3.35 – 3.27 (m, 1H), 1.78 (d, *J* = 13.0 Hz, 2H), 1.67 (d, *J* = 12.0 Hz, 1H), 1.42 – 1.31 (m, 5H), 1.26 (td, *J* = 7.1, 1.6 Hz, 5H); LC/MS (APCI+, *M+1*) 297.47.



SI, Scheme 5. Synthesis of Ferrostation analog SRS14-58.

Synthesis of ethyl 5-amino-4-(cyclohexylamino)-2-methylbenzoate (SRS14-58. SI, Scheme 5)

Following the above general **procedure A and B** and starting from the ethyl ester (SRS14-43, SI, Scheme 5), which was prepared from the corresponding acid, the crude reaction mixture was purified by column chromatography (dichloromethane: methanol = 40:1) to provide the desired ethyl 5-amino-4-(cyclohexylamino)-2-methylbenzoate (SRS14-58, SI, Scheme 5) (18 mg, 0.064 mmol, 83% (2 steps)). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (d, *J* = 2.2 Hz, 1H), 6.41 (s, 1H), 4.30 (dd, *J* = 9.5, 4.6 Hz, 2H), 3.40 – 3.28 (m, 2H), 2.56 (d, *J* = 2.6 Hz, 3H), 2.09 (d, *J* = 9.3 Hz, 2H), 1.81 (d, *J* = 10.0 Hz, 2H), 1.70 (d, *J* = 8.6 Hz, 1H),

1.39 (ddd, *J* = 14.9, 9.3, 8.1 Hz, 5H), 1.29 – 1.16 (m, 3H); LC/MS (APCI+, *M+1*) 277.16.

[1] Blois, M. S. *Nature* **1958**, *181*, 1199.
[2] Beaulieu, P. L.; Hache, B.; Von Moos, E. *Synthesis*, **2003**, *11*, 1683.
[3] Abdel-Magid, A. F.; Carson, K. G.; Harris, B. D.; Maryanoff, C. A.; Shah, R. D. *J. Org. Chem.* **1996,** *61*, 3849.