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Novel ferroptosis inhibitors with improved potency and ADME properties

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Keywords: ferroptosis, regulated necrosis

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ABSTRACT

Ferroptosis is a non-apoptotic, iron-catalysed form of regulated necrosis that is critically dependent on glutathione peroxidase 4 (GPX4). It has been shown to contribute to liver and kidney ischemia reperfusion injury in mice. A chemical inhibitor discovered by high-throughput screening displayed inhibition of ferroptosis with nanomolar activity and was dubbed ferrostatin-1 (fer-1). Ferrostatins inhibit oxidative lipid damage, but suffer from inherent stability problems due to the presence of an ester moiety. This limits the application of these molecules *in vivo*, due to rapid hydrolysis of the ester into the inactive carboxylic acid. Previous studies highlighted the importance of the ethyl ester and suggested steric modifications of the ester for generating improved molecules. In this study, we report the synthesis of novel ferroptosis inhibitors containing amide and sulfonamide moieties with improved stability, single digit nanomolar anti-ferroptotic activity and good ADME properties suitable for application in *in vivo* disease models.

INTRODUCTION

Cell death research was revitalized by the understanding that necrosis can occur in a highly regulated and genetically controlled manner. Although necroptosis is the best understood form of regulated necrosis[1], other important necrotic signalling pathways are emerging.[2] A new type of regulated necrosis induced by erastin, an oncogenic toxic small molecule, was recently described. Erastin acts, at least partially, through inhibition of the System X_C^- Cys/Glu antiporter (Figure 1). This type of erastin-induced cell death was defined as ferroptosis because it critically depends on intracellular iron metabolism and gluthathione peroxidase 4 (GPX4) inactivity.[3] Ferroptosis was recently found to be a detrimental process in kidney and liver ischemia reperfusion injury.[4,5,6] It is inhibited by iron chelators, lipophilic anti-oxidants and/or ferrostatin-1 (fer-1, **1**, figure 2).[7,8] GPX4 inhibits the accumulation of lipid peroxides and depends on the functionality of System X_C^- Cys/Glu antiporter in the plasma membrane. Treatment with erastin blocks uptake of cystine, the oxidized form of Cys, which reduces the levels of intracellular Cys required for gluthatione (GSH) synthesis. Subsequently, this drop in GSH levels leads to the inactivation of GPX4,[8] which uses GSH as a cofactor.[9] In addition, the redox-

active iron pools are capable of directly catalysing formation of damaging free radical via e.g. Fenton reactions. The aberrant accumulation of iron, reactive oxygen species (ROS) or the combination of both are linked to a staggering number of traumas and chronic degenerative conditions including stroke, sepsis, ischemia reperfusion injury, traumatic brain injury and neurodegenerative diseases.[4,5,10] For this reason, iron chelators have been implemented or proposed as treatments for diverse pathologies caused by iron and/or ROS accumulation.[3]

Ferroptosis may be blocked *in vitro* by the small molecule inhibitor ferrostatin-1 (fer-1, **1**, Figure 2). Ferrostatins are believed to act by preventing oxidative damage to membrane lipids.[6,7] Fer-1, an arylalkylamine with anti-oxidative properties, was identified as one of the first inhibitors of ferroptosis.[3] Fer-1 attenuates oxidative, iron-dependent cell death in cancer cells treated with small molecules such as erastin, however the exact target of fer-1 remains unclear. A study by Skouta *et al*. observed that fer-1 treatment did not significantly change the levels of cysteine or glutathione (system X_C^-), in contrast it did preserve normal levels of polyunsaturated fatty acids (PUFAs) and PUFA-derivatives. PUFAs and derivatives are susceptible to oxidation either through enzymatic (e.g. lipoxygenase-mediated) or non-enzymatic (iron-dependent Fenton chemistry-mediated) processes.[11] Fer-1 is able to prevent depletion of oxidized PUFAs and their fragments by inhibiting their oxidative destruction. Thus fer-1 acts as a lipid peroxide reductant to intercept and scavenge a lipid radical through hydrogen atom transfer or direct reduction.[6] Similar data was obtained by Dixon *et al*. This study hypothesized as well that fer-1 is a lipid ROS scavenger, implying that the *N*-cyclohexyl moiety on the central aromatic core serves as a lipophilic anchor within the biological membranes of the cell.[7]

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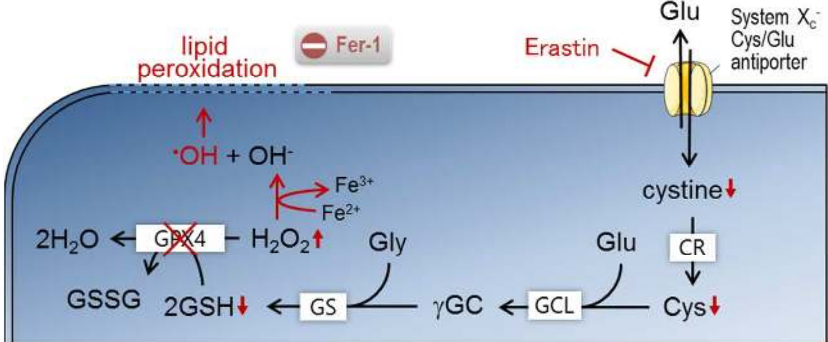

Figure 1. Schematic overview of System Xc- Cys/Glu antiporter inhibition by erastin. System X_c^- Cys/Glu antiporter is an amino acid antiporter that typically mediates the exchange of extracellular cystine and intracellular Glu across the cellular plasma membrane. The import of cystine through this transporter is critical for the production of glutathione (GSH) and protection against oxidative stress. Cystine reductase (CR) rapidly catalyzes cystine to Cys. Glutamate cysteine ligase (GCL) catalyzes the synthesis of γ-glutamyl cysteine (γGC) from Glu and Cys, and gluthathione synthase (GS) generates GSH by adding Gly. GSH reduces hydrogenperoxide (H_2O_2) catalyzed by gluthatione peroxidase 4 (GPX4), and is thereby converted to GSH disulfide (GSSG). Inhibition of System X_c^- Cys/Glu antiporter by erastin results in a sequential drop of intracellular cysteine, Cys and GSH levels. The consequent loss of GPX4 activity due to the lack of GSH, reduces the anti-oxidant capacity of the cell. This results in accumulation of H_2O_2 that upon reaction with Fe^{2+} produces highly reaction hydroxyl radicals (Fenton reaction). This increase in free radicals can initiate a lipid peroxidation chain reaction resulting in membrane damage, and consequent cell death (referred to as ferroptosis). A chemical inhibitor discovered by high-throughput screening displayed inhibition of ferroptosis with nanomolar activity and was dubbed ferrostatin-1 (fer-1). Fer-1 is believed to act by preventing oxidative damage to membrane lipids.[3,7,8,9] GCL: glutamate cysteine ligase; GS: gluthathione synthetase; CR: cysteine reductase

So far only a few analogues of fer-1 have been reported to inhibit ferroptosis (Figure 2). An initial structure-activity relationship (SAR) was established by Stockwell *et al*.[3] Substitution of the primary aromatic amine of fer-1 for a nitro group or elimination of the *N*-cyclohexyl moiety abolished the antioxidant capability of fer-1 as well as its ability to prevent erastin-induced death in HT-1080 cells. In addition ten other fer-1 analogues were synthesised, in which the number of carbons in the *N*-

substituted cycloalkyl moiety varied. A significant correlation between the predicted lipophilicity and erastin-death suppressing ability of each molecule was observed. In general, compounds containing a large, lipophilic *N*-substituted cycloalkyl moiety were more potent.[7] A second SAR-study was reported by Skouta *et al*.[6] A library of 67 analogues of fer-1 was synthesised, designing mono- and disubstituted amines. Secondly, insertion of heteroatoms into the *N*-cyclohexyl moiety decreased potency, suggesting that hydrophobicity of this part in the scaffold is essential for anchoring with lipophilic membrane environments. Also, the ethyl ester of fer-1 was modified and it was observed that elongation of the ester was well tolerated. However, replacement of the ester with an amide as shown in SRS9-11 (**2**, figure 2, IC_{50} = 950 nM) resulted in a 10-fold decrease in potency when compared to the original fer-1 (IC_{50} = 95 nM).[13] It was also observed that tertiary amines resulted in a significant decrease in potency when compared to primary and secondary amine analogues. It became also clear that substitution of the aryl group at *N*-3 with different electron withdrawing or electron donating groups and heteroatoms resulted in decreased potency. Finally, modification of the central aromatic core of the parent compound resulted in additional fer-1 analogues with a decrease in potency. Conclusively, this study generated additional fer-1 analogues with improved properties. For example, SRS11-92 (**3**, Figure 2, IC_{50} = 6 nM) was about 16-fold more potent than the original fer-1.[6] These two studies mainly focussed on increasing potency, rather than improving the poor plasma stability due to the presence of an ethyl ester. Linkermann *et al*. recently published a study reporting fer-1 analogues with improved stability. This study disclosed that variants of fer-1 should be ester analogues. In an effort to enhance plasma stability the ethyl ester moiety was replaced by a *tert*-butyl ester. An imine moiety was additionally introduced to increase microsomal stability. The most promising compound in that study, SRS16-86 (**4**, Figure 2), displayed a high plasma stability ($t_{1/2} \geq$ 120 min) and a high metabolic stability towards mouse microsomes (intrinsic clearance (CLint), mL/min per g liver < 0.5).[5] However, **4** did show a decrease in potency (IC_{50} ~ 350 nM) when compared to fer-1.[12]

It has become clear that the development of analogues with high potency and specificity for ferroptotic cell death is beneficial for numerous therapies. In this study we describe the synthesis and evaluation

of amide and sulfonamide analogues of fer-1. Despite earlier reports on the negative effect of replacing the ester with an amide, we will describe highly potent compounds with excellent plasma and microsomal stability.

1
Ferrostatin-1
IC_{50} = 95 nM

2
SRS9-11
IC_{50} = 950 nM

3
SRS11-92
IC_{50} = 6 nM

4
SRS16-86
IC_{50} ~ 350 nM

Figure 2. Reported analogues of fer-1.

RESULTS AND DISCUSSION

Compound design

In order to obtain novel inhibitors with increased stability and potency, three types of modifications were introduced in this series: (1) replacement of the ester bond by an amide or sulfonamide, (2) increase of lipophilicity by variation of the number of carbon atoms in the cycloalkyl moeity of R_2, (3) addition of benzylic and pyridinylic substituents to the amine of R_3.

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Ferrostatin-1 Novel amide and sulfonamide analogues

Figure 3. Structural comparison of ferrostatin-1 and novel inhibitors with improved stability.

Chemistry

A total of 38 novel inhibitors were synthesized for this study. All compounds were prepared following the general strategies in Schemes 1, 2 and 3.

Prior to the synthesis of the amide and sulfonamide analogues of fer-1, the methyl ester and carboxylic acid were synthesized as a reference to fer-1. The molecules were synthesized from the commercially available 4-chloro-3-nitrobenzoic acid **5** and the methyl 4-chloro-3-nitrobenzoate **6**. Nucleophilic aromatic substitution with cyclohexylamine in basic conditions provided molecules **7-8** respectively. Palladium-catalyzed hydrogenation of the 3-nitro group lead to the corresponding 3-amino analogs **9-10**.

Scheme 1: Reagents and conditions: (a) cyclohexylamine, K_2CO_3, DMSO, 17h, 60°C; (b) Palladium hydroxide, H_2 gas, methanol, 17h, rt;

The synthesis of ethylamide analogues of fer-1 is outlined in Scheme 2. Starting from the commercially available 4-chloro-3-nitrobenzoyl chloride **11**, *N*-ethylamide formation was realized by treatment of the starting material with ethylamine in basic conditions. Nucleophilic aromatic substitution of 4-chloro-*N*-ethyl-3-nitrobenzamide **12** with cyclohexylamine or 2-adamantylamine in basic conditions provided molecules **13-14**. Palladium-catalysed hydrogenation of the 3-nitro group lead to the corresponding 3-amino analogs **15-16**. Treatment of respectively **15-16** with benzyl bromide or 4-(bromomethyl)pyridine hydrobromide resulted in target compounds **17-20**.

Scheme 2: Reagents and conditions: (a) ethylamine HCl-salt, triethylamine, DCM, 4h, rt; (b) cyclohexylamine or 2-adamantylamine HCl-salt, K_2CO_3, DMSO, 17h, 60°C; (c) Palladium hydroxide, H_2 gas, methanol, 17h, rt;[7] (d) benzyl bromide or 4-(bromomethyl)pyridine hydrobromide, K_2CO_3, DMF, 1-4h, 60°C[13]

Scheme 3 shows the synthesis of sulfonamide analogues of ferrostatin-1. The derivatives were synthesized from 4-chloro-3-nitrobenzenesulfonyl chloride **21** in a similar way as described in scheme 2. Reaction of the appropriate amine analogue with sulfonylchloride delivered **22-24**. Nucleophilic

aromatic substitution of **22-24** with different cycloalkylamines in basic conditions resulted in **25-34**. Palladium-catalyzed hydrogenation of the 3-nitro group resulted in **35-44** and coupling of the resulting 3-amines with benzyl bromide or 4-(bromomethyl)pyridine hydrobromide resulted in target compounds **45-64**.

Scheme 3: Reagents and conditions: (a) ethylamine HCl-salt, triethylamine, THF, 1h, -40°C to rt; (b) cyclohexylamine or 2-adamantylamine HCl-salt, K_2CO_3, DMSO, 17h, 60°C; (c) Palladium hydroxide, H_2 gas, methanol, 17h, rt;[7] (d) benzyl bromide or 4-(bromomethyl)pyridine hydrobromide, K_2CO_3, DMF, 1-4h, 60°C[13]

Inhibition of erastin-induced ferroptosis

Compounds **9-10**, **15-20** and **35-64** were evaluated for prevention of erastin-induced ferroptotic cell death and IC_{50}-values are presented in table 1. The 95% confidence interval for all IC_{50}-values is reported in the supplementary information (S2-3).

9-10 **15-20** **35-64**

Compound	R_1	R_2	R_3	IC_{50} (nM)	cLogP [a]
1 (Fer-1)	Ethyl	Cyclohexyl	H	18	3.7
9	H	Cyclohexyl	H	210	2.6
10	Methyl	Cyclohexyl	H	34	3.1
15	Ethyl	Cyclohexyl	H	88	2.1
16	Ethyl	2-Adamantyl	H	14	3.8
17	Ethyl	Cyclohexyl	Benzyl	21	4.3
18	Ethyl	Cyclohexyl	Pyridinyl	26	2.8
19	Ethyl	2-Adamantyl	Benzyl	6.5	6.0
20	Ethyl	2-Adamantyl	Pyridinyl	3.4	4.5
35	Ethyl	Cyclopropyl	H	76	0.8
36	Ethyl	Cyclopentyl	H	82	1.2
37	Ethyl	Cyclohexyl	H	67	2.2
38	Ethyl	Norbornyl	H	28	2.5
39	Ethyl	Cyclooctyl	H	9.3	3.4
40	Ethyl	2-Adamantyl	H	17	3.9
41	Methyl	Cyclohexyl	H	76	1.7
42	Methyl	2-Adamantyl	H	11	3.4
43	*tert*-Butyl	Cyclohexyl	H	27	2.9
44	*tert*-Butyl	2-Adamantyl	H	6.0	4.6
45	Ethyl	Cyclopropyl	Benzyl	61	3.9

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46	Ethyl	Cyclopropyl	Pyridinyl	128	1.5
47	Ethyl	Cyclopentyl	Benzyl	8.2	3.9
48	Ethyl	Cyclopentyl	Pyridinyl	36	2.4
49	Ethyl	Cyclohexyl	Benzyl	4.0	4.4
50	Ethyl	Cyclohexyl	Pyridinyl	23	2.9
51	Ethyl	Norbornyl	Benzyl	3.0	4.7
52	Ethyl	Norbornyl	Pyridinyl	9.4	3.2
53	Ethyl	Cyclooctyl	Benzyl	2.4	5.5
54	Ethyl	Cyclooctyl	Pyridinyl	22	4.0
55	Ethyl	2-Adamantyl	Benzyl	3.5	6.1
56	Ethyl	2-Adamantyl	Pyridinyl	3.9	4.6
57	Methyl	Cyclohexyl	Benzyl	3.7	3.9
58	Methyl	Cyclohexyl	Pyridinyl	15	2.4
59	Methyl	2-Adamantyl	Benzyl	4.9	5.6
60	Methyl	2-Adamantyl	Pyridinyl	6.4	4.1
61	*tert*-Butyl	Cyclohexyl	Benzyl	6.2	5.1
62	*tert*-Butyl	Cyclohexyl	Pyridinyl	11	3.6
63	*tert*-Butyl	2-Adamantyl	Benzyl	3.4	6.8
64	*tert*-Butyl	2-Adamantyl	Pyridinyl	5.2	5.3

Table 1. Anti-ferroptotic activity of synthesized fer-1 analogs library in response to erastin-induced ferroptosis in IMR-32 neuroblastoma cells. In this research IMR-32 neuroblastoma cells were used instead of HT-1080 cells because IMR-32 neuroblastoma were found to be more sensitive for erastin-induced ferroptosis.

[a] Calculated LogP using PerkinElmer Informatics ChemBioDraw software Version 14.0

Based on the anti-ferroptotic activities of the synthesized fer-1 analogues, we established a structure-activity relationship (SAR).

The presence of a carboxylic acid (**9**) is clearly unfavourable, since it decreases activity 10-fold. This demonstrates that ester hydrolysis leads to a significant decrease in potency. Comparing fer-1 to its corresponding amide and sulfonamide analogue, **15** and **37** respectively, it can be concluded that amide and sulfonamide moieties are tolerated in this position and are only slightly less potent than the original ester derivate. Methyl, ethyl and *tert*-butyl groups were coupled to the sulfonamide moiety in R_1, resulting in similar IC_{50}-values.

The importance of cycloalkyl groups at R_2 for ester analogues was already suggested by Skouta *et al.*[6] It was clear that the sulfonamide analogues containing small lipophilic substituents in R_2, such as cyclopropyl, were less active than compounds containing a large, bulky lipophilic group such as 2-adamantyl. In general, increasing the number of carbon atoms in the cycloalkyl moiety in R_2 increases potency of the inhibitor as this can be viewed in **35-40** and **45-56**.

At the R_3-position, we observed that addition of a benzyl group resulted in compounds with a higher potency than those containing a primary amine. Substitution with pyridinyl was also investigated in an attempt to reduce lipophilicity of the compounds. In general it can be stated that compounds containing a pyridinyl moiety are less potent than compounds containing a benzyl moiety (2 to 10-fold) with the exception of compounds that are combined with 2-adamantyl at R_2. This is exemplified with the equipotent compounds pairs **19** and **20**, **55** and **56**, **59** and **60** and finally **63** and **64**.

We observed a general trend between anti-ferroptotic activity and lipophilicity (Figure 4). Compounds with a high cLogP are more likely to display a low IC_{50}-value. This supports the earlier stated hypothesis that the potency of these inhibitors is correlated with their ability to interact with cellular lipid structures. Further increase in cLogP above 4 only yields marginal improvements to the IC_{50}. We thus propose that compounds with single digit nanomolar activity and a cLogP around 4 represent an optimal balance between potency and lipophilicity. These observations will prove useful in the development of compounds with good drug-like properties, suitable for use in *in vivo* disease models.

In conclusion, we were able to generate amide and sulfonamide analogues of the ferrostatin esters with single digit nanomolar anti-ferroptotic activity.

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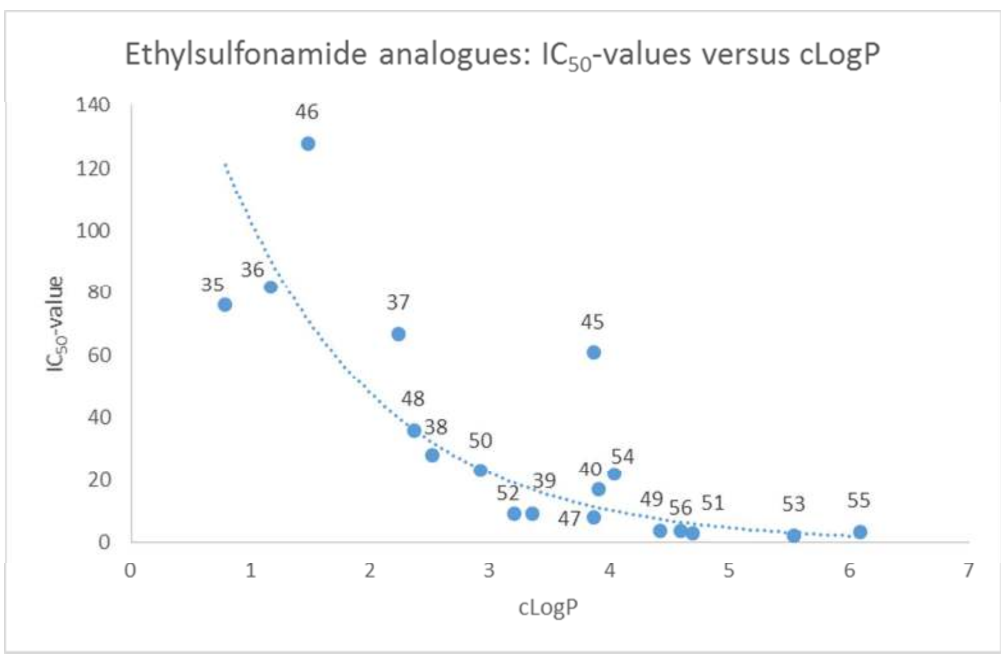

Figure 4. Comparison between IC_{50}-values and cLogP of all ethylsulfonamide analogues.

ADME assays

Five of the most potent amide and sulfonamide analogues were compared with fer-1 for their *in vitro* ADME properties. We determined kinetic solubility at pH 7.4, microsomal (human and mouse) and plasma (human and mouse) stability (Table 2) (supplementary information S4-8). The original ester (fer-1) was rapidly metabolised by human and mouse microsomes with half-life's of approximately 7 and 2 minutes respectively. As anticipated, fer-1 was also extremely unstable in mouse plasma, with complete degradation after 30 minutes. Surprisingly, fer-1 was not degraded in human plasma after 6 hours. This initial result was confirmed in different plasma samples containing different anticoagulants (supplementary information S8-9) and by a third party (supplementary information S9). These data demonstrate that fer-1 is not a suitable tool compound to demonstrate the potential of anti-ferroptotic activity in mouse disease models.

Compared to fer-1, a significant increase in microsomal and plasma stability of the amide and sulfonamide analogues was observed. The amide analogue **20** showed a half-life of about 16 minutes in human and around 30 min in mouse microsomes. The corresponding sulfonamide analogue **56**

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showed a slower metabolism by human microsomes (4-fold). The most stable compound of this subset is the sulfonamide analogue **49**, displaying a half-life of about 90 minutes in human and over 180 minutes in mouse microsomes, with complete stability in human and mouse plasma for up to 6 hours.

As we anticipated, the presence of lipophilic groups at the R_1 and R_2 positions caused a decrease in kinetic solubility in comparison to the original fer-1.

In conclusion combining the results from both IC_{50}-determination and ADME assays, a general SAR can be reported and is shown in figure 5.

Compound	Solubility [a] (μM)	Microsomal stability Half-life ($t_{1/2}$) (min) [b]		Plasma stability % recovery after 6h [c]	
		Human	Mouse	Human	Mouse
1 (Fer-1)	> 200	6.9 ± 0.2	1.9 ± 0 .6	100	0
20	25-50	16 ± 2	30 ± 3	100	100
37	12.5-25	37 ± 3	31 ± 5	81.9	86.2
49	12.5-25	88 ± 16	187 ± 41	100	98.6
55	12.5-25	36 ± 11	31 ± 6	100	99.4
56	12.5-25	64 ± 10	26 ± 5	100	100

Table 2: *in vitro* ADME of selected compounds

[a] Final test compound concentration range of between 3.125 μM and 200 μM [4 μM DMSO solution in 196 μM buffer solution (10 mM PBS pH 7.4)]

[b] Metabolism by microsomes (CYP450 and other NADPH -dependent enzymes) was monitored and expressed as half-life (min)

[c] Percentage of remaining parent compound

Figure 5. General structure-activity relationship (SAR) from combining anti-ferroptotic activity and the results from the ADME assays.

CONCLUSION

In this paper we demonstrate that ferrostatin-1, a frequently used compound to inhibit ferroptosis, is not suitable for use in disease mouse models, because of its very low metabolic stability. For the first time, we report on potent ferroptosis inhibitors in which the labile ester is replaced with amide and sulfonamide moieties. Investigation of the SAR around 3 positions of this scaffold afforded single digit nanomolar ferroptosis inhibitors with improved ADME properties. Analogues of fer-1 containing a sulfonamide show a significant increase in microsomal and plasma stability. Compound **49** inhibits erastin-induced ferroptotic cell death with an IC_{50} of 4 nM and has a half-life of 3 hours upon incubation with mouse microsomes and is completely stable in mouse plasma up to 6 hours. These excellent properties will allow the use of this compound in animal models to demonstrate the therapeutic potential of ferroptosis inhibition. Further optimization of this compound series towards improved solubility is ongoing and will be reported in due course.

EXPERIMENTAL SECTION

Unless otherwise stated, laboratory reagent grade solvents were used. Reagents were obtained from Sigma-Aldrich, Acros Organics or Fluorochem and were used without further purification. Characterization of all compounds was done with ^{1}H and ^{13}C NMR and mass spectrometry. ^{1}H and ^{13}C NMR spectra were recorded on a 400 MHz Bruker Avance III Nanobay spectrometer with Ultrashield and analysed by use of MestReNova analytical chemistry software. Chemical shifts are in ppm, and coupling constants are in hertz (Hz). ES mass spectra were obtained from an Esquire 3000plus ion trap mass spectrometer from Bruker Daltonics. Purities were determined with two diverse HPLC systems based either on mass determination or on UV detection. A Waters acquity UPLC system coupled to a Waters TQD ESI mass spectrometer (HPLC System A) or a Waters SQD ESI mass spectrometer (HPLC system B) was used both in combination with a Waters TUV detector. The same methods on both HPLC system A and B was used for compound detection and purity determination. Water (A) and CH_3CN (B) were used as eluents. Waters Acquity UPLC BEH C18 1.7 µm, 2.1 mm × 50 mm column was used. Solvent A consisted of water with 0.1% formic acid. Solvent B consisted of acetonitrile with 0.1% formic acid. Method I involved the following: 0.15 min 95% A, 5% B, then in 1.85 min from 95% A, 5% B to 95% B, 5% A, then 0.25 min (0.350 mL/min), 95% B, 5% A. The wavelength for UV detection was 254 nm. Method II involved the following: flow 0.4 mL/min, 0.25 min 95% A, 5% B, then in 4.75 min to 95% B, 5% A, then 0.25 min 95% B, 5% A, followed by 0.75 min 95% A, 5% B. The wavelength for UV detection was 214 nm. Where necessary, flash purification was performed on a Biotage ISOLERA One flash system equipped with an internal variable dualwavelength diode array detector (200−400 nm). For normal phase purifications SNAP cartridges (10−340 g, flow rate of 10−100 mL/min) were used, and reversed phase purifications were done making use of KP-C18 containing cartridges. Dry sample loading was done by self-packing samplet cartridges using silica and Celite 545, respectively, for normal and reversed phase purifications. Gradients used varied for each purification. Mouse and rat plasma came from Innovative Research. Liver microsomes were obtained from Corning B.V. Life Sciences. The turbidity in the kinetic

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solubility experiments was measured using the UV/vis spectrophotometer Synergy MX, Biotek with Gen5. [14]

The following section comprises the synthetic procedures and analytical data for all compounds reported in this manuscript. Several synthesis procedures that were used in the preparation of intermediates and final products are summarized here as "General Procedures". The purities of all final products were found to be > 95%, unless stated otherwise.

General procedure A

To a solution of ethylamine HCl-salt (1 equiv.) and triethylamine (2 equiv.) in DCM was added 4-chloro-3-nitrobenzoyl chloride **11** (1 equiv.) under an argon atmosphere. The resulting mixture was washed with water and purified by flash-column chromatography on silica gel (10% methanol in DCM) to provide the desired 4-chloro-*N*-ethyl-3-nitrobenzamide analog **12**.

General procedure B

A solution of 4-chloro-3-nitrobenzene-1-sulfonyl chloride **21** (1 equiv.) in THF was cooled down to -40°C before being treated with the appropriate amine analog (1 equiv.) followed by triethylamine (2 equiv.). The reaction mixture was stirred and allowed to reach room temperature over 1h. The reaction mixture was diluted with ethyl acetate, washed twice with brine and dried. The residue was purified by flash-column chromatography on silica gel (20-40% ethyl acetate in heptane) to provide the desired sulfonamide analogues **22-24**.

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General procedure C

To the appropriate 4-chloro-3-nitrobenzoic acid **5** (1 equiv.), methyl 4-chloro-3-nitrobenzoate **6** (1 equiv.), 4-chloro-*N*-ethyl-3-nitrobenzamide **12** (1 equiv.) or 4-chloro-3-nitrobenzenesulfonamide intermediate **22-24** (1 equiv.) in dry DMSO was added K_2CO_3 (2-4 equiv.) and the appropriate cycloalkylamine analog (1.2 equiv.). The mixture was stirred for 17h at 60°C. The solution was poured in water, extracted with 3x ethyl acetate and dried with $MgSO_4$. The solvent was removed under reduced pressure. The residue was purified by flash-column chromatography on silica gel to respectively provide the desired 4-(cyclohexyl)-3-nitrobenzoic acid **7**, methyl 4-(cyclohexyl)-3-nitrobenzoate **8**, 4-(cycloalkylamino)- *N*-ethyl-3-nitrobenzamide analogues **13-14** and 4-(cycloalkylamino)-3-nitrobenzenesulfonamide analogues **25-34**.

General procedure D

The appropriate 4-(cyclohexyl)-3-nitrobenzoic acid intermediate **7** (1 equiv.), methyl 4-(cyclohexylamino)-3-nitrobenzoate intermediate **8** (1 equiv.), 4-(cycloalkylamino)-*N*-ethyl-3-nitrobenzamide intermediate **13-14** (1 equiv.) or 4-(cycloalkylamino)-3-nitrobenzenesulfonamide intermediate **25-34** (1 equiv.) was dissolved in methanol, flushed with argon and hydrogenated (H_2 gas) over 10% Palladium hydroxide (1.5 equiv.) for 17 h at room temperature. The solution was filtered through a pad of celite and volatiles were removed under reduced pressure. If necessary, the residue was purified by flash-column chromatography on silica gel to respectively provide the desired 3-amino-4-(cyclohexylamino)benzoic acid **9**, methyl 3-amino-4-(cyclohexylamino)benzoate **10**, 4-(cycloalkylamino)- 3-aminobenzamide analogues **15-16** or 4-(cycloalkylamino)- 3-aminobenzenesulfonamide analog **35-44**.

General procedure E

To the 4-(cycloalkylamino)- 3-aminobenzamide intermediate **15-16** (1 equiv.) or 4-(cycloalkylamino)-3-aminobenzenesulfonamide intermediate **35-44** (1 equiv.) in DMF was added the appropriate benzyl

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3 or pyridinylderivate (1-2 equiv.) and K_2CO_3 (2-4 equiv.). The mixture was stirred at 60°C for 1-4
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5 hours then poured in water. The organic layer was extracted with 3x ethyl acetate and the solvent was
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7 removed under reduced pressure. The residue was purified by flash-column chromatography on silica
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9 gel to respectively provide target amide **17-20** and sulfonamide compounds **45-64**.
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14 **4-(cyclohexyl)-3-nitrobenzoic acid (7)**
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16 Following **general procedure C,** using 4-chloro-3-nitrobenzoic acid **5** (2 g, 9.92 mmol) and
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18 cyclohexylamine (1.362 ml, 11.91 mmol). The reaction was purified by flash-column chromatography
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20 on silica gel (5% methanol in DCM) to afford 4-(cyclohexyl)-3-nitrobenzoic acid (1.4 g, 5.46 mmol).
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22 (Yield: 55%)
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24 **^{1}H NMR (400 MHz, DMSO-d_6)** δ 1.18 - 1.32 (m, 1H), 1.34 - 1.49 (m, 4H), 1.55 - 1.66 (m, 1H), 1.65
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26 - 1.75 (m, 2H), 1.90 - 2.03 (m, 2H), 3.69 (td, J = 4.06, 8.73, 9.56 Hz, 1H), 7.18 (d, J = 9.24 Hz, 1H),
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28 7.94 (ddd, J = 0.68, 2.14, 8.99 Hz, 1H), 8.26 (d, J = 7.70 Hz, 1H), 8.60 (d, J = 2.09 Hz, 1H), 12.93 (s,
29
30 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 24.48, 25.44, 32.26, 51.07, 115.39, 117.50, 129.08, 130.64,
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32 136.54, 146.82, 166.34.
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34 MS (ESI) m/z 265 [M +H] (HPLC System B)
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39 **Methyl 4-(cyclohexylamino)-3-nitrobenzoate (8)**
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42 Following **general procedure C,** using methyl 4-chloro-3-nitrobenzoate **6** (1.5 g, 6.96 mmol) and
43
44 cyclohexylamine (0.955 ml, 8.35 mmol). The reaction was purified by flash-column chromatography
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46 on silica gel (5% methanol in DCM) to afford methyl 4-(cyclohexylamino)-3-nitrobenzoate (1.7 g,
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48 6.11 mmol). (Yield: 88%).
49
50 **^{1}H NMR (400 MHz, DMSO-d_6)** δ 1.19 - 1.29 (m, 1H), 1.33 - 1.50 (m, 4H), 1.54 - 1.65 (m, 1H), 1.68
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52 - 1.76 (m, 2H), 1.88 - 2.02 (m, 2H), 3.69 (dt, J = 5.41, 9.32 Hz, 1H), 3.83 (s, 3H), 7.19 (d, J = 9.21 Hz,
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54 1H), 7.94 (ddd, J = 0.72, 2.18, 9.14 Hz, 1H), 8.27 (d, J = 7.72 Hz, 1H), 8.60 (d, J = 2.12 Hz, 1H). **^{13}C**
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3 **NMR (101 MHz, DMSO-d_6)** δ 24.48, 25.42, 32.23, 51.12, 52.48, 115.56, 116.22, 129.02, 130.68,
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5 136.14, 146.95, 165.23.
6
7 MS (ESI) m/z 279 [M +H] (HPLC System A)
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12 **3-amino-4-(cyclohexylamino)benzoic acid (9)**
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15 Following **general procedure D**, using 4-(cyclohexylamino)-3-nitrobenzoic acid **7** (0.500 g, 1.892
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17 mmol) to afford the desired 3-amino-4-(cyclohexylamino)benzoic acid (0.180 g, 0.768 mmol) as an
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19 amorphous powder. (Yield: 41%)
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21 **^{1}H NMR (400 MHz, MeOD)** δ 1.16 - 1.29 (m, 3H), 1.46 - 1.32 (m, 2H), 1.60 - 1.70 (m, 1H), 1.70 -
22
23 1.84 (m, 2H), 2.00 - 2.09 (m, 2H), 3.25 - 3.39 (m, 1H), 6.56 (dd, J = 0.68, 8.44 Hz, 1H), 7.39 (d, J =
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25 2.03 Hz, 1H), 7.47 (dd, J = 2.04, 8.40 Hz, 1H). **^{13}C NMR (101 MHz, MeOD)** δ 24.82, 25.66, 32.75,
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27 51.23, 108.79, 117.30, 117.48, 123.55, 132.14, 141.37, 169.94.
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29 Remark: amine protons were exchanged with solvent.
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32 t_R 1.86 min, MS (ESI) m/z 235 [M +H] (100%) (HPLC System A)
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37 **Methyl 3-amino-4-(cyclohexylamino)benzoate (10)**
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40 Following **general procedure D**, using methyl 4-(cyclohexylamino)-3-nitrobenzoate **8** (1.7 g, 6,11
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42 mmol) to afford the desired methyl 3-amino-4-(cyclohexylamino)benzoate (0.7 g, 2.82 mmol) as an
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44 amorphous powder. (Yield: 46%)
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46 **^{1}H NMR (400 MHz, DMSO-d_6)** δ 1.15 - 1.26 (m, 3H), 1.31 - 1.44 (m, 2H), 1.57 - 1.67 (m, 1H), 1.70
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48 - 1.79 (m, 2H), 1.90 - 2.01 (m, 2H), 3.25 - 3.33 (m, 1H), 3.72 (s, 3H), 4.76 (s, 2H), 4.92 (d, J = 7.48
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50 Hz, 1H), 6.46 (d, J = 8.34 Hz, 1H), 7.15 - 7.22 (m, 2H). **^{13}C NMR (101 MHz, MeOD)** δ 24.85, 25.70,
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52 32.75, 50.73, 51.17, 108.76, 116.81, 117.04, 123.19, 132.54, 141.43, 168.34.
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55 t_R 1.79 min, MS (ESI) m/z 249 [M +H] (100%) (HPLC System B)
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4-chloro-*N*-ethyl-3-nitrobenzamide (12)

Following **general procedure B** and ethylamine hydrochloride as the corresponding amine, the reaction was purified by flash-column chromatography on silica gel (10% methanol in DCM) to afford the desired afford 4-chloro-*N*-ethyl-3-nitrobenzamide (3 g, 13.12 mmol). (Yield: 58%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.14 (t, J = 7.23 Hz, 3H), 3.30 (td, J = 5.43, 7.24 Hz, 2H), 7.89 (d, J = 8.40 Hz, 1H), 8.15 (dd, J = 2.13, 8.43 Hz, 1H), 8.50 (d, J = 2.11 Hz, 1H), 8.85 (s, 1H).

MS (ESI) m/z 229 [M +H] (HPLC System A)

4-(cyclohexylamino)-*N*-ethyl-3-nitrobenzamide (13)

Following **general procedure C,** using 4-chloro-*N*-ethyl-3-nitrobenzamide **12** (2 g, 8.75 mmol) and cyclohexylamine (1.201 ml, 10.50 mmol). The reaction was purified by flash-column chromatography on silica gel (50% ethyl acetate in heptane) to afford the desired 4-(cyclohexyamino)-*N*-ethyl-3-nitrobenzamide (1g, 3.43 mmol). (Yield: 78%)

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.27 (t, J = 7.26 Hz, 3H), 1.30 - 1.52 (m, 6H), 1.70 (ddd, J = 3.04, 5.44, 13.10 Hz, 1H), 1.79 - 1.88 (m, 2H), 1.99 - 2.13 (m, 2H), 3.50 (qd, J = 5.36, 7.28 Hz, 2H), 6.26 (s, 1H), 6.92 (d, J = 9.12 Hz, 1H), 7.96 (ddd, J = 0.67, 2.28, 8.99 Hz, 1H), 8.35 (d, J = 7.34 Hz, 1H), 8.55 (d, J = 2.23 Hz, 1H). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 8.63, 14.93, 24.47, 25.47, 32.61, 35.00, 45.84, 51.30, 76.73, 77.05, 77.25, 77.37, 114.37, 121.07, 125.46, 130.40, 135.10, 146.20, 165.43.

MS (ESI) m/z 292 [M +H] (HPLC System A)

4-(adamantan-2-ylamino)-*N*-ethyl-3-nitrobenzamide (14)

Following **general procedure C,** using 4-chloro-*N*-ethyl-3-nitrobenzamide **12** (2.05 g, 8.97 mmol) and 2-adamantylamine hydrochloride (3.37 g, 17.93 mmol). The reaction was purified by flash-column chromatography on silica gel (8% methanol in DCM) to afford the desired 4-(adamantan-2-ylamino)-*N*-ethyl-3-nitrobenzamide (2.9 g, 8.44 mmol). (Yield: 94%).

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^{1}H NMR (400 MHz, $CDCl_3$) δ 1.25 (td, J = 4.21, 7.26 Hz, 3H), 2.11 - 1.65 (m, 15H), 3.40 - 3.56 (m, 2H), 3.82 (dt, J = 2.96, 6.86 Hz, 1H), 6.62 (t, J = 5.54 Hz, 1H), 6.85 (d, J = 9.13 Hz, 1H), 8.57 (d, J = 2.23 Hz, 1H), 8.84 (d, J = 7.45 Hz, 1H). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 14.89, 27.00, 31.57, 35.00, 36.99, 37.33, 56.68, 114.37, 121.10, 125.69, 130.49, 135.02, 146.15, 165.56,

MS (ESI) m/z 344 [M +H] (HPLC System A)

3-amino-4-(cyclohexylamino)-*N*-ethylbenzamide (15)

Following **general procedure D**, using 4-(cyclohexylamino)-*N*-ethyl-3-nitrobenzamide **13** (1.6 g, 5.49 mmol) to afford the desired 3-amino-4-(cyclohexylamino)-*N*-ethylbenzamide (0.600 g, 2.296 mmol) as an amorphous powder. (Yield: 42%)

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.25 (td, J = 4.21, 7.26 Hz, 3H), 1.65 - 2.11 (m, 15H), 3.40 - 3.56 (m, 2H), 3.82 (dt, J = 2.96, 6.86 Hz, 1H), 6.62 (t, J = 5.54 Hz, 1H), 6.85 (d, J = 9.13 Hz, 1H), 8.57 (d, J = 2.23 Hz, 1H), 8.84 (d, J = 7.45 Hz, 1H). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 14.89, 27.00, 31.57, 35.00, 36.99, 37.33, 56.68, 114.37, 121.10, 125.69, 130.49, 135.02, 146.15, 165.56.

t_R 1.66 min, MS (ESI) m/z 262 [M +H] (100%) (HPLC System A)

4-(adamantan-2-ylamino)-3-amino-*N*-ethylbenzamide (16)

Following **general procedure D**, using 4-(adamantan-2-ylamino)-*N*-ethyl-3-nitrobenzamide **14** (2.9 g, 8.44 mmol) to afford the desired 4-(adamantan-2-ylamino)-3-amino-*N*-ethylbenzamide (1.9 g, 6.06 mmol) as an amorphous powder. (Yield: 72%)

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.17 - 1.30 (m, 3H), 1.55 - 2.13 (m, 16H), 3.31 (br.s, 1H), 3.41 - 3.52 (m, 2H), 3.61 (t, J = 2.84 Hz, 1H), 6.02 (s, 1H), 6.52 - 6.59 (m, 1H), 7.18 - 7.24 (m, 1H), 7.25 - 7.30 (m, 1H). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 15.09, 27.31, 31.65, 34.70, 37.31, 37.65, 56.48, 109.82, 116.53, 120.12, 123.01, 132.75, 140.61, 167.52.

t_R 1.89 min, MS (ESI) m/z 314 [M +H] (95%) (HPLC System A)

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3-(benzylamino)-4-(cyclohexylamino)-*N*-ethylbenzamide (17)

Following **general procedure E**, using 3-amino-4-(cyclohexylamino)-*N*-ethylbenzamide **15** (0.150 g, 0.574 mmol) and benzyl bromide (0.069 ml, 0.574 mmol). The residue was purified by flash-column chromatography (0 to 100% methanol in water) on C-18 to afford the 3-(benzylamino)-4-(cyclohexylamino)-*N*-ethylbenzamide (0.0026 g, 7,40 µmol) as an amorphous powder. (Yield: 1.3%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.20 - 1.29 (m, 4H), 1.35 - 1.48 (m, 2H), 1.59 (s, 3H), 1.66 - 1.74 (m, 1H), 1.80 (dt, J = 3.83, 13.31 Hz, 2H), 2.08 (d, J = 12.50 Hz, 2H), 3.26 – 3.37 (m, 1H), 3.49 (qd, J = 5.52, 7.21 Hz, 3H), 4.34 (s, 2H), 5.92 (s, 1H), 6.65 (d, J = 8.24 Hz, 1H), 7.21 (dd, J = 2.05, 8.24 Hz, 1H), 7.27 (d, J = 2.00 Hz, 1H), 7.31 – 7.47 (m, 5H).

MS (ESI) m/z 352 [M +H] (95%) (HPLC System A)

4-(cyclohexylamino)-*N*-ethyl-3-((pyridin-4-ylmethyl)amino)benzamide (18)

Following **general procedure E**, using 3-amino-4-(cyclohexylamino)-*N*-ethylbenzamide **15** (0.150 g, 0.574 mmol) and 4-(bromomethyl)pyridine hydrobromide (0.128 g, 0.746 mmol). The residue was purified by preparative HPLC to afford the 4-(cyclohexylamino)-*N*-ethyl-3-((pyridin-4-ylmethyl)amino)benzamide (0.0204 g, 0.058 mmol) as an amorphous powder. (Yield: 10%)

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.19 (t, J = 7.26 Hz, 3H), 1.22 - 1.32 (m, 3H), 1.34 - 1.48 (m, 2H), 1.65 - 1.74 (m, 1H), 1.75 - 1.85 (m, 2H), 2.03 - 2.13 (m, 2H), 3.28 - 3.37 (m, 1H), 3.42 (qd, J = 5.46, 7.25 Hz, 2H), 4.36 (s, 2H), 6.16 (t, J = 5.54 Hz, 1H), 6.30 (s, 1H), 6.64 (d, J = 8.27 Hz, 1H), 7.13 (d, J = 2.03 Hz, 1H), 7.18 (dd, J = 1.99, 8.23 Hz, 1H), 7.33 - 7.37 (m, 2H), 8.24 (s, 1H), 8.52 - 8.54 (m, 2H). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 14.96, 24.96, 25.88, 33.34, 34.82, 47.51, 51.66, 110.30, 112.34, 119.27, 122.98, 123.49, 134.82, 140.13, 148.31, 150.24, 167.92.

t_R 1.38 min, MS (ESI) m/z 353 [M +H] (100%) (HPLC System A)

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4-(adamantan-2-ylamino)-3-(benzylamino)-*N*-ethylbenzamide (19)

Following **general procedure E**, using 4-(adamantan-2-ylamino)-3-amino-*N*-ethylbenzamide **16** (0.200 g, 0.638 mmol) and benzyl bromide (0.076 ml, 0.638 mmol). The residue was purified by preparative HPLC to afford the 4-(adamantan-2-ylamino)-3-(benzylamino)-*N*-ethylbenzamide (0.011 g, 0.027 mmol) as an amorphous powder. (Yield: 4%)

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.24 (t, J = 7.26 Hz, 3H), 1.47 - 1.52 (m, 2H), 1.59 - 1.66 (m, 2H), 1.78 - 1.95 (m, 10H), 2.05 - 2.09 (m, 2H), 3.47 (qd, J = 5.53, 7.26 Hz, 2H), 3.62 (t, J = 2.76 Hz, 1H), 4.36 (s, 2H), 5.95 (t, J = 5.25 Hz, 1H), 6.60 (d, J = 8.26 Hz, 1H), 7.23 (dd, J = 2.04, 8.23 Hz, 1H), 7.28 - 7.46 (m, 6H). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 15.04, 27.30, 27.95, 31.65, 34.78, 37.30, 37.64, 49.42, 56.61, 109.89, 113.34, 119.76, 123.32, 127.41, 127.87, 128.69, 135.41, 139.21, 140.75, 167.99.

t_R 2.33 min, MS (ESI) m/z 404 [M +H] (100%) (HPLC System A)

4-(adamantan-2-ylamino)-*N*-ethyl-3-((pyridin-4-ylmethyl)amino)benzamide (20)

Following **general procedure E**, using 4-(adamantan-2-ylamino)-3-amino-*N*-ethylbenzamide **16** (0.150 g, 0.479 mmol) and 4-(bromomethyl)pyridine hydrobromide (0.182 g, 0.718 mmol). The residue was purified by by flash-column chromatography on silica gel (20% methanol in ethyl acetate) to afford the 4-(adamantan-2-ylamino)-*N*-ethyl-3-((pyridin-4-ylmethyl)amino)benzamide (0.049 g, 0.121 mmol) as an amorphous powder. (Yield: 25%)

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.18 (t, J = 7.25 Hz, 3H), 1.57 - 2.09 (m, 15H), 3.42 (qd, J = 5.51, 7.22 Hz, 2H), 3.61 (s, 1H), 4.24 (s, 1H), 4.30 - 4.39 (m, 2H), 6.09 - 6.18 (m, 1H), 6.54 - 6.62 (m, 1H), 7.16 - 7.25 (m, 2H), 7.26 - 7.32 (m, 2H), 8.51 - 8.58 (m, 2H). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 15.03, 27.29, 31.68, 34.72, 37.29, 47.82, 56.59, 110.02, 113.33, 119.74, 122.43, 123.47, 134.85, 140.63, 148.56, 149.96, 167.63.

t_R 1.62 min, MS (ESI) m/z 405 [M +H] (100%) (HPLC System A)

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4-chloro-*N*-methyl-3-nitrobenzenesulfonamide (22)

Following **general procedure B** and methylamine hydrochloride as the corresponding amine, the reaction was purified by flash-column chromatography on silica gel (50% ethyl acetate in heptane) to afford the desired afford 4-chloro-*N*-ethyl-3-nitrobenzenesulfonamide (2.98 g, 11.99 mmol). (Yield: 61%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 2.50 (s, 4H), 7.86 (s, 1H), 8.03 - 8.05 (m, 2H), 8.42 (dd, J = 0.89, 1.64 Hz, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 29.10, 124.55, 129.82, 131.95, 133.60, 140.05, 147.94.

MS (ESI) m/z 499 [M+] (HPLC System A)

4-chloro-*N*-ethyl-3-nitrobenzenesulfonamide (23)

Following **general procedure B** and ethylamine hydrochloride as the corresponding amine, the reaction was purified by flash-column chromatography on silica gel (10% methanol in DCM) to afford the desired afford 4-chloro-*N*-ethyl-3-nitrobenzenesulfonamide (3.5 g, 13.22 mmol). (Yield: 72%)

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.19 (t, J = 7.24 Hz, 3H), 3.12 (qd, J = 5.71, 7.23 Hz, 2H), 4.71 (s, 1H), 7.75 (dd, J = 0.37, 8.39 Hz, 1H), 8.02 (dd, J = 2.16, 8.43 Hz, 1H), 8.38 (dd, J = 0.55, 2.02 Hz, 1H). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 15.20, 38.51, 124.40, 131.07, 131.55, 133.01, 140.53, 147.91.

MS (ESI) m/z 265 [M +H] (HPLC System A)

4-chloro-*N*-(*tert*-butyl)-3-nitrobenzenesulfonamide (24)

Following **general procedure B** and *tert*-butylamine as the corresponding amine, the reaction was purified by flash-column chromatography on silica gel (50% ethyl acetate in heptane) to afford the desired afford 4-chloro-*N*-(*tert*-butyl)-3-nitrobenzenesulfonamide (2.1 g, 7.17 mmol). (Yield: 92%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.14 (s, 9H), 7.93 (s, 1H), 8.00 (d, J = 8.47 Hz, 1H), 8.10 (dd, J = 2.19, 8.45 Hz, 1H), 8.48 (d, J = 2.15 Hz, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 30.18, 54.45, 124.12, 129.19, 131.52, 133.47, 144.96, 147.78.

MS (ESI) m/z 291 [M+] (HPLC System A)

4-(cyclopropylamino)-*N*-ethyl-3-nitrobenzenesulfonamide (25)

Following **general procedure C,** using 4-chloro-*N*-ethyl-3-nitrobenzenesulfonamide **23** (0.500 g, 1.889 mmol) and cyclopropylamine (0.159 ml, 2.267 mmol). The reaction was purified by flash-column chromatography on silica gel (35% ethyl acetate in heptane) to afford the desired 4-(cyclopropylamino)-*N*-ethyl-3-nitrobenzenesulfonamide (0.437 g, 1.532 mmol). (Yield: 81%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 0.67 - 0.72 (m, 2H), 0.89 - 0.94 (m, 2H), 0.99 (t, J = 7.21 Hz, 3H), 2.69 - 2.74 (m, 1H), 2.79 (td, J = 5.67, 7.23 Hz, 2H), 7.53 - 7.58 (m, 2H), 7.89 (ddd, J = 0.68, 2.29, 9.09 Hz, 1H), 8.37 - 8.39 (m, 1H), 8.42 (d, J = 2.22 Hz, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 8.01, 15.14, 25.43, 37.98, 117.22, 125.95, 127.50, 130.70, 133.62, 148.14.

MS (ESI) m/z 286 [M +H] (HPLC System A)

4-(cyclopentylamino)-*N*-ethyl-3-nitrobenzenesulfonamide (26):

Following **general procedure C,** using 4-chloro-*N*-ethyl-3-nitrobenzenesulfonamide **23** (1 g, 3.78 mmol) and cyclopentylamine (0.449 ml, 4.53 mmol). The reaction was purified by flash-column chromatography on silica gel (70% ethyl acetate in heptane) to afford the desired 4-(cyclopentylamino)-*N*-ethyl-3-nitrobenzenesulfonamide (1.003 g, 3.20 mmol). (Yield: 85%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 0.99 (t, J = 7.21 Hz, 3H), 1.54 - 1.66 (m, 4H), 1.70 - 1.78 (m, 2H), 2.05 - 2.15 (m, 2H), 2.77 (q, J = 7.22 Hz, 2H), 4.12 (p, J = 6.04, 6.52 Hz, 1H), 7.29 (d, J = 9.19 Hz, 1H), 7.54 (s, 1H), 7.81 (ddd, J = 0.67, 2.33, 9.19 Hz, 1H), 8.27 (d, J = 6.73 Hz, 1H), 8.43 (d, J = 2.24 Hz, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 15.13, 24.05, 33.07, 37.97, 54.42, 116.77, 126.34, 126.68, 130.27, 133.75, 146.77.

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MS (ESI) m/z 314 [M +H] (HPLC System A)

4-(cyclohexylamino)-*N*-ethyl-3-nitrobenzenesulfonamide (27)

Following **general procedure C,** using 4-chloro-*N*-ethyl-3-nitrobenzenesulfonamide **23** (1 g, 3.78 mmol) and cyclohexylamine (0.519 ml, 4.53 mmol). The reaction was purified by flash-column chromatography on silica gel (5% methanol in DCM) to afford the desired 4-(cyclohexylamino)-*N*-ethyl-3-nitrobenzenesulfonamide (0.95 g, 2.90 mmol). (Yield: 77%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.13 (t, J = 7.24 Hz, 3H), 1.29 - 1.52 (m, 5H), 1.63 - 1.72 (m, 1H), 1.77 - 1.88 (m, 2H), 2.00 - 2.13 (m, 2H), 3.01 (qd, J = 5.92, 7.21 Hz, 2H), 3.52 - 3.65 (m, 1H), 5.04 (t, J = 5.98 Hz, 1H), 6.99 (d, J = 9.17 Hz, 1H), 7.83 (ddd, J = 0.74, 2.37, 9.22 Hz, 1H), 8.43 (d, J = 7.51 Hz, 1H), 8.67 (s, 1H). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 15.09, 24.40, 25.39, 32.51, 38.24, 51.52, 114.96, 125.61, 127.66, 130.43, 133.52, 146.47

MS (ESI) m/z 328 [M +H] (HPLC System A)

4-(bicyclo[2.2.1]heptan-2-ylamino)-*N*-ethyl-3-nitrobenzenesulfonamide (28)

Following **general procedure C,** using 4-chloro-*N*-ethyl-3-nitrobenzenesulfonamide **23** (1 g, 3.78 mmol) and bicyclo[2.2.1]hept-2-ylamine (0.504 g, 4.53 mmol). The reaction was purified by flash-column chromatography on silica gel (50% ethyl acetate in heptane) to afford the desired 4-(bicyclo[2.2.1]heptan-2-ylamino)-*N*-ethyl-3-nitrobenzenesulfonamide (0.9 g, 2.65 mmol). (Yield: 70%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 0.99 (t, J = 7.22 Hz, 3H), 1.13 - 1.25 (m, 2H), 1.30 - 1.41 (m, 2H), 1.44 - 1.60 (m, 3H), 1.92 (ddd, J = 2.33, 7.63, 12.85 Hz, 1H), 2.29 - 2.34 (m, 2H), 2.77 (qd, J = 5.63, 7.21 Hz, 2H), 3.60 (ddd, J = 2.51, 5.54, 10.75 Hz, 1H), 7.19 (d, J = 9.19 Hz, 1H), 7.55 (t, J = 5.71 Hz, 1H), 7.82 (ddd, J = 0.66, 2.26, 9.18 Hz, 1H), 8.13 (d, J = 6.12 Hz, 1H), 8.43 (d, J = 2.23 Hz, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 15.12, 26.17, 28.30, 35.81, 35.86, 37.96, 41.79, 56.05, 116.72, 126.34, 126.89, 130.44, 133.80, 146.17.

MS (ESI) m/z 340 [M +H] (HPLC System A)

4-(cyclooctylamino)-*N*-ethyl-3-nitrobenzenesulfonamide (29)

Following **general procedure C,** using 4-chloro-*N*-ethyl-3-nitrobenzenesulfonamide **23** (1 g, 3.78 mmol) and cyclooctylamine (0.577 g, 4.53 mmol). The reaction was purified by flash-column chromatography on silica gel (40% ethyl acetate in heptane) to afford the desired 4-(cyclooctylamino)-*N*-ethyl-3-nitrobenzenesulfonamide (1.1 g, 3.09 mmol). (Yield: 82%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 0.99 (t, J = 7.21 Hz, 3H), 1.52 - 1.64 (m, 8H), 1.66 - 1.79 (m, 4H), 1.84 - 1.92 (m, 2H), 2.77 (qd, J = 5.59, 7.19 Hz, 2H), 3.87 - 3.94 (m, 1H), 7.22 (d, J = 9.26 Hz, 1H), 7.54 (t, J = 5.69 Hz, 1H), 7.81 (ddd, J = 0.66, 2.34, 9.11 Hz, 1H), 8.34 (d, J = 7.76 Hz, 1H), 8.43 (d, J = 2.25 Hz, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 15.13, 23.48, 25.40, 27.05, 31.70, 37.96, 52.49, 116.54, 126.49, 126.56, 130.11, 133.90, 146.07.

MS (ESI) m/z 356 [M +H] (HPLC System A)

4-(adamantan-2-ylamino)-*N*-ethyl-3-nitrobenzenesulfonamide (30):

Following **general procedure C,** using 4-chloro-*N*-ethyl-3-nitrobenzenesulfonamide **23** (2.380 g, 8.99 mmol) and 2-adamantylamine hydrochloride (1.688 g, 8.99 mmol). The reaction was purified by flash-column chromatography on silica gel (10% methanol in DCM) to afford the desired 4-(adamantan-2-ylamino)-*N*-ethyl-3-nitrobenzenesulfonamide (3.05 g, 8.04 mmol). (Yield: 89%).

1H NMR (400 MHz, $CDCl_3$) δ 1.04 (t, J = 7.23 Hz, 3H), 1.57 - 2.04 (m, 14H), 2.90 (qd, J = 5.77, 7.20 Hz, 2H), 3.79 (dt, J = 2.89, 6.46 Hz, 1H), 5.68 (t, J = 5.91 Hz, 1H), 6.92 (d, J = 9.30 Hz, 1H), 7.76 (ddd, J = 0.62, 2.31, 9.13 Hz, 1H), 8.58 (d, J = 2.26 Hz, 1H), 8.86 (d, J = 7.46 Hz, 1H). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 14.88, 26.96, 31.49, 36.80, 37.21, 38.16, 56.71, 115.24, 125.75, 127.34, 130.25, 133.61, 146.36.

MS (ESI) m/z 380 [M +H] (HPLC System A)

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4-(cyclohexylamino)-*N*-methyl-3-nitrobenzenesulfonamide (31)

Following **general procedure C,** using 4-chloro-*N*-methyl-3-nitrobenzenesulfonamide **22** (1 g, 3.99 mmol) and cyclohexylamine (0.548 ml, 4.79 mmol). The reaction was purified by flash-column chromatography on silica gel (30% ethyl acetate in heptane) to afford the desired 4-(cyclohexylamino)-*N*-methyl-3-nitrobenzenesulfonamide (0.600 g, 1.915 mmol). (Yield: 48%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.20 - 1.32 (m, 1H), 1.37 - 1.47 (m, 4H), 1.56 - 1.66 (m, 1H), 1.68 - 1.76 (m, 2H), 1.92 - 2.01 (m, 2H), 2.41 (s, 3H), 3.62 - 3.80 (m, 1H), 7.33 (d, J = 9.27 Hz, 1H), 7.45 (s, 1H), 7.77 (ddd, J = 0.68, 2.29, 9.15 Hz, 1H), 8.29 (d, J = 7.74 Hz, 1H), 8.42 (d, J = 2.24 Hz, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 24.47, 25.42, 29.06, 32.22, 51.16, 116.50, 125.23, 126.78, 130.07, 133.88, 146.38.

MS (ESI) m/z 314 [M +H] (HPLC System A)

4-(adamantan-2-ylamino)-*N*-methyl-3-nitrobenzenesulfonamide (32)

Following **general procedure C,** using 4-chloro-*N*-methyl-3-nitrobenzenesulfonamide **22** (1 g, 3.99 mmol) and 2-adamantanylamine hydrochloride (0.749 ml, 3.99 mmol). The reaction was purified by flash-column chromatography on silica gel (30% ethyl acetate in heptane) to afford the desired 4-(adamantan-2-ylamino)-*N*-methyl-3-nitrobenzenesulfonamide (0.670 g, 1.833 mmol). (Yield: 46%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.63 - 1.71 (m, 2H), 1.73 - 1.77 (m, 2H), 1.83 - 1.94 (m, 8H), 2.01 - 2.07 (m, 2H), 2.41 (s, 3H), 3.96 - 4.07 (m, 1H), 7.30 (d, J = 9.27 Hz, 1H), 7.46 (s, 1H), 7.78 (dd, J = 2.26, 9.09 Hz, 1H), 8.44 (d, J = 2.25 Hz, 1H), 8.79 (d, J = 7.60 Hz, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 26.95, 29.06, 31.39, 31.66, 36.58, 37.26, 56.00, 116.55, 125.44, 126.80, 130.28, 133.98, 146.37.

MS (ESI) m/z 329 [M +H] (HPLC System A)

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N-(*tert*-butyl)-4-(cyclohexylamino)-3-nitrobenzenesulfonamide (33)

Following **general procedure C,** using *N*-(*tert*-butyl)-4-chloro-3-nitrobenzenesulfonamide **24** (0.950 g, 3.25 mmol) and cyclohexylamine (0.445 ml, 3.89 mmol). The reaction was purified by flash-column chromatography on silica gel (30% ethyl acetate in heptane) to afford the desired *N*-(*tert*-butyl)-4-(cyclohexylamino)-3-nitrobenzenesulfonamide (1.08 g, 3.04 mmol). (Yield: 94%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.11 (s, 9H), 1.21 - 1.31 (m, 1H), 1.35 - 1.49 (m, 4H), 1.56 - 1.64 (m, 1H), 1.68 - 1.76 (m, 2H), 1.92 - 1.99 (m, 2H), 3.65 - 3.78 (m, 1H), 7.31 (d, J = 9.28 Hz, 1H), 7.51 (s, 1H), 7.83 (ddd, J = 0.69, 2.29, 9.21 Hz, 1H), 8.26 (d, J = 7.68 Hz, 1H), 8.47 (d, J = 2.26 Hz, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 24.50, 25.42, 30.22, 32.25, 51.18, 53.74, 116.33, 125.98, 129.87, 130.50, 133.80, 146.07.

MS (ESI) m/z 356 [M +H] (HPLC System A)

N-(*tert*-butyl)-4-(adamantan-2-ylamino)-3-nitrobenzenesulfonamide (34)

Following **general procedure C,** using *N*-(*tert*-butyl)-4-chloro-3-nitrobenzenesulfonamide **24** (1, 3.42 mmol)) and 2-adamantylamine hydrochloride (0.641g, 3.42 mmol). The reaction was purified by flash-column chromatography on silica gel (50% ethyl acetate in heptane) to afford the desired *N*-(*tert*-butyl)-4-(adamantan-2-ylamino)-3-nitrobenzenesulfonamide (1.11 g, 2.72 mmol). (Yield: 80%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.12 (s, 9H), 1.62 - 1.71 (m, 2H), 1.73 - 1.77 (m, 2H), 1.81 - 1.95 (m, 8H), 2.01 - 2.06 (m, 2H), 3.99 - 4.02 (m, 1H), 7.28 (d, J = 9.28 Hz, 1H), 7.53 (s, 1H), 7.83 (ddd, J = 0.64, 2.30, 9.27 Hz, 1H), 8.49 (d, J = 2.26 Hz, 1H), 8.77 (d, J = 7.58 Hz, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 26.95, 30.23, 31.40, 31.67, 36.59, 37.27, 55.99, 116.37, 125.98, 130.06, 130.71, 133.90, 146.06.

MS (ESI) m/z 408 [M +H] (HPLC System A)

3-amino-4-(cyclopropylamino)-*N*-ethylbenzenesulfonamide (35)

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Following **general procedure D**, using 4-(cyclopropylamino)-*N*-ethyl-3-nitrobenzenesulfonamide **25** (0.765 g, 2.68 mmol) to afford the desired 3-amino-4-(cyclopropylamino)-*N*-ethylbenzenesulfonamide (0.500 g, 1.958 mmol) as an oil. (Yield: 73%)

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.43 - 0.51 (m, 2H), 0.68 - 0.74 (m, 2H), 0.97 (t, J = 7.26 Hz, 3H), 2.31 - 2.39 (m, 1H), 2.83 (q, J = 7.27 Hz, 2H), 3.70 (s, 2H), 4.63 (s, 1H), 4.70 (s, 1H), 6.93 (d, J = 8.43 Hz, 1H), 7.14 (d, J = 2.21 Hz, 1H), 7.25 - 7.30 (m, 1H). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 7.25, 14.74, 24.96, 38.16, 110.47, 114.17, 120.54, 126.87, 133.41, 142.25.

t_R 1.34 min, MS (ESI) m/z 256 [M +H] (100%) (HPLC System A)

3-amino-4-(cyclopentylamino)-*N*-ethylbenzenesulfonamide (36)

Following **general procedure D**, using 4-(cyclopentylamino)-*N*-ethyl-3-nitrobenzenesulfonamide **26** (1 g, 3.19 mmol) to afford the desired 3-amino-4-(cyclopentylamino)-*N*-ethylbenzenesulfonamide (0.760 g, 2.68 mmol) as an oil. (Yield: 84%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 0.96 (t, J = 7.23 Hz, 3H), 1.46 - 1.59 (m, 4H), 1.67 - 1.73 (m, 2H), 1.93 - 2.01 (m, 2H), 2.70 (q, J = 7.23 Hz, 2H), 3.75 - 3.80 (m, 1H), 4.97 (s, 2H), 4.98 (s, 1H), 6.49 (d, J = 8.04 Hz, 1H), 6.93 (d, J = 7.74 Hz, 2H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 15.16, 24.34, 33.00, 37.93, 54.17, 108.87, 111.63, 117.54, 126.77, 135.05, 139.08.

t_R 1.64 min, MS (ESI) m/z 284 [M +H] (100%) (HPLC System A)

3-amino-4-(cyclohexylamino)-*N*-ethylbenzenesulfonamide (37)

Following **general procedure D**, using 4-(cyclohexylamino)-*N*-ethyl-3-nitrobenzenesulfonamide **27** (1.760 g, 5.38 mmol) The reaction was purified by flash-column chromatography on silica gel (10% methanol in DCM) to afford the desired 3-amino-4-(cyclohexylamino)-*N*-ethylbenzenesulfonamide (1.412 g, 4.75 mmol) as an amorphous powder. (Yield: 88%)

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.10 (t, J = 7.23 Hz, 3H), 1.17 - 1.51 (m, 6H), 1.70 (dt, J = 3.81, 12.74 Hz, 1H), 1.75 - 1.87 (m, 2H), 2.02 - 2.13 (m, 2H), 2.96 (qd, J = 5.93, 7.18 Hz, 2H), 3.33 (tt, J = 3.76,

10.20 Hz, 1H), 3.49 (s, 2H), 4.46 (t, J = 6.14 Hz, 1H), 6.64 (dd, J = 0.60, 8.40 Hz, 1H), 7.22 (d, J = 2.18 Hz, 1H), 7.34 (dd, J = 2.17, 8.42 Hz, 1H). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 14.99, 24.90, 25.81, 33.16, 38.21, 51.53, 109.71, 115.64, 121.55, 126.26, 132.71, 141.27.

t_R 1.75 min, MS (ESI) m/z 298 [M +H] (100%) (HPLC System A)

3-amino-4-(bicyclo[2.2.1]heptan-2-ylamino)-*N*-ethylbenzenesulfonamide (38)

Following **general procedure D**, using 4-((1S,4S)-bicyclo[2.2.1]heptan-2-ylamino)-*N*-ethyl-3-nitrobenzenesulfonamide **28** (1.044 g, 3.08 mmol) to afford the desired 3-amino-4-(bicyclo[2.2.1]heptan-2-ylamino)-*N*-ethylbenzenesulfonamide (0.700 g, 2.262 mmol) as an oil. (Yield: 74%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 0.96 (t, J = 7.22 Hz, 3H), 1.09 - 1.15 (m, 2H), 1.21 - 1.28 (m, 1H), 1.37 - 1.44 (m, 1H), 1.50 (tp, J = 2.79, 3.55, 6.90 Hz, 3H), 1.71 - 1.77 (m, 1H), 2.20 - 2.27 (m, 2H), 2.69 (q, J = 7.22 Hz, 2H), 3.19 - 3.25 (m, 1H), 4.85 (d, J = 5.64 Hz, 1H), 4.99 (s, 2H), 6.41 (d, J = 8.07 Hz, 1H), 6.92 (d, J = 2.28 Hz, 1H), 6.94 (d, J = 2.20 Hz, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 15.14, 21.23, 26.30, 28.70, 35.49, 35.51, 37.91, 56.30, 108.82, 111.57, 117.45, 126.85, 135.06, 138.51.

t_R 1.83 min, MS (ESI) m/z 310 [M +H] (94%) (HPLC System A)

3-amino-4-(cyclooctylamino)-*N*-ethylbenzenesulfonamide (39)

Following **general procedure D**, using 4-(cyclooctylamino)-*N*-ethyl-3-nitrobenzenesulfonamide **29** (1.1 g, 3.09 mmol) to afford the desired 3-amino-4-(cyclooctylamino)-*N*-ethylbenzenesulfonamide (0.700 g, 2.151 mmol) as an oil. (Yield: 70%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 0.96 (t, J = 7.20 Hz, 3H), 1.42 - 1.83 (m, 15H), 2.69 (q, J = 7.21 Hz, 2H), 3.51 (qt, J = 3.41, 7.64 Hz, 1H), 4.83 (d, J = 7.39 Hz, 1H), 4.96 (s, 2H), 6.37 - 6.42 (m, 1H), 6.91 - 6.96 (m, 2H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 15.14, 24.07, 25.89, 27.18, 32.09, 37.91, 60.23, 108.56, 111.93, 117.67, 126.44, 135.05, 138.33.

t_R 1.98 min , MS (ESI) m/z 326 [M +H] (100%) (HPLC System A)

4-(adamantan-2-ylamino)-3-amino-*N*-ethylbenzenesulfonamide (40)

Following **general procedure D**, using 4-(adamantan-2-ylamino)-*N*-ethyl-3-nitrobenzenesulfonamide **30** (3.05 g, 8.04 mmol). The reaction was purified by flash-column chromatography on silica gel (10% methanol in DCM) to afford the desired 4-(adamantan-2-ylamino)-3-amino-*N*-ethylbenzenesulfonamide (1.2 g, 3.43 mmol) as an amorphous powder. (Yield: 43%)

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.12 (t, J = 7.24 Hz, 3H), 1.61 - 2.12 (m, 15H), 2.98 (qd, J = 6.10, 7.26 Hz, 2H), 3.37 (s, 2H), 3.63 (s, 1H), 4.29 (t, J = 6.09 Hz, 1H), 6.59 (dd, J = 0.68, 8.43 Hz, 1H), 7.23 (d, J = 2.17 Hz, 1H), 7.35 (dd, J = 2.17, 8.42 Hz, 1H). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 15.03, 27.25, 31.59, 37.24, 37.57, 38.21, 56.49, 109.47, 115.80, 121.80, 126.02, 132.63, 141.71.

t_R 2.05 min, MS (ESI) m/z 350 [M +H] (83%) (HPLC System A)

3-amino-4-(cyclohexylamino)-*N*-methylbenzenesulfonamide (41)

Following **general procedure D**, using 4-(cyclohexylamino)-*N*-methyl-3-nitrobenzenesulfonamide **31** (0.600 g, 1.915 mmol) to afford the desired 3-amino-4-(cyclohexylamino)-*N*-methylbenzenesulfonamide (0.523 g, 1.846 mmol) as an amorphous powder. (Yield: 96%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.14 - 1.27 (m, 3H), 1.29 - 1.42 (m, 2H), 1.58 - 1.67 (m, 1H), 1.69 - 1.78 (m, 2H), 1.93 - 2.00 (m, 2H), 2.34 (s, 3H), 3.23 - 3.35 (m, 1H), 4.85 (d, J = 7.45 Hz, 1H), 4.95 (s, 2H), 6.42 - 6.57 (m, 1H), 6.91 (d, J = 7.04 Hz, 2H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 25.16, 26.05, 29.20, 33.05, 51.19, 108.37, 112.14, 117.88, 125.18, 134.94, 138&.61.

t_R 1.67 min, MS (ESI) m/z 284 [M +H] (100%) (HPLC System A)

4-(adamantan-2-ylamino)-3-amino-*N*-methylbenzenesulfonamide (42)

Following **general procedure D,** using 4-(adamantan-2-ylamino)-*N*-methyl-3-nitrobenzenesulfonamide **32** (0.67 g, 1.833 mmol) to afford the desired 4-(adamantan-2-ylamino)-3-amino-*N*-methylbenzenesulfonamide (0.550 g, 1.640 mmol) as an amorphous powder. (Yield: 89%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.46 - 1.56 (m, 2H), 1.67 - 1.75 (m, 3H), 1.78 - 1.90 (m, 6H), 1.95 - 2.00 (m, 2H), 2.05 - 2.12 (m, 2H), 2.34 (s, 3H), 3.55 - 3.61 (m, 1H), 4.72 (d, J = 6.33 Hz, 1H), 5.06 (s, 2H), 6.46 (d, J = 8.39 Hz, 1H), 6.93 (dd, J = 2.24, 8.33 Hz, 1H), 6.97 (d, J = 2.18 Hz, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 27.27, 29.20, 31.33, 37.21, 37.68, 56.48, 108.72, 112.62, 118.05, 125.56, 135.29, 138.92.

t_R 1.97 min, MS (ESI) m/z 336 [M +H] (100%) (HPLC System A)

3-amino-*N*-(*tert*-butyl)-4-(cyclohexylamino)benzenesulfonamide (43)

Following **general procedure D**, using *N*-(*tert*-butyl)-4-(cyclohexylamino)-3-nitrobenzenesulfonamide **33** (1.05 g, 2.95 mmol) to afford the desired 3-amino-*N*-(*tert*-butyl)-4-(cyclohexylamino)benzenesulfonamide (0.850 g, 2.61 mmol) as an amorphous powder. (Yield: 88%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.07 (s, 9H), 1.12 - 1.25 (m, 3H), 1.29 - 1.41 (m, 2H), 1.63 (t, J = 3.78, 12.66 Hz, 1H), 1.68 - 1.79 (m, 2H), 1.91 - 2.01 (m, 2H), 3.22 - 3.32 (m, 1H), 4.78 (d, J = 7.47 Hz, 1H), 4.89 (s, 2H), 6.43 - 6.53 (m, 1H), 6.90 (s, 1H), 6.94 - 6.99 (m, 2H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 25.18, 26.07, 30.21, 33.07, 51.23, 53.07, 108.35, 112.15, 117.63, 130.44, 134.69, 138.19.

t_R 1.93 min, MS (ESI) m/z 326 [M +H] (100%) (HPLC System A)

4-(adamantan-2-ylamino)-3-amino-*N*-(*tert*-butyl)benzenesulfonamide (44)

Following **general procedure D**, using *N*-(*tert*-butyl)-4-(adamantan-2-ylamino)-3-nitrobenzenesulfonamide **34** (1.11 g, 2.72 mmol) to afford the desired 4-(adamantan-2-ylamino)-3-amino-*N*-(*tert*-butyl)benzenesulfonamide (0.917 g, 2.429 mmol) as an amorphous powder. (Yield: 89%)

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^{1}H NMR (400 MHz, DMSO-d_6) δ 1.08 (s, 9H), 1.45 - 1.56 (m, 2H), 1.70 - 1.75 (m, 2H), 1.82 - 1.91 (m, 6H), 1.95 - 2.02 (m, 2H), 2.03 - 2.14 (m, 2H), 3.52 - 3.60 (m, 1H), 4.66 (d, J = 6.26 Hz, 1H), 5.01 (s, 2H), 6.44 (d, J = 8.41 Hz, 1H), 6.93 (s, 1H), 6.98 (dd, J = 2.24, 8.32 Hz, 1H), 7.01 (d, J = 2.22 Hz, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 27.28, 27.46, 30.23, 31.37, 37.22, 37.70, 56.51, 108.64, 112.62, 117.80, 130.82, 135.01, 138.53.

t_R 2.22 min, MS (ESI) m/z 378 [M +H] (100%) (HPLC System A)

3-(benzylamino)-4-(cyclopropylamino)-*N*-ethylbenzenesulfonamide (45)

Following **general procedure E**, using 3-amino-4-(cyclopropylamino)-*N*-ethylbenzenesulfonamide (0.200 g, 0.783 mmol) **35** and benzyl bromide (0.094 ml, 0.783 mmol). The residue was purified by flash-column chromatography (35% ethyl acetate in heptane) on silica gel to afford the desired 3-(benzylamino)-4-(cyclopropylamino)-*N*-ethylbenzenesulfonamide (0.120 g, 0.347 mmol) as an amorphous powder. (Yield: 44%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 0.43 - 0.50 (m, 2H), 0.73 - 0.81 (m, 2H), 0.83 - 0.90 (m, 3H), 2.41 - 2.50 (m, 3H), 4.31 (d, J = 2.94 Hz, 2H), 5.51 (s, 1H), 5.91 (s, 1H), 6.71 (d, J = 2.11 Hz, 1H), 6.88 (dd, J = 7.06, 12.41 Hz, 2H), 7.00 (dd, J = 2.06, 8.24 Hz, 1H), 7.21 - 7.27 (m, 1H), 7.32 - 7.38 (m, 4H). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 7.34, 14.95, 25.11, 38.18, 48.75, 110.68, 120.18, 127.57, 128.11, 128.69, 135.29, 138.40, 142.23.

t_R 1.85 min, MS (ESI) m/z 346 [M +H] (87%) (HPLC System A)

4-(cyclopropylamino)-*N*-ethyl-3-((pyridin-4-ylmethyl)amino)benzenesulfonamide (46)

Following **general procedure E**, using 3-amino-4-(cyclopropylamino)-*N*-ethylbenzenesulfonamide (0.250 g, 0.979 mmol) **35** and 4-(bromomethyl)pyridine hydrobromide (0.867 g, 3.43 mmol). The residue was purified by flash-column chromatography (100% ethyl acetate) on silica gel to afford the 4-(cyclopropylamino)-*N*-ethyl-3-((pyridin-4-ylmethyl)amino)benzenesulfonamide (0.0139 g, 0.040 mmol) as an amorphous powder. (Yield: 4%)

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3 ^{1}**H NMR (400 MHz, DMSO-*d*$_{6}$)** δ 0.44 - 0.54 (m, 2H), 0.76 - 0.80 (m, 3H), 0.83 (t, J = 7.22 Hz, 2H),
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5 2.41 - 2.49 (m, 3H), 4.39 (d, J = 5.08 Hz, 2H), 5.67 (t, J = 5.63 Hz, 1H), 5.90 (d, J = 1.94 Hz, 1H),
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7 6.58 (d, J = 2.06 Hz, 1H), 6.87 - 6.93 (m, 2H), 7.02 (dd, J = 2.02, 8.23 Hz, 1H), 7.37 - 7.40 (m, 2H),
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9 8.51 - 8.54 (m, 2H). ^{13}**C NMR (101 MHz, MeOD)** δ 6.35, 13.08, 13.65, 19.48, 24.51, 37.42, 45.99,
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11 60.14, 108.30, 109.56, 118.33, 122.92, 127.24, 134.25, 141.14, 147.83, 171.59.MS (ESI) m/z 347
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13 t_R 1.25 min, MS (ESI) m/z 347 [M +H] (87%) (HPLC System A)
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19 **3-(benzylamino)-4-(cyclopentylamino)-*N*-ethylbenzenesulfonamide (47)**
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21 Following **general procedure E**, using 3-amino-4-(cyclopentylamino)-*N*-ethylbenzenesulfonamide **36**
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23 (0.250 g, 0.882 mmol) and benzyl bromide (0.106 ml, 0.882 mmol). The residue was purified by flash-
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25 column chromatography (30% ethyl acetate in heptane) on silica gel to afford the desired 3-
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27 (benzylamino)-4-(cyclopentylamino)-*N*-ethylbenzenesulfonamide (0.0608 g, 0.163 mmol) as an
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29 amorphous powder. (Yield: 19%)
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31 ^{1}**H NMR (400 MHz, DMSO-*d*$_{6}$)** δ 0.85 (t, J = 7.26 Hz, 3H), 1.45 - 1.66 (m, 4H), 1.65 - 1.77 (m, 2H),
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33 1.92 - 2.05 (m, 2H), 2.46 (d, J = 8.07 Hz, 2H), 3.82 (p, J = 5.97 Hz, 1H), 4.33 (s, 2H), 5.12 (s, 1H),
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35 5.90 (s, 1H), 6.54 (d, J = 8.38 Hz, 1H), 6.69 (d, J = 2.10 Hz, 1H), 6.83 - 6.91 (m, 1H), 6.97 (dd, J =
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37 2.09, 8.28 Hz, 1H), 7.20 - 7.28 (m, 1H), 7.31 - 7.38 (m, 4H). ^{13}**C NMR (101 MHz, CDCl$_3$)** δ 14.92,
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39 24.21, 32.99, 38.16, 49.18, 55.51, 112.41, 112.99, 121.02, 127.76, 128.07, 128.40, 128.69, 134.11,
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41 137.19, 139.86.
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43 t_R 2.02 min, MS (ESI) m/z 374 [M +H] (64%) (HPLC System A)
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49 **4-(cyclopentylamino)-*N*-ethyl-3-((pyridin-4-ylmethyl)amino)benzenesulfonamide (48)**
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51 Following **general procedure E**, using 3-amino-4-(cyclopentylamino)-*N*-ethylbenzenesulfonamide **36**
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53 (0.250 g, 0.882 mmol) and 4-(bromomethyl)pyridine hydrobromide (0.781 g, 3.09 mmol). The residue
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55 was purified by flash-column chromatography (100% ethyl acetate) on silica gel to afford the 4-
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(cyclopentylamino)-*N*-ethyl-3-((pyridin-4-ylmethyl)amino)benzenesulfonamide (0.0475 g, 0.127 mmol) as an amorphous powder. (Yield: 15%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 0.81 (t, J = 7.23 Hz, 3H), 1.48 - 1.63 (m, 5H), 1.68 - 1.78 (m, 2H), 1.97 - 2.08 (m, 2H), 2.43 (qd, J = 5.73, 7.23 Hz, 2H), 3.83 (s, 1H), 4.35 - 4.49 (m, 2H), 5.22 (s, 1H), 5.88 (t, J = 5.36 Hz, 1H), 6.52 - 6.59 (m, 1H), 6.87 (t, J = 5.82 Hz, 1H), 6.98 (dd, J = 2.05, 8.31 Hz, 1H), 7.33 - 7.42 (m, 2H), 8.51 - 8.54 (m, 2H). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 14.86, 24.27, 33.52, 38.07, 47.19, 54.51, 110.09, 110.45, 120.46, 122.67, 127.06, 134.80, 141.41, 149.40.

t_R 1.44 min, MS (ESI) m/z 375 [M +H] (100%) (HPLC System A)

3-(benzylamino)-4-(cyclohexylamino)-*N*-ethylbenzenesulfonamide (49)

Following **general procedure E**, using 3-amino-4-(cyclohexylamino)-*N*-ethylbenzenesulfonamide **37** (0.200 g, 0.672 mmol) and benzyl bromide (0.080 ml, 0.672 mmol). The residue was purified by flash-column chromatography (85% methanol in water) on silica gel to afford the desired 3-(benzylamino)-4-(cyclohexylamino)-*N*-ethylbenzenesulfonamide (0.0403 g, 0.104 mmol) as an amorphous powder. (Yield: 16%)

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.04 (t, J = 7.23 Hz, 3H), 1.19 - 1.50 (m, 5H), 1.67 - 1.75 (m, 1H), 1.76 - 1.85 (m, 2H), 2.03 - 2.15 (m, 2H), 2.85 (qd, J = 6.08, 7.23 Hz, 2H), 3.27 - 3.43 (m, 2H), 3.83 (d, J = 6.24 Hz, 1H), 4.20 (t, J = 6.14 Hz, 1H), 4.32 (d, J = 5.42 Hz, 2H), 6.67 (dd, J = 0.66, 8.33 Hz, 1H), 7.15 (d, J = 2.11 Hz, 1H), 7.30 - 7.44 (m, 6H). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 14.98, 24.96, 25.84, 33.29, 38.16, 48.96, 51.57, 109.40, 111.52, 120.64, 126.77, 127.53, 128.02, 128.71, 135.30, 138.62, 141.09.

t_R 2.14 min, MS (ESI) m/z 388 [M +H] (100%) (HPLC System A)

4-(cyclohexylamino)-*N*-ethyl-3-((pyridin-4-ylmethyl)amino)benzenesulfonamide (50)

Following **general procedure E**, using 3-amino-4-(cyclohexylamino)-*N*-ethylbenzenesulfonamide **37** (0.200g, 0.672 mmol) and 4-(bromomethyl)pyridine hydrobromide (0.340 g, 1.345 mmol). The

residue was purified by flash-column chromatography (95% ethyl acetate in heptane) on silica gel to afford the desired 4-(cyclohexylamino)-*N*-ethyl-3-((pyridin-4-ylmethyl)amino)benzenesulfonamide (0.0371 g, 0.095 mmol) as an amorphous powder. (Yield: 14%)

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.00 (t, J = 7.24 Hz, 3H), 1.20 - 1.34 (m, 3H), 1.37 - 1.50 (m, 2H), 1.67 - 1.75 (m, 1H), 1.77 - 1.87 (m, 2H), 2.06 - 2.14 (m, 2H), 2.80 (qd, J = 6.04, 7.25 Hz, 2H), 3.29 - 3.41 (m, 1H), 3.61 (t, J = 5.77 Hz, 1H), 3.91 (d, J = 6.27 Hz, 1H), 4.32 - 4.42 (m, 3H), 6.65 - 6.72 (m, 1H), 7.02 (d, J = 2.04 Hz, 1H), 7.27 - 7.31 (m, 2H), 7.36 (dd, J = 2.08, 8.38 Hz, 1H), 8.53 - 8.60 (m, 2H). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 14.93, 24.91, 25.81, 33.28, 38.09, 47.51, 51.59, 109.79, 111.63, 120.97, 122.54, 126.95, 134.52, 141.13, 147.81, 150.03.

t_R 1.51 min , MS (ESI) m/z 389 [M +H] (100%) (HPLC System A)

3-(benzylamino)-4-(bicyclo[2.2.1]heptan-2-ylamino)-*N*-ethylbenzenesulfonamide (51)

Following **general procedure E**, using 3-amino-4-(bicyclo[2.2.1]heptan-2-ylamino)-*N*-ethylbenzenesulfonamide **38** (0.200 g, 0.646 mmol) and benzyl bromide (0.077 ml, 0.646 mmol). The residue was purified by flash-column chromatography (35% ethyl acetate in heptane) on silica gel to afford the desired 3-(benzylamino)-4-(bicyclo[2.2.1]heptan-2-ylamino)-*N*-ethylbenzenesulfonamide (0.0525 g, 0.131 mmol) as an amorphous powder. (Yield: 20%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 0.85 (t, J = 7.22 Hz, 3H), 1.12 - 1.18 (m, 2H), 1.22 - 1.30 (m, 1H), 1.37 - 1.57 (m, 4H), 1.71 - 1.81 (m, 1H), 2.26 (q, J = 4.05 Hz, 2H), 2.43 - 2.49 (m, 2H), 3.22 - 3.29 (m, 1H), 4.33 (s, 2H), 5.12 (s, 1H), 5.76 (s, 1H), 6.44 (d, J = 8.37 Hz, 1H), 6.69 (d, J = 2.11 Hz, 1H), 6.87 (t, J = 5.86 Hz, 1H), 6.96 (dd, J = 2.08, 8.29 Hz, 1H), 7.21 - 7.27 (m, 1H), 7.30 - 7.39 (m, 4H). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 14.94, 26.41, 28.44, 35.56, 35.73, 38.16, 40.80, 41.33, 48.88, 56.70, 110.21, 111.20, 120.53, 127.25, 127.54, 128.10, 128.69, 135.25, 138.41, 141.09.

t_R 2.13 min, MS (ESI) m/z 400 [M +H] (100%) (HPLC System A)

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4-(bicyclo[2.2.1]heptan-2-ylamino)-*N*-ethyl-3-((pyridin-4-ylmethyl)amino)benzenesulfonamide (52)

Following **general procedure E**, using 3-amino-4-(bicyclo[2.2.1]heptan-2-ylamino)-*N*-ethylbenzenesulfonamide **38** (0.200 g, 0.646 mmol) and 4-(bromomethyl)pyridine hydrobromide (0.572 g, 2.262 mmol). The residue was purified by flash-column chromatography (100% ethyl acetate) on silica gel to afford the 4-(bicyclo[2.2.1]heptan-2-ylamino)-*N*-ethyl-3-((pyridin-4-ylmethyl)amino)benzenesulfonamide (0.0582 g, 0.145 mmol) as an amorphous powder. (Yield: 23%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 0.81 (t, J = 7.23 Hz, 3H), 1.13 - 1.21 (m, 2H), 1.22 - 1.33 (m, 1H), 1.39 - 1.61 (m, 4H), 1.72 - 1.83 (m, 1H), 2.24 - 2.32 (m, 2H), 2.43 (qd, J = 5.73, 7.23 Hz, 2H), 3.29 (s, 1H), 4.33 - 4.47 (m, 2H), 5.12 (d, J = 5.49 Hz, 1H), 5.92 (s, 1H), 6.47 (d, J = 8.35 Hz, 1H), 6.55 (d, J = 2.11 Hz, 1H), 6.88 (t, J = 5.81 Hz, 1H), 6.98 (dd, J = 2.04, 8.32 Hz, 1H), 7.36 - 7.42 (m, 2H), 8.49 - 8.56 (m, 2H). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 14.82, 26.38, 28.43, 35.55, 35.71, 38.05, 40.71, 41.30, 47.11, 56.65, 109.97, 110.27, 120.31, 122.66, 126.96, 134.80, 140.88, 148.52, 149.47.

t_R 1.53 min, MS (ESI) m/z 401 [M +H] (100%) (HPLC System A)

3-(benzylamino)-4-(cyclooctylamino)-*N*-ethylbenzenesulfonamide (53)

Following **general procedure E**, using 3-amino-4-(cyclooctylamino)-*N*-ethylbenzenesulfonamide **39** (200 mg, 0.614 mmol) and benzyl bromide (0.073 ml, 0.614 mmol). The residue was purified by flash-column chromatography (40% ethyl acetate in heptane) on silica gel to afford the desired 3-(benzylamino)-4-(cyclooctylamino)-*N*-ethylbenzenesulfonamide (0.0729 g, 0.175 mmol) as an amorphous powder. (Yield: 26%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 0.85 (t, J = 7.23 Hz, 3H), 1.47 - 1.88 (m, 14H), 2.44 (qd, J = 5.77, 7.32 Hz, 2H), 3.55 (s, 1H), 4.33 (s, 2H), 5.09 (s, 1H), 5.68 - 5.80 (m, 1H), 6.44 (d, J = 8.44 Hz, 1H), 6.69 (d, J = 2.12 Hz, 1H), 6.85 (t, J = 5.85 Hz, 1H), 6.96 (dd, J = 2.10, 8.29 Hz, 1H), 7.21 - 7.39 (m, 5H). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 14.97, 24.10, 25.95, 26.93, 32.68, 38.16, 48.90, 52.90, 110.16, 111.61, 120.54, 127.51, 128.01, 128.69, 135.45, 138.49, 140.51.

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3 t_R 2.24 min, MS (ESI) m/z 416 [M +H] (100%) (HPLC System A)
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8 **4-(cyclooctylamino)-*N*-ethyl-3-((pyridin-4-ylmethyl)amino)benzenesulfonamide (54)**
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11 Following **general procedure E**, using 3-amino-4-(cyclooctylamino)-*N*-ethylbenzenesulfonamide **39**
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13 (0.200 g, 0.614 mmol) and 4-(bromomethyl)pyridine hydrobromide (0.544 g, 2.151 mmol). The
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15 residue was purified by flash-column chromatography (100% ethyl acetate) on silica gel to afford the
16
17 4-(cyclooctylamino)-*N*-ethyl-3-((pyridin-4-ylmethyl)amino)benzenesulfonamide (0.0583 g, 0.140
18
19 mmol) as an amorphous powder. (Yield: 23%)
20
21 **^{1}H NMR (400 MHz, DMSO-d_6)** δ 0.81 (t, J = 7.23 Hz, 3H), 1.49 - 1.90 (m, 14H), 2.34 - 2.50 (m, 2H),
22
23 3.57 (s, 1H), 4.41 (d, J = 5.09 Hz, 2H), 5.08 (d, J = 7.03 Hz, 1H), 5.89 (t, J = 5.74 Hz, 1H), 6.47 (d, J =
24
25 8.42 Hz, 1H), 6.56 (d, J = 2.09 Hz, 1H), 6.86 (t, J = 5.83 Hz, 1H), 6.98 (dd, J = 2.08, 8.31 Hz, 1H),
26
27 7.32 - 7.43 (m, 2H), 8.48 - 8.57 (m, 2H). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 14.87, 24.09, 25.93, 26.95,
28
29 32.72, 38.07, 47.31, 52.58, 109.69, 111.10, 120.79, 122.68, 126.69, 134.62, 140.86, 148.60, 149.42.
30
31 MS (ESI) m/z 417 [M +H] (95%) (HPLC System A)
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37 **4-(adamantan-2-ylamino)-3-(benzylamino)-*N*-ethylbenzenesulfonamide (55)**
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39 Following **general procedure E**, using 4-(adamantan-2-ylamino)-3-amino-*N*-
40
41 ethylbenzenesulfonamide **40** (0.200 g, 0.572 mmol) and benzyl bromide (0.082 ml, 0.687 mmol). The
42
43 residue was purified by flash-column chromatography (10% methanol in DCM) on silica gel to afford
44
45 the desired 4-(adamantan-2-ylamino)-3-(benzylamino)-*N*-ethylbenzenesulfonamide (0.0723 g, 0.164
46
47 mmol) as an amorphous powder. (Yield: 29%)
48
49 **^{1}H NMR (400 MHz, $CDCl_3$)** δ 1.03 (t, J = 7.22 Hz, 3H), 1.62 - 1.83 (m, 6H), 1.92 - 2.09 (m, 9H),
50
51 2.82 (qd, J = 5.88, 7.15 Hz, 2H), 3.46 (t, J = 5.83 Hz, 1H), 3.64 (dt, J = 2.73, 5.96 Hz, 1H), 4.35 (d, J =
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53 5.96 Hz, 2H), 4.40 (d, J = 6.08 Hz, 1H), 6.62 (d, J = 8.43 Hz, 1H), 7.17 (d, J = 2.08 Hz, 1H), 7.28 -
54
55 7.33 (m, 1H), 7.35 - 7.43 (m, 5H). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 14.95, 27.27, 31.62, 37.25, 37.58,
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57 38.15, 49.01, 56.57, 109.31, 112.49, 121.07, 126.50, 127.41, 127.72, 128.69, 135.17, 138.80, 141.61.
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t_R 2.50 min, MS (ESI) m/z 440 [M +H] (100%) (HPLC System A)

4-(adamantan-2-ylamino)-*N*-ethyl-3-((pyridin-4-ylmethyl)amino)benzenesulfonamide (56)

Following **general procedure E**, using 4-(adamantan-2-ylamino)-3-amino-*N*-ethylbenzenesulfonamide **40** (0.200 g, 0.572 mmoland 4-(bromomethyl)pyridine hydrobromide (0.217 g, 0.858 mmol). The residue was purified by flash-column chromatography (40% ethyl acetate in heptane) on silica gel to afford the desired 4-(adamantan-2-ylamino)-*N*-ethyl-3-((pyridin-4-ylmethyl)amino)benzenesulfonamide (0.0483 g, 0.110 mmol) as an amorphous powder. (Yield: 19%)

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.99 (t, J = 7.23 Hz, 3H), 1.62 - 2.12 (m, 15H), 2.78 (qd, J = 5.97, 7.22 Hz, 2H), 3.64 (d, J = 2.82 Hz, 2H), 4.39 (s, 2H), 4.44 (t, J = 6.08 Hz, 1H), 6.64 (d, J = 8.87 Hz, 1H), 7.05 (d, J = 2.09 Hz, 1H), 7.29 - 7.32 (m, 2H), 7.37 (dd, J = 2.09, 8.39 Hz, 1H), 8.53 - 8.60 (m, 2H). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 14.91, 31.62, 31.85, 37.22, 37.53, 38.07, 47.59, 56.58, 109.67, 112.49, 121.42, 122.40, 126.70, 134.34, 141.61, 147.99, 149.99.

t_R 1.75 min, MS (ESI) m/z 441 [M +H] (100%) (HPLC System A)

3-(benzylamino)-4-(cyclohexylamino)-*N*-methylbenzenesulfonamide (57)

Following **general procedure E**, using 3-amino-4-(cyclohexylamino)-*N*-methylbenzenesulfonamide **41** (0.170 g, 0.600 mmol) and benzyl bromide (0.072 ml, 0.600 mmol). The residue was purified by flash-column chromatography (30% ethyl acetate in heptane) on silica gel to afford the desired 3-(benzylamino)-4-(cyclohexylamino)-*N*-methylbenzenesulfonamide (0.05 g, 0.134 mmol) as an amorphous powder. (Yield: 22%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.16 - 1.28 (m, 3H), 1.32 - 1.44 (m, 2H), 1.60 - 1.68 (m, 1H), 1.71 - 1.80 (m, 2H), 1.95 - 2.05 (m, 2H), 2.17 (d, J = 5.16 Hz, 3H), 3.34 (s, 1H), 4.28 - 4.34 (m, 2H), 5.12 (d, J = 6.84 Hz, 1H), 5.66 (t, J = 5.28 Hz, 1H), 6.55 (d, J = 8.43 Hz, 1H), 6.70 (d, J = 2.09 Hz, 1H), 6.79 (q, J = 5.11 Hz, 1H), 6.95 (dd, J = 2.08, 8.34 Hz, 1H), 7.22 - 7.27 (m, 1H), 7.31 - 7.40 (m, 4H).

^{13}C NMR (101 MHz, MeOD) δ 24.67, 24.86, 25.36, 25.67, 27.83, 32.71, 33.36, 51.41, 108.23, 109.51, 118.54, 124.48, 126.61, 127.21, 128.09, 134.97, 139.36, 139.90.

t_R 2.12 min , MS (ESI) m/z 374 [M +H] (100%) (HPLC System A)

4-(cyclohexylamino)-*N*-methyl-3-((pyridin-4-ylmethyl)amino)benzenesulfonamide (58)

Following **general procedure E**, using 3-amino-4-(cyclohexylamino)-*N*-methylbenzenesulfonamide **41** (0.150 g, 0.614 mmol) and 4-(bromomethyl)pyridine hydrobromide (0.268 g, 1.059 mmol). The residue was purified by flash-column chromatography (100% ethyl acetate) on silica gel to afford the 4-(cyclohexylamino)-*N*-methyl-3-((pyridin-4-ylmethyl)amino)benzenesulfonamide (0.06 g, 0.160 mmol) as an amorphous powder. (Yield: 30%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.16 - 1.31 (m, 3H), 1.32 - 1.45 (m, 2H), 1.60 - 1.68 (m, 1H), 1.71 - 1.80 (m, 2H), 1.96 - 2.05 (m, 2H), 2.14 (d, J = 5.25 Hz, 3H), 3.31 (d, J = 0.80 Hz, 1H), 4.38 (d, J = 5.33 Hz, 2H), 5.10 (d, J = 7.15 Hz, 1H), 5.80 (t, J = 5.71 Hz, 1H), 6.55 - 6.61 (m, 2H), 6.79 (q, J = 5.11 Hz, 1H), 6.97 (dd, J = 2.02, 8.34 Hz, 1H), 7.32 - 7.38 (m, 2H), 8.45 - 8.54 (m, 2H). **^{13}C NMR (101 MHz, MeOD)** δ 24.83, 25.67, 27.72, 32.74, 46.16, 51.40, 108.40, 109.21, 118.93, 122.68, 124.58, 134.25, 139.89, 148.63, 150.71.

t_R 1.47 min, MS (ESI) m/z 375 [M +H] (100%) (HPLC System A)

4-(adamantan-2-ylamino)-3-(benzylamino)-*N*-methylbenzenesulfonamide (59)

Following **general procedure E**, using 4-(adamantan-2-ylamino)-3-amino-*N*-methylbenzenesulfonamide **42** (0.200 g, 0.596 mmol) and benzyl bromide (0.071 ml, 0.596 mmol). The residue was purified by flash-column chromatography (30% ethyl acetate in heptane) on silica gel to afford the desired 4-(adamantan-2-ylamino)-3-(benzylamino)-*N*-methylbenzenesulfonamide (0.096 g, 0.226 mmol) as an amorphous powder. (Yield: 38%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.49 - 1.57 (m, 2H), 1.66 - 1.76 (m, 3H), 1.81 - 1.93 (m, 6H), 1.99 - 2.06 (m, 2H), 2.08 - 2.16 (m, 4H), 3.63 (s, 1H), 4.36 (s, 2H), 4.97 (s, 1H), 5.97 (s, 1H), 6.52 (d, J =

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3 8.41 Hz, 1H), 6.73 (d, J = 2.11 Hz, 1H), 6.79 (q, J = 5.10 Hz, 1H), 6.97 (dd, J = 2.09, 8.39 Hz, 1H),
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5 7.19 - 7.26 (m, 1H), 7.30 - 7.36 (m, 2H), 7.36 - 7.41 (m, 2H). **^{13}C NMR (101 MHz, DMSO-*d_6*)** δ
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7 27.29, 27.46, 28.92, 31.25, 31.54, 37.19, 37.66, 47.24, 56.60, 108.55, 109.05, 118.02, 125.81, 127.16,
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9 127.58, 128.76, 135.14, 139.25, 140.15.
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11 t_R 2.41 min, MS (ESI) m/z 426 [M +H] (100%) (HPLC System A)
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16 **4-(adamantan-2-ylamino)-*N*-methyl-3-((pyridin-4-ylmethyl)amino)benzenesulfonamide (60)**
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19 Following **general procedure E**, using 4-(adamantan-2-ylamino)-3-amino-*N*-
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21 methylbenzenesulfonamide **42** (0,150 g, 0.447 mmol) and 4-(bromomethyl)pyridine hydrobromide
22
23 (0.170 g, 0.671 mmol). The residue was purified by flash-column chromatography (100% ethyl
24
25 acetate) on silica gel to afford the 4-(adamantan-2-ylamino)-*N*-methyl-3-((pyridin-4-
26
27 ylmethyl)amino)benzenesulfonamide (0.043 g, 0,101 mmol) as an amorphous powder. (Yield: 23%)
28
29 **^{1}H NMR (400 MHz, DMSO-*d_6*)** δ 1.50 - 1.59 (m, 2H), 1.71 - 1.77 (m, 2H), 1.82 - 1.92 (m, 6H), 2.02
30
31 - 2.06 (m, 2H), 2.08 - 2.16 (m, 5H), 3.65 (dd, J = 3.03, 6.04 Hz, 1H), 4.41 (d, J = 5.39 Hz, 2H), 4.95
32
33 (d, J = 5.92 Hz, 1H), 6.07 (t, J = 5.76 Hz, 1H), 6.54 (d, J = 8.41 Hz, 1H), 6.60 (d, J = 2.07 Hz, 1H),
34
35 6.80 (q, J = 5.10 Hz, 1H), 6.98 (dd, J = 2.05, 8.31 Hz, 1H), 7.32 - 7.41 (m, 2H), 8.45 - 8.56 (m, 2H).
36
37 **^{13}C NMR (101 MHz, DMSO-*d_6*)** δ 27.28, 27.44, 28.92, 31.26, 31.53, 37.17, 37.66, 46.18, 56.60,
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39 106.02, 108.72, 118.27, 122.70, 124.49, 125.80, 134.73, 139.26, 147.14, 149.92.
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41 t_R 1.67 min ,MS (ESI) m/z 427 [M +H] (100%) (HPLC System A)
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47 **3-(benzylamino)-*N*-(*tert*-butyl)-4-(cyclohexylamino)benzenesulfonamide (61)**
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50 Following **general procedure E**, using 3-amino-*N*-(*tert*-butyl)-4-
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52 (cyclohexylamino)benzenesulfonamide **43** (0.200 g, 0.614 mmol) and benzyl bromide (0.073 ml,
53
54 0.614 mmol). The residue was purified by flash-column chromatography (25% ethyl acetate in
55
56 heptane) on silica gel to afford the desired 3-(benzylamino)-*N*-(*tert*-butyl)-4-
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58 (cyclohexylamino)benzenesulfonamide (0.211 g, 0.508 mmol) as an amorphous powder. (Yield: 83%)
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^{1}H NMR (400 MHz, DMSO-d_6) δ 0.93 (s, 9H), 1.15 - 1.29 (m, 3H), 1.30 - 1.45 (m, 2H), 1.60 - 1.70 (m, 1H), 1.70 - 1.79 (m, 2H), 1.95 - 2.04 (m, 2H), 3.31 (s, 1H), 4.33 (d, J = 5.41 Hz, 2H), 5.04 (d, J = 7.18 Hz, 1H), 5.63 (t, J = 5.73 Hz, 1H), 6.52 (d, J = 8.45 Hz, 1H), 6.77 (d, J = 2.11 Hz, 1H), 6.84 (s, 1H), 6.99 (dd, J = 2.09, 8.31 Hz, 1H), 7.17 - 7.26 (m, 1H), 7.28 - 7.40 (m, 4H). **^{13}C NMR (101 MHz, MeOD)** δ 24.87, 25.70, 28.87, 32.73, 51.47, 52.84, 108.24, 109.53, 118.28, 126.55, 126.99, 128.13, 129.69, 134.92, 139.39.

t_R 2.38 min, MS (ESI) m/z 416 [M +H] (95%) (HPLC System A)

N-(*tert*-butyl)-4-(cyclohexylamino)-3-((pyridin-4-ylmethyl)amino)benzenesulfonamide (62)

Following **general procedure E**, using 3-amino-*N*-(*tert*-butyl)-4-(cyclohexylamino)benzenesulfonamide **43** (0.150 g, 0,461 mmol) and 4-(bromomethyl)pyridine hydrobromide (0.233 g, 0.922 mmol). The residue was purified by flash-column chromatography (100% ethyl acetate) on silica gel to afford the *N*-(*tert*-butyl)-4-(cyclohexylamino)-3-((pyridin-4-ylmethyl)amino)benzenesulfonamide (0.073 g, 0.175 mmol) as an amorphous powder. (Yield: 38%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 0.87 (s, 9H), 1.19 - 1.31 (m, 3H), 1.33 - 1.46 (m, 2H), 1.60 - 1.69 (m, 1H), 1.72 - 1.80 (m, 2H), 1.97 - 2.06 (m, 2H), 3.31 (s, 1H), 4.39 (d, J = 5.53 Hz, 2H), 5.03 (d, J = 7.14 Hz, 1H), 5.79 (t, J = 5.76 Hz, 1H), 6.55 (d, J = 8.46 Hz, 1H), 6.62 (d, J = 2.10 Hz, 1H), 6.83 (s, 1H), 7.00 (dd, J = 2.07, 8.32 Hz, 1H), 7.27 - 7.34 (m, 2H), 8.44 - 8.51 (m, 2H). **^{13}C NMR (101 MHz, MeOD)** δ 24.86, 25.71, 28.87, 28.90, 32.77, 51.47, 52.77, 108.42, 109.25, 118.61, 122.60, 129.77, 134.04, 139.49, 148.63, 150.79.

t_R 1.62 min, MS (ESI) m/z 417 [M +H] (100%) (HPLC System A)

4-(adamantan-2-ylamino)-3-(benzylamino)-*N*-(*tert*-butyl)benzenesulfonamide (63)

Following **general procedure E**, using 4-(adamantan-2-ylamino)-3-amino-*N*-(*tert*-butyl)benzenesulfonamide **44** (0.150 g, 0.397 mmol) and benzyl bromide (0.048 ml, 0.397 mmol). The residue was purified by flash-column chromatography (25% ethyl acetate in heptane) on silica gel to

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afford the desired 4-(adamantan-2-ylamino)-3-(benzylamino)-*N*-(*tert*-butyl)benzenesulfonamide (0.09 g, 0.192 mmol) as an amorphous powder. (Yield: 48%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 0.91 (s, 9H), 1.48 - 1.58 (m, 2H), 1.72 - 1.76 (m, 2H), 1.80 - 1.90 (m, 6H), 2.02 (d, J = 3.67 Hz, 2H), 2.12 (d, J = 12.73 Hz, 2H), 3.62 (d, J = 4.45 Hz, 1H), 4.31 - 4.41 (m, 2H), 4.89 (d, J = 5.77 Hz, 1H), 5.92 (s, 1H), 6.48 (d, J = 8.46 Hz, 1H), 6.78 (d, J = 2.16 Hz, 1H), 6.85 (s, 1H), 7.00 (dd, J = 2.10, 8.36 Hz, 1H), 7.17 - 7.25 (m, 1H), 7.29 - 7.39 (m, 4H). **^{13}C NMR (101 MHz, MeOD)** δ 27.48, 27.63, 28.88, 31.24, 31.50, 36.99, 37.42, 52.86, 56.71, 108.49, 110.92, 118.93, 126.52, 126.90, 128.13, 129.85, 135.01, 139.54, 140.33.

t_R 2.61 min, MS (ESI) m/z 468 [M +H] (100%) (HPLC System A)

4-(adamantan-2-ylamino)-*N*-(*tert*-butyl)-3-((pyridin-4-ylmethyl)amino)benzenesulfonamide (64)

Following **general procedure E**, using 4-(adamantan-2-ylamino)-3-amino-*N*-(*tert*-butyl)benzenesulfonamide **44** (0.150 g, 0,397 mmol) and 4-(bromomethyl)pyridine hydrobromide (0.151 g, 0.596 mmol). The residue was purified by flash-column chromatography (100% ethyl acetate) on silica gel to afford the 4-(adamantan-2-ylamino)-*N*-(*tert*-butyl)-3-((pyridin-4-ylmethyl)amino)benzenesulfonamide (0.04 g, 0.085 mmol) as an amorphous powder. (Yield: 22%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 0.86 (s, 9H), 1.54 (d, J = 12.17 Hz, 2H), 1.75 (d, J = 2.84 Hz, 2H), 1.81 - 1.92 (m, 6H), 2.03 (d, J = 3.72 Hz, 2H), 2.13 (d, J = 12.73 Hz, 2H), 3.64 (s, 1H), 4.41 (d, J = 5.56 Hz, 2H), 4.88 (d, J = 5.89 Hz, 1H), 6.04 (t, J = 5.74 Hz, 1H), 6.51 (d, J = 8.48 Hz, 1H), 6.65 (d, J = 2.10 Hz, 1H), 6.85 (s, 1H), 7.01 (dd, J = 2.09, 8.38 Hz, 1H), 7.28 - 7.35 (m, 2H), 8.45 - 8.50 (m, 2H). **^{13}C NMR (101 MHz, MeOD)** δ 27.49, 27.62, 28.85, 31.25, 31.51, 36.98, 37.41, 46.11, 52.77, 56.73, 108.63, 110.28, 119.09, 122.59, 129.96, 134.14, 140.10, 148.62, 150.88.

t_R 1.85 min, MS (ESI) m/z 469 [M +H] (100%) (HPLC System A)

Inhibition of erastin-induced ferroptosis in IMR-32 neuroblastoma cells

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In order to determine IC_{50}-values, human neuroblastoma cells (IMR-32) were seeded in a 96-well plate at a density of 25,000 cells/well. The next day, the cells were pretreated for 1h (in triplicates) with a $^{1}/_{3}$ dilution series of ferrostatin-1 analogues ranging from 5µM to 0.68nM and Sytox Green (1.6 µM). After stimulating the cells with erastin (10µM) the plate was transferred to a temperature- and CO_2-controled FLUOstar Omega fluorescence plate reader (BMG Labtech). Sytox Green intensity was measured after 13h using an excitation filter of 485 nm and an emission filter of 520 nm. In each setup, Triton-X100 (0.05%) was used to induce lyses of the cells in 6 wells/plate, and was used as 100% cell death reference. The percentage of the cell death was calculated by the formula ((AVG[erastin] - AVG[background]) / (AVG[Triton-X100] - AVG[background])) × 100. Cell death percentage was plotted in GraphPad Prism 6, and IC_{50}-values were calculated using a sigmoidal dose-response (variable slope) curve.

ASSOCIATED CONTENT

Supporting Information. Detailed protocols, extended data series, molecular formula strings, additional charts and graphs for *in vitro* ADME analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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ABBREVIATIONS

GPX4, Glutathione peroxidase 4; fer-1, ferrostatin-1; GSH, glutathione; PUFA, polyunsaturated fatty acids; CR, cystine reductase; GCL, glutamate cysteine lipase; γGC, γ-glutamyl cysteine; GS, glutathione synthase; GSSG, glutathione disulfide.

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Table of contents graphic

49

IC_{50}(Ferroptosis)= 3.4 nM

Microsomal stability:
- $T_{1/2}$(mouse)= 187 min
- $T_{1/2}$(human)= 88 min

Plasma stability after 6h:
- %recovery(mouse)= 99
- %recovery(human)= 100

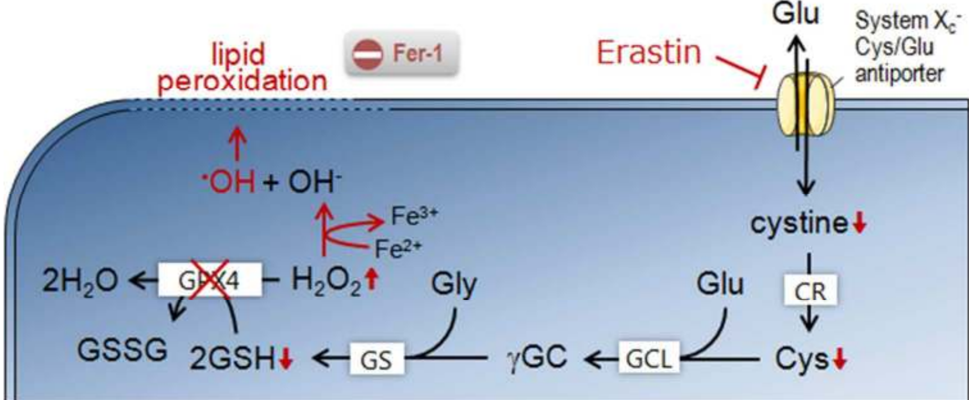

Figure 1. Biological mechanisms of ferroptosis
51x21mm (300 x 300 DPI)

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Ferrostatin-1
IC_{50} = 95 nM

2
SRS9-11
IC_{50} = 950 nM

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SRS11-92
IC_{50} = 6 nM

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SRS16-86
IC_{50} ~ 350 nM

Figure 2. reported analogues of fer-1
93x72mm (600 x 600 DPI)

Figure 3. Structural comparison of ferrostatin-1
38x10mm (600 x 600 DPI)

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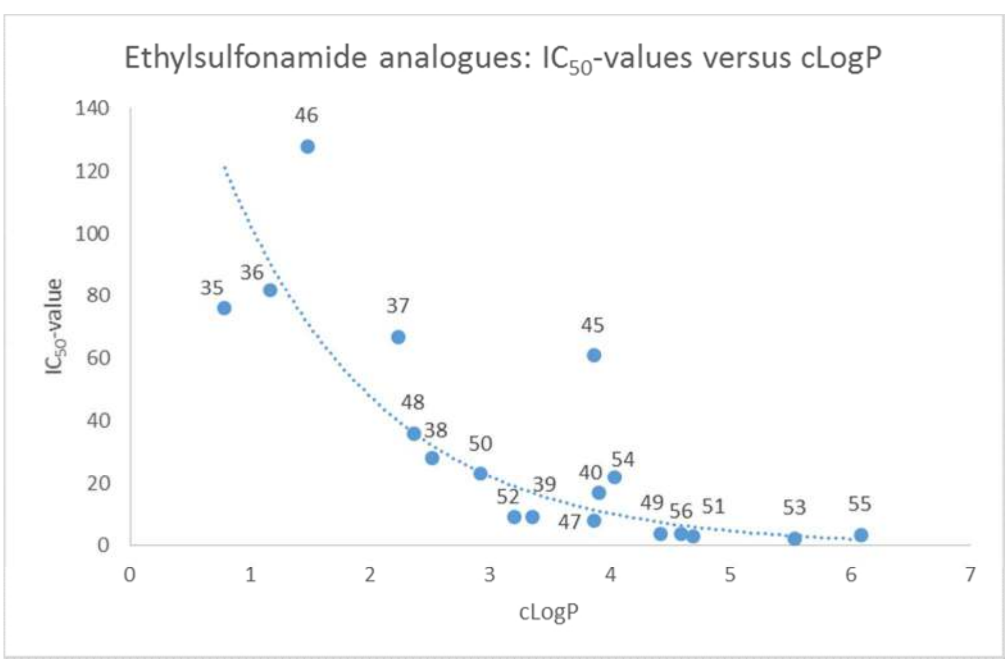
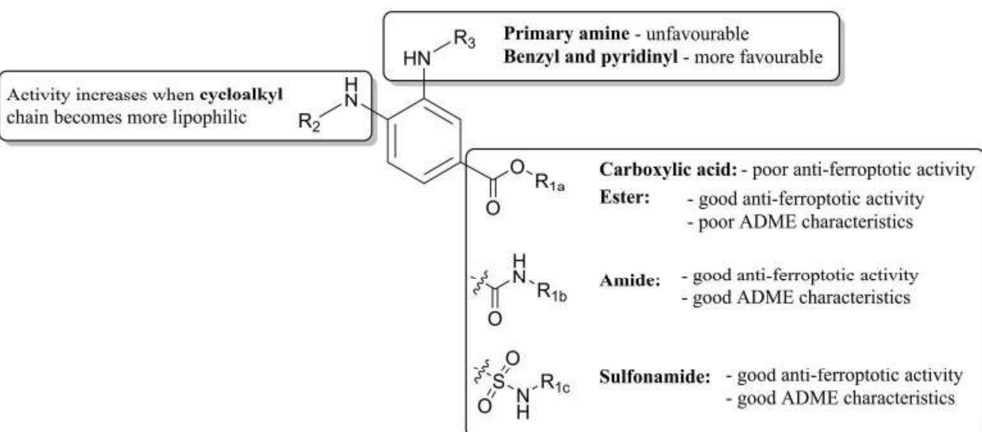


Figure 4. IC50-values versus cLogP
140x90mm (150 x 150 DPI)



R_1 = (a) H, methyl; (b) ethyl; (c) methy, ethyl and *tert*-butyl
R_2 = H, cyclopropyl, cyclopentyl, cyclohexyl, norbornyl, cyclooctyl, 2-adamantyl
R_3 = H, benzyl, pyridinyl

Figure 5. General structure-activity relationship (SAR)
98x53mm (600 x 600 DPI)

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NO_2 Cl 5-6 O O R_1 a H N NO_2 7-8 O O R_1 b H N NH_2 9-10 O O R_1

R_1 = H

Scheme 1
37x8mm (600 x 600 DPI)

Scheme 2
108x86mm (600 x 600 DPI)

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Scheme 3
118x109mm (600 x 600 DPI)

9-10 **15-20** **35-64**

Table 1 figure
33x8mm (600 x 600 DPI)

HN
H
N
O
S
NH
O
49

IC_{50}(Ferroptosis)= 3.4 nM

Microsomal stability:

- $T_{1/2}$(mouse)= 187 min
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Plasma stability after 6h:

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Table of Contents (TOC) Graphic
31x11mm (600 x 600 DPI)