Soft drug-inhibitors for the epigenetic targets Lysine-Specific Demethylase 1 (LSD1) and Histone Deacetylases (HDACs)

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Abstract

Epigenetic modulators such as Lysine-specific Demethylase 1 (LSD1) and Histone Deacetylases (HDACs), are drug targets for cancer, neuropsychatric disease or inflammation but inhibitors of these enzymes exhibit considerable side effects. For a potential local treatment with reduced systemic toxicity, we present here soft drugs as new LSD1 and HDAC inhibitors. A soft drug is a compound that is degraded *in vivo* to less active metabolites, after having achieved its therapeutic function. This has been successfully applied for corticosteroids in the clinic but soft drugs targeting epigenetic enzymes are scarce, with the HDAC inhibitor remetinostat being the only example. We have developed new methyl ester containing inhibitors targeting LSD1 respectively HDACs and compared their biological activity to the one of their respective carboxylic acids cleavage products. *In vitro* activity assays, cellular experiments, and a stability assay identified potent HDAC and LSD1 soft drugs that are superior to their corresponding carboxylic acids as potential candidates for local therapy with minimized side effects.

Introduction

Epigenetic processes regulate the expression of genes and carry on the information over cell division. Cell types can hence differ from each other in their phenotype despite of their identical genotype. An important epigenetic mechanism is the posttranslational modification of histones. Among them, reversible methylation and acetylation are the most prominent. Enzymes that remove these modifications are Lysine Demethylases (KDMs), among them the Lysine-specific Demethylase 1 (LSD1) and Histone Deacetylases (HDACs). LSD1 and HDACs exhibit increased expression levels in many diseases and are validated as promising targets for therapy. Mainly in various cancers, LSD1 and HDACs are disregulated, for example in prostate cancer, breast cancer (LSD1 and HDACs), T-cell lymphoma or Melanoma (HDACs), acute myeloid leukemia (AML) or non-small-cell lung cancer (NSCLC) (LSD1). [2-7] In addition, LSD1 and HDACs also contribute to inflammatory diseases of the skin or the mammae. [8-10] LSD1 is also discussed to be a potential target in lung fibrosis.[11] It is an FAD-dependent demethylase and mono- and dimethylated lysines 4 and 9 of Histone H3 are its main substrates.[12] A milestone in the development of LSD1 inhibitors was the discovery that Tranylcypromine (TCP, **1a**) which had already been approved as an antidepressant targeting the homologous FAD-dependent enzymes monoamine oxidases A and B (MAO A / B) also blocks LSD1. TCP binds covalently to the cofactor FAD and thus inhibits these enzymes irreversibly.[13] Starting from this, numerous potent and selective inhibitors with low nanomolar IC_{50} values against LSD1 on the basis of TCP have been developed.[14-15] However, although clinical studies have been pursued, limits for these clinical candidates have emerged: The nanomolar inhibitor ORY-1001, currently in clinical phase IIa for the treatment of AML and SCLC, manifests toxicities such as thrombocytopenia and neutropenia which are dose-limiting side-effects. A second example is the inhibitor GSK2879552, where studies needed to be stopped due to an unfavorable risk-benefit ratio (NCT02034123; NCT02177812).

Among HDACs, Class I, IIa/b, and IV are zinc-dependent enzymes that remove acetyl-groups from the Nε of lysines. They are ubiquitously expressed and are mostly located in the nucleus, influence DNA-replication, DNA-repairing mechanisms, splicing and are involved in cell proliferation. Research in the field of HDAC inhibitors, mostly zinc-chelating hydroxamic acids, have brought FDA-approvement for potent drugs like Vorinostat (**2**) or Panobinostat.[16-17] The available HDAC inhibitors are unselective which brings about dose limiting toxicities.[1]

These examples demonstrate that there is still a need for drugs discriminating between damaged and healthy tissue/cells against HDACs and LSD1. One approach is the development of prodrugs that are only activated in certain cell types or tissues. This has been exemplified in the field of epigenetics for hypoxia activated epigenetic prodrugs for HDACs as well as LSD1.[18-19]

An alternative strategy is the use of so-called soft drugs (also termed ante drugs) that are active per se and are degraded *in vivo* to predictable nontoxic and inactive metabolites, after having achieved its therapeutic role respectively for the fraction of drug that bypasses the desired tissue (see **Figure 1**). In general, the advantage here is that by a local application of the soft drug, for example on the skin, the eyes or in the lungs, a systemic distribution of the active compound is averted. Soft drugs have already been used in the clinics, for example remifentanil, which is an opioid given as infusions during anaesthesia, or loteprednol etabonate, a corticosteroid for local treatment of eye diseases. Both drugs incorporate methyl ester moieties and undergo a fast hydrolysis and hence metabolic inactivation.[20-22]

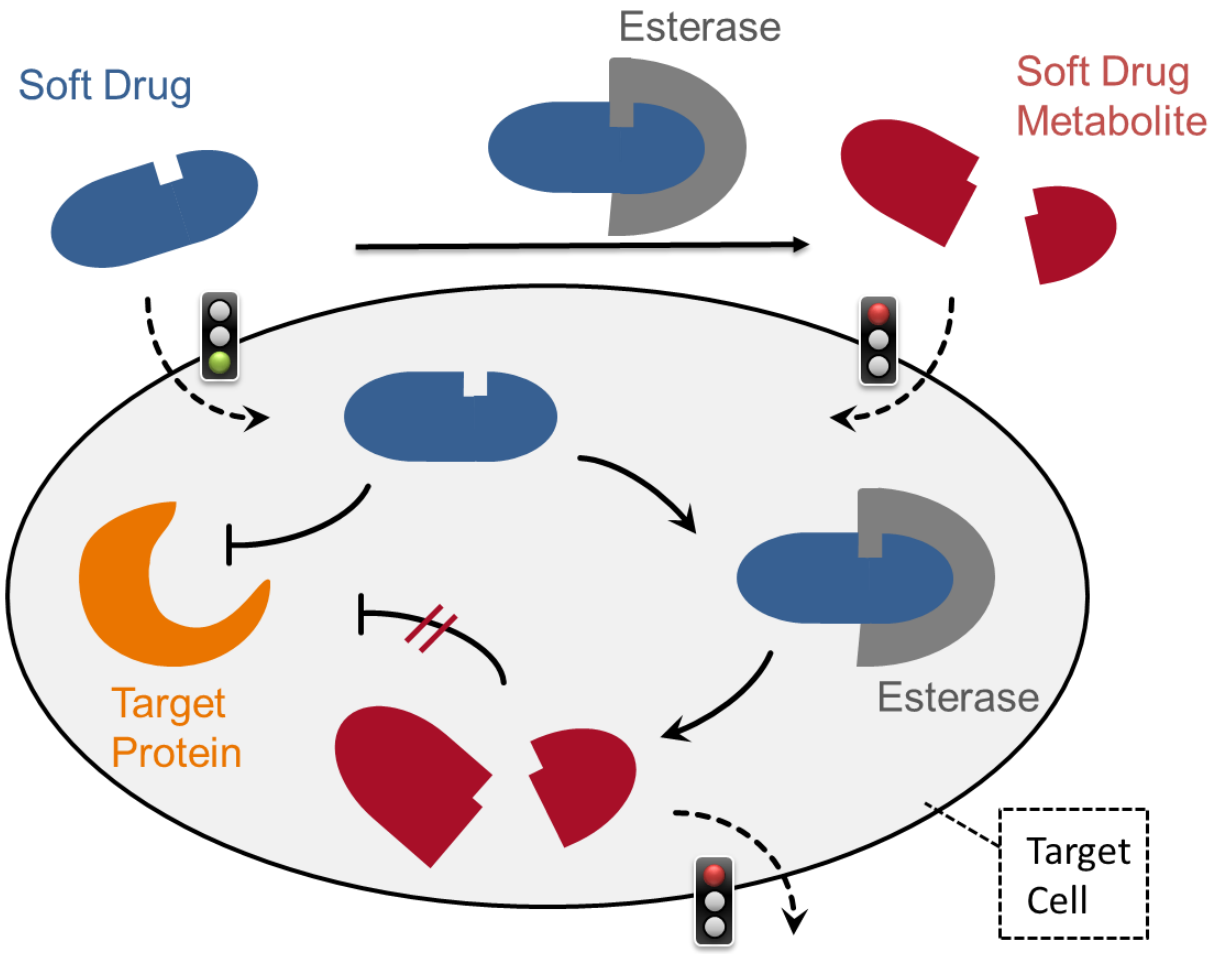


Figure 1. Soft drug principle. The active soft drug inhibits its target protein and gets metabolized into an inactive metabolite afterwards, for example soft drugs designed as methyl esters get hydrolyzed by esterases. The soft drug metabolite then is either less potent against the target or incapable of the penetration of cell membranes, which both contributes to a prevention of systemic side effects.

The only approach for epigenetic soft drugs so far was performed on HDAC inhibitors. There, the modification of vorinostat (SAHA) lead to the soft drug remetinostat (SHP-141, **3**), which is also a methyl ester and was investigated in phase II clinical study as topical treatment in patients with basal cell carcinoma.[23] There were no serious side events and a reduction in skin lesion was observed (NCT03180528).

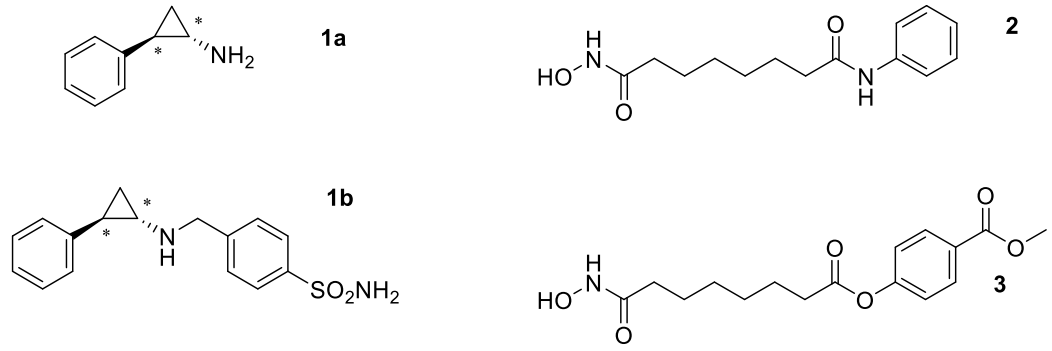


Figure 2. Reference inhibitors for LSD and HDACs relevant for this manuscript. Tranylcypromine (**1a)** was among the first discovered LSD1 inhibitors. **1b** is a potent TCP derivative from our group (cpd **8c** from ref.[24]). Vorinostat (**2**) is an approved HDAC inhibitor. Remetinostat (**3**) was the first soft drug for HDACs.

As indicated, a common structural basis for the design of a soft drug is the incorporation of a carboxylic ester moiety into the active drug, which gets hydrolysed by ubiquitous carboxylesterases in tissues and blood. As a result, the drug is inactivated if the carboxylic acid metabolite may exhibit altered pharmacodynamic properties. Alternatively or also in addition, the resulting carboxylic acid might be less permeable for membranes. Depending on whether the hydrolysis takes place in cells that the original drugs have penetrated or in the plasma, this leads to either an accumulation inside the target cells or to a reduced penetration into other cells.

Here, to obtain epigenetic soft drugs, we prepared a set of methyl ester derivatives on the basis of the pharmacophore of the LSD1 inhibitor tranylcypromine (**1**) respectively the HDAC inhibitor vorinostat (**2**). For both target classes optimized inhibitors with cellular activity and desired metabolic conversion were identified.

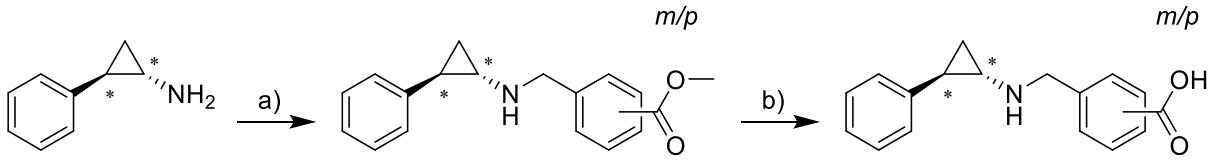
Results

LSD1 inhibitor soft drugs

In order to incorporate carboxylic ester moieties into potent LSD1 and HDAC inhibitors, we built up on established compounds. For LSD1, we extended N-benzylated TCP derivatives that had proven to be potent and selective inhibitors respectively prodrugs before.[18, 24] We started with simple benzoic acid methyl ester moieties and coupled this part to tranylcypromine directly (**4**–**7**, **Scheme 1**). For further SAR, an ester containing group was attached to a phenyl core first, which then was attached to tranylcypromine (**8**-**33**) via a methylene bridge as N-substituent as shown in **Scheme 2**.

A reductive amination of *meta* and *para* methyl-formylbenzoate with TCP was performed for the final soft drugs **4** and **6** that have the ester moiety directly attached to the phenyl ring of the N-substituent. *Via* alkaline hydrolysis, the methyl esters were cleaved in a second step, resulting in the carboxylic acids **5** and **7**.

Scheme 1. Synthesis route for first soft drugs analogues against LSD1 – esters and corresponding carboxylic acids.*

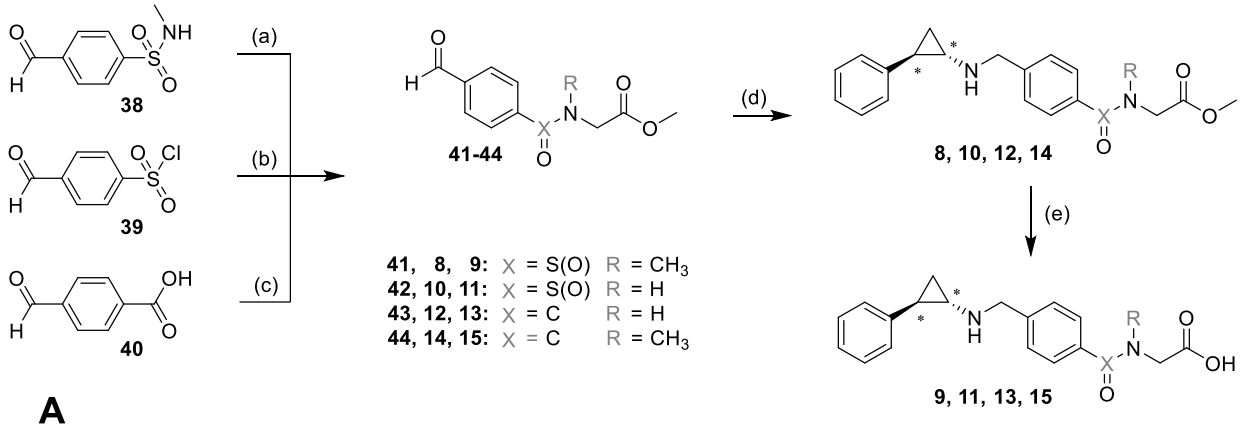


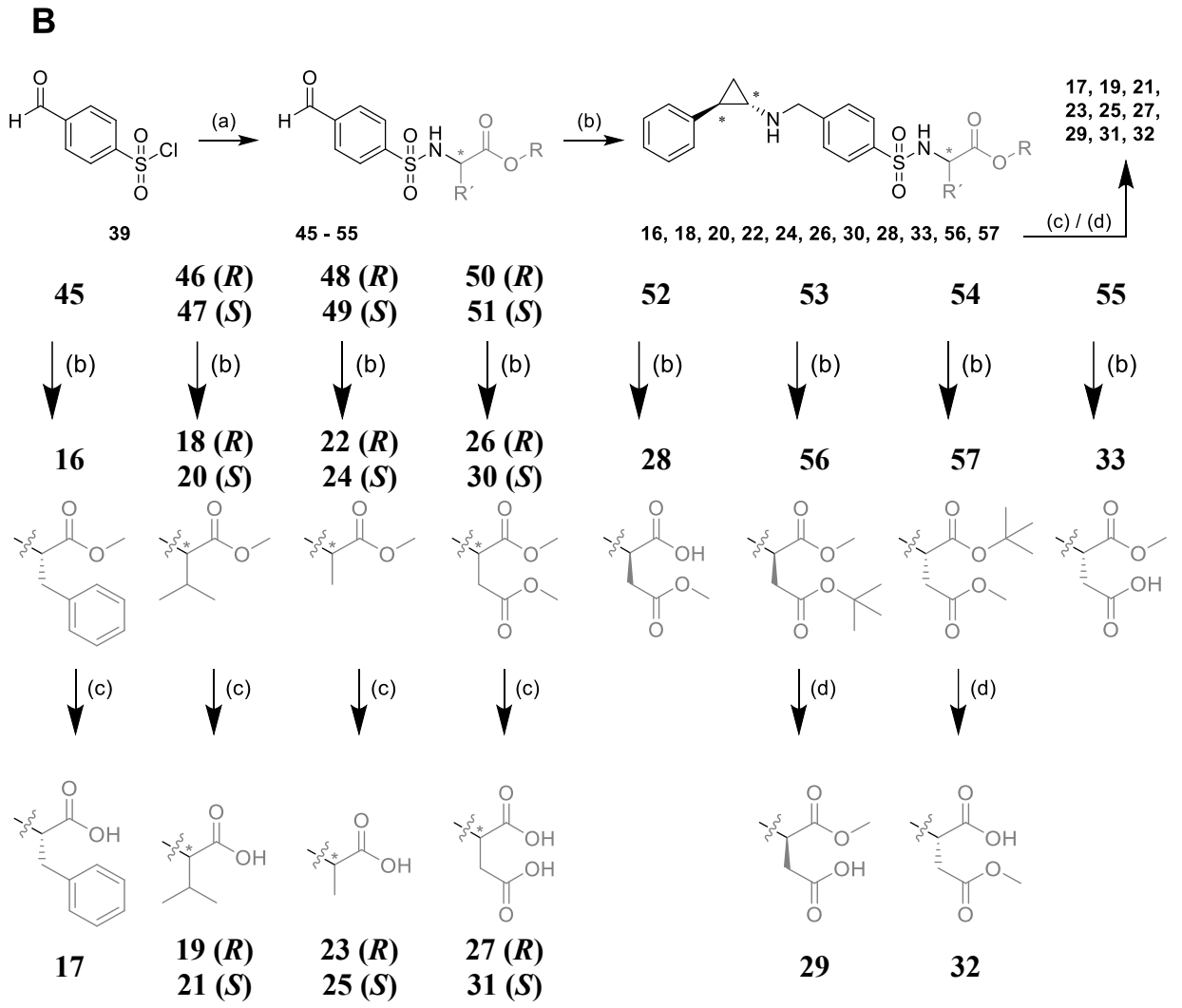
1 *m*: **4** *p*: **6** *m*: **5** *p*: **7**

*Reagents and conditions: (a) **2**: methyl 3-formylbenzoate/ **4**: methyl 4-formylbenzoate, sodium triacetoxyborohydride, DCE, rt, 2 h, 27-65%; (b) KOH 1 M aq., MeOH, rt, overnight 46–60 %.

As the LSD1 active site and esterase acceptance have to be addressed simultaneously for softdrugs we choose an exploratory approach for further SAR. We modifed the arylester moiety of our initial compounds by insertion of a sulfonamide or carboxylamide group, which had led to potent and selective inhibitors in our previous studies[24] and attachment of amino acids that bridge to the methyl ester. To this end, we first synthesized amide linked building blocks from an amino acid methyl ester and a formylphenyl sulfonic respectively carboxylic acid derivative. For the synthesis of **41**, nucleophilic substitution of methyl 2-bromoacetate with 4-formyl-*N*-methylbenzenesulfonamide (**38**) was performed. Compound **42** was obtained by coupling of methyl glycinate and 4-formylbenzensulfonyl chloride (**39**), whereas the carboxylic acid derivatives **43** and **44** originated from the coupling of 4-formylbenzoic acid (**40**) with methyl glycinate. In a common subsequent step, reductive amination of the formyl group with TCP led to the esters **8**, **10**, **12**, and **14** and saponification to the corresponding acids **9**, **11**, **13**, and **15**. For further SAR we concentrated on the sulfonamides, as the inhibition in the methyl glycinate series was better for the sulfonamide **10** as compared to the carboxylic acid amide in **14** (see Table 1). As the -SO_2NH- compound **10** was slightly more potent and selective than the methylated derivative **8**, we kept the -NH- for further derivatives. We synthesized further analogues by varying the amino acid ester part with regard to steric bulk (e.g. benzyl in **16** vs. isopropyl in **20** and methyl in **24**) and lipophilicity (e.g. methyl in **24** vs. ester in **30** vs. acid in **33**) of the side chain as well as the stereochemistry (e.g. **18** vs. **20** or **22** vs. **24**) to probe their effects on both LSD1 inhibition and esterase acceptance. For the synthesis of the different mono methyl aspartates (e.g. **32** vs. **33**), a *t*butyl ester protecting group was employed. The different aspartate di- (**26** and **30**) and mono- (**28**, **29**, **32** and **33**) esters were prepared to study the inhibitors potency and esterase preference for main chain vs. side chain methyl ester groups.

Scheme 2. Synthesis route for extended soft drugs analogues against LSD1 – esters and corresponding carboxylic acids: **8**–**57**.*





*Reagents and conditions: Part A: (a) methyl 2-bromoacetate, K_2CO_3, DMF, 60 °C, 21 h, 62 %; (b) methyl glycinate, NaOH, H_2O/EtOAc 0 °C–rt, 17 h, 88 %; (c) **43**: methyl glycinate / **44**: methyl methyl glycinate, bis(2-oxooxazolidin-3-yl)phosphinic chloride, Et_3N, DMF/DCM 2:5 (v/v), 60 °C, overnight, 29 %; (d) sodium triacetoxyborohydride, DCE, rt–70 °C, 6.5 h–overnight, 40–84%; (e) NaOH 1 M aq., MeOH, 50 °C, overnight 12–72%.

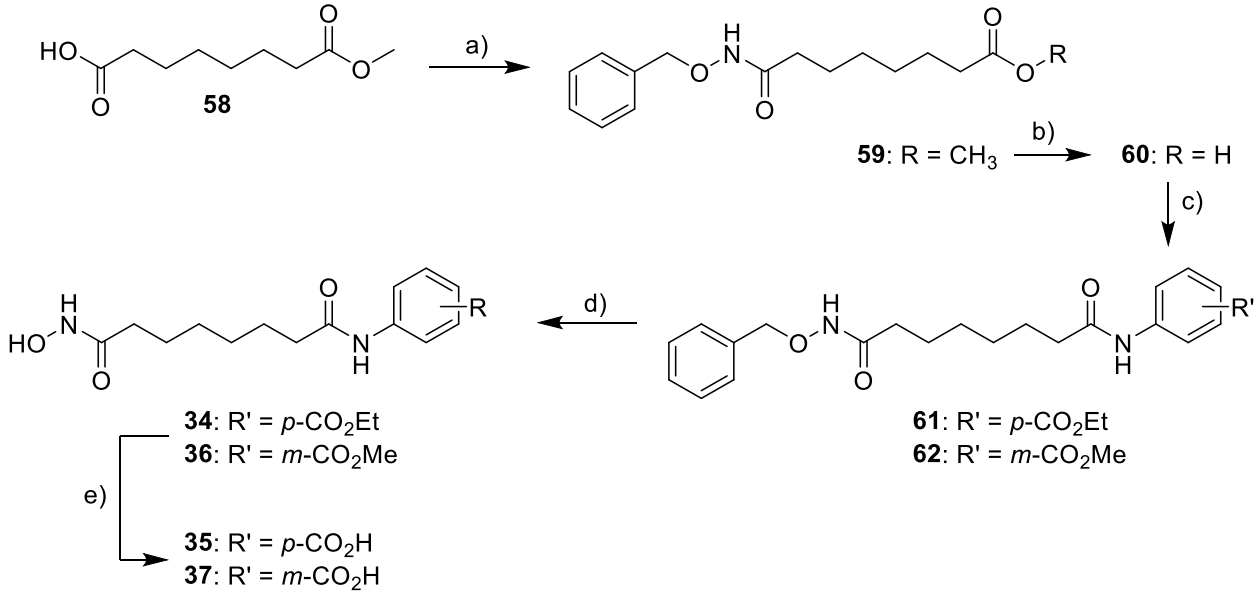
Part B: (a) **45**: L-phenylalanine methylester / **46**: D-valine methylester / **47**: L-valine methylester / **48**: D-alanine methylester / **49**: L-alanine methylester / **50**: D-asparatic acid dimethylester / **51**: L-asparatic acid dimethylester / **52**: D-asparatic acid-4-methylester / **53**: D-asparatic acid-1-methylester-4-*tert*-butylester / **54**: L-asparatic acid-4-methylester-1-*tert*-butylester / **55**: L-aspartic acid-1-methylester, aq. NaOH 1 M, H_2O/EtOAc, 0° C-rt., 3–23 h, 32–91 %; (b) sodium triacetoxyborohydride, DCE, 70° C, 4–25 h, 46–77 %; (c) NaOH, MeOH, 50° C, 3–48 h, 1–72 %; (d) H_2O/TFA 1:4 (v/v), 40° C, 10 h, 2–84 %.

HDACs

Our approach towards potential HDAC soft drugs was carried out on the basis of vorinostat (**2**). In contrast to **3** where the internal amide bond of **2** is replaced by an ester, we wanted to keep the amide structure. Thus, while in **3** ester hydrolysis breaks down the pharmacophore, we wanted to keep this intact after hydrolysis. Thus, residual potency was expected and a potential soft-drug effect in vivo would more rely on pharmacokinetic properties.

Stolfa *et al.* had already synthesized a derivative of **2** which includes an additional carboxylic acid on the phenyl ring.[25] Here, we adapted this synthetic route for our new compounds at the step of the amide formation. Instead of using *tert*-butyl-3-aminobenzoate as published, methyl-3-aminobenzoate and ethyl-4-aminobenzoate were applied to generate the desired esters (**Scheme 3**). An additional deprotection and alkylation step could be avoided by this route. With a subsequent hydrolysis, the corresponding acids **35** and **37** were obtained.

Scheme 3. Synthesis route for soft drugs analogues of HDAC inhibitor Vorinostat (**2**) – esters and corresponding carboxylic acids.*



*Reagents and conditions: (a) O-benzylhydroxylamine·HCl, bis(2-oxooxazolidin-3-yl)phosphinic chloride, *N*,*N*-Diisopropylethylamine, dry CH_2Cl_2, rt, 19 h, 10–75 %; (b) LiOH 1 M aq., THF, rt–40 °C, 4 h, 97 %; (c) **61**: ethyl-4-aminobenzoate / **62**: methyl-3-aminobenzoate, bis(2-oxooxazolidin-3-yl)phosphinic chloride, *N*,*N*-diisopropylethylamine, dry CH_2Cl_2, rt, 19 h, 10–75%; (d) H_2, Pd/C, dry MeOH, 20–57%; (e) LiOH 1 M aq., THF, rt–40 °C, 3–5 h, 47 %

Starting from suberic acid monomethyl ester **58**, a O-benzyl-protected hydroxamic acid was incorporated to facilitate purification and handling of the intermediates. Subsequently, the methyl ester **59** was hydrolysed under basic conditions resulting in the free carboxylic acid **60**. The next step was the amide bond formation between **60** and the requisite aniline derivative. The carboxylic acid was activated with BOP-Cl for amide coupling. Finally, the hydroxamic acid was deprotected with H_2 and a catalytic amount of Pd/C, yielding the compounds **34** and **36**. The free carboxylic acids **35** and **37** were obtained by hydrolysis of the esters.

Biology

In vitro assay results

LSD1

All final compounds, carboxylic esters and acids, were investigated in *in vitro* assays. We used the standard Peroxidase coupled assay to determine IC_{50} values for LSD1 and compared esters (potential soft drugs) vs. acids (predicted metabolites). The assay uses a peptide featuring the first 20 amino acids of Histone 3 with dimethylation on lysine 4 (H3K4(me_2)aa-1-20) as a substrate. LSD1 catalyzed demethylation generates hydrogen peroxide upon regeneration of the cofactor FAD. H_2O_2 and added 10-acetyl-3,7-dihydroxyphenoxazine (Amplex Red reagent) are converted to a fluorescent compound by a horseradish peroxidase. Inhibition of MAOs was checked with the MAO-Glo assay kit (Promega). **Table 1** shows that the newly synthesized esters are potent inhibitors of LSD1 and that the corresponding amino acids were significantly less active. Most methyl esters exhibited an IC_{50} value below 500 nM, except for the valine derivative **20** and the aspartic acid mono esters **27**, **28** and **31**, **32**. All free acids inhibit LSD1 only in the micromolar range. Furthermore, all compounds were selective over MAOs. For MAO-B the selectivity was very high in all instances. For MAO-A, the selectivity was lower in many cases but still very pronounced for most of the aspartate derivatives, e.g. **30**.

Table 1. In vitro evaluation of LSD1 and MAO inhibition by LSD1 compounds. Values are given as mean ± SD (n=2) Methyl esters (soft drugs) and carboxylic acids (metabolites) were both tested in a Peroxidase coupled assay for LSD1 and a luminescence assay for MAOs.* **1b** was used as a reference inhibitor.

	IC_{50} [μM]		
	LSD1	MAO-A	MAO-B
1b	0.15 ± 0.01	21	>200
4	0.13 ± 1.53	41 %[a]	n.i.
5	14.6 ± 0.01	n.i.	n.i.
6	0.07 ± 0.006	55 %[a]	n.i.
7	1.39 ± 0.22	n.i.	n.i.
8	0.34 ± 0.02	11.3	19 %[b]
9	4.14 ± 0.19	n.i.	n.i.
10	0.258 ± 0.01	28.9	n.i.
11	2.32 ± 0.23	n.i.	n.i.
12	0.461 ± 0.02	8.27	n.i.
13	2.88 ± 0.10	n.i.	n.i.
14	1.25 ± 0.10	16.4	10 %[b]
15	9.98 ± 0. 99	n.i.	n.i.
16	0.307 ± 0.02	4.97	15 %[b]
17	1.65 ± 0.06	14.5	n.i.
18	0.358 ± 0.01	8.63	n.i.
19	2.68 ± 0.10	n.i.	n.i.
20	1.73 ± 0.13	24.2	n.i.
21	6.14 ± 0.21	n.i.	n.i.
22	0.406 ± 0.01	91 %[b]	n.i.
23	3.59 ± 0.04	21 %[b]	n.i.
24	0.326 ± 0.01	83 %[b]	n.i.
25	4.71 ± 0.16	30 %[b]	13.2
26	0.484 ± 0.03	81 %[b]	9 %[b]
27	12.1 ± 0.70	n.i.	n.i.
28	3.74 ± 0.13	n.i.	n.i.
29	4.43 ± 0.19	n.i.	n.i.
30	0.499 ± 0.03	n.i.	n.i.
31	18.05 ± 2.07	n.i.	n.i.
32	2.38 ± 0.07	12 %[b]	13 %[b]
33	4.87 ± 0.16	n.i.	n.i.

*Inhibition below 10 % at 31 μM is labelled as not inhibiting (n.i.). (-) were not tested. [a]Percentage inhibition at 1 μM. [b]Percentage inhibition at 31 μM.

HDACs

The compounds designed for HDACs (**34**–**37)** were tested on purified HDAC1, 6 and 8 as representative isoforms to assess their inhibitory effects and possible isoform selectivity in biochemical conversion assays. IC_{50} values were determined either by a trypsin assay using a lysine derivative[26] for HDAC1 and 6 respectively by the commercially available Fluor de Lys® assay for HDAC8.[27] The inhibitory activities are shown reported in **Table 2**.

Table 2. In vitro evaluation of HDAC inhibition. Methyl ester (soft drug) and carboxylic acid (metabolite) were both tested, as well as the parent drug **2**. Values are mean ± SD (n=3)

Compound	IC_{50} (μM)		
	HDAC1	HDAC6	HDAC8
2	0.15 ± 0.03	0.05 ± 0.05	0.4 ± 0.1[28]
34	0.10 ± 0.03	0.05 ± 0.05	0.43 ± 0.04
35	0.56 ± 0.03	0.38 ± 0.10	10.6 ± 0.04
36	0.05 ± 0.01	0.03 ± 0.02	1.25 ± 0.12
37	0.82 ± 0.03	0.55 ± 0.27	10.1 ± 0.05

The ester derivatives **34** and **36** possess a similar activity to the clinically used inhibitor **2**. **36** is even more potent on HDAC1 and 6 than compound **2**, implicating that meta-substituted capping groups are favourable. The activity of the two carboxylic acids **35** and **37** is weaker but still in the nanomolar range as expected. As mentioned above we wanted to focus on differences in cellular permability in the HDAC series. The parent structure **2** and the methyl ester derivatives **34** and **36** as well as the carboxylic acids **35** and **37** show a weaker effect on HDAC8 compared to HDAC1 and 6.

Cellular activity

LSD1

The majority of our methyl esters were potent inhibitors *in vitro*. Next, we performed experiments in cultured cancer cells. LSD1 not only has catalytic activity on methylated lysines, but also has scaffolding functions that can be interrupted by TCP binding, for example in THP1 cells, a leukemic cell line, where LSD1 regulates GFI1-target genes. As a result of inhibition the *cd86* gene is upregulated.[29] CD86 can be used as a surrogate biomarker for LSD1 inhibition in THP1 cells.[30] We determined a cellular LSD1 inhibition for our LSD1 compounds *via* quantification of the CD86 expression of THP-1 cells after treatment *via* FACS analysis. The THP-1 cell line was also used for the assessment of the inhibition of viability of our compounds in an MTS assay format. The amount of metabolically active cells can be determined by the conversion of MTS ([3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)]-2H-tetrazolium, 54) to the dye Formazan. The results are displayed in **Table 3**.

Table 3. Cellular activity of LSD1 inhibitors. Methyl ester (soft drug) and carboxylic acid (metabolite) were both evaluated. FACS analysis of CD86-positive cells for cellular LSD1 inhibition and an MTS assay to assess inhibition of cell viability (GI_{50}) were performed.*

	$CD86^+$ [%]				GI_{50} [μM]
	Inhibitor [μM]				
	0.05	0.1	0.5	1.0	
4	-	-	44.7	-	-
5	-	-	26.8	-	-
6	-	-	13.0	-	-
7	-	-	n.i.	-	-
8	10.6	6.8	33.8	24.6	9.3
9	n.i.	n.i.	n.i.	15.6	n.i.
10	17.3	11.3	38.6	32.1	6.0
11	n.i.	n.i.	21.4	30.6	n.i.
12	42.7	39.7	61.8	73.7	20.2
13	n.i.	n.i.	42.1	54.1	n.i.
14	32.6	26.2	52.3	56.2	32.1
15	n.i.	n.i.	10.6	20.2	n.i.
1b			58		

	$CD86^+$ [%]			GI_{50} [μM]
	Inhibitor [μM]			
	0.1	0.5	1.0	
16	-	-	65.1	10.6
17	-	38.4	55.6	n.i.
18	71.7	-	80.2	n.i.
19	23.2	-	65.5	n.i.
20	-	-	61.8	n.i.
21	-	-	64.5	n.i.
22	30.5	68.1	-	4.8
23	10.2	22.7	-	n.i.
24	-	51.1	-	3.1
25	-	39.7	-	92 %[a]
26	34.4	57.6	-	1.8
27	n.i.	12.6	-	n.i.
28	-	-	-	n.i.
29	-	-	-	n.i.
30	42.3	62.5	-	1.4
31	11.8	37.0	-	n.i.
32	-	-	-	n.i.
33	-	-	-	n.i.

*Inhibition below 10 % at 50 μM were registered as no inhibition (n.i.). (-) were not tested. [a]Percentage inhibition at 50 μM.

A surface presentation level of CD86 higher than 50 % were seen after treatment at 1, 0.5 μM or even 0.1 μM with the esters **12**, **13**, **14**, **16**, **18**, **20**, **24**, **26** and **30** but also the acids **19** and **21** at 1 μM. Among the most potent compounds, the biggest difference between the ester and acid was achieved for **18**/**19**, the D-valine derivatives, with 71.7 % vs. 23.3 % at 0.1 μM and **22**/**23**, the D-alanine congeners, with 68.1 % vs. 22.7 % at 0.5 μM. Also, **26**/**27** (D-aspartates) with 57.6 % vs. 12.6 % at 0.5 μM performed well in terms of reduction of activity. The compounds **12** and **20**, even if potent at 1.0 μM, also have metabolites that are quite potent at this concentration. It seems that the effect of LSD1 inhibition is limited at higher concentrations, which is why esters and acids converge in some cases, see e.g. **16**/**17** at 1.0 μM.

HDACs

To determine the cellular inhibition of global HDAC enzymatic activity, the trypsin assay was carried out on living cells from the SK-N-SH neuroblastoma cell line. The cell toxicity of the designed compounds was determined on an additional cell line, HL-60 cells.[31] HL-60 is a human myeloid cell line with mainly neutrophilic promyelocytes but up to 10 % of the cultured cells spontaneously differentiate.[32] IC_{50} and GI_{50} values are reported in **Table 4**. The soft drugs **5** and **6** exhibit a cellular IC_{50} of 2.93 μM respectively 5.27 μM in the adherent SK-N-SH cells and hence, similar activity as the model compound vorinostat (**1**). In contrast to the esters, the acids **6** and **8** do not show any activity at 50 μM. This can be attributed to a lack of cell permeability.[33]

Table 4. Cytotoxicity data and cellular inhibition of HDAC by soft drugs and the parent drug **2**.[1]

	GI_{50} [μM]		IC_{50} cellular HDAC activity
	HL-60	SK-N-SH	SK-N-SH
2	0.54 ± 0.03[34]	5.75 ± 0.04	4.01 ± 0.12
34	1.13 ± 0.02	3.61 ± 0.11	5.27 ± 0.05
35	n.i.	n.i.	n.i.
36	0.51 ± 0.01	1.39 ± 0.04	2.93 ± 0.05
37	n.i.	n.i.	n.i.

[1]Inhibition below 10 % at 50 μM were described as not inhibiting (n.i.). (-) were not tested. [a]Percentage inhibition at 50 μM.

Ultimately, we investigated our selected soft drugs for the capability of getting metabolised by human enzymes. For this, we set up a stability assay with human serum.

Degradation in human serum

We wanted to confirm that our methyl esters are degraded into the corresponding carboxylic acids by plasmatic esterases. For that, we used human serum, incubated our methyl esters in it for a day at 37° C, and then analyzed it *via* HPLC. Our synthesized carboxylic acids allowed us to identify whether the emerged degradation products were the results of ester hydrolysis. A control experiment was done in water at pH 7.5. As shown in **Figure 4**, an accelerated degradation in serum can be observed for most of our compounds, among them two of the inhibitors from the cellular experiments with a high selectivity window, **16** and **22**. Interestingly, the compounds with a valine residue, **18** and **20**, were stable against hydrolysis which invalidates them as soft drug candidates. It was also observed that aspartate diesters **26** and **30** do only degrade to the monoacids with the γ-ester being intact. and not to the dicarboxylic aspartic acids. The accelerated hydrolysis

of the methyl ester into the carboxylic acids in human serum shows that the soft drug design was successfully applied for our LSD1 inhibitors.

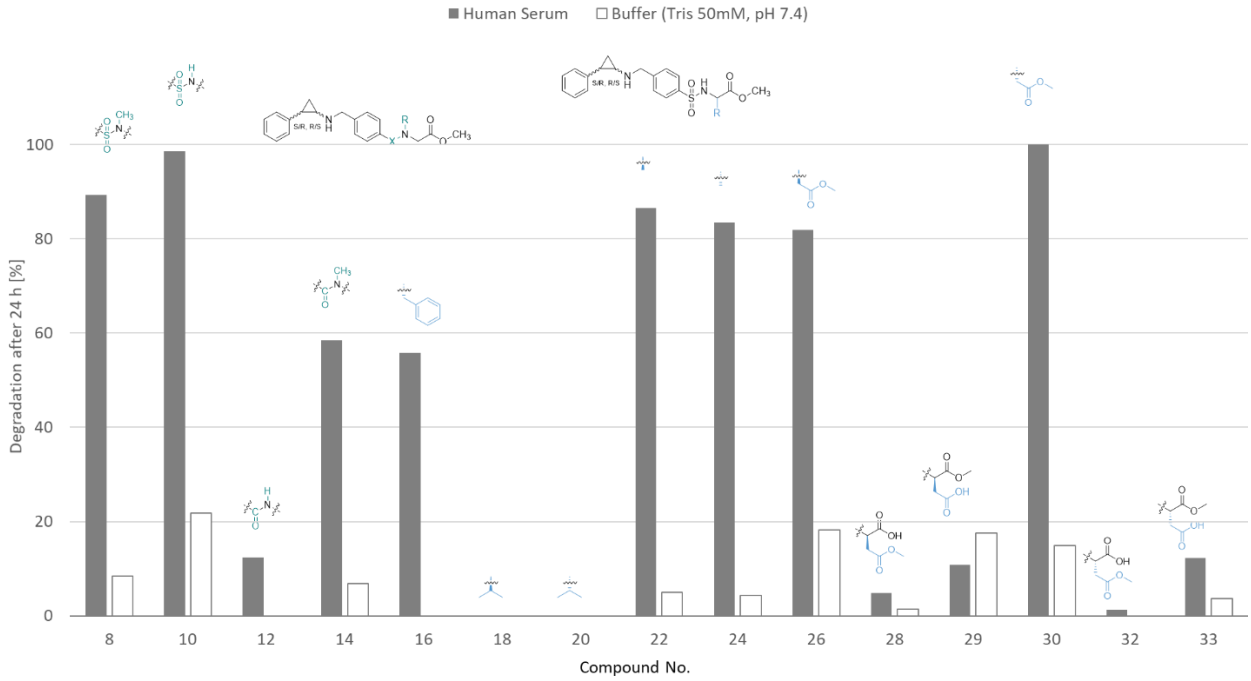


Figure 3. Degradation of LSD1 soft drugs in human serum and buffer after 24 h. Most methyl esters undergo enzymatic hydrolysis in serum except for the valine-moiety containing compounds (**18**, **20**) that do not get cleaved in either condition. Some background non-enzymatic hydrolysis is observed in buffer. **8**, **10**, **22**, **24**, **26** and **30** were metabolized by the largest extent. **26** and **30** mainly hydrolyzed to the monoesters with the γ-ester being intact.

Discussion and Conclusion

We synthesized a set of LSD1 and HDAC inhibitors designed as methyl esters, of which most of them turned out to be active *in vitro* and in cells. The comparison of HDAC soft drugs **34** and **36** to their metabolites **35** and **37** indicates the effectiveness of the soft drug approach. **35** and **37** seem not to penetrate cells, whereas **34** and **36** are potently active on cells. For both, we showed that the methyl esters were more potent enzyme inhibitors than the corresponding carboxylic acids: **34** is superior to **35** and **36** to **37**. **36** with a cellular IC_{50} of 2.93 µM even shows a lower inhibition

against the isoenzymes HDAC1 and HDAC6 than the parent drug **2** with 4.01 µM**.** Thus, as intended, in contrast to **3** where the pharmacophore is destroyed by esterase activity, a soft-drug effect in-vivo of our compounds would be driven mostly by differential cell permeation.

Among the LSD1 soft drugs which all incorporate the tranylcypromine pharmacophore, **16** (S-phenylalanine derivative) stands out in terms of cellular potency and selectivity (58% $CD86^+$ cells in the ester at 100 nM vs. 10.7% for the acid **17**) and good esterase driven hydrolysis, yet has limited selectivity over MAO-A (16fold). The aspartate **30** shows excellent enzymatic selectivity over both MAO-A and -B

Thus, we successfully established new soft drugs for the epigenetic targets LSD1 and HDACs that are in principal suitable for in-vivo investigation of pharmacodynamics and pharmacokinetics in appropriate models. There is no precedence for such inhibitors of LSD1 so far and only one other study on HDAC inhibitor soft drugs had been published before. For the LSD1 inhibitors both esters and acids enter the cells, the major difference in cellular activity seems to stem from a mixture of pharmacodynamics properties (reduced enzyme inhibition) and permeation. For the HDAC the big gap between the cellular activity of esters and acids is mainly driven by the pharmacokinetics (permeation) of the drugs.

Experimental Section

General procedures. The reactions were carried out in glassware under inert (nitrogen) atmosphere. Used reagents and solvents were purchased from commercial sources and used without further purification. Brine refers to a saturated aqueous solution of sodium chloride. Cyclohexane (CH) and ethyl acetate (EtOAc) were obtained in technical grade and were distilled prior to use. Water for buffers and solutions was double distilled to *Milli-Q* purity. Reactions were monitored by thin-layer chromatography (TLC) was carried out on Merck silica gel 60 F_{254} and 60 RP-18 F_{254}s aluminium-supported thin layer chromatography sheets. The visualization done was by fluorescence quenching of UV light (λ_{max} 254 or 365 nm) or thermal development after staining with ninhydrin (for primary and secondary amines). Yields were not optimized. Flash column chromatography was performed on a Biotage® Isolera Prime/One purification system using pre-packed silica gel columns (40–60 μM) from Biotage (SNAP) or Telos and the purifications were followed by TLC. Purification of several final compounds was performed on either an Agilent Technologies 1260 Infinity system or an Agilent 1260 Infinity II analytical-scale LC purification system using UV detection. NMR spectroscopy and mass spectrometry were used for product identification. ^{1}H and ^{13}C NMR spectral data were recorded on a Bruker Advance II+ 400 MHz spectrometer using as solvents DMSO-d_6, Chloroform-*d*, and Methanol-d_4. Chemical shifts (δ) are referenced to a residual solvent peak. Mass spectra were recorded on an Advion expression CMS mass spectrometer (LRMS: low-resolution MS) and on a Thermo Scientific Exactive mass spectrometer (HRMS) using ASAP® (Atmospheric Solids Analysis Probe; *aka* APCI: Atmospheric Pressure Chemical Ionization) and electrospray ionization (ESI) as ion sources. HPLC analysis was performed to determine the purity of all final compounds on an Agilent Technologies 1260 Infinity system using UV detection at 210 nm and a Phenomenex Kinetex 5u

XB-C18 100 Å 250×4.60 mm column. Eluent A was water containing 0.05 % TFA and eluent B was acetonitrile containing 0.05 % TFA (Method 1) or Eluent A containing 0.05 % formic acid and eluent B containing 0.05 % formic acid (Method 2). Linear gradient conditions were as follows: 0–4 min: A/B (90 : 10); 4–29 min: linear increase to 100 % of B; 29–31 min: 100 % B; 31–40 min: A/B (90 : 10). All final compounds displayed a chemical purity of >95 % at the wavelength of 210 nm. The stereochemical descriptors *R* and *S* are complemented with (*) to show their interchangeability, for example, that R*S*represents both *RS* and *SR* isomers.

Materials.

General Synthetic Procedures

Method A: Preparation of a sulfonamide by sulfonylation of an amine (for compounds **42** and **45**–**55**)

The secondary amine (5.0 eq) was dissolved in H_2O (5 mL) and the addition of a NaOH solution (1.0 eq, 1 M, or 6.0 eq, 1 M, if the HCl salt of the amine was used). The solution was stirred and cooled to 0° C. A solution of 4-formylbenzenesulfonyl chloride (**39**) was prepared in EtOAc (10 mL) and added dropwise via dropping funnel over a period of 30 min. The resulting biphasic mixture was stirred for five additional minutes at 0° C and then stirred at room temperature for 1.5 h–22 h. The reaction was quenched with HCl solution (1 M) to acidify the reaction mixture. Extraction of the sulfonamide was done with EtOAc (20 mL x 3). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified via silica gel column chromatography with CH/EtOAc (90:10 to 15:85, v/v).

Method B: Reductive amination of *trans*-2-Phenylcyclopropylamine (for compounds **4**, **6**, **8**, **10**, **12**, **14**, **16**, **18**, **20**, **22**, **24**, **26**, **28**, **30**, **33**, **56**, and **57**)

Trans-2-Phenylcyclopropylamine hydrochloride (TCP HCl, **1**, 1.25–2.22 eq.) and the aldehyde (1.0 eq.) were stirred in 10 mL 1,2-Dichloroethane and glacial acetic acid (1.1–3.4 eq.) and the desiccant Na_2SO_4 anhydrous were added. The reaction mixture was heated up to 70° C for 3 h stirring intensively. The mixture was then cooled down to room temperature passively and sodium triacetoxyborohydride slowly added. The reaction was stirred for several more hours (reaction times from beginning given for every compound). The reaction was quenched by adding 10 mL of a 5 % $NaHCO_3$ solution. Extraction of the amine was done with DCM (20 mL x 3). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified via silica gel column chromatography with CH/EtOAc (90:10 to 15:85, v/v).

Method C: Saponification of a carboxylic ester (for compounds **5**, **7**, **17**, **19**, **21**, **23**, **25**, **27**, **31**)

The carboxylic ester (1.0 eq) was dissolved in 5–10 mL MeOH. An aqueous solution of NaOH, LiOH, or KOH (1–6 eq., 1 M) was added and stirred at 50° C or reflux up to 48 h. After termination, the methanol was evaporated. The aqueous layer was then extracted with EtOAc (20 mL x 3). Optionally, the product was purified either via reversed phase silica gel column chromatography with H_2O/ACN (90:10 to 10:90, v/v) or via semi-preparative RP-HPLC with H_2O/ACN.

Methyl 4-((((1*S,2*R**)-2-phenylcyclopropyl)amino)methyl)benzoate (4).** $C_{18}H_{19}NO_2$, M_r: 281.36. Procedure Method B: TCP HCl (195 mg, 1.15 mmol, 1.1 eq.), methyl 4-formylbenzoate (171 mg, 1.04 mmol, 1.0 eq.), Sodium triacetoxyborohydride (500 mg, 2.36 mmol, 2.27 eq.),

Glacial acetic acid (65 µL, 1.15 mmol, 1.1 eq), 4.0 h; yield: 87 mg colorless oil, 0.28 mmol, 27 %. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.91 – 7.83 (m, 2H, 3,5-H$_{benz}$), 7.47 – 7.43 (m, 2H, 2,6-H$_{benz}$), 7.23 – 7.14 (m, 2H), 7.12 – 7.05 (m, 1H), 7.00 – 6.92 (m, 2H), 3.84 (s, 2H), 3.83 (s, 3H), 3.02 (s, 1H), 2.24 – 2.16 (m, 1H), 1.86 – 1.78 (m, 1H), 1.04 – 0.96 (m, 1H), 0.96 – 0.89 (m, 1H). **^{13}C NMR** (100 MHz, DMSO-d_6) δ 166.2, 146.9, 142.5, 129.0, 128.2, 128.1, 127.8, 125.4, 125.1, 52.3, 52.0, 41.7, 24.6, 16.8. LRMS (pos. ESI): *m/z* 282.6 $[M+H]^+$. HPLC (Method 1): t_R 15.42 min.

4-((((1*S,2*R**)-2-phenylcyclopropyl) amino)methyl)benzoic acid (5).** $C_{17}H_{17}NO_2$, M_r: 267.33. Procedure Method C: **4** (55 mg, 0.20 mmol, 1.0 eq.), NaOH (1000 µL (1 M), 1.0 mmol, 5.0 eq.), rt, 16 h, Purification: reversed phase silica gel column chromatography with H_2O/ACN (90:10 to 10:90, v/v); yield: 32 mg white solid, 0.12 mmol, 60 %. ^{1}H NMR (400 MHz, D_2O) δ 7.78 – 7.74 (m, 2H), 7.36 – 7.31 (m, 2H), 7.30 – 7.24 (m, 2H), 7.21 – 7.14 (m, 1H), 6.99 – 6.94 (m, 2H), 3.89 (d, *J* = 13.2 Hz, 1H), 3.83 (d, *J* = 13.2 Hz, 1H), 2.27 (ddd, *J* = 7.7, 4.5, 3.3 Hz, 1H), 1.81 (ddd, *J* = 9.3, 6.0, 3.3 Hz, 1H), 1.05 (ddd, *J* = 9.3, 5.6, 4.5 Hz, 1H), 1.03 – 0.97 (m, 1H). ^{13}C NMR (100 MHz, D_2O) δ 175.4, 142.3, 142.1, 134.9, 128.9, 128.5, 128.4, 125.6, 125.5, 51.9, 40.2, 23.5, 15.1. LRMS (neg. ESI): *m/z* 266.1 $[M–H]^–$. HPLC (Method 1): t_R 13.54 min.

Methyl 3-((((1*S,2*R**)-2-phenylcyclopropyl)amino)methyl)benzoate (6).** $C_{18}H_{19}NO_2$, M_r: 281.36. Procedure Method B: TCP HCl (200 mg, 1.18 mmol, 1.7 eq.), methyl 3-formylbenzoate (114 mg, 0.69 mmol, 1.0 eq.), Sodium triacetoxyborohydride (500 mg, 2.36 mmol, 3.42 eq.), Glacial acetic acid (67 µL, 1.18 mmol, 1.7 eq), 4.0 h; yield: 126 mg colorless oil, 0.45 mmol, 65 %. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.92 – 7.89 (m, 1H), 7.84 – 7.78 (m, 1H), 7.60 – 7.54 (m, 1H), 7.47 – 7.40 (m, 1H), 7.22 – 7.14 (m, 2H), 7.12 – 7.05 (m, 1H), 6.98 – 6.91 (m, 2H), 3.83 (s, 3H), 3.82 (s, 2H), 3.01 (s, 1H), 2.18 (ddd, *J* = 7.2, 4.3, 3.1 Hz, 1H), 1.78 (ddd, *J* = 9.1, 5.8, 3.1 Hz, 1H), 1.05 – 0.96 (m, 1H), 0.96 – 0.87 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.4, 142.5,

141.8, 133.0, 129.4, 128.7, 128.5, 128.1, 127.4, 125.5, 125.1, 52.2, 52.1, 41.7, 24.6, 16.7. LRMS (pos. ESI): *m/z* 282.1 $[M+H]^+$. HPLC (Method 1): t_R 15.36 min.

3-((((1*S,2*R**)-2-phenylcyclopropyl) amino)methyl)benzoic acid (7).** $C_{17}H_{17}NO_2$, M_r: 267.33. Procedure Method C: **6** (68 mg, 0.24 mmol, 1.0 eq.), NaOH (1210 µL (1 M), 1.21 mmol, 5.0 eq.), rt, 16 h, Purification: reversed phase silica gel column chromatography with H_2O/ACN (90:10 to 10:90, v/v); yield: 28 mg white solid, 0.11 mmol, 46 %. ^{1}H NMR (400 MHz, D_2O) δ 7.76 – 7.72 (m, 1H), 7.71 (s, 1H), 7.40 – 7.32 (m, 2H), 7.27 – 7.20 (m, 2H), 7.19 – 7.11 (m, 1H), 6.96 – 6.88 (m, 2H), 3.85 (d, *J* = 13.1 Hz, 1H), 3.78 (d, *J* = 13.1 Hz, 1H), 2.25 – 2.17 (m, 1H), 1.76 (ddd, *J* = 9.3, 6.0, 3.3 Hz, 1H), 1.06 – 0.99 (m, 1H), 0.99 – 0.93 (m, 1H). ^{13}C NMR (100 MHz, D_2O) δ 175.3, 142.0, 138.9, 136.4, 131.6, 129.2, 128.4, 128.3, 127.6, 125.6, 125.5, 52.1, 40.2, 23.5, 15.0. LRMS (neg. ESI): *m/z* 266.1 $[M–H]^–$. HPLC (Method 1): t_R 13.79 min.

Methyl *N*-((4-formylphenyl)sulfonyl)-*N*-methylglycinate (41). $C_{11}H_{13}NO_5S$, M_r: 271.29. A mixture of 4-formyl-*N*-methylbenzenesulfonamide (**38**, 300 mg, 1.49 mmol, 1.0 eq) and methyl 2-bromoacetate (282 µL, 2.982 mmol, 2.0 eq.), K_2CO_3 (309 mg, 2.24 mmol, 1.5 eq.), and KI (49 mg, 0.30 mmol, 0.2 eq.) were stirred in 3.7 mL dry DMF at 60° C for 3 h. DMF was removed by evaporation *in vacuo*. The resulting mixture was diluted with EtOAc (20 mL) and an equivalent amount of water. The aqueous layer was extracted with EtOAc (20 mL x 3). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified via silica gel column chromatography with CH/EtOAc (90:10 to 15:85, v/v) to obtain **47** (300 mg, 1.491, 74 %). R_f 0.37 (Toluene/Acetone 10:1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.12 (s, 1H), 8.14 – 8.09 (m, 2H), 8.04 – 7.99 (m, 2H), 4.09 (s, 2H), 3.57 (s, 3H), 2.83 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 192.7, 168.8, 142.6, 138.8, 130.2, 127.8, 52.0, 50.5, 35.6.

Methyl *N*-methyl-*N*-((4-((((1*S,2*R**)-2-phenylcyclopropyl)amino)methyl)phenyl)sulfonyl)-glycinate (8).** $C_{20}H_{24}N_2O_4S$, M_r: 388.48. Procedure Method B: **41** (396 mg, 1.46 mmol, 1.0 eq.), TCP HCl (550 mg, 3.24 mmol, 2.22 eq.), Sodium triacetoxyborohydride (928 mg, 4.38 mmol, 3.0 eq.), Glacial acetic acid (93 µL, 1.620 mmol, 1.11 eq), 5.5 h; yield: 297 mg yellow oil, 0.76 mmol, 52 %. R_f 0.36 (EtOAc/CH 2:1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.74 – 7.66 (m, 2H), 7.58 – 7.48 (m, 2H), 7.24 – 7.13 (m, 2H), 7.13 – 7.02 (m, 1H), 7.00 – 6.89 (m, 2H), 3.97 (s, 2H), 3.85 (s, 2H), 3.57 (s, 3H), 3.07 (s, 1H), 2.76 (s, 3H), 2.21 (dd, *J* = 3.5, 6.9 Hz, 1H), 1.80 (ddd, *J* = 3.0, 5.8, 9.1 Hz, 1H), 1.00 (dt, *J* = 4.6, 9.2 Hz, 1H), 0.93 (ddd, *J* = 4.8, 5.8, 7.0 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.0, 146.6, 142.4, 135.7, 128.7, 128.1, 126.9, 125.5, 125.1, 52.1, 51.9, 50.6, 41.7, 35.6, 24.7, 16.7. LRMS (pos. APCI): *m/z* 388.6 $[M+H]^+$. HPLC (Method 1): t_R 15.23 min.

***N*-Methyl-*N*-((4-((((1*S**,2*R**)-2-phenylcyclopropyl)amino)methyl)phenyl)sulfonyl)glycine (9).** $C_{19}H_{22}N_2O_4S$, M_r: 374.46. Procedure Method C: **8** (294 mg, 0.76 mmol, 1.0 eq.), NaOH (1514 µL (1 M), 1.514 mmol, 2.0 eq.), 50° C, 18 h, Purification: RP-HPLC; yield: 217 mg white solid, 0.55 mmol, 72 %. R_f 0.47 (ACN/H_2O 1:1); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.80 – 7.59 (m, 2H), 7.55 – 7.45 (m, 2H), 7.25 – 7.14 (m, 2H), 7.12 – 7.03 (m, 1H), 6.99 – 6.87 (m, 2H), 3.92 – 3.76 (m, 2H), 3.18 (s, 2H), 3.04 (td, *J* = 3.5, 6.6 Hz, 1H), 2.63 (s, 3H), 2.24 – 2.17 (m, 1H), 1.79 (ddd, *J* = 3.0, 5.8, 9.0 Hz, 1H), 1.00 (dt, *J* = 4.7, 9.2 Hz, 1H), 0.93 (ddd, *J* = 4.8, 5.8, 7.1 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.8, 145.9, 142.4, 135.6, 128.4, 128.1, 127.0, 125.5, 125.1, 53.7, 52.2, 41.8, 35.2, 24.7, 16.6. LRMS (neg. APCI): *m/z* 372.5 $[M–H]^–$. HPLC (Method 1): t_R 14.06 min.

Methyl ((4-formylphenyl)sulfonyl)glycinate (42). $C_{10}H_{11}NO_5S$, M_r: 257.26. Procedure Method A: 4-formylbenzenesulfonyl chloride (**39**, 200 mg, 0.97 mmol, 1.0 eq.), Methyl glycinate

hydrochloride (607 mg, 4.84 mmol, 5.0 eq.), NaOH (4838 µL (1 M), 4.838 mmol, 5.0 eq), 15 h; yield: 220 mg pale yellow powder, 0.86 mmol, 88 %. R_f 0.59 (EtOAc/CH 2:1).

Methyl ((4-((((1*S,2*R**)-2-phenylcyclopropyl)amino)methyl)phenyl)sulfonyl)glycinate (10).** $C_{19}H_{22}N_2O_4S$, M_r: 374.46. Procedure Method B: **42** (84 mg, 0.33 mmol, 1.0 eq.), TCP HCl (69 mg, 0.41 mmol, 1.25 eq.), Sodium triacetoxyborohydride (347 mg, 1.64 mmol, 5.0 eq.), Glacial acetic acid (64 µL, 1.112 mmol, 3.4 eq.), 20 h; yield: 102 mg white solid, 0.33 mmol, 83 %. R_f 0.23 (EtOAc/CH 2:1). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.74 (d, *J* = 8.0 Hz, 2H), 7.51 (d, *J* = 7.9 Hz, 2H), 7.31 – 7.18 (m, 3H), 7.03 – 6.95 (m, 2H), 4.28 (s, 2H), 3.81 – 3.67 (m, 2H), 3.61 (s, 3H), 2.75 (dt, *J* = 4.0, 8.0 Hz, 1H), 2.58 (ddd, *J* = 3.4, 6.4, 10.1 Hz, 1H), 1.58 (ddd, *J* = 4.2, 6.6, 10.8 Hz, 1H), 1.23 (q, *J* = 6.9 Hz, 1H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 169.5, 140.9, 137.5, 135.4, 130.8, 128.9, 127.8, 127.3, 126.5, 52.7, 51.6, 44.0, 38.2, 21.7, 12.9. LRMS (pos. APCI): *m/z* 374.7 $[M+H]^+$. HPLC (Method 1): t_R 15.18 min.

((4-((((1*S,2*R**)-2-Phenylcyclopropyl)amino)methyl)phenyl)sulfonyl)glycine (11).** $C_{18}H_{20}N_2O_4S$, M_r: 360.43. Procedure Method C: **10** (101 mg, 0.27 mmol, 1.0 eq.), NaOH (809 µL (1 M), 0.809 mmol, 3.0 eq.), 50° C, 21 h, Purification: RP-HPLC; yield: 87 mg white solid, 0.23 mmol, 84 %. R_f 0.49 (ACN/H_2O 1:1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.89 (s, 1H), 7.75 – 7.66 (m, 2H), 7.54 – 7.46 (m, 2H), 7.24 – 7.15 (m, 2H), 7.14 – 7.04 (m, 1H), 7.04 – 6.92 (m, 2H), 3.85 (d, *J* = 1.8 Hz, 2H), 3.51 (s, 2H), 2.24 (ddd, *J* = 3.0, 4.3, 7.2 Hz, 1H), 1.83 (ddd, *J* = 3.1, 5.9, 9.1 Hz, 1H), 1.02 (dt, *J* = 4.7, 9.2 Hz, 1H), 0.99 – 0.90 (m, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.6, 145.8, 142.6, 139.2, 128.9, 128.5, 126.8, 125.9, 125.6, 52.4, 44.3, 41.9, 24.9, 16.9. LRMS (neg. APCI) *m/z* 358.7 $[M–H]^–$. HPLC (Method 1) t_R 12.98 min.

Methyl (4-formylbenzoyl)glycinate (43). $C_{11}H_{11}NO_4$, M_r: 221.21. 4-formylbenzoic acid (**40**, 297 mg, 1.98 mmol, 1.0 eq), BOP-Cl (755 mg, 2.97 mmol, 1.5 eq), and trimethylamine (607 µL,

4.352 mmol, 2.2 eq.) were mixed in a dried flask and diluted in 5 mL dry DMF and 2 mL dry DCM. The reaction was stirred under nitrogen atmosphere for 20 min. After this, methyl glycinate hydrochloride (497 mg, 3.96 mmol, 2.0 eq) was added and an immediate change of color from bright yellow to a dark yellow tone was observed. The reaction was stopped after 26 h. The resulting mixture was diluted with DCM (20 mL) and an equivalent amount of water. The mixture was acidified with HCl solution (1 M). The aqueous layer was extracted with DCM (20 mL x 3). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified via silica gel column chromatography with CH/EtOAc (90:10 to 15:85, v/v). to obtain a white powder (129 mg, 0.58, 29 %). R_f 0.38 (EtOAc/CH 2:1).

Methyl (4-((((1*S,2*R**)-2-phenylcyclopropyl)amino)methyl)benzoyl)glycinate (12).** $C_{20}H_{22}N_2O_3$, M_r: 338.41. Procedure Method B: **23** (128 mg, 0.58 mmol, 1.0 eq.), TCP HCl (196 mg, 1.16 mmol, 2.0 eq.), Sodium triacetoxyborohydride (489 mg, 2.31 mmol, 4.0 eq.), Glacial acetic acid (41 µL, 0.72 mmol, 1.25 eq.), 24 h; yield: 164 mg yellow solid, 0.49 mmol, 84 %. R_f 0.11 (EtOAc/CH 2:1). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 9.07 (t, *J* = 5.8 Hz, 1H), 7.93 – 7.86 (m, 2H), 7.70 – 7.58 (m, 2H), 7.33 – 7.23 (m, 2H), 7.25 – 7.14 (m, 1H), 7.14 – 7.02 (m, 2H), 4.35 (s, 2H), 4.10 – 3.94 (m, 2H), 3.66 (s, 2H), 2.89 (t, *J* = 4.4 Hz, 1H), 2.58 – 2.51 (m, 1H), 1.56 (ddd, *J* = 4.4, 6.2, 10.4 Hz, 1H), 1.28 (dt, *J* = 6.3, 7.8 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 170.3, 166.0, 138.7, 135.3, 133.9, 130.1, 128.4, 127.4, 126.5, 126.4, 51.8, 50.0, 41.2, 37.5, 20.6, 12.5. LRMS (pos. APCI): *m/z* 338.8 $[M+H]^+$. HPLC (Method 1): t_R 13.28 min.

(4-((((1*S,2*R**)-2-Phenylcyclopropyl)amino)methyl)benzoyl)glycine (13).** $C_{19}H_{20}N_2O_3$, M_r: 324.38. Procedure Method C: **12** (129 mg, 0.38 mmol, 1.0 eq.), NaOH (190 µL (1 M), 0.190 mmol, 0.5 eq.), 50° C, 21 h, Purification: RP-HPLC; yield: 25 mg , 0.08 mmol, 20 %. R_f 0.43 (ACN/H_2O 1:1). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 8.81 (t, *J* = 5.9 Hz, 1H), 7.85 – 7.74 (m,

2H), 7.49 – 7.42 (m, 2H), 7.28 – 7.16 (m, 2H), 7.19 – 7.07 (m, 1H), 7.04 – 6.96 (m, 2H), 3.94 (s, 2H), 3.91 (d, *J* = 5.9 Hz, 2H), 2.36 (dt, *J* = 4.0, 7.7 Hz, 1H), 1.98 (ddd, *J* = 3.2, 6.1, 9.4 Hz, 1H), 1.13 (dq, *J* = 4.8, 9.5 Hz, 1H), 1.01 (dt, *J* = 5.5, 7.2 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.4, 166.2, 142.5, 141.6, 132.5, 128.3, 128.2, 127.2, 125.7, 125.4, 51.8, 41.2, 40.7, 23.7, 15.9. LRMS (neg. APCI): *m/z* 322.8 $[M–H]^-$. HPLC (Method 1): t_R 12.24 min.

Methyl *N*-(4-formylbenzoyl)-*N*-methylglycinate (44). $C_{12}H_{13}NO_4$, M_r: 235.24. 4-formylbenzoic acid (**40**, 297 mg, 1.98 mmol, 1.0 eq), BOP-Cl (755 mg, 2.97 mmol, 1.5 eq), and trimethylamine (827 µL, 5.935 mmol, 3.0 eq.) were mixed in a dried flask and diluted in 5 mL dry DMF and 2 mL dry DCM. The reaction was stirred under nitrogen atmosphere for 30 min. After this, methyl sarcosinate hydrochloride (552 mg, 3.96 mmol, 2.0 eq) was added and an immediate change of color from colorless to a yellow tone was observed. The reaction was stopped after 24 h. The mixture was concentrated *in vacuo* in order to remove DMF.The resulting oil was diluted with EtOAc (20 mL) and an equivalent amount of water. The mixture was acidified with HCl solution (1 M). The aqueous layer was extracted with EtOAc (20 mL x 3). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified via silica gel column chromatography with CH/EtOAc (90:10 to 15:85, v/v) to obtain a colorless oil (189 mg, 0.80 mmol, 41 %). R_f 0.44 (EtOAc/CH 2:1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.07 (s, 1H), 8.06 – 7.98 (m, 2H), 7.66 – 7.59 (m, 2H), 4.27 (s, 2H), 3.71 (s, 3H), 2.94 (s, 3H).

Methyl *N*-methyl-*N*-(4-((((1*S,2*R**)-2-phenylcyclopropyl)amino)methyl)benzoyl)glycinate (14).** $C_{21}H_{24}N_2O_3$, M_r: 352.43. Procedure Method B: **44** (168 mg, 0.72 mmol, 1.0 eq.), TCP HCl (243 mg, 1.43 mmol, 2.0 eq.), Sodium triacetoxyborohydride (607 mg, 2.86 mmol, 4.0 eq.), Glacial acetic acid (51 µL, 0.894 mmol, 1.25 eq.), 18 h; yield: 191 mg pale yellow oil, 0.54 mmol, 54 %. R_f 0.12 (EtOAc/CH 2:1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.42 – 7.27 (m,

2H), 7.27 – 7.14 (m, 2H), 7.13 – 7.03 (m, 1H), 7.02 – 6.92 (m, 2H), 4.22 (s, 1H), 3.79 (d, *J* = 9.2 Hz, 2H), 3.69 (s, 2H), 2.96 (d, *J* = 4.3 Hz, 3H), 2.21 (dt, *J* = 3.7, 7.5 Hz, 1H), 1.81 (ddd, *J* = 3.0, 5.2, 8.5 Hz, 1H), 1.00 (dt, *J* = 4.6, 9.2 Hz, 1H), 0.96 – 0.88 (m, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.8, 169.6, 142.9, 142.5, 133.6, 128.1, 127.9, 126.8, 125.5, 125.1, 52.3, 51.8, 48.8, 41.7, 38.4, 24.6, 16.7. LRMS (pos. APCI): *m/z* 352.8 $[M+H]^+$. HPLC (Method 1): t_R 13.83 min. Purity: 93 %

***N*-Methyl-*N*-(4-((((1*S**,2*R**)-2-phenylcyclopropyl)amino)methyl)benzoyl)glycine (15).** $C_{20}H_{22}N_2O_3$, M_r: 338.41. Procedure Method C: **14** (59 mg, 0.17 mmol, 1.0 eq.), NaOH (335 µL (1 M), 0.335 mmol, 2.0 eq.), reflux, 27 h, Purification: RP-HPLC; yield: 8 mg white solid, 0.02 mmol, 12 %. R_f 0.46 (ACN/H_2O 1:1). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.50 – 7.35 (m, 4H), 7.24 – 7.15 (m, 2H), 7.14 – 7.05 (m, 1H), 7.02 – 6.94 (m, 2H), 3.88 (d, *J* = 10.3 Hz, 2H), 3.77 (s, 2H), 2.34 – 2.23 (m, 1H), 1.97 – 1.88 (m, 1H), 1.89 (s, 3H), 1.08 (ddd, *J* = 4.4, 5.2, 9.5 Hz, 1H), 1.03 – 0.92 (m, 1H). ^{13}C NMR (101 MHz, Methanol-d_4) δ 175.7, 174.2, 143.2, 142.7, 136.3, 129.7, 129.2, 128.1, 126.8, 126.5, 56.8, 53.8, 41.8, 25.3, 24.2, 16.8. LRMS (neg. APCI): *m/z* 337.7 $[M–H]^–$. HPLC (Method 1): t_R 12.55 min. Purity: 93 %

Methyl ((4-formylphenyl)sulfonyl)-*L*-phenylalaninate (45). $C_{17}H_{17}NO_5S$, M_r: 347.39. Procedure Method A: 4-formylbenzenesulfonyl chloride (**39,** 103 mg, 0.50 mmol, 1.0 eq.), Methyl L-phenylalaninate hydrochloride (187 mg, 0.87 mmol, 1.74 eq.), NaOH (1246 µL (1 M), 1.246 mmol, 2.5 eq.), 23 h; yield: 121 mg pale yellow powder, 0.35 mmol, 70 %. R_f 0.85 (EtOAc/CH 2:1).

Methyl ((4-((((1*S,2*R**)-2-phenylcyclopropyl)amino)methyl)phenyl)sulfonyl)-*L*-phenylalaninate (16).** $C_{26}H_{28}N_2O_4S$, M_r: 464.58. Procedure Method B: **45** (119 mg, 0.34 mmol, 1.0 eq.), TCP HCl (116 mg, 0.68 mmol, 2.0 eq.), Sodium triacetoxyborohydride (290 mg,

1.37 mmol, 4.0 eq.), Glacial acetic acid (24 µL, 0.427 mmol, 1.25 eq.), 5 h; yield: 73 mg pale yellow powder, 0.16 mmol, 46 %. **R_f** 0.37 (EtOAc/CH 2:1). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.70 – 7.51 (m, 2H), 7.49 – 7.32 (m, 2H), 7.31 – 7.11 (m, 5H), 7.15 – 7.02 (m, 3H), 7.07 – 6.87 (m, 2H), 4.05 (ddd, *J* = 2.1, 6.6, 8.1 Hz, 1H), 3.90 (s, 2H), 3.36 (d, *J* = 5.3 Hz, 3H), 2.97 (ddd, *J* = 1.8, 6.5, 13.6 Hz, 1H), 2.84 (ddd, *J* = 4.2, 8.1, 13.7 Hz, 1H), 2.24 (ddt, *J* = 3.0, 4.4, 7.3 Hz, 1H), 1.94 – 1.84 (m, 1H), 1.08 (ddd, *J* = 4.4, 5.3, 9.6 Hz, 1H), 0.99 (dddd, *J* = 1.1, 5.3, 6.3, 7.1 Hz, 1H). ^{13}C NMR (101 MHz, Methanol-d_4) δ 173.0, 146.0, 143.1, 140.6, 137.5, 130.3, 130.1, 129.5, 129.3, 128.1, 128.0, 126.8, 126.6, 58.9, 53.5, 52.5, 41.8, 39.8, 25.4, 16.7. LRMS (pos. APCI): *m/z* 464.7 $[M+H]^+$. HPLC (Method 1): t_R 17.38 min.

((4-((((1*S,2*R**)-2-Phenylcyclopropyl)amino)methyl)phenyl)sulfonyl)-*L*-phenylalanine (17).** $C_{25}H_{26}N_2O_4S$, M_r: 450.55. Procedure Method C: **16** (55 mg, 0.118 mmol, 1.0 eq.), NaOH (237 µL (1 M), 0.237 mmol, 2.0 eq.), reflux, 48 h, Purification: reversed phase silica gel column chromatography with H_2O/ACN (90:10 to 10:90, v/v); yield: 20 mg pale yellow powder, 0.04 mmol, 37 %. R_f 0.40 (ACN/H_2O 1:1). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.73 – 7.56 (m, 2H), 7.48 – 7.31 (m, 2H), 7.29 – 7.05 (m, 6H), 7.06 – 6.88 (m, 2H), 3.89 (s, 2H), 3.86 – 3.74 (m, 1H), 3.01 (dd, *J* = 5.0, 13.6 Hz, 1H), 2.91 (ddd, *J* = 2.2, 6.5, 13.4 Hz, 1H), 2.28 (dddd, *J* = 1.7, 3.2, 4.5, 7.4 Hz, 1H), 1.92 – 1.85 (m, 1H), 1.08 (ddd, *J* = 4.4, 5.3, 9.5 Hz), 0.98 (dt, *J* = 5.6, 7.2 Hz, 1H). ^{13}C NMR (101 MHz, Methanol-d_4) δ 177.0, 145.8, 143.2, 140.5, 139.0, 131.0, 130.1, 129.3, 128.9, 128.2, 127.2, 126.8, 126.5, 60.6, 53.6, 41.9, 40.6, 25.5 , 24.2. LRMS (neg. APCI): *m/z* 448.5 $[M–H]^–$. HPLC (Method 1): t_R 15.95 min.

Methyl ((4-formylphenyl)sulfonyl)-*D*-valinate (46). $C_{13}H_{17}NO_5S$, M_r: 299.34. Procedure Method A: 4-formylbenzenesulfonyl chloride (**39,** 149 mg, 0.73 mmol, 1.0 eq.), Methyl D-valinate

hydrochloride (608 mg, 3.63 mmol, 5.0 eq.), NaOH (4354 µL (1 M), 4.354 mmol, 6.0 eq.), 4 h; yield: 207 mg white powder, 0.69 mmol, 95 %. R_f 0.59 (EtOAc/CH 2:1).

Methyl ((4-((((1*S,2*R**)-2-phenylcyclopropyl)amino)methyl)phenyl)sulfonyl)-*D*-valinate (18).** $C_{22}H_{28}N_2O_4S$, M_r: 416.54. Procedure Method B: **46** (205 mg, 0.69 mmol, 1.0 eq.), TCP HCl (232 mg, 1.37 mmol, 2.0 eq.), Sodium triacetoxyborohydride (580 mg, 2.74 mmol, 4.0 eq.), Glacial acetic acid (49 µL, 0.856 mmol, 1.25 eq.), 19 h; yield: 151 mg colorless oil, 0.36 mmol, 53 %. R_f 0.12 (EtOAc/CH 1:1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.19 (s, 1H), 7.73 – 7.56 (m, 2H), 7.56 – 7.39 (m, 2H), 7.26 – 7.14 (m, 2H), 7.14 – 7.04 (m, 1H), 7.06 – 6.87 (m, 2H), 3.82 (s, 2H), 3.50 (d, *J* = 7.0 Hz, 1H), 3.28 (d, *J* = 2.8 Hz, 3H), 3.05 (s, 1H), 2.16 (ddd, *J* = 3.1, 4.3, 7.2 Hz, 1H), 1.95 – 1.75 (m, 2H), 1.04 – 0.95 (m, 1H), 0.97 – 0.84 (m, 1H), 0.79 (d, *J* = 6.7 Hz, 3H), 0.76 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.2, 145.9, 142.4, 138.9, 128.2, 128.1, 126.4, 125.5, 125.1, 61.4, 52.0, 51.5, 41.5, 30.4, 24.5, 18.8, 18.3, 16.7. LRMS (pos. APCI): *m/z* 416.7 $[M+H]^+$. HPLC (Method 1): t_R 16.46 min.

((4-((((1*S,2*R**)-2-Phenylcyclopropyl)amino)methyl)phenyl)sulfonyl)-*D*-valine (19).** $C_{21}H_{26}N_2O_4S$, M_r: 402.51. Procedure Method C: **18** (86 mg, 0.22 mmol, 1.0 eq.), NaOH (660 µL (1 M), 0.660 mmol, 3.0 eq.), reflux, 48 h, Purification: reversed phase silica gel column chromatography with H_2O/ACN (90:10 to 10:90, v/v); yield: 22 mg white solid, 0.06 mmol, 25 %. R_f 0.46 (ACN/H_2O 1:1). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.86 – 7.69 (m, 2H), 7.54 – 7.37 (m, 2H), 7.26 – 7.12 (m, 2H), 7.16 – 7.04 (m, 1H), 7.07 – 6.93 (m, 2H), 3.89 (s, 2H), 3.41 (dd, *J* = 2.8, 4.4 Hz, 1H), 2.35 – 2.19 (m, 1H), 2.03 (pd, *J* = 4.5, 6.8 Hz, 1H), 1.94 – 1.84 (m, 1H), 1.08 (dddd, *J* = 0.9, 4.3, 5.3, 9.6 Hz, 1H), 1.06 – 0.96 (m, 1H), 0.94 (d, *J* = 6.9 Hz, 3H), 0.83 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (101 MHz, Methanol-d_4) δ 177.3, 145.8, 143.2, 140.5, 130.1, 129.2, 128.4, 126.7,

126.5, 64.8, 53.6, 42.0, 32.7, 25.4, 20.2, 18.0, 16.9. LRMS (neg. APCI): *m/z* 400.7 $[M–H]^-$. HPLC (Method 1): t_R 15.23 min.

Methyl ((4-formylphenyl)sulfonyl)-*L*-valinate (47). $C_{13}H_{17}NO_5S$, M_r: 299.34. Procedure Method A: 4-formylbenzenesulfonyl chloride (**39**, 149 mg, 0.73 mmol, 1.0 eq.), Methyl L-valinate hydrochloride (608 mg, 3.63 mmol, 5.0 eq.), NaOH (4354 µL (1 M), 4.354 mmol, 6.0 eq.), 4 h; yield: 154 mg colorless oil, 0.51 mmol, 71 %. R_f 0.59 (EtOAc/CH 2:1).

Methyl ((4-((((1*S,2*R**)-2-phenylcyclopropyl)amino)methyl)phenyl)sulfonyl)-*L*-valinate (20).** $C_{22}H_{28}N_2O_4S$, M_r: 416.54. Procedure Method B: **47** (152 mg, 0.51 mmol, 1.0 eq.), TCP HCl (173 mg, 1.02 mmol, 2.0 eq.), Sodium triacetoxyborohydride (432 mg, 2.04 mmol, 4.0 eq.), Glacial acetic acid (36 µL, 0.637 mmol, 1.25 eq.), 19 h; yield: 105 mg colorless oil, 0.252 mmol, 50 %. R_f 0.13 (EtOAc/CH 1:1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.21 (d, *J* = 9.1 Hz, 1H), 7.71 – 7.57 (m, 2H), 7.57 – 7.40 (m, 2H), 7.25 – 7.12 (m, 2H), 7.16 – 7.05 (m, 1H), 7.07 – 6.88 (m, 2H), 3.83 (s, 2H), 3.51 (dd, *J* = 7.1, 9.2 Hz, 1H), 3.29 (d, *J* = 2.8 Hz, 3H), 3.06 (s, 1H), 2.17 (dt, *J* = 3.9, 7.4 Hz, 1H), 1.96 – 1.77 (m, 2H), 1.01 (dt, *J* = 4.7, 9.1 Hz, 1H), 0.93 (ddd, *J* = 4.8, 5.8, 7.1 Hz, 1H), 0.90 – 0.69 (m, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.6, 146.3, 142.8, 139.2, 128.6, 128.5, 126.8, 125.9, 125.5, 61.8, 52.4, 51.9, 41.9, 30.8, 24.9, 19.2, 18.7, 17.1. LRMS (pos. APCI): *m/z* 416.6 $[M+H]^+$. HPLC (Method 1): t_R 16.45 min.

((4-((((1*S,2*R**)-2-Phenylcyclopropyl)amino)methyl)phenyl)sulfonyl)-*L*-valine (21).** $C_{21}H_{26}N_2O_4S$, M_r: 402.51. Procedure Method C: **20** (52 mg, 0.13 mmol, 1.0 eq.), NaOH (502 µL (1 M), 0.502 mmol, 4.0 eq.), reflux, 48 h, Purification: reversed phase silica gel column chromatography with H_2O/ACN (90:10 to 10:90, v/v); yield: 36 mg white solid, 0.02 mmol, 72 %. R_f 0.47 (ACN/H_2O 1:1). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.95 – 7.83 (m, 2H), 7.76 – 7.59 (m, 2H), 7.35 – 7.24 (m, 2H), 7.27 – 7.14 (m, 1H), 7.18 – 7.02 (m, 2H), 4.46 (s, 2H), 3.67 (dd, *J* = 2.2,

5.5 Hz, 1H), 3.06 – 2.94 (m, 1H), 2.48 (dddd, *J* = 1.6, 3.6, 6.6, 10.3 Hz, 1H), 2.13 – 1.97 (m, 1H), 1.53 (dddd, *J* = 1.2, 4.4, 6.9, 10.3 Hz, 1H), 1.37 (dt, *J* = 6.7, 7.9 Hz, 1H), 0.95 (d, *J* = 6.8 Hz, 3H), 0.88 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (101 MHz, Methanol-d_4) δ 174.0, 143.5, 139.3, 136.8, 131.8, 129.7, 128.9, 128.0, 127.3, 62.8, 52.0, 39.1, 32.4, 22.6, 19.7, 18.0, 13.7. LRMS (neg. APCI): *m/z* 400.6 $[M–H]^-$. HPLC (Method 1): t_R 15.21 min.

Methyl ((4-formylphenyl)sulfonyl)-*D*-alaninate (48). $C_{11}H_{13}NO_5S$, M_r: 271.29. Procedure Method A: 4-formylbenzenesulfonyl chloride (**39**, 149 mg, 0.73 mmol, 1.0 eq.), Methyl D-alaninate hydrochloride (506 mg, 3.63 mmol, 5.0 eq.), NaOH (4.354 mL (1 M), 4.354 mmol, 6.0 eq.), 5 h; yield: 159 mg, 0.57 mmol, 79 %. R_f 0.41 (EtOAc/CH 1:1).

Methyl ((4-((((1*S,2*R**)-2-phenylcyclopropyl)amino)methyl)phenyl)sulfonyl)-*D*-alaninate (22).** $C_{20}H_{24}N_2O_4S$, M_r: 388.48. Procedure Method B: **48** (144 mg, 0.53 mmol, 1.0 eq.), TCP HCl (180 mg, 1.06 mmol, 2.0 eq.), Sodium triacetoxyborohydride (449 mg, 2.12 mmol, 4.0 eq.), Glacial acetic acid (30 µL, 0.529 mmol, 1.0 eq.), 23 h; yield: 138 mg pale yellow solid, 0.35 mmol, 63 %. R_f 0.13 (EtOAc/CH 1:1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.26 (d, *J* = 6.9 Hz, 1H), 7.73 – 7.63 (m, 2H), 7.53 – 7.45 (m, 2H), 7.23 – 7.14 (m, 2H), 7.13 – 7.04 (m, 1H), 6.99 – 6.91 (m, 2H), 3.89 – 3.78 (m, 3H), 3.39 (d, *J* = 2.9 Hz, 3H), 2.18 (ddd, *J* = 3.1, 4.3, 7.3 Hz, 1H), 1.81 (ddd, *J* = 3.1, 5.8, 9.0 Hz, 1H), 1.13 (d, *J* = 7.2 Hz, 3H), 1.00 (dt, *J* = 4.6, 9.2 Hz, 1H), 0.97 – 0.82 (m, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.1, 146.0, 142.4, 139.1, 128.4, 128.1, 126.3, 125.5, 125.1, 52.1, 51.8, 51.1, 41.6, 24.6, 18.1, 16.7. LRMS (pos. APCI): *m/z* 388.7 $[M+H]^+$. HPLC (Method 2): t_R 12.84 min.

((4-((((1*S,2*R**)-2-Phenylcyclopropyl)amino)methyl)phenyl)sulfonyl)-*D*-alanine (23).** $C_{19}H_{22}N_2O_4S$, M_r: 374.46. Procedure Method C: **22** (53 mg, 0.14 mmol, 1.0 eq.), LiOH (273 µL (1 M), 0.273 mmol, 2.0 eq.), reflux, 27 h, Purification: RP-HPLC; yield: 6 mg pale yellow solid,

0.02 mmol, 12 %. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.91 (d, J = 8.1 Hz, 2H), 7.65 (d, J = 8.1 Hz, 2H), 7.35 – 7.26 (m, 2H), 7.26 – 7.18 (m, 1H), 7.13 – 7.06 (m, 2H), 4.47 (s, 2H), 3.92 (q, J = 7.2 Hz, 1H), 3.00 (t, J = 4.7 Hz, 1H), 2.42 (ddd, J = 3.6, 6.5, 10.2 Hz, 1H), 1.53 – 1.44 (m, 1H), 1.40 (q, J = 7.0 Hz, 1H), 1.34 – 1.27 (m, 3H). ^{13}C NMR (101 MHz, Methanol-d_4) δ 175.1, 143.8, 139.1, 136.8, 131.8, 129.7, 128.8, 128.1, 127.3, 52.7, 52.0, 39.1, 22.6, 19.6, 13.7. LRMS (neg. APCI): *m/z* 372.7 $[M–H]^-$. HPLC (Method 1): t_R 11.99 min.

Methyl ((4-formylphenyl)sulfonyl)-*L*-alaninate (49). $C_{11}H_{13}NO_5S$, M_r: 271.29. Procedure Method A: 4-formylbenzenesulfonyl chloride (**39**, 150 mg, 0.73 mmol, 1.0 eq.), Methyl L-alaninate hydrochloride (506 mg, 3.63 mmol, 5.0 eq.), NaOH (4354 µL (1 M), 4.354 mmol, 6.0 eq.), 5 h; yield: 180 mg, 0.66 mmol, 91 %. R_f 0.41 (EtOAc/CH 1:1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.10 (s, 1H), 8.13 – 8.05 (m, 2H), 8.01 – 7.93 (m, 2H), 3.97 (q, J = 7.2 Hz, 1H), 3.41 (s, 3H), 1.18 (d, J = 7.2 Hz, 3H).

Methyl ((4-((((1*S,2*R**)-2-phenylcyclopropyl)amino)methyl)phenyl)sulfonyl)-*L*-alaninate (24).** $C_{20}H_{24}N_2O_4S$, M_r: 388.48. Procedure Method B: **49** (178 mg, 0.66 mmol, 1.0 eq.), TCP HCl (223 mg, 1.31 mmol, 2.0 eq.), Sodium triacetoxyborohydride (557 mg, 2.63 mmol, 4.0 eq.), Glacial acetic acid (47 µL, 0.821 mmol, 1.25 eq, 25 h); yield: 150 mg white solid, 0.39 mmol, 59 %. R_f 0.13 (EtOAc/CH 1:1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.71 – 7.63 (m, 2H), 7.53 – 7.45 (m, 2H), 7.26 – 7.14 (m, 2H), 7.13 – 7.04 (m, 1H), 7.05 – 6.91 (m, 2H), 3.89 – 3.77 (m, 1H), 3.83 (s, 2H), 3.39 (d, J = 2.9 Hz, 3H), 3.06 (s, 1H), 2.18 (ddd, J = 3.1, 4.3, 7.3 Hz, 1H), 1.81 (ddd, J = 3.0, 5.8, 9.0 Hz, 1H), 1.13 (d, J = 7.1 Hz, 3H), 1.05 – 0.94 (m, 2H), 0.94 – 0.82 (m, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.1, 146.0, 142.4, 139.1, 128.4, 128.1, 126.3, 125.5, 125.1, 52.1, 51.8, 51.1, 41.6, 24.6, 18.1, 16.7. LRMS (pos. APCI): *m/z* 388.6 $[M+H]^+$. HPLC (Method 2): t_R 13.11 min.

((4-((((1*S,2*R**)-2-Phenylcyclopropyl)amino)methyl)phenyl)sulfonyl)-*L*-alanine (25).** $C_{19}H_{22}N_2O_4S$, M_r: 374.46. Procedure Method C: **24** (40 mg, 0.10 mmol, 1.0 eq.), LiOH (206 µL (1 M), 0.206 mmol, 2.0 eq.), 24 h, Purification: RP-HPLC; yield: 5 mg pale yellow solid, 0.01 mmol, 11 %. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.96 – 7.88 (m, 2H), 7.68 – 7.61 (m, 2H), 7.36 – 7.28 (m, 2H), 7.28 – 7.20 (m, 1H), 7.14 – 7.07 (m, 2H), 4.47 (s, 2H), 3.92 (q, *J* = 7.2 Hz, 1H), 3.02 (dddd, *J* = 1.8, 3.6, 4.5, 8.0 Hz, 1H), 2.41 (ddd, *J* = 3.6, 6.7, 10.4 Hz, 1H), 1.53 – 1.43 (m, 1H), 1.43 – 1.34 (m, 1H), 1.37 – 1.24 (d, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, Methanol-d_4) δ 175.1, 144.0, 139.1, 136.8, 131.8, 129.8, 128.9, 128.1, 127.3, 52.7, 52.1, 39.2, 22.7, 19.7, 13.7. LRMS (neg. APCI): *m/z* 372.6 $[M–H]^-$. HPLC (Method 2): t_R 11.79 min.

Dimethyl ((4-formylphenyl)sulfonyl)-*D*-aspartate (50). $C_{13}H_{15}NO_7S$, M_r: 329.32. Procedure Method A: 4-formylbenzenesulfonyl chloride (**39**, 198 mg, 0.97 mmol, 1.0 eq.), Dimethyl D-aspartate hydrochloride (392 mg, 1.98 mmol, 2.05 eq.), NaOH (2903 µL (1 M), 2.903 mmol, 3.0 eq.), 4 h; yield: 108 mg, 0.33 mmol, 34 %. **R_f** 0.39 (EtOAc/CH 1:1).

Dimethyl ((4-((((1*S,2*R**)-2-phenylcyclopropyl)amino)methyl)phenyl)sulfonyl)-*D*-aspartate (26).** $C_{22}H_{26}N_2O_6S$, M_r: 446.52. Procedure Method B: **50** (107 mg, 0.33 mmol, 1.0 eq.), TCP HCl (110 mg, 0.65 mmol, 2.0 eq.), Sodium triacetoxyborohydride (275 mg, 1.30 mmol, 4.0 eq.), Glacial acetic acid (23 µL, 1.275 mmol, 1.25 eq.), 5 h, Purification: RP-HPLC; yield: 35 mg white powder, 0.08 mmol, 24 %. R_f 0.18 (EtOAc/CH 1:1). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 10.14 (s, 2H), 7.84 (d, *J* = 7.8 Hz, 2H), 7.73 (d, *J* = 7.9 Hz, 2H), 7.22 (ddd, *J* = 1.7, 7.2, 12.4 Hz, 3H), 7.01 – 6.94 (m, 2H), 6.06 (d, *J* = 8.3 Hz, 1H), 4.27 (s, 2H), 4.20 (t, *J* = 4.2 Hz, 1H), 3.69 – 3.59 (m, 3H), 3.63 – 3.54 (m, 3H), 2.96 (dd, *J* = 4.1, 17.3 Hz, 1H), 2.84 (dd, *J* = 4.8, 17.2 Hz, 1H), 2.78 – 2.73 (m, 1H), 2.65 – 2.60 (m, 1H), 1.84 – 1.73 (m, 1H), 1.26 – 1.18 (m, 1H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 170.9, 170.5, 141.4, 137.5, 135.0, 131.5, 128.9, 127.9, 127.3, 126.5, 53.3,

52.4, 52.3, 51.2, 37.8, 37.5, 21.8, 13.2. LRMS (pos. APCI): *m/z* 446.7 $[M+H]^+$. HPLC (Method 2): t_R 13.24 min.

((4-((((1*S,2*R**)-2-Phenylcyclopropyl)amino)methyl)phenyl)sulfonyl)-*D*-aspartic acid (27).** $C_{20}H_{22}N_2O_6S$, M_r: 418.46. Procedure Method C: **26** (129 mg, 0.29 mmol, 1.0 eq.), KOH (865 µL (1 M), 0.865 mmol, 3.0 eq.), reflux, 4 h, Purification: RP-HPLC; yield: 32 mg white solid, 0.08 mmol, 27 %. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.03 – 7.88 (m, 2H), 7.72 – 7.62 (m, 2H), 7.35 – 7.26 (m, 2H), 7.28 – 7.14 (m, 1H), 7.11 (dt, *J* = 1.5, 7.0 Hz, 2H), 4.47 (s, 2H), 4.22 (t, *J* = 5.8 Hz, 1H), 3.01 (ddt, *J* = 3.4, 4.4, 7.8 Hz, 1H), 2.74 (dd, *J* = 1.2, 5.8 Hz, 2H), 2.42 (dddd, *J* = 2.3, 3.6, 6.3, 10.3 Hz, 1H), 1.54 – 1.41 (m, 1H), 1.39 (dt, *J* = 6.8, 7.7 Hz, 1H). ^{13}C NMR (101 MHz, Methanol-d_4) δ 173.4, 173.1, 143.6, 139.1, 136.8, 131.8, 129.8, 129.0, 128.1, 127.3, 53.7, 52.1, 39.1, 38.8, 22.7, 13.7. LRMS (neg. APCI): *m/z* 413.8 $[M–H]^–$. HPLC (Method 2): t_R 11.51 min.

(*R*)-2-((4-Formylphenyl)sulfonamido)-4-methoxy-4-oxobutanoic acid (52). $C_{12}H_{13}NO_7S$, M_r: 315.30. Procedure Method A: 4-formylbenzenesulfonyl chloride (**39**, 298 mg, 0.97 mmol, 1.0 eq.), (*R*)-2-amino-4-methoxy-4-oxobutanoic acid (712 mg, 4.84 mmol, 5.0 eq.), NaOH (1451 µL (1 M), 1.451 mmol, 1.5 eq.), 4 h; yield: 257 mg, 0.82 mmol, 84 % (not pure). R_f 0.70 (ACN/H_2O 1:1).

(*R*)-4-Methoxy-4-oxo-2-((4-((((1*S,2*R**)-2-phenylcyclopropyl)amino)methyl)phenyl)sulfonamido)butanoic acid (28).** $C_{21}H_{24}N_2O_6S$, M_r: 432.49. Procedure Method B: **52** (238 mg, 0.72 mmol, 1.0 eq.), TCP HCl (245 mg, 1.44 mmol, 2.0 eq.), Sodium triacetoxyborohydride (612 mg, 2.89 mmol, 4.0 eq.), Glacial acetic acid (83 µL, 1.443 mmol, 2.0 eq.), 21 h, Purification: RP-HPLC; yield: 4 mg pale yellow oil, 0.01 mmol, 1 %. R_f 0.42 (ACN/H2O 1:1). ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.02 – 7.85 (m, 2H), 7.74 – 7.57 (m,

2H), 7.36 – 7.26 (m, 2H), 7.30 – 7.19 (m, 1H), 7.11 (dt, *J* = 1.5, 8.0 Hz, 2H), 4.47 (s, 2H), 4.24 (t, *J* = 6.0 Hz, 1H), 3.62 (d, *J* = 0.9 Hz, 3H), 3.05 – 2.96 (m, 1H), 2.87 – 2.69 (m, 2H), 2.41 (ddt, *J* = 3.1, 6.3, 10.1 Hz, 1H), 1.48 (ddd, *J* = 3.4, 6.9, 13.1 Hz, 1H), 1.40 (dtd, *J* = 0.9, 6.8, 7.8 Hz, 1H). ^{13}C NMR (101 MHz, Methanol-d_4) δ 191.1, 179.6, 170.6, 135.4, 130.3, 128.3, 127.5, 126.6, 125.9, 52.3, 51.0, 50.6, 37.6, 37.4, 21.2, 12.2. 1 aromatic carbon atom not visible. LRMS (neg. APCI): *m/z* 430.6 $[M–H]^-$. HPLC (Method 2): t_R 11.94 min.

4-(*tert*-Butyl) 1-methyl ((4-formylphenyl)sulfonyl)-*D*-aspartate (53). $C_{16}H_{21}NO_7S$, M_r: 371.40. Procedure Method A: 4-formylbenzenesulfonyl chloride (**39**, 84 mg, 0.41 mmol, 1.0 eq.), 4-(*tert*-butyl) 1-methyl D-aspartate (493 mg, 2.06 mmol, 5.0 eq.), NaOH (822 µL (1 M), 0.822 mmol, 2 eq.), 24 h; yield: 94 mg white solid, 0.25 mmol, 62 %.

4-(*tert*-Butyl) 1-methyl ((4-((((1*S,2*R**)-2-phenylcyclopropyl)amino)methyl)phenyl)sulfonyl)-D -aspartate (56).** $C_{25}H_{32}N_2O_6S$, M_r: 488.60. Procedure Method B: **53** (92 mg, 0.25 mmol, 1.0 eq.), TCP HCl (84 mg, 0.50 mmol, 2.0 eq.), Sodium triacetoxyborohydride (210 mg, 0.99 mmol, 4.0 eq.), Glacial acetic acid (28 µL, 0.496 mmol, 2.0 eq.), 19 h; yield: 80 mg, 0.16 mmol, 66 %. R_f 0.25 (EtOAc/CH 1:1). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.84 – 7.73 (m, 2H), 7.54 – 7.46 (m, 2H), 7.27 – 7.15 (m, 2H), 7.14 – 7.03 (m, 1H), 6.97 (dt, *J* = 1.4, 8.1 Hz, 2H), 4.23 (t, *J* = 6.5 Hz, 1H), 3.93 (s, 2H), 3.41 (d, *J* = 3.4 Hz, 3H), 2.66 (dd, *J* = 6.3, 16.0 Hz, 1H), 2.57 (ddd, *J* = 3.6, 6.7, 16.0 Hz, 1H), 2.27 (dddd, *J* = 2.2, 3.2, 4.4, 7.5 Hz, 1H), 1.90 (dddd, *J* = 1.8, 3.3, 5.5, 9.3 Hz, 1H), 1.42 (d, *J* = 3.5 Hz, 9H), 1.09 (ddd, *J* = 4.4, 5.3, 9.5 Hz, 1H), 1.06 – 0.94 (m, 1H). ^{13}C NMR (101 MHz, Methanol-d_4) δ 172.1, 170.5, 146.2, 143.1, 140.8, 130.2, 129.3, 128.3, 126.8, 126.6, 82.7, 54.0, 53.5, 52.8, 41.9, 40.0, 28.2, 25.4, 16.8.

(*R*)-4-Methoxy-4-oxo-3-((4-((((1*S,2*R**)-2-phenylcyclopropyl)amino)methyl)phenyl)sulfonamido)butanoic acid (29).** $C_{21}H_{24}N_2O_6S$, M_r: 432.49. **56** (74 mg, 0.15 mmol, 1.0 eq) was suspended in 25 mL of a 20 % aqueous solution of TFA (v/v). The reaction was heated to 40° C in a water bath for 10 h. The reaction was finished by evaporation of TFA and H_2O. The crude product was purified via RP-HPLC with H_2O/ACN (85:15 to 30:70, v/v) to obtain the colorless solid (55 mg, 0.13 mmol, 84 %). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.98 – 7.89 (m, 2H), 7.70 – 7.62 (m, 2H), 7.35 – 7.25 (m, 2H), 7.28 – 7.19 (m, 1H), 7.15 – 7.07 (m, 2H), 4.48 (d, *J* = 1.4 Hz, 2H), 4.28 (t, *J* = 6.0 Hz, 1H), 3.50 (d, *J* = 1.1 Hz, 3H), 3.00 (dddd, *J* = 0.9, 3.6, 4.4, 7.9 Hz, 1H), 2.74 (dd, *J* = 1.4, 6.1 Hz, 2H), 2.42 (ddd, *J* = 3.4, 6.5, 10.2 Hz, 1H), 1.55 – 1.41 (m, 1H), 1.45 – 1.36 (m, 1H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 173.1, 172.1, 143.7, 139.1, 136.9, 131.7, 129.8, 129.0, 128.1, 127.3, 53.8, 52.9, 52.0, 39.1, 38.5, 22.7, 13.6. LRMS (neg. APCI): *m/z* 430.7 $[M–H]^-$. HPLC (Method 2): t_R 11.94 min.

Dimethyl ((4-formylphenyl)sulfonyl)-*L*-aspartate (51). $C_{13}H_{15}NO_7S$, M_r: 329.32. Procedure Method A: 4-formylbenzenesulfonyl chloride (**39**, 331 mg, 1.62 mmol, 1.0 eq.), Dimethyl L-aspartate hydrochloride (1598 mg, 8.09 mmol, 5.0 eq.), NaOH (9701 µL (1 M), 9.701 mmol, 6.0 eq.), 3 h; yield: 339 mg colorless oil, 1.03 mmol, 64 %.R_f 0.39 (EtOAc/CH 1:1).

Dimethyl ((4-((((1*S,2*R**)-2-phenylcyclopropyl)amino)methyl)phenyl)sulfonyl)-*L*-aspartate (30).** $C_{22}H_{26}N_2O_6S$, M_r: 446.52. Procedure Method B: **51** (336 mg, 1.02 mmol, 1.0 eq.), TCP HCl (346 mg, 2.04 mmol, 2.0 eq.), Sodium triacetoxyborohydride (865 mg, 4.08 mmol, 4.0 eq.), Glacial acetic acid (73 µL, 1.275 mmol, 1.25 eq.), 4 h; yield: 289 mg pale yellow powder, 0.65 mmol, 63 %. R_f 0.29 (EtOAc/CH 3:1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.60 (d, *J* = 8.7 Hz, 1H), 7.85 – 7.75 (m, 2H), 7.78 – 7.62 (m, 2H), 7.35 – 7.26 (m, 2H), 7.28 – 7.17 (m, 1H), 7.14 (dt, *J* = 1.4, 8.1 Hz, 2H), 4.43 (s, 2H), 4.22 (dtd, *J* = 1.0, 6.9, 7.8 Hz, 1H), 3.53 (s, 3H), 3.41 (d, *J* = 1.1

Hz, 3H), 2.95 (dd, *J* = 3.9, 7.9 Hz, 1H), 2.75 (dd, *J* = 6.6, 16.4 Hz, 1H), 2.66 – 2.53 (m, 1H), 2.49 – 2.38 (m, 1H), 1.46 (ddd, *J* = 4.5, 6.4, 10.5 Hz, 1H), 1.33 (dt, *J* = 6.5, 7.8 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.2, 169.7, 141.5, 138.6, 136.4, 130.5, 128.4, 126.7, 126.5, 126.3, 52.2, 52.2, 51.8, 50.0, 37.7, 36.7, 20.8, 12.7. LRMS (pos. APCI): *m/z* 446.6 $[M+H]^+$. HPLC (Method 1): t_R 15.25 min.

((4-((((1*S,2*R**)-2-Phenylcyclopropyl)amino)methyl)phenyl)sulfonyl)-*L*-aspartic acid (45).** $C_{20}H_{22}N_2O_6S$, M_r: 418.46. Procedure Method C: **30** (221 mg, 0.49 mmol, 1.0 eq.), KOH (1483 µL (1 M), 1.483 mmol, 3.0 eq.), reflux, 3 h, Purification: RP-HPLC; yield: 3 mg white powder, 0.01 mmol, 1 %. R_f 0.57 (ACN/H_2O 1:1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.30 (d, *J* = 8.5 Hz, 1H), 7.84 (d, *J* = 8.0 Hz, 2H), 7.66 (d, *J* = 8.1 Hz, 2H), 7.30 (t, *J* = 7.4 Hz, 2H), 7.22 (t, *J* = 7.2 Hz, 1H), 7.12 (d, *J* = 7.7 Hz, 2H), 4.41 (s, 2H), 4.08 (q, *J* = 7.0 Hz, 1H), 2.97 (d, *J* = 6.8 Hz, 1H), 2.59 (dt, *J* = 7.9, 15.7 Hz, 1H), 2.49 – 2.40 (m, 1H), 2.43 – 2.35 (m, 1H), 1.44 (dt, *J* = 5.4, 10.4 Hz, 1H), 1.31 (q, *J* = 6.9 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.5, 171.1, 141.8, 138.5, 136.1, 130.5, 128.4, 126.8, 126.6, 126.4, 52.4, 50.0, 37.6, 37.3, 20.8, 12.8. LRMS (neg. APCI): *m/z* 416.7 $[M–H]^–$. HPLC (Method 1): t_R 11.71 min.

1-(*tert*-Butyl) 4-methyl ((4-formylphenyl)sulfonyl)-*L*-aspartate (54). $C_{16}H_{21}NO_7S$, M_r: 371.40. Procedure Method A: 4-formylbenzenesulfonyl chloride (**39**, 198 mg, 0.97 mmol, 1.0 eq.), 1-(*tert*-butyl) 4-methyl L-aspartate (983 mg, 4.84 mmol, 5.0 eq.), NaOH (1451 µL (1 M), 1.451 mmol, 1.5 eq.), 23 h; yield: 170 mg white solid, 0.458 mmol, 47 %; R_f 0.51 (EtOAc/CH 1:1).

1-(*tert*-Butyl) 4-methyl ((4-((((1*S,2*R**)-2-phenylcyclopropyl)amino)methyl)phenyl)-sulfonyl)-*L*-aspartate (57).** $C_{25}H_{32}N_2O_6S$, M_r: 488.60. Procedure Method B: **54** (168 mg, 0.45 mmol, 1.0 eq.), TCP HCl (154 mg, 0.91 mmol, 2.0 eq.), Sodium triacetoxyborohydride (384 mg, 1.81 mmol, 4.0 eq.), Glacial acetic acid (52 µL, 0.906 mmol, 2.0 eq.), 8 h, Purification:

silica gel column chromatography with CH/EE (90:10 to 10:90, v/v); yield: 137 mg, 0.28 mmol, 62 %. R_f 0.34 (EtOAc/CH 1:1).

(*S*)-4-Methoxy-4-oxo-2-((4-((((1*S,2*R**)-2-phenylcyclopropyl)amino)methyl)phenyl)sulfonamido)butanoic acid (32).** $C_{21}H_{24}N_2O_6S$, M_r: 432.49. **57** (131 mg, 0.27 mmol, 1.0 eq) was suspended in 25 mL of a 20 % aqueous solution of TFA (v/v). The reaction was heated to 40° C in a water bath for 10 h. The reaction was finished by evaporation of TFA and H_2O. The crude product was purified via RP-HPLC with H_2O/ACN (85:15 to 30:70, v/v) to obtain a colorless oil (2 mg, 0.01 mmol, 2 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.03 (s, 1H), 7.73 – 7.66 (m, 2H), 7.49 (d, *J* = 8.2 Hz, 2H), 7.20 (dd, *J* = 6.9, 8.2 Hz, 2H), 7.15 – 7.05 (m, 1H), 6.98 (dt, *J* = 1.4, 8.0 Hz, 2H), 3.97 (s, 1H), 3.87 (s, 2H), 3.47 (d, *J* = 1.3 Hz, 3H), 2.72 – 2.61 (m, 1H), 2.56 – 2.48 (m, 1H), 2.26 (dt, *J* = 3.6, 7.3 Hz, 1H), 1.86 (tt, *J* = 3.0, 5.8 Hz, 1H), 1.03 (dt, *J* = 4.7, 9.3 Hz, 1H), 0.95 (dt, *J* = 5.4, 7.0 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.2, 170.1, 145.1, 142.1, 139.3, 128.5, 128.1, 126.4, 125.6, 125.2, 52.5, 51.9, 51.5, 45.7, 37.3, 24.3, 16.5. LRMS (neg. APCI): *m/z* 430.5 $[M–H]^–$. HPLC (Method 2): t_R 12.30 min.

(*S*)-3-((4-Formylphenyl)sulfonamido)-4-methoxy-4-oxobutanoic acid (55). $C_{12}H_{13}NO_7S$, M_r: 315.30. Procedure Method A: 4-formylbenzenesulfonyl chloride (**39**, 198 mg, 0.97 mmol, 1.0 eq.), (*S*)-3-amino-4-methoxy-4-oxobutanoic acid (712 mg, 4.84 mmol, 5.0 eq.), NaOH (1451 µL (1 M), 1.451 mmol, 1.5 eq.), 4 h, Purification: silica gel column chromatography with H_2O + 5 % AcOH/ACN +5 % AcOH (90:10 to 10:90, v/v); yield: 98 mg, 0.31 mmol, 32 %. R_f 0.70 (ACN/H_2O 1:1).

(*S*)-4-Methoxy-4-oxo-3-((4-((((1*S,2*R**)-2-phenylcyclopropyl)amino)methyl)phenyl)sulfonamido)butanoic acid (33).** $C_{21}H_{24}N_2O_6S$, M_r: 432.49. Procedure Method B: **55** (98 mg, 0.30 mmol, 1.0 eq.), TCP HCl (101 mg, 0.60 mmol,

2.0 eq.), Sodium triacetoxyborohydride (252 mg, 1.19 mmol, 4.0 eq.), Glacial acetic acid (34 µL, 0.595 mmol, 2.0 eq.), 21 h, Purification: RP-HPLC; yield: 6 mg pale yellow oil, 0.01 mmol, 5 %. R_f 0.42 (ACN/H2O 1:1). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.96 – 7.89 (m, 2H), 7.70 – 7.63 (m, 2H), 7.36 – 7.25 (m, 2H), 7.28 – 7.18 (m, 1H), 7.11 (dt, *J* = 1.4, 8.0 Hz, 2H), 4.48 (d, *J* = 1.5 Hz, 2H), 4.28 (t, *J* = 6.0 Hz, 1H), 3.49 (d, *J* = 1.2 Hz, 3H), 2.99 (dddd, *J* = 0.9, 3.5, 4.4, 7.9 Hz, 1H), 2.74 (dd, *J* = 1.5, 6.1 Hz, 2H), 2.43 (ddd, *J* = 3.6, 6.6, 10.3 Hz, 1H), 1.50 (ddd, *J* = 4.4, 6.9, 10.3 Hz, 1H), 1.41 (dt, *J* = 6.8, 7.9 Hz, 1H). ^{13}C NMR (101 MHz, Methanol-d_4) δ 173.2, 172.1, 143.7, 139.1, 136.9, 131.7, 129.8, 129.0, 128.1, 127.3, 53.8, 52.9, 52.0, 39.1, 38.5, 22.7, 13.6. LRMS (neg. APCI): *m/z* 430.6 $[M–H]^-$. HPLC (Method 2): t_R 12.30 min.

Methyl 8-((benzyloxy)amino)-8-oxooctanoate (59). $C_{16}H_{23}NO_4$, M_r: 293.36. 8-methoxy-8-oxooctanoic acid (**58**, 0.86 g, 4.57 mmol) and BOP-Cl (1.30 g, 5.02 mmol) were dissolved in dry DCM (8 mL) and DIPEA (0.80 mL, 4.59 mmol) was added. The solution was stirred for 10 min, then *O*-benzylhydroxylamine hydrochloride (0.73 g, 4.58 mmol) and DIPEA (1.80 mL, 10.3 mmol) were added. After 4 h of stirring, the reaction was quenched by adding $NaHCO_3$ (10 % m/v aqueous solution). The organic phase was washed with citric acid (10 % m/v aqueous solution, 2 × 25 mL) and brine (1 × 25 mL). The organic phase was concentrated *in vacuo* and the target compound was purified by flash chromatography, yielding a colorless oil (0.77 g, 57 %). R_f 0.33 (CH/EtOAc 4:6); 0.60 (DCM/MeOH 95:5). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.94 (s, 1H), 7.45 – 7.32 (m, 5H), 4.78 (s, 2H), 3.58 (s, 3H), 2.28 (t, *J* = 7.3 Hz, 2H), 1.94 (t, *J* = 7.3 Hz, 2H), 1.48 (ddt, *J* = 13.8, 11.1, 7.3 Hz, 4H), 1.31 – 1.15 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.8, 169.7, 136.5, 129.2, 128.7, 128.6, 77.1, 51.6, 33.6, 32.5, 28.52, 28.49, 25.2, 24.7.

8-((benzyloxy)amino)-8-oxooctanoic acid (60). $C_{15}H_{21}NO_4$, M_r: 279.34. To a solution of methyl ester **59** (0.75 g, 2.56 mmol) in THF (10 mL), LiOH (10 mL, 1 M aq.) was added dropwise at 0° C.

The mixture was stirred for 4 h. The crude was concentrated *in vacuo* and diluted with $NaHCO_3$ (10 % m/v in aqueous solution, 1 × 15 mL). The water phase was washed with EtOAc (1 × 30 mL) and afterwards acidified with HCl (1 M aq., 10 mL), then extracted with EtOAc (3 × 20 mL). The organic phases were combined, dried over Na_2SO_4 and evaporated under reduced pressure, resulting in a white solid (0.69 g, 97 %). R_f 0.15 (DCM/MeOH 95:5). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.99 (s, 1H), 10.94 (s, 1H), 7.41 – 7.32 (m, 5H), 4.78 (s, 2H), 2.18 (t, J = 7.4 Hz, 2H), 2.00 – 1.89 (m, 2H), 1.53 – 1.41 (m, 4H), 1.29 – 1.16 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.9, 169.7, 136.5, 129.2, 128.7, 128.6, 77.1, 34.0, 32.6, 28.6, 25.2, 24.8.

Ethyl 4-(8-((benzyloxy)amino)-8-oxooctanamido)benzoate (61). $C_{24}H_{30}N_2O_5$, M_r: 426.51. Carboxylic Acid **60** (284 mg, 1.01 mmol) and BOP-Cl (437 mg, 1.72 mmol) were dissolved in dry DCM (8 mL) and stirred together with TEA (280 µL, 2.02 mmol) at rt for 10 min. The remaining amount of TEA (280 µL, 2.02 mmol) and ethyl-4-aminobenzoate (260 mg, 1.57 mmol) were added and stirred overnight at rt. The reaction was quenched with $NaHCO_3$ (10 % m/v in aqueous solution, 25 mL) and washed with DCM (2 × 20 mL). The organic layers were combined and washed with citric acid (10 % m/v in aqueous solution, 2 × 20 mL) and brine (2 × 20 mL). The organic phase was concentrated under reduced pressure and chromatographed. The target compound was purified by chromatography resulting in a colourless solid (64 mg, 10 %). R_f 0.29 (CH/EtOAc 4:6). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.94 (s, 1H), 10.22 (s, 1H), 7.93 – 7.87 (m, 2H), 7.76 – 7.70 (m, 2H), 7.41 – 7.31 (m, 5H), 4.77 (s, 2H), 4.28 (q, J = 7.1 Hz, 2H), 2.37 – 2.30 (m, 2H), 1.95 (t, J = 7.3 Hz, 2H), 1.62 – 1.45 (m, 4H), 1.35 – 1.20 (m, 4H), 1.31 (t, J = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.9, 169.8, 164.4, 144.1, 136.5, 130.6, 129.2, 128.7, 128.6, 124.5, 118.7, 77.1, 60.8, 36.9, 32.6, 28.8, 28.7, 25.2, 14.6. LRMS (pos. ESI, MeOH): *m/z* 449.5 $[M+Na]^+$. HPLC (Method 1): t_R 20.07 min.

Ethyl 4-(8-(hydroxyamino)-8-oxooctanamido)benzoate (34). $C_{17}H_{24}N_2O_5$, M_r: 336.39. To a solution of ethyl ester **61** (64 mg, 1.0 mmol) in dry EtOH (10 mL) a catalytic amount of Pd/C (35 mg, 10 % loading) was added. The flask was flushed with H_2 and stirred overnight. The reaction was filtered and purified with flash chromatography yielding in a white solid (29 mg, 57 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.34 (d, J = 1.7 Hz, 1H), 10.23 (s, 1H), 8.68 (d, J = 1.8 Hz, 1H), 8.00 – 7.84 (m, 2H), 7.84 – 7.67 (m, 2H), 4.28 (q, J = 7.2 Hz, 2H), 2.34 (t, J = 7.2 Hz, 2H), 1.94 (t, J = 7.4 Hz, 2H), 1.65 – 1.44 (m, 4H), 1.35 – 1.20 (m, 4H), 1.31 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.2, 169.5, 164.4, 144.1, 130.6, 126.7, 118.7, 60.8, 36.9, 32.6, 28.8, 28.7, 25.2, 14.6. LRMS (pos. ESI, MeOH): *m/z* 359.5 $[M+Na]^+$. HPLC (Method 1): t_R 15.75 min.

4-(8-(hydroxyamino)-8-oxooctanamido)benzoic acid (35). $C_{15}H_{20}N_2O_5$, M_R: 308.33. Ethyl ester **34** (28.6 mg, 0.09 mmol) was dissolved in THF (3.5 mL) and LiOH (700 µL, 1 M aq.) was added. The solution was stirred for 5 h at 40 °C under reflux. Excess of THF was removed *in vacuo* and the residue was diluted with H_2O (10 mL). The water phase was washed with EtOAc (3 × 15 mL), then acidified with HCl (1 M aq., 15 mL) and washed with EtOAc (2 × 10 mL). The organic layer was concentrated under reduced pressure and purified by flash chromatography, resulting in a red solid (11 mg, 47 %). R_f 0.73 (H_2O/ACN 1:1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.34 (s, 1H), 10.19 (s, 1H), 8.67 (s, 1H), 7.87 (d, J = 8.6 Hz, 2H), 7.70 (d, J = 8.6 Hz, 2H), 2.34 (t, J = 7.4 Hz, 2H), 1.94 (t, J = 7.5 Hz, 2H), 1.63 – 1.42 (m, 4H), 1.35 – 1.20 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.2, 169.5, 165.8, 144.1, 130.6, 126.8, 118.7, 77.2, 36.9, 32.6, 28.8, 28.7, 25.3. HRMS (neg. ESI, MeOH): *m/z* 307.1300 [MH]. HPLC (Method 1): t_R 11.78 min.

Methyl 3-(8-((benzyloxy)amino)-8-oxooctanamido)benzoate (62). $C_{23}H_{28}N_2O_5$, M_r: 412.49. Carboxylic acid **60** (306 mg, 1.07 mmol), BOP-Cl (467 mg, 1.10 mmol) and TEA (304 µL,

2.19 mmol) were dissolved in dry DCM (8 mL) and stirred at rt for 10 min. To the solution the remaining amount of TEA (304 µL, 2.19 mmol) and methyl-3-aminobenzoate (480 mg, 3.17 mmol) were added and stirred overnight at rt. The reaction was quenched with $NaHCO_3$ (10 % m/v in aqueous solution, 25 mL) and the water phase was washed with DCM (3 × 15 mL). The organic layer was washed with citric acid (10 % m/v in aqueous solution, 2 × 25 mL) and brine (1 × 25 mL). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The target compound was purified by chromatography, yielding a colorless solid (0.34 mg, 75 %). R_f 0.29 (CH/EtOAc 6:4). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.95 (s, 1H), 10.11 (s, 1H), 8.29 (dd, J = 2.3, 1.7 Hz, 1H), 7.84 (ddd, J = 8.1, 2.3, 1.1 Hz, 1H), 7.62 (ddd, J = 7.7, 1.7, 1.1 Hz, 1H), 7.44 (t, J = 8.1 Hz, 1H), 7.40 – 7.32 (m, 5H), 4.78 (s, 2H), 3.85 (s, 3H), 2.31 (t, J = 7.4 Hz, 2H), 1.95 (t, J = 7.3 Hz, 2H), 1.57 (p, J = 7.2 Hz, 2H), 1.50 (p, J = 7.2 Hz, 2H), 1.36 – 1.20 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.0, 169.7, 166.5, 140.1, 136.5, 130.4, 129.6, 129.2, 128.7, 128.6, 124.0, 123.8, 119.9, 77.1, 52.6, 36.8, 32.6, 28.8, 28.7, 25.3, 25.3. LRMS (pos. ESI, MeOH): *m/z* 435.5 $[M+Na]^+$.. HPLC (Method 1): t_R 20.59 min.

Methyl 3-(8-(hydroxyamino)-8-oxooctanamido)benzoate (36). $C_{16}H_{22}N_2O_5$, M_r: 322.36. Methyl ester **62** (332 mg, 0.80 mmol) was dissolved in dry MeOH (12 mL) and a catalytic amount of Pd/C (66 mg, 10 % loading) was added. The flask was flushed with H_2 and stirred overnight. The reaction was filtered and purified with flash chromatography, resulting in a white solid (53 mg, 20 %). R_f 0.38 (DCM/MeOH 95:5). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.34 (s, 1H), 10.11 (s, 1H), 8.67 (s, 1H), 8.29 (dd, J = 2.2, 1.8 Hz, 1H), 7.84 (ddd, J = 8.0, 2.2, 1.1 Hz, 1H), 7.65 – 7.58 (m, 1H), 7.44 (dd, J = 8.0, 8.0 Hz, 1H), 3.85 (s, 3H), 2.39 – 2.25 (m, 2H), 1.96 (dd, J = 18.1, 10.8 Hz, 2H), 1.68 – 1.41 (m, 4H), 1.37 – 1.18 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.0, 169.5,

166.5, 140.1, 130.4, 129.6, 124.0, 123.9, 119.9, 52.6, 36.8, 32.7, 28.8, 25.4, 25.3. LRMS (pos. ESI, MeOH): *m/z* 345.6 $[M+Na]^+$. HPLC (Method 1): t_R 14.40 min.

3-(8-(hydroxyamino)-8-oxooctanamido)benzoic acid (37). $C_{15}H_{20}N_2O_5$, M_r: 308.33. Methyl Ester **36** (25.2 mg, 0.08 mmol) was dissolved in THF (3 mL) and LiOH (700 µL, 1 M aq.) was added dropwise. After stirring for 1 h at rt, the solution was stirred for 2 h at 40° C. Excess of THF was removed *in vacuo* and the residue was diluted with H_2O (10 mL). The water phase was washed with CH_2Cl_2 (15 mL), then acidified with HCl (1 M aq., 15 mL) and washed with EtOAc (4 × 10 mL). The organic layers were combined, concentrated under reduced pressure and chromatographed, yielding a red solid (11 mg, 47 %). R_f 0.71 (H_2O/ACN 1:1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.95 (s, 1H), 10.34 (s, 1H), 10.07 (s, 1H), 8.67 (s, 1H), 8.23 (dd, *J* = 2.0, 1.8 Hz, 1H), 7.92 – 7.79 (m, 1H), 7.66 – 7.56 (m, 1H), 7.41 (t, *J* = 7.9 Hz, 1H), 2.30 (d, *J* = 7.2 Hz, 2H), 1.94 (t, *J* = 7.3 Hz, 2H), 1.57 (t, *J* = 7.2 Hz, 2H), 1.54 (t, *J* = 7.4 Hz, 2H) 1.36 – 1.21 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.9, 169.5, 167.6, 139.9, 131.6, 129.3, 125.2, 124.2, 120.1, 36.8, 32.7, 28.8, 25.44, 25.37. HRMS (neg. ESI, MeOH): *m/z* 307.1300 [MH]. HPLC (Method 1): t_R 12.13 min.

Peroxidase based LSD1 assay

Determination of enzyme activity and inhibition was performed in an established HRP-coupled assay system based on the Amplex Red protocol from Invitrogen (*BPS Bioscience*). The assay was conducted in a white OptiPlate-384 microtiter plate (*PerkinElmer*) using a 45 mM HEPES buffer at pH 8.5 containing 40 mM NaCl. LSD1 enzyme (8 µL; final concentration 0.045 µg/µL; expressed in Sf9 cells as published elsewhere[35]), was incubated with inhibitor solutions of varying concentration (2 µL in DMSO; final DMSO concentration 10 %) for 20 min at RT. Demethylation

reaction was initiated by the addition of H3K4(me2)aa$_{1–20}$ (10 µL; final concentration 20 µM; sequence: ARTK(me2)QTARKSTGGKAPRKQL; from *Peptide Specialty Laboratories GmbH*). As control for 0 % LSD1 activity, buffer solution was added instead of peptide solution, whereas for 100 % reference value, DMSO was used without inhibitor. After the plate was incubated for 60 min at RT, the Amplex Red reagent/Horseradish Peroxidase (HRP) mixture (20 µL; final concentration 50 µM Amplex Red reagent (Ampliflu™ Red, *Sigma-Aldrich*) and 1 U/mL HRP (*Sigma-Aldrich*, P8125) in reaction buffer) were added. Immediately after addition, fluorescence intensity of the forming product resorufin was measured at λ_{ex} = 510 nm and λ_{em} = 615 nm on a POLARstar Optima microplate reader (BMG Labtech, Germany). Values were blank-corrected. Inhibition in [%] is in comparison to compoundfree DMSO control and no-substrate negative control. Inhibition curves were analysed by sigmoidal curve fitting using OriginPro 2018b and IC_{50} values are given as mean ± SD from two independent experiments.

Luminescence based MAO assay

Determination of enzyme activity and inhibition was performed in an established Luciferase-coupled assay system with all reagents from the commercial MAO-Glo^{TM} assay kit (*Promega*) in a final volume of 20 µL. 13 µL (0.62 mg MAO-A or 3.25 mg MAO-B enzyme, *Sigma-Aldrich*) of MAO enzyme and the inhibitor in 2 µL DMSO (or DMSO as a control without inhibitor) were incubated at r.t. in a white OptiPlate-384 microtiter plate (*PerkinElmer*) for 20 min, before the reaction was conducted at 37° C for 60 min after addition of 5 µL of a luminogenic MAO substrate (40 mM for MAO-A or 4 mM for MAO-B as final concentration). 20 µL of a luciferin detection reagent was added to stop the enzymatic activity. The assay read-out has been executed in an EnVision 2102 Multilabel Reader (*Perkin Elmer*) 20 min after the reaction was mixed with the detection reagent. Luminescence intensity was measured without emission filter. Inhibition in [%]

is in comparison to compoundfree DMSO control and no-substrate negative control. Values were blank-corrected. Inhibition curves were analysed by sigmoidal curve fitting using OriginPro 2018b and IC_{50} values are given as mean ± SD from two independent experiments.

Stability assay

Determination of ester stability was performed in human serum purchased from *PAN-Biotech*. Prepared deep-frozen aliquots were thawed slowly in a fridge before usage. 99 µL of at 37° C pre-incubated serum or 99 µL of at 37° C pre-incubated buffer solution (50 mM Tris, pH 7,5) was mixed with the inhibitor in 1 µL DMSO (100 mM) in an Eppendorf tube^{TM} for each measurement. After 60 min, 100 µL of ice-cold ACN was added. The suspension was shortly vortexed and then centrifuged for 10 min at 10.000 rpm. The supernatant was taken, filtered and transferred to an HPLC vial. HPLC analysis was conducted according to the standard analytical HPLC method 1. Quantification of either the original compound or metabolite was performed.

Cell culture

THP1 cell line (RRID:CVCL_0006) was used, which was a kind gift of Prof. Lubbert from the University Hospital, Freiburg. The cell line was cultivated in RPMI1640 medium supplemented with 10 % (*v/v*) FCS, 2 mM L-glutamine, 1 % penicillin/streptomycin at 37°C in a humidified atmosphere with 5 % CO_2.

Cell viability (MTS) assay

Cells were diluted to $7*10^4$ cells*mL^{-1} and mixed with compounds to a final DMSO concentration of 0.5 % and seeded at 100 µL in 96-well plates in triplicates. After 72 h incubation, the CellTiter

96® AQueous Non-Radioactive Cell Proliferation Assay from Promega was performed according to the manufacturer's instructions. Assay plates were measured at 492 nm on a POLARstar Optima microplate reader (*BMG Labtech*, Germany).

Flow cytometry

Cells were treated with compounds to a final DMSO concentration of 0.1 % for 72 h and stained with the antibody APC Mouse Anti-Human CD86 (BD Bioscience, cat: 555660, lot: 8018951) and 7-AAD (*BD Bioscience*). Measurement was done using a CyAn (Beckmann Coulter) with 10000 events per sample. *Z*i values were calculated from Equation (1), values from DMSO treated cells were set as min(x) and values from cells treated with 500 nM *rac-trans-N*-((2-methoxypyridin-3-yl)methyl)-2-phenylcyclopropan-1-amine were set as max(x).

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