SUPPLEMENTARY INFORMATION

A Selective Phenelzine Analog Inhibitor of Histone Demethylase LSD1

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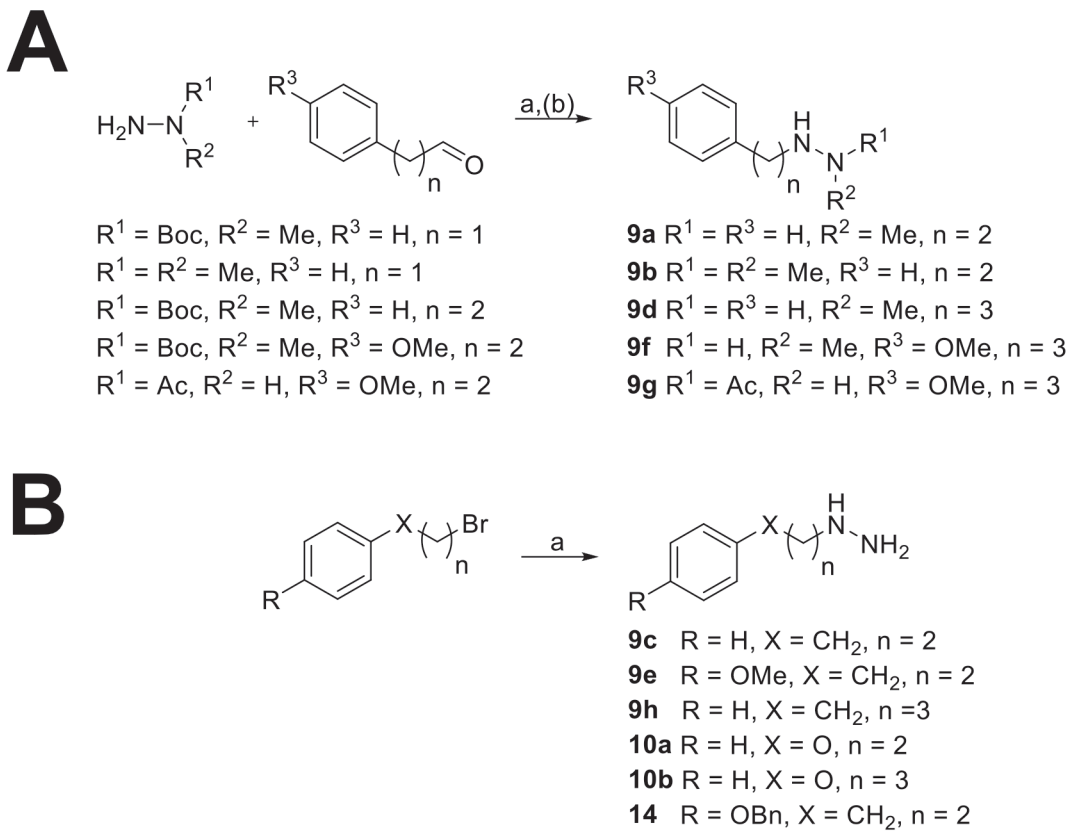
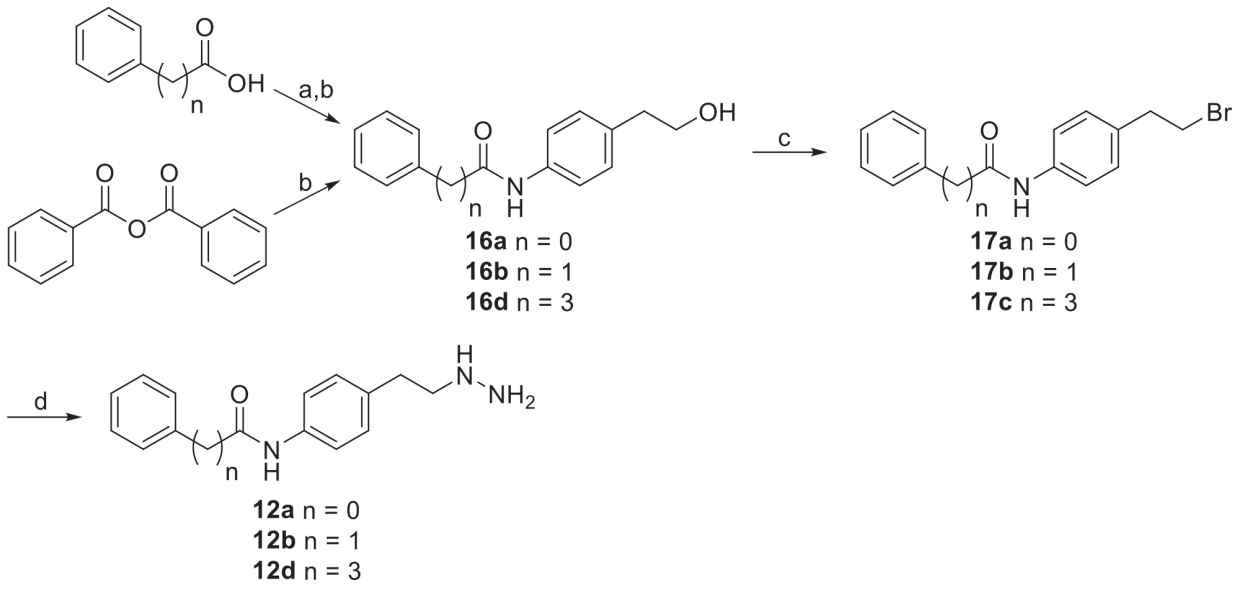
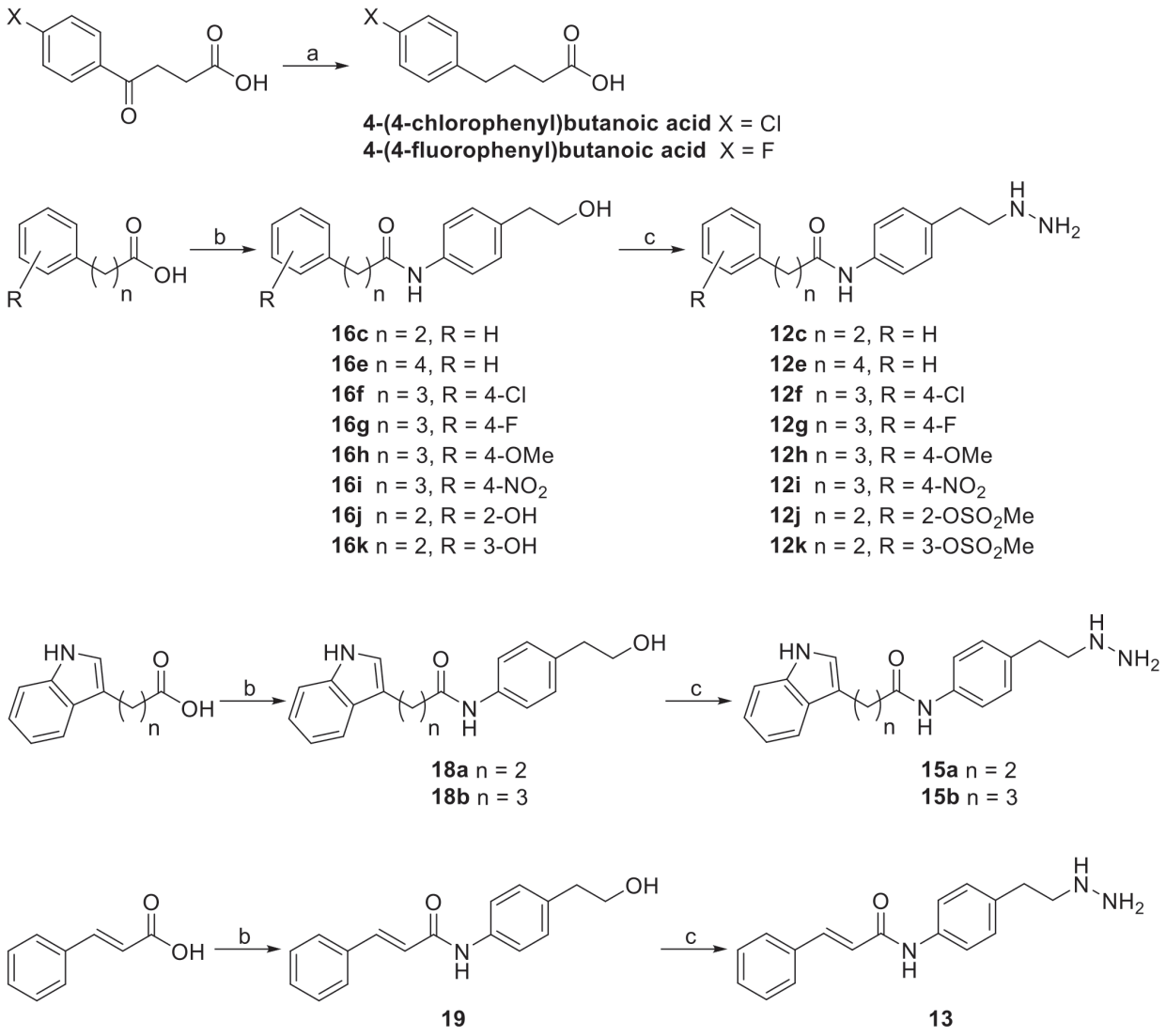
SUPPLEMENTARY FIGURES AND TABLES



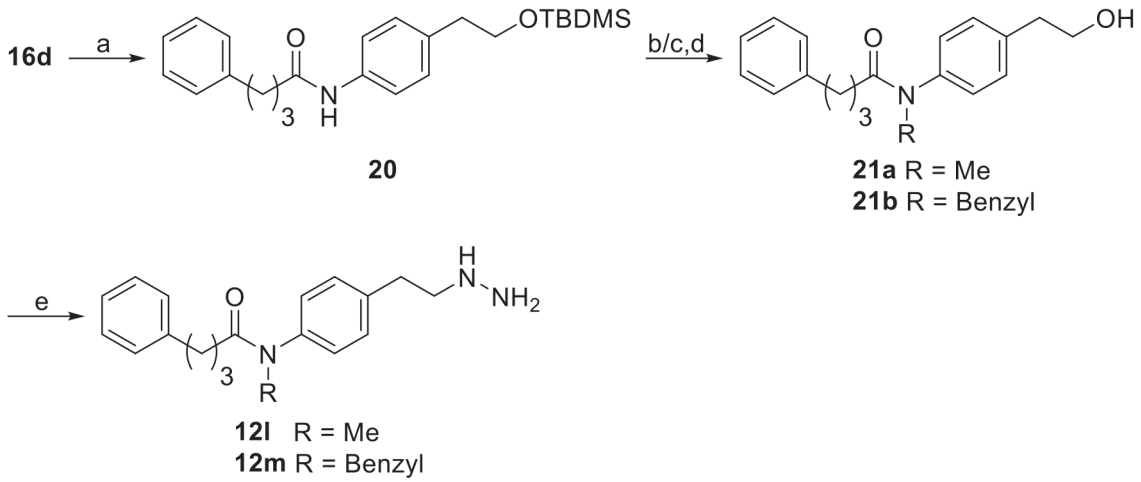
Supplementary Figure 1. Synthesis of LSD1 inhibitors with modifications to the alkyl chain and substitutions to the hydrazine moiety. (A) Reagents and conditions: a) AcOH, $NaBH_3CN$, MeCN, 0 °C to RT, 16 h; b) HCl, EtOAc, RT, 20 min - 2 h. (B) Reagents and conditions: a) N_2H_4, EtOH, 80 °C, 16 h.



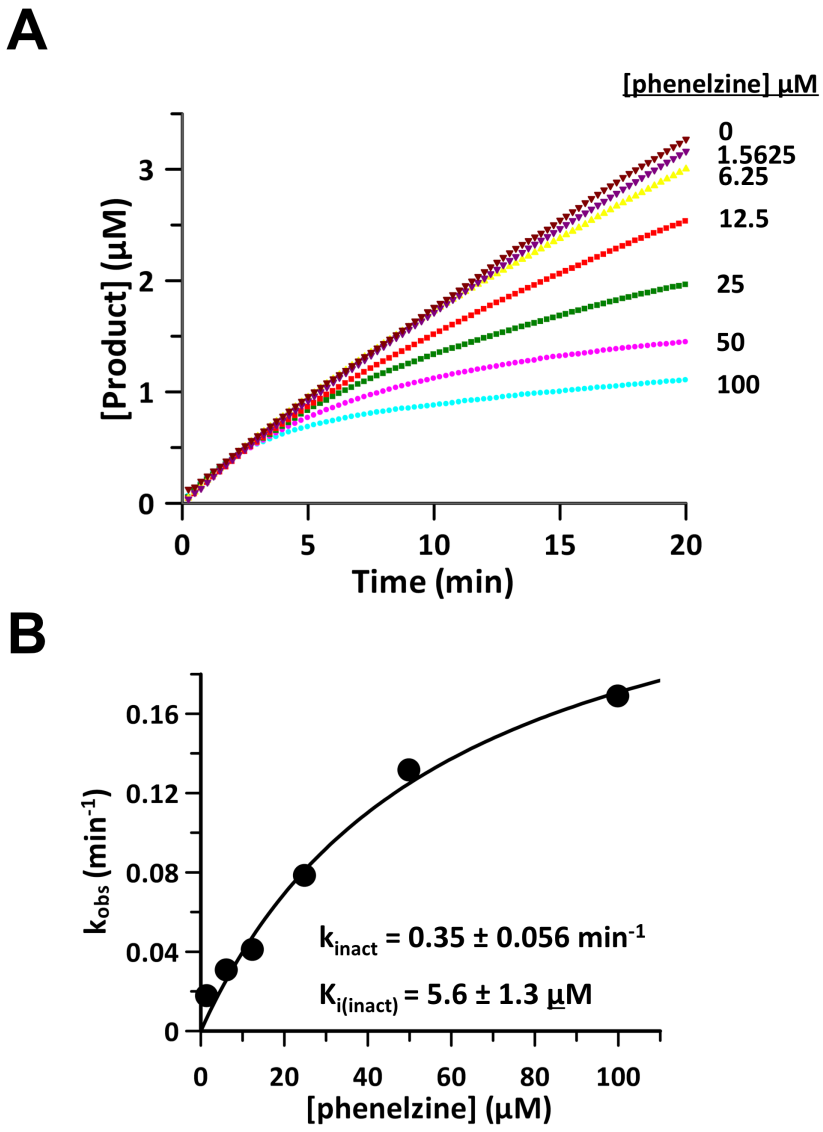
Supplementary Figure 2. Synthesis of LSD1 inhibitors with variations in the length of the alkyl chain connecting the distal phenyl moiety to the phenelzine scaffold. Reagents and conditions: a) $SOCl_2$, Et_3N, DCM, 0 °C to 55 °C, 8 h; b) i) 2-(4-aminophenyl)ethanol, DIPEA, DCM, 0 °C to RT, 16 h; ii) NaOH, MeOH, RT, 6 h; c) PPh_3, CBr_4, DCM, RT, 6 h; d) N_2H_4, EtOH, 80 °C, 1 h.



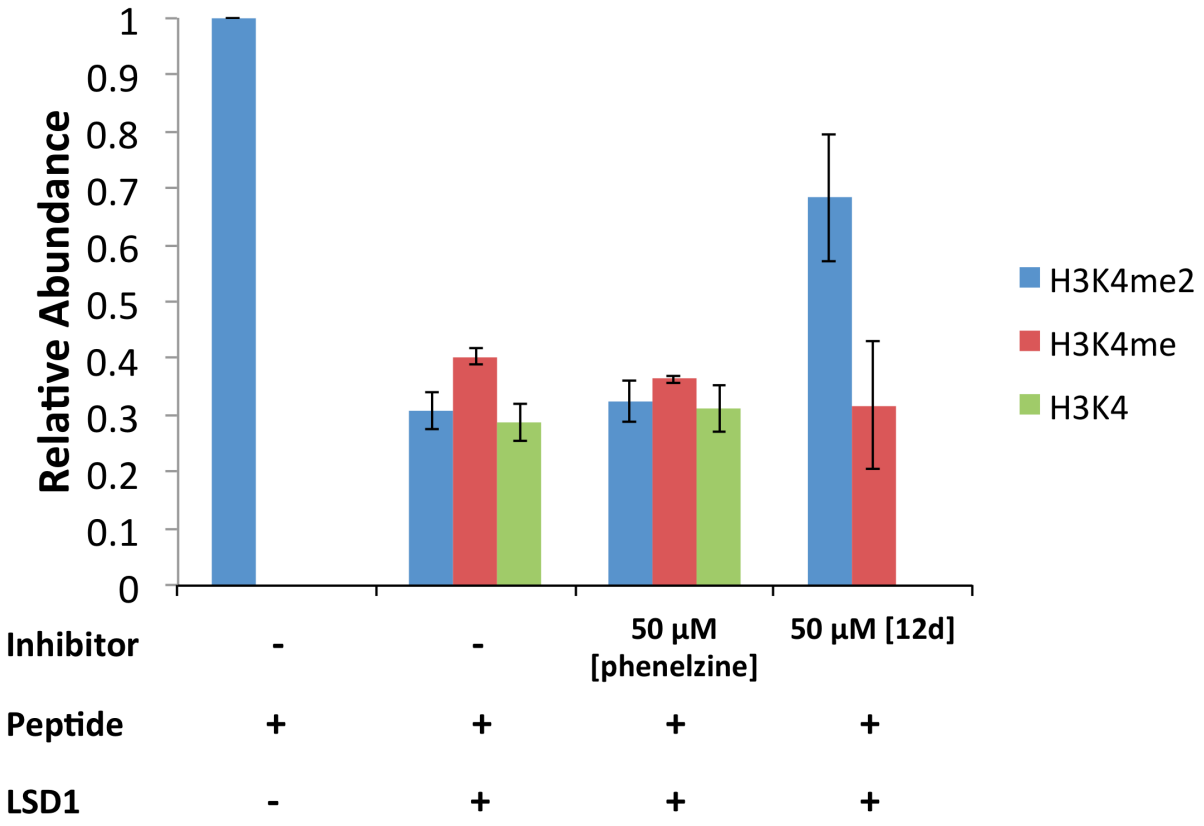
Supplementary Figure 3. Synthesis of LSD1 inhibitors possessing substitutions on the distal phenyl ring of **12d** (bizine). Reagents and conditions: a) KOH, $N_2H_4 \cdot H_2O$, diethylene glycol, 120–130 °C, 2 h; b) 2-(4-aminophenyl)ethanol, EDC, DMAP, DCM, RT, 16 h; c) i) CH_3SO_2Cl, Et_3N, DCM 0 °C to RT, 1–3 h; ii) N_2H_4, EtOH, 80 °C, 2 h.



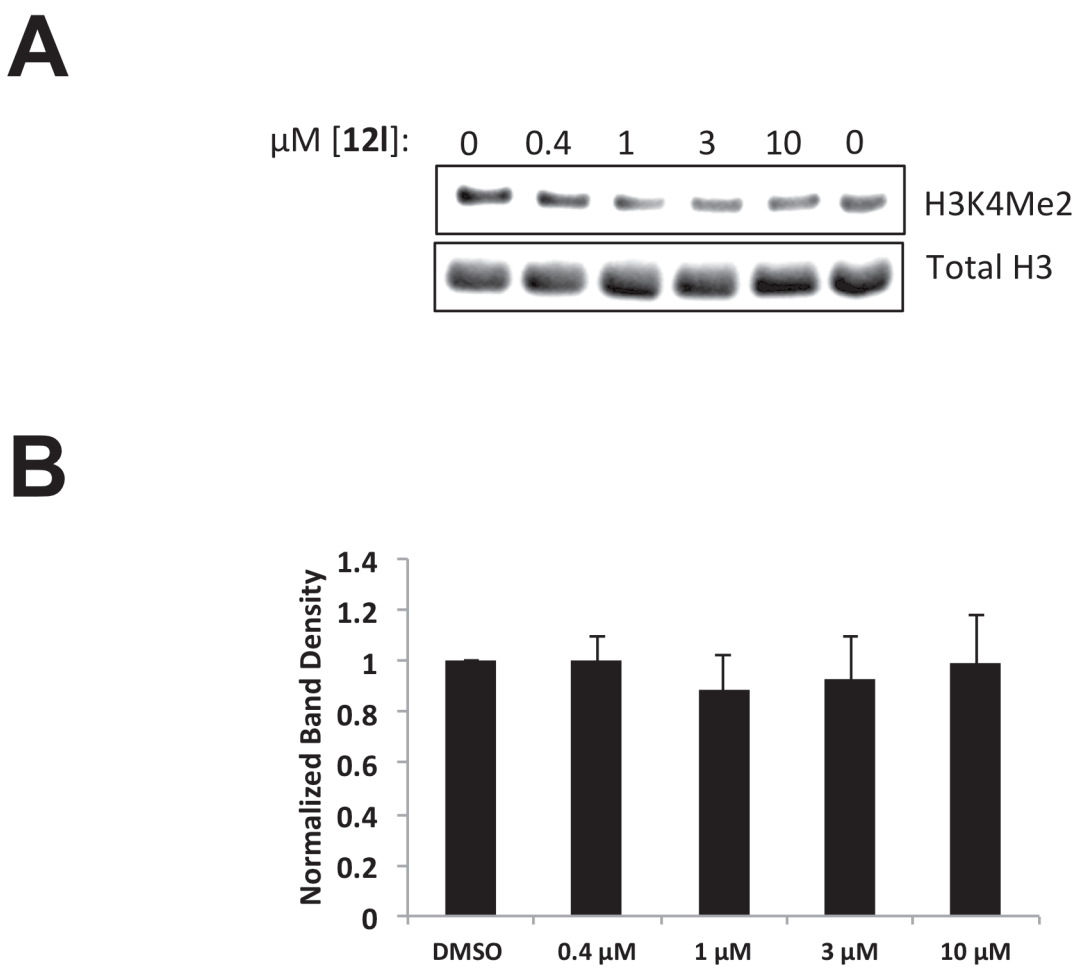
Supplementary Figure 4. Synthesis of *N*-substituted **12d** (bizine) derivatives. Reagents and conditions: a) TBDMSCl, Et_3N, DMAP, DCM, RT, 2 h; b) NaH, MeI, THF, 0 °C to RT, 4 h; c) KO^tBu, benzyl bromide, DCM/DMF, 0 °C to 60 °C, 16 h; d) TBAF, THF, RT, 24 h; e) i) CH_3SO_2Cl, Et_3N, DCM, 0 °C to RT, 1–3 h; ii) N_2H_4, EtOH, 80 °C, 2 h.



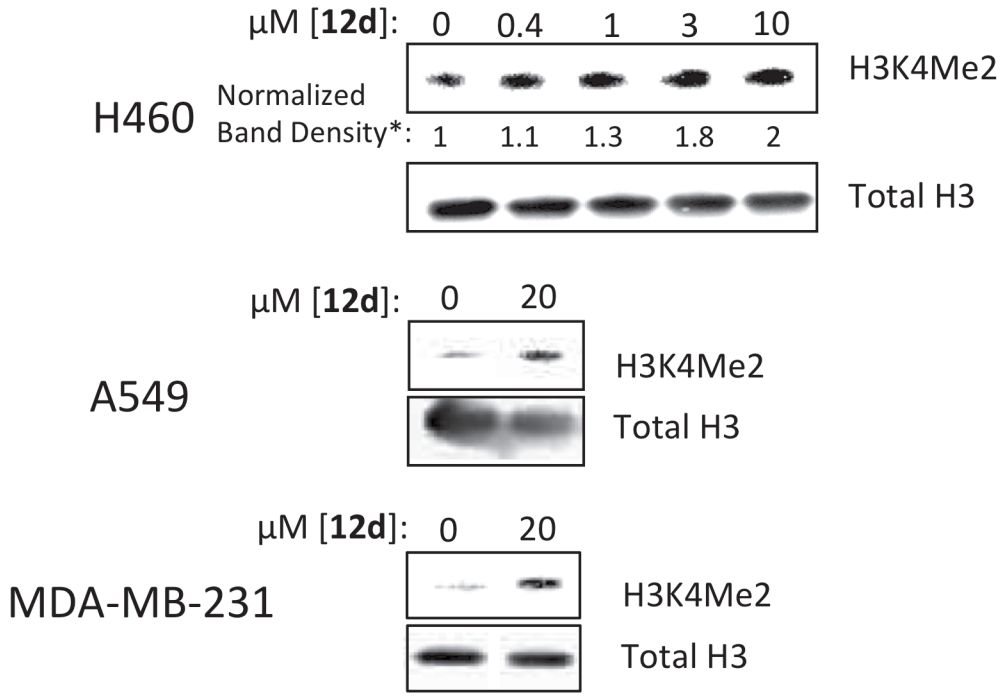
Supplementary Figure 5. Inhibition of LSD1 by phenelzine. (A) Steady-state progress curve of LSD1 inactivation by phenelzine ranging from 0 to 100 µM. (B) k_{obs} values obtained from steady-state data plotted against inhibitor concentration to determine k_{inact} and $K_{i(inact)}$ values.



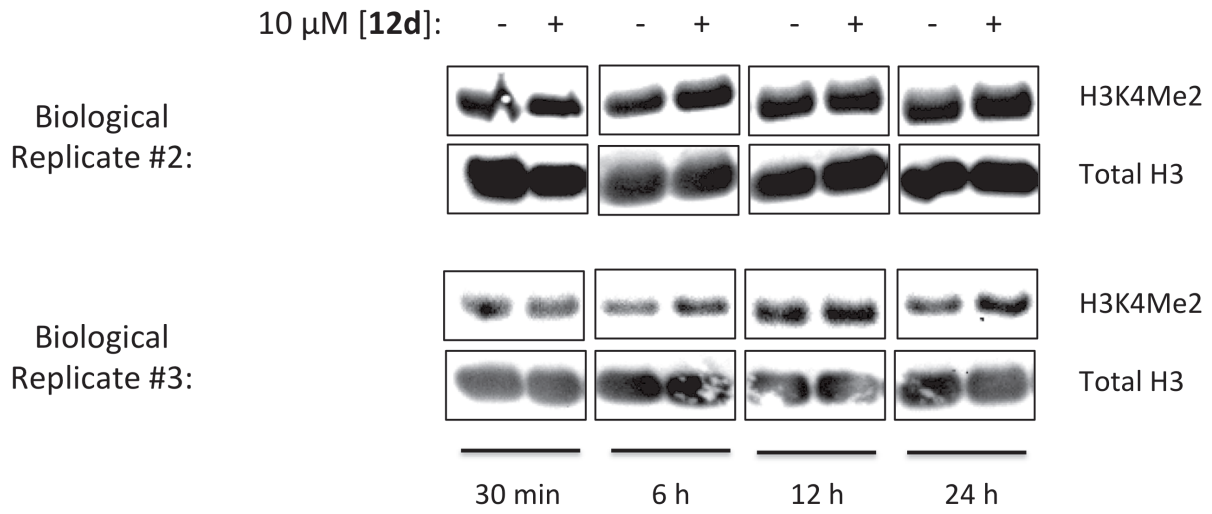
Supplementary Figure 6. Quantification of methylation states of H3K4 as a result of LSD1 inhibition by phenelzine or **12d** (bizine) as determined by the MassSQUIRM technique.



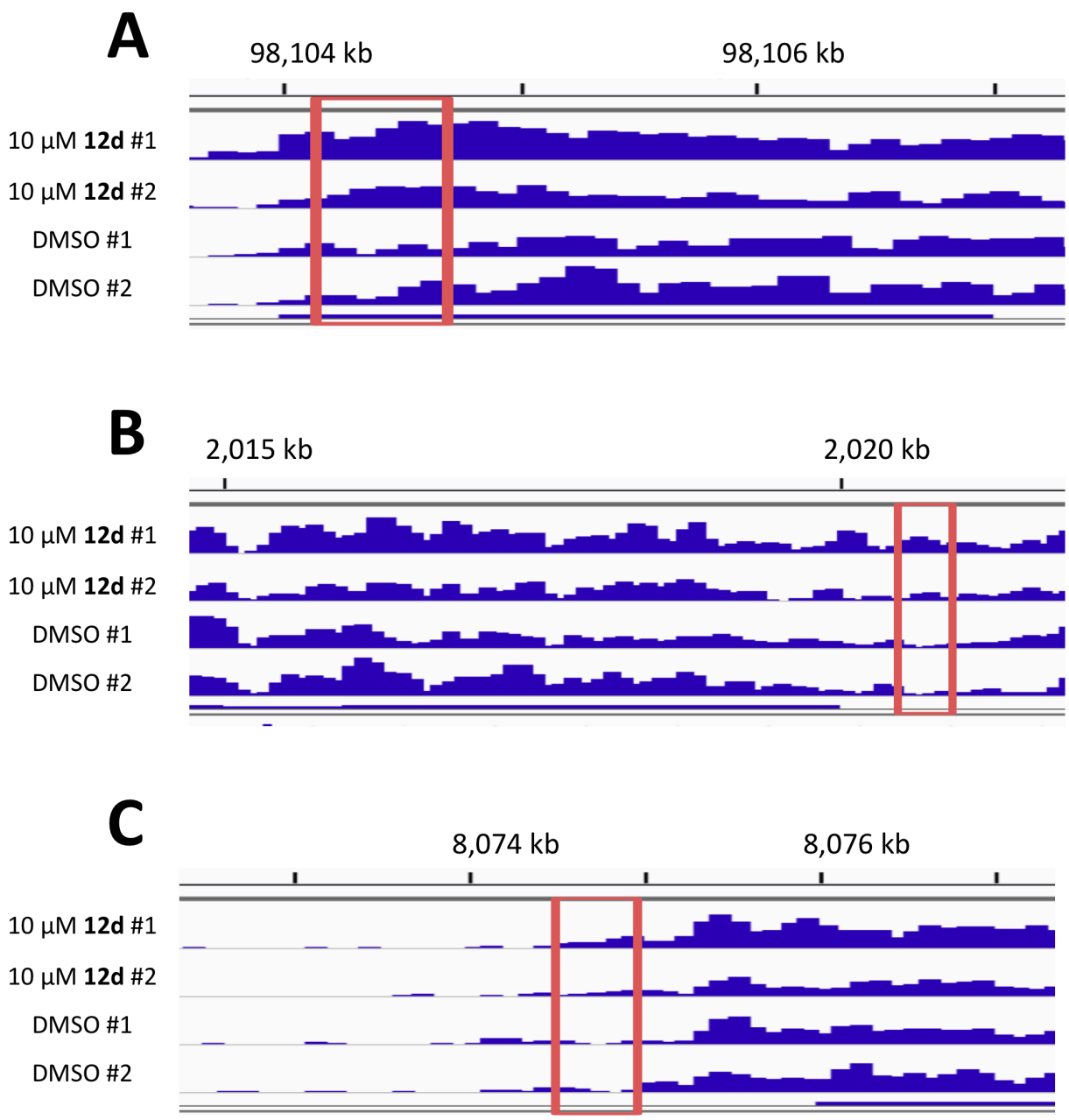
Supplementary Figure 7. LSD1 inhibition by compound **12l** in LNCaP cells. (A) Cells were treated with **12l** (0.4–10 μM) for 48 h and blotted against H3K4Me2 and total H3. (B) H3K4Me2 band density quantification plot as determined by 2 biological replicates.



Supplementary Figure 8. H460, A549, and MDA-MB-231 cell lines were treated with compound **12d** (bizine) (0.4–10 μM or 20 μM) for 48 h and blotted against H3K4Me2 and total H3. *Determined using biological triplicates.

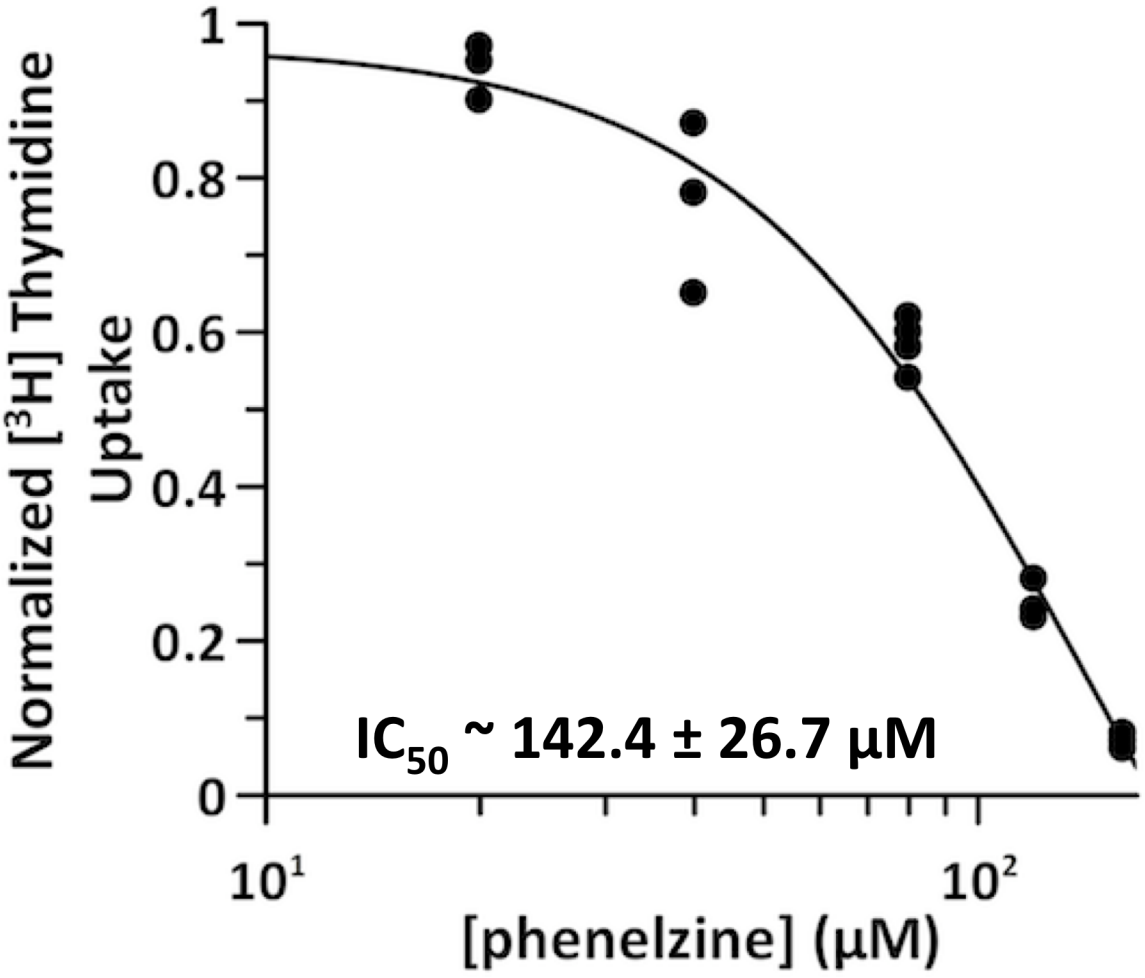


Supplementary Figure 9. LNCaP cells were treated with 10 µM compound **12d** (bizine) for 30 min, 6 h, 12 h, and 24 h and blotted against H3K4Me2 and total H3 (with additional two biological replicates).

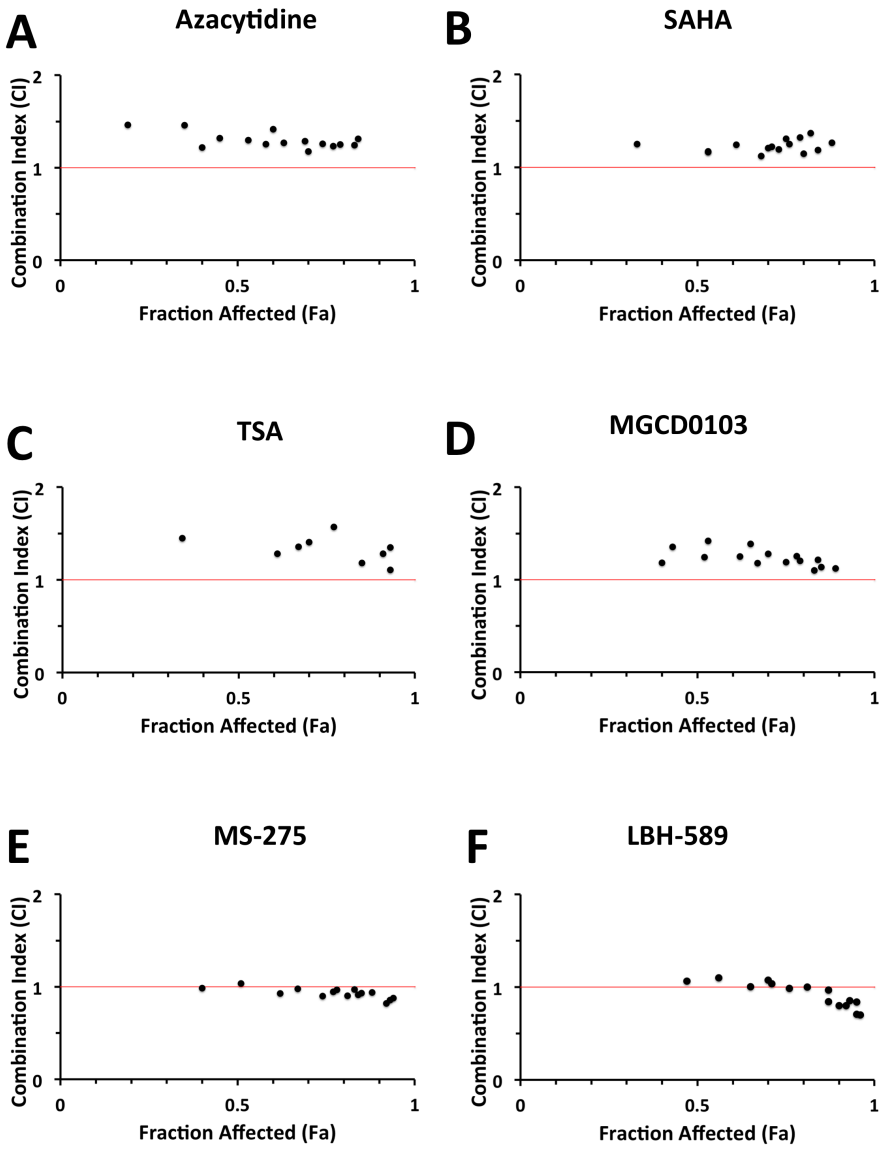


Supplementary Figure 10. Shown are representative examples of three genes' Integrative Genomics Viewer (IGV)[1,2] tracks from the list of 2,432 genes identified through the ChIP-seq experiment that showed an increase in H3K4Me2 with LSD1 inhibition by **12d** (bizine) (with two biological replicates): (A) RGMB (chr5:98,079,869-98,189,371); (B) SMARCA2 (chr9:1,999,116-2,177,398); (C) ERRFI1 (chr1:7,902,135-8,201,537). Red boxes mark statistically significant peak increases with **12d** (bizine)

treatment. Scale indicated by tick marks.



Supplementary Figure 11. DNA replication dose response curves using a [^{3}H] thymidine assay in H460 cells after 48 h treatment with phenelzine.



Supplementary Figure 12. The H460 cell line was treated simultaneously with compound **12d** (bizine) and (A) azacytidine, (B) SAHA, (C) TSA, (D) MGCD0103, (E) MS-275, (F) LBH-589 for 48 h and DNA replication was monitored using the [^{3}H] thymidine assay. Synergy was determined by CompuSyn using a non-constant ratio approach. CI > 1, CI = 1, or CI < 1 indicates antagonism, additivity, or synergy, respectively. For example, points above, on, or under the red line indicate antagonism, additivity, or synergy, respectively. Fa indicates the fraction of cells affected by a given dose of drugs.

Supplementary Table 1. Phenelzine and **12d** (bizine) selectivity profile for LSD1 vs. MAO A, MAO B, and LSD2.

Enzyme Tested	Inhibitor	$K_{i(inact)}$ (μM)	$k_{(inact)}$ (min^{-1})	$k_{(inact)}/K_{i(inact)}$ ($\mu M^{-1}min^{-1}$)	Selectivity for LSD1 vs. Enzyme Tested
MAO A	**phenelzine**	0.82±0.47	0.24±0.057	0.29±0.18	0.217
	12d	2.6±2.3	0.30±0.11	0.11±0.11	22.7
MAO B	**phenelzine**	3.9±1.7	0.20±0.040	0.051±0.025	1.24
	12d	6.5±4.6	0.26±0.14	0.040±0.036	62.5
LSD2	**phenelzine**	N/A	N/A	N/A	N/A
	12d	N/A	N/A	N/A	>100

Supplementary Table 2. Using ChIP-seq, 17,542 differential H3K4Me2 peaks were found between cells treated with 10 μM compound **12d** (bizine) versus vehicle. (See additional supplementary file.)

Supplementary Table 3. Using ChIP-seq, 2,432 genes were identified that showed a H3K4Me2 increase with LSD1 inhibition by compound **12d** (bizine) near the genes' promoter regions. (See additional supplementary file.)

Supplementary Table 4. Gene ontology (GO) analysis performed on 2,432 genes found to show a H3K4Me2 increase by ChIP-seq. (See additional supplementary file.)

Supplementary Table 5. 146 genes that overlapped in the chemical inhibition and LSD1 knockout ChIP-seq experiments, with 26 tumor suppressor genes highlighted in yellow. (See additional supplementary file.)

Supplementary Table 6. Gene ontology (G) analysis performed on 146 genes that overlapped in the chemical inhibition and LSD1 knockout ChIP-seq experiments. (See

additional supplementary file.)

Supplementary Table 7. 26 tumor suppressor genes (p-value = 5.80E-9), with original p-values, of the 146 genes that overlapped in the chemical inhibition and LSD1 knockout ChIP-seq experiments. Tumor suppressor genes were identified using two data sets; one data set used was from Vanderbilt University (http://bioinfo.mc.vanderbilt.edu/TSGene/Human_716_TSGs.txt), and the other data set used was from Memorial Sloan-Kettering Cancer Center (http://cbio.mskcc.org/CancerGenes/).

Symbol	Full Name	p-val
Cdh1	E-cadherin	2.76E-10
Pkn3	protein kinase N3	9.16E-09
Sash1	SAM and SH3 domain containing 1	1.67E-08
Phlpp2	PH domain and leucine rich repeat protein phosphatase 2	2.36E-08
Trim13	tripartite motif containing 13	6.77E-07
Smarca2	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 2	9.44E-07
App	amyloid beta (A4) precursor protein	1.72E-06
Ercc6	excision repair cross-complementing rodent repair deficiency, complementation group 6	1.88E-06
Errfi1	ERBB receptor feedback inhibitor 1	3.36E-06

Hint1	histidine triad nucleotide binding protein 1	4.98E-06
Hic1	hypermethylated in cancer 1	1.08E-05
Abl1	c-abl oncogene 1, non-receptor tyrosine kinase	1.27E-05
Raf1	v-raf-1 murine leukemia viral oncogene homolog 1	1.47E-05
Mxi1	MAX interactor 1, dimerization protein	1.53E-05
Ptprj	protein tyrosine phosphatase, receptor type, J	1.97E-05
Mef2c	myocyte enhancer factor 2C	2.52E-05
Ppapdc1b	phosphatidic acid phosphatase type 2 domain containing 1B	3.25E-05
Dusp22	dual specificity phosphatase 22	3.71E-05
Yeats4	YEATS domain containing 4	4.12E-05
Cdkn2a	cyclin-dependent kinase inhibitor 2A	4.42E-05
Dab2	Dab, mitogen-responsive phosphoprotein, homolog 2	4.99E-05
Fam120a	family with sequence similarity 120A	5.00E-05
Tcf4	transcription factor 4	5.77E-05

Acta2	actin, alpha 2, smooth muscle, aorta	7.31E-05
Etv6	ets variant 6	8.14E-05
Lox	lysyl oxidase	8.86E-05

METHODS

Overview of the Synthetic Schemes. The compounds investigated in this study were synthesized from commercially available or readily prepared starting materials. A series of compounds containing substitutions to the hydrazine moiety was prepared via reductive amination with commercially available aldehydes and either substituted or protected hydrazines.[3] Subsequent deprotection of the hydrazine was carried out in the presence of hydrochloric acid as necessary to yield compounds **9a-b**, **9d**, and **9f-g**, which were isolated as free bases or as dihydrochloride salts (Supplementary Figure 1). Additionally, phenelzine derivatives possessing heteroatom substitutions in the alkyl chain and variations in the overall chain length, as well as substitutions to the *para* position of the phenyl ring, were easily prepared in one step from commercially available starting materials (Supplementary Figure 2). Nucleophilic substitution of various alkyl bromides with excess anhydrous hydrazine resulted in the desired compounds **9c**, **9e**, **9h**, **10a**-**b**, and **14**.[4,5]

In addition, a series of compounds with larger hydrophobic groups attached to the *para* position of the phenyl ring of phenelzine was prepared (Scheme 2). Excess benzoic anhydride was treated directly with 2-(4-aminophenyl)ethanol resulting in acylation of the aryl amine and aliphatic alcohol. Alternatively, an excess amount of various phenyl alkyl substituted acids differing in alkyl linker length were converted to acid chlorides using thionyl chloride and then treated with 2-(4-aminophenyl)ethanol which yielded diacylated products similar to those obtained from the anhydride reaction. The esters were subsequently saponified with sodium hydroxide to provide the desired alcohols **16a-b** and **16d**. The Appel reaction was employed using triphenylphosphine and carbon tetrabromide to convert the alcohols to their respective alkyl bromides **17a-c**. Then, the alkyl bromides were treated with excess anhydrous hydrazine to produce the desired final products **12a-b** and **12d**, which were isolated as hydrochloride salts as described in detail in the experimental section.

Additional variations in the alkyl linker and substitutions to the phenyl ring distal to the hydrazine of **12d** were also explored. 4-(4-Chlorophenyl)butanoic acid and 4-(4-

fluorophenyl)butanoic acid were obtained from their respective keto acids via a Wolff-Kishner reduction (Scheme 3).[6] Amide bond formation was achieved using standard carbodiimide coupling conditions to generate intermediate alcohols **16c**, **16e-k**, **18a-b**, and **19** from the respective acid and 2-(4-aminophenyl)ethanol. Subsequent conversion to the mesylate[7] followed by nucleophilic substitution with excess anhydrous hydrazine yielded the desired products which were isolated as either sulfate or oxalate salts **12c**, **12e-k**, **15a-b**, and **13** as indicated in the experimental section (Supplementary Figure 3).

Preparation of *N*-substituted amides was achieved by first protecting the alcohol of **16d** as a silyl ether[8] to generate common intermediate **20**. Substitution of the amide nitrogen with methyl iodide or benzyl chloride using either sodium hydride[9] or potassium *tert*-butoxide as the base, respectively, followed by deprotection in the presence of TBAF[10] resulted in the generation of intermediate alcohols **21a-b**. Alcohol to hydrazine conversion was carried out as previously described and the final products were isolated as oxalate salts **12l-m** (Supplementary Figure 4).

General. NMR spectra were recorded on either a Bruker 400 MHz (^{1}H, 400 MHz; ^{13}C, 101 MHz), a Varian 400 MHz (^{1}H, 400 MHz), or a Bruker 500 MHz (^{1}H, 500 MHz; ^{13}C, 125 MHz) spectrometer. Chemical shifts (δ) are expressed in parts per million relative to internal tetramethylsilane; coupling constants (*J*) are in hertz (Hz). The following abbreviations were used to describe multiplicity: br (broad), s (singlet), d (doublet), t (triplet), quin (quintet), m (multiplet), dd (double doublet), td (triple doublet), dt (double triplet). NMR spectra were processed using ACD/NMR Processor Academic Addition, version 12.01 (Advanced Chemistry Development, Inc., Toronto, On, Canada, www.acdlabs.com, 2013). When DMSO-d_6 was used as the sole NMR solvent, the hydrazine protons were visible; however, the peaks were very broad and could not be accurately integrated. High resolution ESI/APCI spectra were recorded on either an Agilent LCTOF instrument at the Mass Spectrometry Facility of the University of California, Riverside (NSF grant CHE-0541848) or a Shimadzu IT-TOF instrument at the Research Resources Center Mass Spectrometry Facility of the University of Illinois at Chicago. Solvents were purchased from Aldrich as anhydrous and used as received.

Starting materials and reagents were purchased from commercial sources and were also used as received. Reaction progress was monitored by thin layer chromatography (TLC) using pre-coated, glass supported silica gel plates (Sigma-Aldrich F254, 60 Å pore size, 250 μM thickness). All final compounds are estimated to be >97% pure as determined by NMR. (See additional supplementary file for all NMR spectra.)

General Procedure A for hydrazine displacement reactions. Under argon, to a stirred solution of the appropriate alkyl bromide (1 mol equiv) in EtOH (1-3 mL/mmol) in a round-bottomed flask was added hydrazine (4-23 mol equiv). The mixture was refluxed overnight after which the volatiles were removed in vacuo and the residual product was dissolved in 1 N NaOH (10 mL). The aqueous layer was extracted with DCM (3 x 15 mL) and the combined organic layers were dried in vacuo. The residue was dissolved in MeOH (1-2 mL/mmol) and a 6 N HCl solution (0.3-0.4 mL/mmol) was added while stirring. After 20 min, the volatiles were removed in vacuo, and the desired product was purified via recrystallization from MeOH/Et_2O.

General Procedure B for reductive hydrazination. Under nitrogen and on ice, the appropriate aldehyde (1 mol equiv) was dissolved in MeOH (10 mL/mmol) in a round-bottomed flask. To this stirred solution was added 1-boc-1-methylhydrazine (1 mol equiv) dropwise. The ice bath was removed after 30 min, and the reaction was left to stir for 2 h. After cooling the reaction on ice, sodium cyanoborohydride (1.75 mol equiv) was slowly added along with acetic acid (150 μL/mmol, 1.5% v/v). EtOH was then removed in vacuo and either saturated sodium bicarbonate or 1 N NaOH (5 mL/mmol) was added. The aqueous layer was extracted with EtOAc (3 x 15 mL) and dried in vacuo. The product was then purified via flash chromatography (SiO_2, 75-90% hexanes/EtOAc). The base was dissolved in EtOAc (0.5 mL/mmol) and a 6 N HCl solution (0.5 mL/mmol) was added while stirring the solution on ice. After 2 h, the reaction was concentrated in vacuo and filtered. The resulting precipitate was washed with cold EtOAc to yield the desired product.

General Procedure C for hydrazine displacement reactions. Under nitrogen, the appropriate alkyl bromide (1 mol equiv) was dissolved in EtOH (3 mL/mmol) in a round-bottomed flask. To this stirred solution was added anhydrous hydrazine (20 mol equiv) dropwise. The solution was then heated to reflux for 0.5-1.75 h with monitoring by TLC. After cooling, EtOH was removed in vacuo and 1 N NaOH (80 mL) was added. The aqueous layer was extracted with DCM (3 x 80 mL) and dried in vacuo. The base was then dissolved in MeOH (10 mL) and 6 N HCl (2.5-3.5 mL/mmol) was added dropwise while stirring the solution on ice. The solution was left to stir on ice for 10-15 min after which the precipitate was filtered and washed with cold Et_2O to yield the desired product.

General Procedure D for amide coupling. The appropriate acid (1 mol equiv) was dissolved in DCM (10 mL, 0.25 mL/mmol). The stirred solution was then placed in an ice bath and thionyl chloride (5 mol equiv) was slowly added. After the addition was complete, the resulting solution was stirred on ice for 10 min and then transferred to an oil bath and heated to 55 °C. The solution was then stirred for 7.25-7.50 h and monitored by TLC. The solution was then cooled to RT and dried to furnish the appropriate acid chloride. While drying the acid chloride, 2-(4-aminophenyl)ethanol (2.00 g, 14.6 mmol) was placed under nitrogen and dissolved in DCM (20 mL). The stirred solution was placed on ice and *N,N*-diisopropylethylamine (18 mL, 102.1 mmol) was slowly added followed by the slow addition of the solid dried acid chloride. After the addition was complete, the resulting solution was stirred overnight and allowed to warm to RT. DCM (100 mL) was added and the organic phase was washed with 1 N HCl (100 mL), saturated sodium bicarbonate (100 mL), brine (100 mL), and dried in vacuo. The solid was then dissolved in MeOH (100 mL) at RT and to this stirred solution, 1 N NaOH (20-50 mL) was added in portions. Stirring was continued for 6 h and the reaction was monitored by TLC. After completion, the solution was concentrated in vacuo and EtOAc (100 mL) was added. The organic layer was washed with 1 N HCl (100 mL), saturated sodium bicarbonate (2 x 100 mL), brine (100 mL), and then dried to furnish the crude product, which was further purified by flash chromatography (SiO_2, 50% hexanes/EtOAc) to yield the desired product.

General Procedure E for bromination. Under nitrogen, the appropriate alcohol (1 mol equiv) was dissolved in DCM (8-20 mL/mmol) in a round-bottomed flask. To this stirred solution was added triphenylphosphine (2 mol equiv) and carbon tetrabromide (2 mol equiv). The resulting solution was stirred for 6 h and monitored by TLC. Upon completion, the solution was concentrated in vacuo to give the crude product, which was further purified by flash chromatography (SiO_2, 20-25% hexanes/EtOAc) to furnish the desired product.

General Procedure F for amide coupling. The appropriate acid, 2-(4-aminophenyl)ethanol (1 mol equiv), EDC (1.2 mol equiv), and DMAP (0.1 mol equiv) were placed in a round-bottomed flask under argon at 0 °C and dissolved in anhydrous DCM (2 mL/mmol). The reaction mixture was allowed to warm to RT and stirred overnight (approximately 16 h). Then, the reaction was poured into H_2O (20 mL) and the pH was adjusted to approximately 4 with an aqueous solution of 1 N HCl. The organic layer was isolated and the aqueous layer was further extracted with DCM (2 x 20 mL). The combined organic extracts were washed with 1 N HCl (15 mL) and brine (15 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The desired product was purified via recrystallization from EtOAc unless otherwise stated.

General Procedure G mesylate formation and hydrazine displacement reactions. The respective alcohol and triethylamine (1.2 mol equiv) were dissolved in anhydrous DCM (4 mL/mmol) under argon and cooled to 0 °C in an ice bath. Then, methanesulfonyl chloride (1.1 mol equiv) was dissolved in anhydrous DCM (1 mL/mmol) and added dropwise. The reaction was stirred for 1 h at 0 °C and then allowed to warm to RT and stirred for an additional 1-3 h or until complete as evidenced by TLC. The reaction was then slowly poured into an aqueous solution of 0.5 N HCl (approximately 10 mL), DCM was added (10 mL), and the organic layer isolated. The aqueous layer was further extracted with DCM (2 x 15 mL). The combined organic extracts were washed with brine (15 mL), dried with anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The residue obtained was placed under argon, taken up in 95% EtOH (4 mL),

and cooled to 0 °C in an ice bath. Hydrazine (20 mol equiv) was dissolved in 95% EtOH (1 mL) and added dropwise to the reaction at 0 °C. The reaction was allowed to warm to RT and then heated at reflux (approximately 80 °C) for 2 h. After the reaction was complete as evidenced by TLC, it was cooled to RT and treated with a 1 N aqueous solution of NaOH (80 mL). DCM (15 mL) was added and the organic layer was isolated. The aqueous layer was further extracted with DCM (2 x 15 mL) and then the combined organic extracts were washed with brine (20 mL), dried with anhydrous Na_2SO_4, filtered, and concentrated in vacuo. See individual compounds for salt formation and purification.

General Procedure H for sulfate salt formation. The crude hydrazine was dissolved in MeOH (10 mL/mmol) and cooled to 0 °C in an ice bath. Concentrated H_2SO_4 (0.55 mL/mmol) was added dropwise to the solution and stirring was continued for 30 min at 0 °C. The resulting precipitate was isolated by filtration, washed with cold MeOH (2 mL), and dried under vacuum. Et_2O can be added dropwise to facilitate precipitation of the desired product.

General procedure I for oxalate salt formation. Oxalic acid (0.90 g, 10 mmol) was dissolved in MeOH (9 mL) and cooled to 0 °C in an ice bath. Then, the crude hydrazide was dissolved in MeOH (1 mL) and added dropwise to the solution of oxalic acid at 0 °C. Stirring was continued for 30 min after which Et_2O was added dropwise to facilitate precipitation of the desired product. The resulting precipitate was isolated by filtration, washed with cold MeOH (2 mL), and dried under vacuum.

1-Methyl-2-(2-phenylethyl)hydrazine dihydrochloride salt (9a): To a stirred solution of phenylacetaldehyde (200 µL, 1.7 mmol) in anhydrous CH_3CN (10 mL) in a round-bottomed flask at 0 °C was added *t*-butyl 1-methylcarboxylate (0.25 g, 1.7 mmol), followed by the addition of acetic acid (0.15 mL, 1.5% v/v). The reaction mixture was allowed to warm to RT and stirred for 2 h. Then, sodium cyanoborohydride (193 mg, 3.1 mmol) was added at 0 °C and stirring was continued overnight at RT. After completion, the volatiles were removed in vacuo and the desired compound was purified via flash

chromatography (SiO_2, 75% hexanes/EtOAc) to yield a colorless oil (168 mg, 67%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.30 (m, 5H), 3.10 (m, 5H), 2.82 (t, *J* = 8 Hz, 2H), 1.50 (s, 9H). This compound was taken up in EtOAc (1 mL) and to it was added a 6 M solution of aqueous HCl (1 mL) at RT. The reaction was stirred for 2 h and then the volatiles were removed in vacuo and the desired product was isolated as a white solid (137 mg, 92%). ^{1}H NMR (400 MHz, MeOD): δ 7.27 (m, 5H), 3.23 (m, 2H), 2.89 (t, *J* = 7.7 Hz, 2H), 2.79 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 138.55, 128.59, 128.40, 126.31, 48.62, 33.97, 32.41.

1,1-Dimethyl-2-(2-phenylethyl)hydrazine (9b): To a stirred solution of phenylacetaldehyde (0.20 mL, 1.7 mmol) in anhydrous CH_3CN (10 mL) in a round-bottomed flask at 0 °C was added *N,N*-dimethylhydrazine (143 μL, 1.88 mmol), followed by the addition of acetic acid (0.15 mL, 1.5% v/v). The reaction mixture was allowed to warm to RT and stirred for 2 h. Then, sodium cyanoborohydride (193 mg, 3.1 mmol) was added at 0 °C and stirring was continued overnight at RT. After completion, the volatiles were removed in vacuo and the desired product was purified via flash chromatography (SiO_2, 2:1 hexanes/EtOAc) and isolated as a colorless oil (100 mg, 36%). ^{1}H NMR (500 MHz, MeOD): δ 7.27 (m, 4H), 7.20 (m, 1H), 3.17 (t, *J* = 7.5 Hz, 2H), 2.83 (s, 6H), 2.75 (t, *J* = 7.5 Hz, 2H). ^{13}C NMR (125 MHz, MeOD): δ 140.45, 129.89, 129.67, 127.59, 51.72, 46.09, 35.12. ESI-LRMS: $[M+H]^+$ = *m/z* 165.2.

(3-Phenylpropyl)hydrazine dihydrochloride salt (9c): The title compound was synthesized from 3-phenylpropyl bromide (380 μL, 2.51 mmol) according to general procedure A and isolated as a white solid (0.256 g, 68%). ^{1}H NMR (400 MHz, MeOD): δ 7.24 (m, 5H), 3.05 (m, 2H), 2.72 (t, *J* = 7.6 Hz, 2H), 1.97 (quin, *J* = 7.7 Hz, 2H). ^{13}C NMR (125 MHz, MeOD): δ 142.00, 129.74, 129.55, 127.46, 52.22, 33.71, 28.04.

1-Methyl-2-(3-phenylpropyl)hydrazine dihydrochloride salt (9d): The title compound was synthesized from hydrocinnamaldehyde (263 μL, 2 mmol) according to general procedure B and isolated as a white powder (0.056 g, 12%). ^{1}H NMR (400 MHz, MeOD): δ 7.22 (m, 5H), 3.01 (t, *J* = 7.6 Hz, 2H), 2.76 (s, 3H), 2.71 (t, *J* = 7.6 Hz, 2H),

1.92 (quin, *J* = 7.8 Hz, 2H). ^{13}C NMR (101 MHz, MeOD): δ 142.36, 129.67, 129.56, 127.32, 49.11, 35.56, 33.88, 29.20. ESI-HRMS: calcd. for $C_{10}H_{16}N_2$: $[M+H]^+$ = *m/z* 165.1391, found: $[M+H]^+$ = *m/z* 165.1386.

[3-(4-Methoxyphenyl)propyl]hydrazine dihydrochloride salt (9e): The title compound was synthesized from 1-(3-bromopropyl)-4-methoxybenzene (524 μL, 3 mmol) according to general procedure A and isolated as a white powder (0.052 g, 7.2%). ^{1}H NMR (400 MHz, MeOD): δ 7.14 (m, 2H), 6.85 (m, 2H), 3.75 (s, 3H), 3.04 (t, *J* = 7.8 Hz, 2H), 2.65 (t, *J* = 7.6 Hz, 2H), 1.94 (quin, *J* = 7.6 Hz, 2H).

1-[3-(4-Methoxyphenyl)propyl]-2-methylhydrazine dihydrochloride salt (9f): The title compound was synthesized from 3-(4-methoxyphenyl)propionaldehyde (317 μL, 2 mmol) according to general procedure B and isolated as a white powder (0.217 g, 56%). ^{1}H NMR (500 MHz, MeOD): δ 7.13 (d, *J* = 8.6 Hz, 2H), 6.85 (d, *J* = 8.6 Hz, 2H), 3.76 (s, 3H), 2.98 (m, 2H), 2.75 (s, 3H), 2.66 (t, *J* = 7.5 Hz, 2H), 1.88 (quin, *J* = 7.5 Hz, 2H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 157.49, 133.00, 129.20, 113.75, 54.97, 46.77, 34.08, 31.32, 27.70. ESI-HRMS: calcd. for $C_{11}H_{18}N_2O$: $[M+H]^+$ = *m/z* 195.1496, found: $[M+H]^+$ = *m/z* 195.1492.

***N*'-[3-(4-Methoxyphenyl)propyl]acetohydrazide (9g):** Under nitrogen on ice, acetylhydrazide (593 mg, 8 mmol) was dissolved in MeOH (20 mL) and 3-(4-methoxyphenyl)propionaldehyde (0.317 mL, 2 mmol) was slowly added. The ice bath was removed after 30 min, and the reaction was left to stir for 2 h. Volatiles were removed in vacuo and saturated sodium bicarbonate (10 mL) was added. The product was extracted with EtOAc (3 x 15 mL) and dried in vacuo. The product was then purified via flash chromatography (2% MeOH/DCM) to yield the intermediate (0.107 g, 24%) as a white powder. Under nitrogen, the intermediate (0.107 g, 0.49 mmol) was dissolved in MeOH (10 mL). Sodium cyanoborohydride (220 mg, 3.5 mmol) was slowly added along with acetic acid (300 μL, 1.5% v/v). The reaction was left to stir overnight. MeOH was then removed in vacuo and saturated sodium bicarbonate (10 mL) was added. The product was extracted with EtOAc (3 x 15 mL) and dried in vacuo. Purification via flash

chromatography (SiO_2, 2% MeOH/DCM) yielded the desired product as a white powder (0.095 g, 88%). ^{1}H NMR (500 MHz, MeOD): δ 7.10 (d, *J* = 8.6 Hz, 2H), 6.81 (d, *J* = 8.6 Hz, 2H), 3.75 (s, 3H), 2.76 (t, *J* = 7.2 Hz, 2H), 2.60 (t, *J* = 7.6 Hz, 2H), 1.89 (s, 3H), 1.75 (quin, *J* = 7.4 Hz, 2H). ^{13}C NMR (125 MHz, MeOD): δ 171.61, 159.50, 135.35, 130.46, 114.92, 55.78, 52.21, 33.43, 30.95, 20.74. ESI-HRMS: calcd. for $C_{12}H_{18}N_2O_2$: $[M+H]^+$ = *m/z* 223.1437, found: $[M+H]^+$ = *m/z* 223.1441.

(4-Phenylbutyl)hydrazine dihydrochloride salt (9h): The title compound was synthesized from 4-bromobutyl benzene (1.00 mL, 5.70 mmol) according to general procedure A and isolated as a white solid (0.640 g, 45%). ^{1}H NMR (400 MHz, MeOD): δ 7.20 (m, 5H), 3.05 (m, 2H), 2.67 (t, *J* = 7.3 Hz, 2H), 1.69 (m, 4H).

(2-Phenoxyethyl)hydrazine dihydrochloride salt (10a): The title compound was synthesized from *beta*-bromophenetole (0.500 g, 2.49 mmol) according to general procedure A and isolated as an off-white solid (0.136 g, 36%). ^{1}H NMR (400 MHz, MeOD): δ 7.30 (dd, J_1 = 8.8 Hz, J_2 = 7.4 Hz, 2H), 6.98 (m, 3H), 4.24 (t, *J* = 5.0 Hz, 2H), 3.43 (t, *J* = 4.4 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$/MeOD): δ 157.60, 129.46, 121.64, 114.45, 62.68, 49.74.

(3-Phenoxypropyl)hydrazine dihydrochloride salt (10b): The title compound was synthesized from (3-bromopropoxy)benzene (366 μL, 2.32 mmol) according to general procedure A and isolated as an off-white solid (0.179 g, 46%). ^{1}H NMR (400 MHz, MeOD): 7.27 (t, *J* = 8.0 Hz, 2H), 6.94 (m, 3H), 4.10 (t, *J* = 5.8 Hz, 2H), 3.26 (t, *J* = 7.2 Hz, 2H), 2.14 (quin, *J* = 7.0 Hz, 2H). ^{13}C NMR (125 MHz, MeOD): δ 160.12, 130.66, 122.25, 115.67, 66.28, 50.30, 26.63.

{3-[4-(Benxyloxy)phenyl]propyl}hydrazine dihydrochloride salt (14): The title compound was synthesized from 1-(3-bromopropyl)-4-(phenylmethoxy)-benzene (400 mg, 1.30 mmol) according to general procedure C and isolated as a white powder (0.152 g, 34%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.37 (m, 5H), 7.12 (d, *J* = 8.3 Hz, 2H), 6.93 (dd, J_1 = 8.6 Hz, J_2 = 3.0 Hz, 2H), 5.06 (s, 2H), 2.87 (t, *J* = 7.3 Hz, 2H), 2.56 (t, *J* =

7.3 Hz, 2H), 1.83 (quin, *J* = 7.1 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 156.68, 137.26, 133.11, 129.31, 128.46, 127.81, 127.69, 114.72, 69.16, 49.83, 31.16, 26.63. ESI-HRMS: calcd. for $C_{16}H_{20}N_2O$: $[M+H]^+$ = *m/z* 257.1654, found: $[M+H]^+$ = *m/z* 257.1648.

4-(3-Hydrazinylpropyl) morpholine (11): Purchased from ChemBridge Screening Library (Catalog #9195784).

***N*-[4-(2-Hydroxyethyl)phenyl]benzamide (16a):** Under nitrogen, 2-(4-aminophenyl)ethanol (2.00 g, 15.0 mmol) was dissolved in DCM (20 mL). The stirred solution was placed on ice and *N,N*-diisopropylethylamine (22.9 mL, 131 mmol) was slowly added followed by the slow addition of benzoic anhydride (14.8 g, 66.0 mmol). After the addition was complete, the solution was stirred overnight and allowed to warm to RT. DCM (100 mL) was added to this solution and the organic phase was washed with 1 N HCl (100 mL), saturated sodium bicarbonate (100 mL), brine (100 mL), and dried in vacuo. The intermediate was then dissolved in MeOH (100 mL). To this stirred solution, 1 N NaOH (50 mL) was added in portions. The resulting solution was stirred at RT for 6 h and monitored by TLC. The solution was then concentrated in vacuo and EtOAc (100 mL) was added. The organic layer was washed with 1 N HCl (100 mL), saturated sodium bicarbonate (2 x 100 mL), brine (100 mL), and then dried to furnish the crude product, which was further purified via flash chromatography (SiO_2, 50% hexanes/EtOAc) to yield the title compound as an off-white solid (0.600 g, 17%). ^{1}H NMR (400 MHz, MeOD): δ 7.92 (m, 2H), 7.54 (m, 5H), 7.24 (m, 2H), 3.75 (t, *J* = 7.1 Hz, 2H), 2.82 (t, *J* = 7.1 Hz, 2H).

***N*-[4-(2-Hydroxyethyl)phenyl]-2-phenylacetamide (16b):** The title compound was synthesized from phenylacetic acid (5.95 g, 43.7 mmol) according to general procedure D and isolated as an off-white solid (3.20 g, 86%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.41 (m, 2H), 7.35 (m, 5H), 7.15 (d, *J* = 8.6 Hz, 2H), 7.09 (s, 1H), 3.81 (t, *J* = 6.6 Hz, 2H), 3.74 (s, 2H), 2.81 (t, *J* = 6.4 Hz, 2H).

***N*-[4-(2-Hydroxyethyl)phenyl]-4-phenylbutanamide (16d):** The title compound was synthesized from 4-phenylbutyric acid (7.18 g, 43.7 mmol) according to general procedure D and isolated to furnish the pure product as an off-white solid (6.20 g, 49%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.61 (s, 1H), 7.41 (d, *J* = 8.6 Hz, 2H), 7.29 (m, 2H), 7.21 (m, 3H), 7.13 (d, *J* = 8.3 Hz, 2H), 3.79 (t, *J* = 6.6 Hz, 2H), 2.80 (t, *J* = 6.6 Hz, 2H), 2.69 (t, *J* = 7.5 Hz, 2H), 2.32 (t, *J* = 7.5 Hz, 2H), 2.04 (quin, *J* = 7.5 Hz, 2H).

***N*-[4-(2-Bromoethyl)phenyl]benzamide (17a):** The title compound was synthesized from *N*-[4-(2-hydroxyethyl)phenyl]benzamide **16a** (0.600 g, 2.49 mmol) according to general procedure E and isolated to furnish the final product as an off-white solid (0.600 g, 79%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.98 (s, 1H), 7.86 (dd, J_1 = 8.2 Hz, J_2 = 1.1 Hz, 2H), 7.61 (d, *J* = 8.6 Hz, 2H), 7.50 (m, 3H), 7.21 (d, *J* = 8.3 Hz, 2H), 3.56 (t, *J* = 7.5 Hz, 2H), 3.15 (t, *J* = 7.5 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 165.76, 136.69, 135.06, 134.82, 131.81, 129.26, 128.72, 126.98, 120.44, 38.74, 33.04.

***N*-[4-(2-Bromoethyl)phenyl]-2-phenylacetamide (17b):** The title compound was synthesized from *N*-[4-(2-hydroxyethyl)phenyl]-2-phenylacetamide **16b** (0.750 g, 2.93 mmol) according to general procedure E and isolated to furnish the desired product as an off-white solid (0.500 g, 49%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.36 (m, 8H), 7.12 (d, *J* = 8.3 Hz, 2H), 3.72 (s, 2H), 3.52 (t, *J* = 7.5 Hz, 2H), 3.11 (t, *J* = 7.6 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 169.12, 136.38, 134.93, 134.34, 129.44, 129.15, 129.10, 127.61, 120.04, 44.68, 38.63, 33.03. ESI-HRMS: calcd. for $C_{16}H_{16}NOBr$: $[M+H]^+$ = *m/z* 318.0497, found: $[M+H]^+$ = *m/z* 318.0488.

***N*-[4-(2-Bromoethyl)phenyl]-4-phenylbutanamide (17c):** The title compound was synthesized from *N*-[4-(2-hydroxyethyl)phenyl]-4-phenylbutanamide **16d** (0.700 g, 2.47 mmol) according to general procedure E and isolated to furnish the pure product as an off-white solid (0.500 g, 58%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.46 (d, *J* = 8.3 Hz, 2H), 7.31 (m, 3H), 7.20 (m, 5H), 3.54 (t, *J* = 7.6 Hz, 2H), 3.12 (t, *J* = 7.6 Hz, 2H), 2.71 (t, *J* = 7.5 Hz, 2H), 2.34 (t, *J* = 7.6 Hz, 2H), 2.07 (quin, *J* = 7.5 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.98, 141.26, 136.63, 134.68, 129.16, 128.46, 128.40, 126.01, 119.99,

38.69, 36.66, 34.99, 33.04, 26.81. ESI-HRMS: calcd. for $C_{18}H_{20}NOBr$: $[M+H]^+$ = *m/z* 346.0808, found: $[M+H]^+$ = *m/z* 346.0801.

***N*-[4-(2-Hydrazinylethyl)phenyl]benzamide dihydrochloride salt (12a):** The title compound was synthesized from *N*-[4-(2-bromoethyl)phenyl]benzamide **17a** (0.400 g, 1.31 mmol) according to general procedure C and isolated to yield the product as a white powder (0.370 g, 91%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.32, (s, 1H), 7.97 (dd, J_1 = 8.5 Hz, J_2 = 1.1 Hz, 2H), 7.74 (d, *J* = 8.6 Hz, 2H), 7.54 (m, 3H), 7.20 (d, *J* = 8.3 Hz, 2H), 3.13 (t, *J* = 7.6 Hz, 2H), 2.85 (t, *J* = 7.3 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 165.53, 137.74, 134.95, 133.26, 131.65, 128.80, 128.46, 127.78, 120.68, 51.37, 30.85. ESI-HRMS: calcd. for $C_{15}H_{17}N_3O$: $[M+H]^+$ = *m/z* 256.1447, found: $[M+H]^+$ = *m/z* 256.1444.

***N*-[4-(2-Hydrazinylethyl)phenyl]-2-phenylacetamide dihydrochloride salt (12b):** The title compound was synthesized from *N*-[4-(2-bromoethyl)phenyl]-2-phenylacetamide **17b** (0.400 g, 1.16 mmol) according to general procedure C and isolated to yield the product as a white powder (0.188 g, 51%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.37 (s, 1H), 7.57 (d, *J* = 8.3 Hz, 2H), 7.32 (m, 4H), 7.24 (m, 1H), 7.14 (d, *J* = 8.6 Hz, 2H), 3.64 (s, 2H), 3.07 (t, *J* = 7.6 Hz, 2H), 2.79 (t, *J* = 7.6 Hz, 2H). ^{13}C NMR (101 MHz, MeOD): δ 172.37, 138.22, 137.00, 136.84, 130.26, 130.15, 129.72, 128.08, 121.69, 57.22, 44.79, 34.39. ESI-HRMS: calcd. for $C_{16}H_{19}N_3O$: $[M+H]^+$ = *m/z* 270.1605, found: $[M+H]^+$ = *m/z* 270.1601.

***N*-[4-(2-Hydrazinylethyl)phenyl]-4-phenylbutanamide dihydrochloride salt (12d):** Under nitrogen, *N*-[4-(2-bromoethyl)phenyl]-4-phenylbutanamide **17c** (0.400 g, 1.15 mmol) was dissolved in EtOH (4 mL). To this stirred solution was added anhydrous hydrazine (0.720 mL, 23.1 mmol) dropwise. The solution was then refluxed for 1 h and monitored by TLC. After cooling, EtOH was removed and 1 N NaOH (80 mL) was added. The aqueous layer was extracted with DCM (3 x 80 mL) and dried in vacuo. The hydrazine free base was then dissolved in MeOH (10 mL) and 6 M HCl (2 mL) was added dropwise while stirring the solution on ice. The solution was left to stir on ice for

10 min after which Et_2O (5 mL) was added and the reaction was concentrated in vacuo to yield a precipitate that was filtered and washed with cold Et_2O. The precipitate was dried to yield the product as a light yellow powder (0.132 g, 33%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.89 (s, 1H), 7.53 (d, *J* = 8.5 Hz, 2H), 7.29 (m, 2H), 7.19 (m, 3H), 7.14 (d, *J* = 8.5 Hz, 2H), 3.07 (t, *J* = 7.8 Hz, 2H), 2.78 (t, *J* = 7.9 Hz, 2H), 2.61 (t, *J* = 7.6 Hz, 2H), 2.31 (t, *J* = 7.5 Hz, 2H), 1.88 (quin, *J* = 7.5 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 170.94, 141.76, 137.90, 132.41, 128.78, 128.39, 128.38, 125.85, 119.29, 51.42, 35.79, 34.70, 30.79, 26.92. ESI-HRMS: calcd. for $C_{18}H_{23}N_3O$: $[M+H]^+$ = *m/z* 298.1913, found: $[M+H]^+$ = *m/z* 298.1914.

4-(4-Chlorophenyl)butanoic acid: 4-(4-Chlorophenyl)-4-oxobutanoic acid (1.06 g, 5 mmol) and KOH (85% by wt., 0.79 g, 12 mmol) were placed in a round-bottomed flask fitted with a Dean-Stark apparatus and a reflux condenser and suspended in diethylene glycol (10 mL) at RT. Then, hydrazine monohydrate (50% by wt., 1.20 g, 12 mmol) was added slowly to the reaction at RT after which it was heated to 120–130 °C for 2 h. The reaction became homogenous after heating for approximately 45 min. After 2 h, the temperature was increased to 180–200 °C and the reaction stirred for an additional 3 h to remove residual hydrazine and water via the Dean–Stark trap. The reaction was then cooled to RT, diluted with H_2O (10 mL), and poured into a 2.5 N aqueous solution of HCl (20 mL). The suspension was cooled in an ice bath and the resulting precipitate was isolated by filtration. To remove residual diethylene glycol, the solid was dissolved in a saturated aqueous solution of K_2CO_3 (20 mL), diluted with H_2O (20 mL), and poured into a 2.5 N aqueous solution of HCl (20 mL). The suspension was again cooled in an ice bath and the precipitate isolated by filtration, washed with cold H_2O (2 x 15 mL), and dried under vacuum. The title compound was isolated as a white solid (0.89 g, 89%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.06 (br, 1H), 7.32 (d, *J* = 8.5 Hz, 2H), 7.21 (d, *J* = 8.5 Hz, 2H), 2.57 (t, *J* = 7.4 Hz, 2H), 2.20 (t, *J* = 7.3 Hz, 2H), 1.77 (q, *J* = 7.5 Hz, 2H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 174.16, 140.57, 130.41, 130.17, 128.20, 33.63, 32.95, 26.11. ESI-LRMS: $[M-H]^-$ = *m/z* 284.3. ESI-HRMS: calcd. for $C_{10}H_{11}ClO_2$: $[M-H]^-$ = *m/z* 197.0375, found: $[M-H]^-$ = *m/z* 197.0379.

4-(4-Fluorophenyl)butanoic acid: 4-(4-Fluorophenyl)-4-oxobutanoic acid (0.98 g, 5 mmol) and KOH (85% by wt., 0.79 g, 12 mmol) were placed in a round-bottomed flask fitted with a Dean-Stark apparatus and a reflux condenser and suspended in diethylene glycol (10 mL) at RT. Then, hydrazine monohydrate (50% by wt., 1.20 g, 12 mmol) was added slowly to the reaction at RT after which it was heated to 120–130 °C for 2 h. The reaction became homogenous after heating for approximately 45 min. After 2 h, the temperature was increased to 180–200 °C and the reaction stirred for an additional 3 h to remove residual hydrazine and water via the Dean–Stark trap. The reaction was then cooled to RT, diluted with H_2O (10 mL), and poured into a 2.5 N aqueous solution of HCl (20 mL). The organic products were extracted with EtOAc (3 x 15 mL), washed with brine (10 mL), dried with anhydrous Na_2SO_4, filtered, and concentrated in vacuo. Purification by column chromatography (30–50% EtOAc/hexanes) afforded the desired product as a clear, viscous oil (0.32 g, 35%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 11.50 (br, 1H), 7.16 (m, 2H), 7.00 (m, 2H), 2.67 (t, *J* = 7.6 Hz, 2H), 2.40 (t, *J* = 7.4 Hz, 2H), 1.97 (quin, *J* = 7.5 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 180.16, 161.34 (d, *J* = 243.4 Hz), 136.74, 129.76 (d, *J* = 7.3 Hz), 115.09 (d, *J* = 20.9 Hz), 34.09, 33.20, 26.24.

***N*-[4-(2-Hydroxyethyl)phenyl]-3-phenylpropanamide (16c):** The title compound was synthesized from 3-phenylpropanoic acid (1.50 g, 10 mmol) according to general procedure F and isolated as a white solid (2.36 g, 88%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.80 (s, 1H), 7.46 (d, *J* = 8.5 Hz, 2H), 7.27 (m, 4H), 7.18 (m, 1H), 7.11 (d, *J* = 8.5 Hz, 2H), 4.59 (t, *J* = 5.2 Hz, 1H), 3.55 (td, J_1 = 7.1 Hz, J_2 = 5.3 Hz, 2H), 2.90 (t, *J* = 7.6 Hz, 2H), 2.65 (t, *J* = 7.2 Hz, 2H), 2.60 (t, *J* = 7.7 Hz, 2H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 170.08, 141.19, 137.12, 134.08, 128.95, 128.27, 128.21, 125.89, 118.99, 62.24, 38.45, 37.88, 30.85. ESI-HRMS: calcd. for $C_{17}H_{19}NO_2$: $[M+H]^+$ = *m/z* 270.1489, found: $[M+H]^+$ = *m/z* 270.1501.

***N*-[4-(2-Hydroxyethyl)phenyl]-5-phenylpentanamide (16e):** The title compound was synthesized from 5-phenylpentanoic acid (0.89 g, 5 mmol) according to general procedure F. Purification by recrystallization from EtOAc facilitated by the dropwise addition of hexanes afforded the desired product as a white, crystalline solid (1.12 g,

75%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.44 (d, *J* = 8.3 Hz, 2H), 7.36 (s, 1H), 7.30 (m, 2H), 7.19 (m, 5H), 3.84 (t, *J* = 6.4 Hz, 2H), 2.84 (t, *J* = 6.5 Hz, 2H), 2.67 (t, *J* = 7.4 Hz, 2H), 2.37 (t, *J* = 7.2 Hz, 2H), 1.79 (m, 2H), 1.72 (m, 2H), 1.65 (br, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.21, 142.07, 136.28, 134.41, 129.45, 128.36, 128.31, 125.78, 120.19, 63.57, 38.53, 37.48, 35.65, 30.97, 25.22. ESI-HRMS: calcd. for $C_{19}H_{23}NO_2$: $[M+H]^+$ = *m/z* 298.1802, found: $[M+H]^+$ = *m/z* 298.1807.

4-(4-Chlorophenyl)-*N*-[4-(2-hydroxyethyl)phenyl]butanamide (16f): The title compound was synthesized from 4-(4-chlorophenyl)butanoic acid (0.57 g, 3 mmol) according to general procedure F. Purification by recrystallization from EtOAc facilitated by the dropwise addition of hexanes afforded the desired product as a white solid (0.89 g, 93%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.78 (s, 1H), 7.47 (d, *J* = 8.5 H, 2H), 7.33 (m, 2H), 7.24 (d, *J* = 8.3 Hz, 2H), 7.11 (d, *J* = 8.5 Hz, 2H), 4.59 (t, *J* = 5.2 Hz, 1H), 3.55 (td, J_1 = 7.1 Hz, J_2 = 5.3 Hz, 2H), 2.65 (t, *J* = 7.2 Hz, 2H), 2.61 (t, *J* = 7.6 Hz, 2H), 2.28 (t, *J* = 7.5 Hz, 2H), 1.87 (q, *J* = 7.5 Hz, 2H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 170.56, 140.68, 137.20, 134.02, 130.37, 130.21, 128.92, 128.18, 119.01, 62.27, 38.46, 35.52, 33.82, 26.57. ESI-HRMS: calcd. for $C_{18}H_{20}ClNO_2$: $[M+H]^+$ = *m/z* 318.1255, found: $[M+H]^+$ = *m/z* 318.1268.

4-(4-Fluorophenyl)-*N*-[4-(2-hydroxyethyl)phenyl]butanamide (16g): The title compound was synthesized from 4-(4-fluorophenyl)butanoic acid (0.32 g, 1.8 mmol) according to general procedure F and isolated as a white solid (0.53 g, 94%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.77 (s, 1H), 7.47 (d, *J* = 8.3 Hz, 2H), 7.24 (m, 2H), 7.10 (m, 4H), 4.59 (t, *J* = 5.2 Hz, 1H), 3.55 (td, J_1 = 7.1 Hz, J_2 = 5.3 Hz, 2H), 2.65 (t, *J* = 7.2 Hz, 2H), 2.60 (t, *J* = 7.5 Hz, 2H), 2.28 (t, *J* = 7.5 Hz, 2H), 1.86 (quin, *J* = 7.5 Hz, 2H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 170.61, 160.60 (d, *J* = 241.6 Hz), 137.76 (d, *J* = 2.7 Hz), 137.20, 134.03, 130.02 (d, *J* = 8.2 Hz), 128.92, 119.01, 114.91 (d, *J* = 20.9 Hz), 62.27, 38.46, 35.58, 33.70, 26.83. ESI-HRMS: calcd. for $C_{18}H_{20}FNO_2$: $[M+H]^+$ = *m/z* 302.1551, found: $[M+H]^+$ = *m/z* 302.1559.

***N*-[4-(2-Hydroxyethyl)phenyl]-4-(4-methoxyphenyl)butanamide (16h):** The title compound was synthesized from 4-(4-methoxyphenyl)butanoic acid (0.58 g, 3 mmol) according to general procedure F. Purification by column chromatography (SiO_2, 25–75% EtOAc/hexanes) afforded the desired product as a white solid (0.74 g, 79%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.42 (m, 3H), 7.15 (d, *J* = 8.3 Hz, 2H), 7.10 (d, *J* = 8.6, 2H), 6.83 (d, *J* = 8.5 Hz, 2H), 3.81 (t, *J* = 6.5 Hz, 2H), 3.78 (s, 3H), 2.81 (t, *J* = 6.5 Hz, 2H), 2.64 (t, *J* = 7.4 Hz, 2H), 2.31 (t, *J* = 7.5 Hz, 2H), 2.02 (quin, *J* = 7.4 Hz, 2H), 1.76 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.17, 157.84, 136.26, 134.42, 133.34, 129.43, 129.35, 120.18, 113.80, 63.53, 55.22, 38.52, 36.59, 34.10, 27.07. ESI-HRMS: calcd. for $C_{19}H_{23}NO_3$: $[M+H]^+$ = *m/z* 314.1751, found: $[M+H]^+$ = *m/z* 314.1763.

***N*-[4-(2-Hydroxyethyl)phenyl]-4-(4-nitrophenyl)butanamide (16i):** The title compound was synthesized from 4-(4-nitrophenyl)butanoic acid (1.05 g, 5 mmol) according to general procedure F and isolated as a white solid (1.49 g, 90%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.79 (s, 1H), 8.16 (d, *J* = 8.8 Hz, 2H), 7.51 (d, *J* = 8.8 Hz, 2H), 7.46 (d, *J* = 8.5 Hz, 2H), 7.11 (d, *J* = 8.6 Hz, 2H), 4.60 (br, 1H), 3.55 (m, 2H), 2.76 (t, *J* = 7.6 Hz, 2H), 2.65 (t, *J* = 7.2 Hz, 2H), 2.31 (t, *J* = 7.4 Hz, 2H), 1.93 (quin, *J* = 7.5 Hz, 2H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 170.44, 150.23, 145.87, 137.16, 134.09, 129.67, 128.95, 123.45, 119.04, 62.29, 38.47, 35.48, 34.35, 26.20. ESI-HRMS: calcd. for $C_{18}H_{20}N_2O_4$: $[M+H]^+$ = *m/z* 329.1496, found: $[M+H]^+$ = *m/z* 329.1501.

***N*-[4-(2-Hydroxyethyl)phenyl]-3-(2-hydroxyphenyl)propanamide (16j):** The title compound was synthesized from 3-(2-hydroxyphenyl)propanoic acid (0.83 g, 5 mmol) according to general procedure F and isolated as a white solid (1.43 g, 82%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.78 (s, 1H), 9.32 (s, 1H), 7.47 (d, *J* = 8.5 Hz, 2H), 7.11 (d, *J* = 8.5 Hz, 2H), 7.08 (dd, *J* = 7.4 Hz, 1.4 Hz, 1H), 7.00 (td, *J* = 7.7 Hz, 1.7 Hz, 1H), 6.78 (dd, J_1 = 8.0 Hz, J_2 = 0.9 Hz, 1H), 6.69 (td, J_1 = 7.4 Hz, J_2 = 1.1 Hz, 1H), 4.58 (br, 1H), 3.55 (t, *J* = 7.2 Hz, 2H), 2.82 (t, *J* = 7.8 Hz, 2H), 2.65 (t, *J* = 7.2 Hz, 2H), 2.55 (t, *J* = 7.8 Hz, 2H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 170.57, 155.07, 137.20, 134.00, 129.63, 128.94, 127.24, 126.98, 119.01, 118.84, 114.84, 62.27, 38.46, 36.25, 25.59. ESI-HRMS: calcd. for $C_{17}H_{19}NO_3$: $[M+H]^+$ = *m/z* 286.1438, found: $[M+H]^+$ = *m/z* 286.1445.

***N*-[4-(2-Hydroxyethyl)phenyl]-3-(3-hydroxyphenyl)propanamide (16k):** The title compound was synthesized from 3-(3-hydroxyphenyl)propanoic acid (0.83 g, 5 mmol) according to general procedure F. Purification by column chromatography (SiO_2, 5% MeOH/DCM) afforded the desired product as a clear, viscous oil that solidified on standing overnight to form a white solid (0.67 g, 47%). ^{1}H NMR (500 MHz, DMSO-d_6): 9.80 (s, 1H), 9.25 (s, 1H), 7.46 (d, J = 8.3 Hz, 2H), 7.12 (d, J = 8.3 Hz, 2H), 7.05 (t, J = 7.8 Hz, 1H), 6.64 (m, 2H), 6.57 (dt, J_1 = 8.0 Hz, J_2 = 1.2 Hz, 1H), 4.59 (t, J = 5.3 Hz, 1H), 3.55 (td, J_1 = 7.1 Hz, J_2 = 5.3 Hz, 2H), 2.80 (t, J = 7.7 Hz, 2H), 2.65 (t, J = 7.1 Hz, 2H), 2.55 (t, J = 7.7 Hz, 2H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 170.15, 157.28, 142.61, 137.16, 134.08, 129.19, 128.96, 119.01, 118.80, 115.24, 112.88, 62.27, 38.47, 37.86, 30.86. ESI-HRMS: calcd. for $C_{17}H_{19}NO_3$: $[M+H]^+$ = *m/z* 286.1438, found: $[M+H]^+$ = *m/z* 286.1449.

***N*-[4-(2-Hydrazinylethyl)phenyl]-3-phenylpropanamide sulfate salt (12c):** The title compound was synthesized from *N*-[4-(2-hydroxyethyl)phenyl]-3-phenylpropanamide **16c** (0.28 g, 1 mmol) according to general procedure G and the sulfate salt was prepared according to general procedure H. The desired product was isolated as a white solid (0.25 g, 67%). ^{1}H NMR (500 MHz, MeOD): δ 7.47 (d, J = 8.2 Hz, 2H), 7.22 (m, 7H), 3.25 (t, J = 7.8 Hz, 2H), 2.99 (t, J = 7.6 Hz, 2H), 2.91 (t, J = 7.8 Hz, 2H), 2.66 (t, J = 7.6 Hz, 2H). ^{13}C NMR (125 MHz, MeOD): δ 173.91, 142.23, 138.76, 134.18, 130.23, 129.62, 129.54, 127.38, 122.03, 53.71, 39.87, 32.93, 32.12. ESI-HRMS: calcd. for $C_{17}H_{21}N_3O$: $[M+H]^+$ = *m/z* 284.1757, found: $[M+H]^+$ = *m/z* 284.1770.

***N*-[4-(2-Hydrazinylethyl)phenyl]-5-phenylpentanamide oxalate salt (12e):** The title compound was synthesized from *N*-[4-(2-hydroxyethyl)phenyl]-5-phenylpentanamide **16e** (0.30 g, 1 mmol) according to general procedure G and the oxalate salt was prepared according to general procedure I. The desired product was isolated as a white solid (0.32 g, 80%). ^{1}H NMR (500 MHz, MeOD): δ 7.51 (d, J = 8.5 Hz, 2H), 7.20 (m, 7H), 3.24 (m, 2H), 2.91 (t, J = 7.7 Hz, 2H), 2.66 (t, J = 7.2 Hz, 2H), 2.39 (t, J = 7.2 Hz, 2H), 1.71 (m, 4H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 171.02, 163.93, 142.04, 137.77,

132.34, 128.74, 128.25, 128.23, 125.63, 119.21, 51.48, 36.19, 34.89, 30.78, 30.63, 24.80. ESI-HRMS: calcd. for $C_{19}H_{25}N_3O$: $[M+H]^+$ = *m/z* 312.2070, found: $[M+H]^+$ = *m/z* 312.2081.

4-(4-Chlorophenyl)-*N*-[4-(2-hydrazinylethyl)phenyl]butanamide sulfate salt (12f): The title compound was synthesized from 4-(4-chlorophenyl)-*N*-[4-(2-hydroxyethyl)phenyl]butanamide **16f** (0.32 g, 1 mmol) according to general procedure G and the sulfate salt was prepared according to general procedure H. The desired product was isolated as a white solid (0.31 g, 73%). ^{1}H NMR (500 MHz, MeOD): δ 7.50 (d, *J* = 8.6 Hz, 2H), 7.23 (m, 6H), 3.25 (m, 2H), 2.92 (t, *J* = 7.8 Hz, 2H), 2.68 (t, *J* = 7.7 Hz, 2H), 2.38 (t, *J* = 7.4 Hz, 2H), 1.99 (quin, *J* = 7.5 Hz, 2H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 170.73, 140.67, 137.77, 132.29, 130.39, 130.22, 128.76, 128.21, 119.27, 51.48, 35.54, 33.82, 30.81, 26.57. ESI-HRMS: calcd. for $C_{18}H_{22}ClN_3O$: $[M+H]^+$ = *m/z* 332.1524, found: $[M+H]^+$ = *m/z* 332.1537.

4-(4-Fluorophenyl)-*N*-[4-(2-hydrazinylethyl)phenyl]butanamide sulfate salt (12g): The title compound was synthesized from 4-(4-fluorophenyl)-*N*-[4-(2-hydroxyethyl)phenyl]butanamide **16g** (0.30 g, 1 mmol) according to general procedure G and the sulfate salt was prepared according to general procedure H. The desired product was isolated as a white solid (0.24 g, 58%). ^{1}H NMR (500 MHz, MeOD): δ 7.51 (d, *J* = 8.5 Hz, 2H), 7.21 (m, 4H), 6.99 (t, *J* = 8.8 Hz, 2H), 3.25 (m, 2H), 2.92 (t, *J* = 7.4 Hz, 2H), 2.37 (t, *J* = 7.7 Hz, 2H), 2.38 (t, *J* = 7.4 Hz, 2H), 1.98 (quin, *J* = 7.5 Hz, 2H). ^{13}C NMR (125 MHz, MeOD): δ 174.39, 162.94 (d, *J* = 242.5 Hz), 139.00 (d, *J* = 3.63 Hz), 138.95, 134.11, 131.23 (d, *J* = 7.3 Hz), 130.23, 121.90, 116.08 (d, *J* = 21.8 Hz), 53.72, 37.29, 35.56, 32.20, 28.79. ESI-HRMS: calcd. for $C_{18}H_{22}FN_3O$: $[M+H]^+$ = *m/z* 316.1820, found: $[M+H]^+$ = *m/z* 316.1825.

***N*-[4-(2-Hydrazinylethyl)phenyl]-4-(4-methoxyphenyl)butanamide sulfate salt (12h):** The title compound was synthesized from *N*-[4-(2-hydroxyethyl)phenyl]-4-(4-methoxyphenyl)butanamide **16h** (0.63 g, 2 mmol) according to general procedure G and the sulfate salt was prepared according to general procedure H. The desired product

was isolated as a white solid (0.57 g, 67%). ^{1}H NMR (500 MHz, MeOD): δ 7.51 (d, *J* = 8.5 Hz, 2H), 7.22 (d, *J* = 8.5 Hz, 2H), 7.12 (d, *J* = 8.6 Hz, 2H), 6.83 (d, *J* = 8.6 Hz, 2H), 3.75 (s, 3H), 3.25 (m, 2H), 2.91 (t, *J* = 7.8 Hz, 2H), 2.62 (t, *J* = 7.5 Hz, 2H), 2.36 (t, *J* = 7.5 Hz, 2H), 1.96 (quin, *J* = 7.5 Hz, 2H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 170.93, 157.44, 137.82, 133.51, 132.30, 129.25, 128.78, 119.29, 113.73, 54.98, 51.53, 35.72, 33.74, 30.82, 27.07. ESI-HRMS: calcd. for $C_{19}H_{25}N_3O_2$: $[M+H]^+$ = *m/z* 328.2020, found: $[M+H]^+$ = *m/z* 328.2026.

***N*-[4-(2-Hydrazinylethyl)phenyl]-4-(4-nitrophenyl)butanamide sulfate salt (12i):** The title compound was synthesized from *N*-[4-(2-hydroxyethyl)phenyl]-4-(4-nitrophenyl)butanamide **16i** (0.33 g, 1 mmol) according to general procedure G and the sulfate salt was prepared according to general procedure H. The desired product was isolated as a white solid (0.29 g, 65%). ^{1}H NMR (500 MHz, MeOD): δ 8.15 (d, *J* = 8.8 Hz, 2H), 7.48 (t, *J* = 9.0 Hz, 4H), 7.21 (d, *J* = 8.5 Hz, 2H), 3.25 (m, 2H), 2.92 (t, *J* = 7.9 Hz, 2H), 2.84 (t, *J* = 8.6 Hz, 2H), 2.42 (t, *J* = 7.4 Hz, 2H), 2.06 (quin, *J* = 7.5 Hz, 2H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 170.63, 150.24, 145.90, 137.75, 132.37, 129.71, 128.80, 123.48, 119.31, 51.52, 35.52, 34.36, 30.83, 26.22. ESI-HRMS: calcd. for $C_{18}H_{22}N_4O_3$: $[M+H]^+$ = *m/z* 343.1765, found: $[M+H]^+$ = *m/z* 343.1768.

2-(3-{[4-(2-Hydrazinylethyl)phenyl]amino}-3-oxopropyl)phenyl methanesulfonate oxalate salt (12j): The title compound was synthesized from *N*-[4-(2-hydroxyethyl)phenyl]-3-(2-hydroxyphenyl)propanamide **16j** (0.29 g, 1 mmol) according to general procedure G and the oxalate salt was prepared according to general procedure I. The desired product was isolated as a white solid (0.16 g, 34%). ^{1}H NMR (500 MHz, MeOD): δ 7.49 (d, *J* = 8.5 Hz, 2H), 7.40 (dd, J_1 = 7.0 Hz, J_2 = 2.3 Hz, 1H), 7.36 (dd, J_1 = 7.6 Hz, J_2 = 1.7 Hz, 1H), 7.28 (m, 2H), 7.21 (d, *J* = 8.6 Hz, 2H), 3.34 (s, 3H), 3.24 (m, 2H), 3.11 (t, *J* = 7.8 Hz, 2H), 2.91 (t, *J* = 7.9 Hz, 2H), 2.70 (t, *J* = 7.6 Hz, 2H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 169.97, 163.85, 147.33, 137.64, 134.27, 132.49, 130.55, 128.78, 127.73, 127.22, 122.09, 119.27, 51.46, 38.30, 36.09, 30.80, 25.03. ESI-HRMS: calcd. for $C_{18}H_{23}N_3O_4S$: $[M+H]^+$ = *m/z* 378.1482, found: $[M+H]^+$ = *m/z* 378.1499.

3-(3-{[4-(2-Hydrazinylethyl)phenyl]amino}-3-oxopropyl)phenyl methanesulfonate oxalate salt (12k): The title compound was synthesized from *N*-[4-(2-hydroxyethyl)phenyl]-3-(3-hydroxyphenyl)propanamide **16k** (0.29 g, 1 mmol) according to general procedure G and the oxalate salt was prepared according to general procedure I. The desired product was isolated as an off-white solid (56 mg, 12%). ^{1}H NMR (500 MHz, DMSO-d_6/MeOD): δ 7.45 (d, J = 8.5 Hz, 2H), 7.33 (t, J = 7.9 Hz, 1H), 7.21 (d, J = 7.7 Hz, 1H), 7.18 (m, 1H), 7.11 (m, 3H), 3.19 (s, 3H), 3.08 (t, J = 7.7 Hz, 2H), 2.94 (t, J = 7.5 Hz, 2H), 2.77 (t, J = 7.7 Hz, 2H), 2.60 (t, J = 7.7 Hz, 2H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 170.00, 164.09, 149.13, 143.76, 137.61, 132.54, 129.92, 128.79, 127.32, 121.93, 119.77, 119.27, 51.48, 37.43, 37.36, 30.79, 30.40. ESI-HRMS: calcd. for $C_{18}H_{23}N_3O_4S$: $[M+H]^+$ = *m/z* 378.1482, found: $[M+H]^+$ = *m/z* 378.1499.

***N*-[4-(2-Hydroxyethyl)phenyl]-3-(1*H*-indol-3-yl)propanamide (18a):** The title compound was synthesized from 3-(1*H*-indol-3-yl)propanoic acid (0.57 g, 3 mmol) according to general procedure F and isolated as a white solid (0.82 g, 89%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.75 (s, 1H), 9.82 (s, 1H), 7.56 (d, J = 7.9 Hz, 1H), 7.48 (d, J = 8.5 Hz, 2H), 7.32 (d, J = 8.0 Hz, 1H), 7.12 (m, 3H), 7.06 (td, J_1 = 7.5 Hz, J_2 = 1.0 Hz, 1H), 6.98 (td, J_1 = 7.5 Hz, J_2 = 0.9 Hz, 1H), 4.60 (t, J = 5.3 Hz, 1H), 3.56 (td, J_1 = 7.2 Hz, J_2 = 5.3 Hz, 2H), 3.01 (t, J = 7.5 Hz, 2H), 2.66 (t, J = 7.4 Hz, 4H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 170.71, 137.23, 136.21, 134.02, 128.95, 127.01, 122.13, 120.90, 119.00, 118.34, 118.14, 113.70, 111.31, 62.27, 38.47, 37.22, 20.83. ESI-HRMS: calcd. for $C_{19}H_{20}N_2O_2$: $[M+H]^+$ = *m/z* 309.1598, found: $[M+H]^+$ = *m/z* 309.1603.

***N*-[4-(2-Hydroxyethyl)phenyl]-4-(1*H*-indol-3-yl)butanamide (18b):** The title compound was synthesized from 4-(1*H*-indol-3-yl)butanoic acid (0.61 g, 3 mmol) according to general procedure F and isolated as a white solid (0.41 g, 43%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.77 (s, 1H), 9.79 (s, 1H), 7.52 (d, J = 7.9 HZ, 1H), 7.49 (d, J = 8.3 Hz, 2H), 7.33 (d, J = 8.2 Hz, 1H), 7.11 (m, 3H), 7.06 (dt, J_1 = 7.1 Hz, J_2 = 0.9 Hz, 1H), 6.97 (dt, J_1 = 7.1 Hz, J_2 = 0.9 Hz, 1H), 4.60 (t, J = 5.2 Hz, 1H), 3.56 (td, J_1 = 7.1 Hz, J_2 = 5.3 Hz, 2H), 2.73 (t, J = 7.4 Hz, 2H), 2.66 (t, J = 7.2 Hz, 2H), 2.35 (t, J = 7.5 Hz, 2H), 1.96 (quin, 7.4 Hz, 2H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 170.97, 137.29, 136.31,

133.97, 128.93, 127.17, 122.28, 120.81, 119.01, 118.29, 118.10, 114.01, 111.31, 62.29, 38.48, 36.14, 25.95, 24.31. ESI-HRMS: calcd. for $C_{20}H_{22}N_2O_2$: $[M+H]^+$ = *m/z* 323.1754, found: $[M+H]^+$ = *m/z* 323.1759.

***N*-[4-(2-Hydrazinylethyl)phenyl]-3-(1*H*-indol-3-yl)propanamide sulfate salt (15a):** The title compound was synthesized from *N*-[4-(2-hydroxyethyl)phenyl]-3-(1*H*-indol-3-yl)propanamide **18a** (0.31 g, 1 mmol) according to general procedure G and the sulfate salt was prepared according to general procedure H. The desired product was isolated as a white solid (0.26 g, 62%). ^{1}H NMR (500 MHz, DMSO-d_6/MeOD): δ 7.53 (d, *J* = 7.9 Hz, 1H), 7.49 (d, *J* = 8.5 Hz, 2H), 7.29 (d, *J* = 8.2 Hz, 1H), 7.12 (d, *J* = 8.5 Hz, 2H), 7.03 (m, 2H), 6.95 (m, 1H), 3.09 (t, *J* = 7.4 Hz, 2H), 3.02 (t, *J* = 7.5 Hz, 2H), 2.78 (t, *J* = 7.8 Hz, 2H), 2.65 (t, *J* = 7.6 Hz, 2H). ^{13}C NMR (125 MHz, MeOD/DMSO-d_6): δ 173.88, 139.09, 138.11, 134.05, 130.30, 128.73, 123.34, 122.56, 121.65, 119.82, 119.68, 115.34, 112.59, 53.55, 39.03, 32.14, 22.48. ESI-HRMS: calcd. for $C_{19}H_{22}N_4O$: $[M+H]^+$ = *m/z* 323.1866, found: $[M+H]^+$ = *m/z* 323.1871.

***N*-[4-(2-Hydrazinylethyl)phenyl]-4-(1*H*-indol-3-yl)butanamide sulfate salt (15b):** The title compound was synthesized from *N*-[4-(2-hydroxyethyl)phenyl]-4-(1*H*-indol-3-yl)butanamide **18b** (0.32 g, 1 mmol) according to general procedure G and the sulfate salt was prepared according to general procedure H. The desired product was isolated as an off-white solid (84 mg, 19%). ^{1}H NMR (500 MHz, MeOD/DMSO-d_6): δ 7.63 (d, *J* = 8.3 Hz, 3H), 7.43 (d, *J* = 8.0 Hz, 1H), 7.27 (d, *J* = 8.5 Hz, 2H), 7.17 (m, 2H), 7.08 (m, 1H), 3.25 (t, *J* = 7.4 Hz, 2H), 2.94 (t, *J* = 7.7 Hz, 2H), 2.88 (t, *J* = 7.5 Hz, 2H), 2.48 (t, *J* = 7.5 Hz, 2H), 2.12 (quin, *J* = 7.4 Hz, 2H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 171.12, 137.86, 136.31, 132.20, 128.76, 127.15, 122.28, 120.81, 119.27, 118.27, 118.09, 113.98, 111.33, 51.48, 36.14, 30.82, 25.95, 24.29. ESI-HRMS: calcd. for $C_{20}H_{24}N_4O$: $[M+H]^+$ = *m/z* 337.2023, found: $[M+H]^+$ = *m/z* 337.2025.

(2*E*)-*N*-[4-(2-Hydroxyethyl)phenyl]-3-phenylprop-2-enamide (19): The title compound was synthesized from (2*E*)-3-phenylprop-2-enoic acid (0.74 g, 5 mmol) according to general procedure F and isolated as a white, crystalline solid (1.34 g,

88%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.13 (s, 1H), 7.61 (m, 5H), 7.42 (m, 3H), 7.18 (d, J = 8.5 Hz, 2H), 6.85 (d, J = 15.6 Hz, 1H), 4.62 (t, J = 5.3 Hz, 1H), 3.59 (td, J_1 = 7.1 Hz, J_2 = 5.2 Hz, 2H), 2.69 (t, J = 7.1 Hz, 2H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 163.30, 139.90, 137.17, 134.76, 134.54, 129.69, 129.13, 128.99, 127.66, 122.37, 119.15, 62.25, 38.51. ESI-HRMS: calcd. for $C_{17}H_{17}NO_2$: $[M+H]^+$ = *m/z* 268.1332, found: $[M+H]^+$ = *m/z* 268.1342.

(2*E*)-*N*-[4-(2-Hydrazinylethyl)phenyl]-3-phenylprop-2-enamide sulfate salt (13): The title compound was synthesized from (2*E*)-*N*-[4-(2-hydroxyethyl)phenyl]-3-phenylprop-2-enamide **19** (0.27 g, 1 mmol) according to general procedure G and the sulfate salt was prepared according to general procedure H. The desired product was isolated as a white solid (0.24 g, 64%).^{1}H NMR (500 MHz, MeOD): δ 7.63 (m, 5H), 7.41 (m, 3H), 7.27 (d, J = 8.5 Hz, 2H), 6.80 (d, J = 15.6 Hz, 1H), 3.27 (m, 2H), 2.94 (t, J = 7.8 Hz, 2H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 163.48, 140.04, 137.80, 134.75, 132.80, 129.75, 129.00, 128.96, 127.76, 122.38, 119.44, 51.49, 30.89. ESI-HRMS: calcd. for $C_{17}H_{19}N_3O$: $[M+H]^+$ = *m/z* 282.1601, found: $[M+H]^+$ = *m/z* 282.1608.

***N*-[4-(2-{[*tert*-Butyl(dimethyl)silyl]oxy}ethyl)phenyl]-4-phenylbutanamide (20):** *N*-[4-(2-hydroxyethyl)phenyl]-4-phenylbutanamide **16d** (0.99 g, 3.5 mmol) was dissolved in anhydrous DCM (8 mL) and to it was added triethylamine (1.22 mL, 8.75 mmol) and DMAP (43 mg, 0.35 mmol) at RT. Upon dissolution of **16d**, *tert*-butyldimethylsilyl chloride (0.63 g, 4.2 mmol) was dissolved in anhydrous DCM (7 mL) and added to the reaction in one portion. The reaction was then stirred at RT for 2 h after which it was poured into H_2O (15 mL) and the organic layer isolated. The aqueous layer was further extracted with DCM (2 x 15 mL). The combined organic fractions were washed with brine (15 mL), dried with anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The residue obtained was dissolved in a 1:1 mixture of EtOAc/hexanes and passed through a 3-inch pad of silica gel (60 Å, 200–400 mesh). The filtrate was concentrated in vacuo which afforded the desired product as a clear, viscous oil (1.29 g, 92%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.43 (m, 3H), 7.30 (m, 2H), 7.21 (m, 3H), 7.15 (d, J = 8.3 Hz, 2H), 3.79 (t, J = 7.1 Hz, 2H), 2.80 (t, J = 7.1 Hz, 2H), 2.71 (t, J = 7.5 Hz, 2H), 2.34 (t, J = 7.5 Hz,

2H), 2.07 (quin, *J* = 7.5 Hz, 2H), 0.90 (s, 9H), 0.01 (s, 6H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.95, 141.31, 136.01, 135.08, 129.52, 128.44, 128.36, 125.95, 119.75, 64.43, 38.94, 36.65, 35.02, 26.84, 25.88, 18.27, 5.42. ESI-HRMS: calcd. for $C_{24}H_{35}NO_2Si$: $[M+H]^+$ = *m/z* 398.2510, found: $[M+H]^+$ = *m/z* 398.2526.

***N*-[4-(2-Hydroxyethyl)phenyl]-*N*-methyl-4-phenylbutanamide (21a):** Sodium hydride (95% by wt., 33 mg, 1.3 mmol) was placed under argon, suspended in anhydrous THF (2 mL), and cooled to 0 °C in an ice bath. Then, *N*-[4-(2-{[*tert*-butyl(dimethyl)silyl]oxy}ethyl)phenyl]-4-phenylbutanamide **20** (0.40 g, 1 mmol) was dissolved in anhydrous THF (3 mL) and added slowly to the reaction at 0 °C. Stirring was continued for 5 min and then methyl iodide (2 M solution in THF, 1.0 mL, 2 mmol) was added dropwise to the reaction. The reaction was stirred at 0 °C for 30 min after which it was warmed to RT and stirred for an additional 16 h. The reaction was then partitioned between saturated aqueous ammonium chloride (15 mL) and EtOAc (15 mL). The organic layer was isolated and the aqueous layer was further extracted with EtOAc (2 x 15 mL). The combined organic extracts were washed with brine (10 mL), dried with anhydrous Na_2SO_4, filtered, and concentrated in vacuo. Purification by column chromatography (SiO_2, 25% EtOAc/hexanes) afforded the desired product as a clear viscous oil (0.34 g, 82%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.24 (m, 4H), 7.15 (m, 1H), 7.11 (d, *J* = 7.2 Hz, 2H), 7.05 (d, *J* = 8.0 Hz, 2H), 3.84 (t, *J* = 6.6 Hz, 2H), 3.25 (s, 3H), 2.84 (t, *J* = 6.7 Hz, 2H), 2.54 (t, *J* = 7.7 Hz, 2H), 2.11 (t, *J* = 7.4 Hz, 2H), 1.91 (quin, *J* = 7.5 Hz, 2H), 0.87 (s, 9H), -0.02 (s, 6H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 172.84, 142.16, 141.78, 139.12, 130.44, 128.33, 128.17, 126.88, 125.68, 64.09, 38.96, 37.28, 35.22, 33.42, 26.99, 25.85, 18.27, -5.45. ESI-HRMS: calcd. for $C_{25}H_{37}NO_2Si$: $[M+H]^+$ = *m/z* 412.2666, found: $[M+H]^+$ = *m/z* 412.2676.

N-[4-(2-{[*tert*-butyl(dimethyl)silyl]oxy}ethyl)phenyl]-*N*-methyl-4-phenylbutanamide (0.32 g, 0.8 mmol) was dissolved in anhydrous THF (5 mL) and to it was added tetra-*n*-butylammonium fluoride (1 M solution in THF, 2.4 mL, 2.4 mmol) at RT. Stirring was continued until the reaction was complete as evidenced by TLC (approximately 24 h). Then, the reaction was poured into H_2O (10 mL) and the organic products were extracted with DCM (3 x 10 mL). The combined organic fractions were washed with

brine (10 mL), dried with anhydrous Na_2SO_4, filtered, and concentrated in vacuo. Purification by column chromatography (SiO_2, 25–50% EtOAc/hexanes) afforded the desired product as a clear, viscous oil that solidified under vacuum (0.22 g, 94%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.23 (m, 4H), 7.15 (m, 1H), 7.09 (d, *J* = 7.7 Hz, 2H), 7.07 (d, *J* = 8.2 Hz, 2H), 3.90 (t, *J* = 6.6 Hz, 2H), 3.25 (s, 3H), 2.90 (t, *J* = 6.6 Hz, 2H), 2.54 (t, *J* = 7.6 Hz, 2H), 2.10 (t, *J* = 7.3 Hz, 2H), 1.90 (quin, *J* = 7.4 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 172.91, 142.30, 141.70, 138.37, 130.20, 128.33, 128.16, 127.19, 125.68, 63.31, 38.62, 37.26, 35.16, 33.35, 26.94. ESI-HRMS: calcd. for $C_{19}H_{23}NO_2$: $[M+H]^+$ = *m/z* 298.1802, found: $[M+H]^+$ = *m/z* 298.1810.

***N*-Benzyl-*N*-[4-(2-hydroxyethyl)phenyl]-4-phenylbutanamide (21b):** Potassium *tert*-butoxide (0.14 g, 1.2 mmol) was placed under argon, suspended in 4 mL of a 1:1 mixture of anhydrous DCM/DMF, and cooled to 0 °C in an ice bath. Then, *N*-[4-(2-{[*tert*-butyl(dimethyl)silyl]oxy}ethyl)phenyl]-4-phenylbutanamide **20** (0.40 g, 1 mmol) dissolved in an additional 4 mL of a 1:1 mixture of anhydrous DCM/DMF was added slowly at 0 °C. The reaction was stirred for 15 min after which benzyl bromide (0.13 mL, 1.1 mmol) dissolved in 2 mL of a 1:1 mixture of anhydrous DCM/DMF was added dropwise to the reaction at 0 °C. The reaction was allowed to warm to RT and then heated to 60 °C for 16 h. The reaction was quenched by the addition of H_2O (30 mL), then DCM (15 mL) was added and the organic layer isolated. The aqueous layer was further extracted with DCM (2 x 10 mL) and the combined organic fractions were washed with H_2O (3 x 30 mL), brine (10 mL), dried with anhydrous Na_2SO_4, filtered, and concentrated in vacuo. Purification by column chromatography (SiO_2, 20% EtOAc/hexanes) afforded the desired product as a clear, viscous oil (0.40 g, 82%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.29 (m, 7H), 7.19 (m, 5H), 6.89 (d, *J* = 8.5 Hz, 2H), 4.92 (s, 2H), 3.86 (t, *J* = 6.6 Hz, 2H), 2.85 (t, *J* = 6.5 Hz, 2H), 2.61 (t, *J* = 7.8 Hz, 2H), 2.16 (t, *J* = 7.4 Hz, 2H), 1.99 (quin, *J* = 7.5 Hz, 2H), 0.91 (s, 9H), 0.00 (s, 6H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 172.63, 141.75, 140.36, 139.31, 137.72, 130.24, 128.79, 128.34, 128.24, 128.17, 127.95, 127.18, 125.67, 63.97, 52.93, 38.92, 35.17, 33.63, 26.98, 25.84, 18.24, -5.47. *N*-Benzyl-*N*-[4-(2-{[*tert*-butyl(dimethyl)silyl]oxy}ethyl)phenyl]-4-phenylbutanamide (0.35 g, 0.7 mmol) was dissolved in anhydrous THF (5 mL) and to it was added tetra-*n*-

butylammonium fluoride (1 M solution in THF, 2.2 mL, 2.2 mmol) at RT. Stirring was continued until reaction was complete as evidenced by TLC (approximately 24 h). Then, the reaction was poured into H_2O (10 mL) and the organic products extracted with DCM (3 x 10 mL). The combined organic fractions were washed with brine (10 mL), dried with anhydrous Na_2SO_4, filtered, and concentrated in vacuo. Purification by column chromatography (SiO_2, 25–50% EtOAc/hexanes) afforded the desired product as a clear, viscous oil (0.25 g, 92%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.26 (m, 7H), 7.17 (m, 3H), 7.11 (d, *J* = 7.2 Hz, 2H), 6.89 (d, *J* = 8.0 Hz, 2H), 4.89 (s, 2H), 3.89 (q, *J* = 6.1 Hz, 2H), 2.88 (t, *J* = 6.6 Hz, 2H), 2.58 (t, *J* = 7.6 Hz, 2H), 2.12 (t, *J* = 7.4 Hz, 2H), 1.96 (quin, *J* = 7.5 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 172.70, 141.73, 140.72, 138.41, 137.67, 129.99, 128.69, 128.38, 128.31, 128.19, 127.24, 125.71, 63.30, 52.95, 38.64, 35.16, 33.63, 26.95. ESI-HRMS: calcd. for $C_{25}H_{27}NO_2$: $[M+H]^+$ = *m/z* 374.2115, found: $[M+H]^+$ = *m/z* 374.2125.

***N*-[4-(2-Hydrazinylethyl)phenyl]-*N*-methyl-4-phenylbutanamide oxalate salt (12l):** The title compound was synthesized from *N*-[4-(2-hydroxyethyl)phenyl]-*N*-methyl-4-phenylbutanamide **21a** (0.20 g, 0.68 mmol) according to general procedure G and the oxalate salt was prepared according to general procedure I. The desired product was isolated as a white solid (0.11 g, 40%).^{1}H NMR (500 MHz, MeOD): δ 7.32 (d, *J* = 8.0 Hz, 2H), 7.19 (m, 4H), 7.13 (m, 1H), 7.05 (d, *J* = 6.9 Hz, 2H), 3.27 (t, *J* = 7.8 Hz, 2H), 3.21 (s, 3H), 2.98 (t, *J* = 7.5 Hz, 2H), 2.50 (t, *J* = 6.9 Hz, 2H), 2.08 (t, *J* = 6.8 Hz, 2H), 1.84 (br, 2H). ^{13}C NMR (125 MHz, MeOD/DMSO-d_6): δ 175.16, 165.52, 144.00, 142.98, 138.91, 131.44, 129.57, 129.50, 128.78, 127.05, 53.20, 37.88, 36.19, 34.35, 32.40, 28.41. ESI-HRMS: calcd. for $C_{19}H_{25}N_3O$: $[M+H]^+$ = *m/z* 312.2070, found: $[M+H]^+$ = *m/z* 312.2079.

***N*-Benzyl-*N*-[4-(2-hydrazinylethyl)phenyl]-4-phenylbutanamide oxalate salt (12m):** The title compound was synthesized from *N*-Benzyl-*N*-[4-(2-hydroxyethyl)phenyl]-4-phenylbutanamide **21b** (0.25 g, 0.66 mmol) according to general procedure G and the oxalate salt was prepared according to general procedure I. The desired product was isolated as an off-white solid (0.23 g, 47%). ^{1}H NMR (500 MHz, MeOD): δ 7.20 (m,

10H), 7.06 (d, *J* = 7.2 Hz, 2H), 6.95 (d, *J* = 8.0 Hz, 2H), 4.88 (s, 2H), 3.23 (m, 2H), 2.93 (t, *J* = 8.2 Hz, 2H), 2.53 (t, *J* = 7.5 Hz, 2H), 2.10 (t, *J* = 7.4 Hz, 2H), 1.88 (quin, *J* = 7.4 Hz, 2H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 171.61, 164.24, 141.57, 140.53, 137.76, 137.57, 129.54, 128.27, 128.20, 128.18, 128.06, 127.74, 126.97, 125.69, 51.95, 50.95, 34.39, 32.78, 30.92, 26.64. ESI-HRMS: calcd. for $C_{25}H_{29}N_3O$: $[M+H]^+$ = *m/z* 388.2383, found: $[M+H]^+$ = *m/z* 388.2396.

cDNA Cloning of Mouse LSD2. Two ovaries from a C57Black6 mouse (a gift from Josh Mendell's lab) were dissected and snap frozen by Raghu Chivukula. RNA was isolated by adding 200 µL of cold Trizol (Invitrogen) and the samples were homogenized with a handheld homogenizer with disposable tips (Fisher). An additional 800µL of Trizol was added and mixed, the sample was clarified 1 min at 12,000 x g, and the supernatant was transferred to a fresh tube, precipitated with isopropanol, washed with ethanol, air dried, and then resuspended in 20 µL DEPC-treated ddH_2O. cDNA was prepared by first digesting with DNase I and then reverse-transcribing using the Superscript III first strand synthesis system (Invitrogen) using Oligo-dT priming according to the manufacturer's instructions. Refseq LSD2 was amplified with primers AGCGCTCTGAGGTTTTCCAA and TGAGGGTCAGTGGTTGCAGA, and a ~2.7 kB product was gel purified and cloned using the StrataClone Blunt PCR Cloning Kit (Agilent). Clones were fully sequenced and one was identified as fully identical to the coding region of Kdm1b, NM_172262.3.

LSD2 Expression and Purification. To express Mouse LSD2 with a C-terminal His tag, we N-terminally truncated Refseq Mouse LSD2 by 25 amino acids and installed it into pET28b between NcoI and XhoI sites. The cDNA was PCR amplified with primers TCGTCGACATGTCTGGGCGGCAGGCAAAGAA and AATAATCTCGAGAAAGGCTGCAATCTTGCTTGCTTC, cut with PciI and XhoI, and ligated into pET28b cut between NcoI and XhoI sites. The Δ25 Mouse LSD2 was then subcloned from the Mouse cDNA library into a pET28b vector and was overexpressed in *E. coli* BL21DE(3) codon plus cells as a C-terminal His6 tagged protein. Cell were grown to an OD_{600} of 0.6 in LB at 37 °C, then induced with 0.25 mM IPTG (final

concentration) and grown for 20 h at 16 °C. Cell pellets were harvested by centrifugation at 5000 g for 20 min and resuspended in cold lysis buffer [280 mM NaCl, 5.4 mM KCl, 20 mM Na_2HPO_4, 3.6 mM KH_2PO_4, 1.3 mM PMSF, 6.8 μg mL^{-1} DNase I and 10% glycerol (pH 7.4)] containing cOmplete, EDTA-free Protease Inhibitor Cocktail Tablets (Roche). The cells were then lysed via single pass on a french press (16000-18000 psi), and the lysates were clarified by centrifugation at 25000 g for 30 min. The clarified lysate from 6 L of culture was incubated with 2 mL nickel sepharose fast flow resin that was pre-equilibrated with resin equilibration buffer [280 mM NaCl, 5.4 mM KCl, 20 mM Na_2HPO_4, 3.6 mM KH_2PO_4 and 10% glycerol (pH 7.4)] for 2 h at 4 °C. The resin was then washed with equilibration buffer (3 x 20 mL). The resin was then washed with equilibration buffer containing 20 mM imidazole (20 mL). The protein was then eluted with equilibration buffer containing sequential steps of 100 mM, 200 mM and 300 mM imidazole (3 x 5 mL). The 200 mM imidazole elution contained the purest fraction of LSD2 as gauged by Coomassie-stained SDSPAGE. This fraction was dialyzed against equilibration buffer (3 x 2 L) containing 1 mM β-mercaptoethanol. The dialyzed LSD2-His_6 was then concentrated to 4.3 μM.

LSD2 Enzymatic Assays. Initial velocity measurements were performed using a peroxidase-coupled assay, which monitors hydrogen peroxide production as previously described[11]. The time courses of the reaction were measured under aerobic conditions using a Beckman Instruments DU series 600 spectrophotometer equipped with a thermostatted cell holder (T = 25 °C). The 100 μL reactions were initiated by addition of enzyme (430 nM LSD2) to reaction mixtures consisting of 50 mM HEPES buffer (pH 7.5), 0.1 mM 4-aminoantipyrine, 1 mM 3,5-dichloro-2-hydroxybenzene-sulfonic acid, 0.76 μM horseradish peroxidase (Worthington Biochemical Corp.), 20 μM phenelzine analog and 100 μM DiMeK4H3-21. Absorbance changes were monitored at 515 nm, and an extinction coefficient of 26,000 M^{-1} cm^{-1} was used to quantify product formation. Progress curves were then fit accordingly to eq 1-3 as previously stated. Each experiment was repeated at least two independent times and repeat measured values were typically within 20% of each other.

MassSQUIRM Assays. MassSQUIRM inhibition experiments were performed in triplicate as described previously[12]. The reaction mixtures containing 13.3 μM H3K4me2-biotin peptide (^{1}ARTKme2QTA RKS TGG KAP RKQ LYKbio), 50 mM HEPES (pH 7.5), and 50 μM phenelzine or **12d**, were incubated at 25 °C for 5 min, prior to initiation with 215 nM GST-LSD1. The demethylase reactions were run at 25 °C for 30 min and then analyzed as reported previously.

Antibodies. H3K4Me was detected using a polyclonal rabbit antibody (abcam ab8895). $H3K4Me_2$ was detected using a monoclonal rabbit antibody (abcam ab32356). $H3K4Me_3$ was detected using a polyclonal rabbit antibody (abcam ab8580). H3K4-Unmodified was detected using a monoclonal mouse antibody (Active Motif 39763). $H3K9Me_2$ was detected using a monoclonal mouse antibody (abcam ab1220). H3K36Me3 was detected using a polyclonal rabbit antibody (abcam ab9050). H3K9Ac was detected using a polyclonal rabbit antibody (abcam ab4441). Total H3 was detected using a polyclonal rabbit antibody (abcam ab1791). LSD1 was detected using a polyclonal rabbit antibody (abcam ab17721). Actin was detected using a monoclonal mouse antibody (Sigma A1978).

ChIP-seq Assay. LNCaP cells were seeded in 2, 150 x 25 mm tissue culture dishes (Corning 430599) per condition. Cells were grown to ~70% confluency, and after washing with phosphate-buffered saline (2 x 10 mL) (PBS, Gibco 10010-023), the cells were treated with either vehicle (DMSO) or 10 μM **12d** (bizine) (>97% purity as determined by NMR) and grown in serum-free media for 48 h. Cells were then cross-linked with 1% formaldehyde for 10 min at 37 °C. Cells were then placed on ice and washed with ice cold PBS (2 x 10 mL), scraped and pelleted. Pellets were then resuspended in PIPES buffer (5 mM PIPES (pH 8.0), 85 mM KCl, 0.5% NP-40, 1x cOmplete, EDTA-free, Protease Inhibitor Cocktail Tablets (Roche)), lysed in lysis buffer (1% SDS, 10 mM EDTA, 50 mM Tris-HCl pH 8.1, 1x cOmplete, EDTA-free, Protease Inhibitor Cocktail Tablets (Roche)), and sonicated to shear cross-linked DNA. Samples were kept in an ice bath at all times. Nucleic acid concentration was then measured using a Nanodrop (Thermo Scientific). The nucleic acid (20-100 μg) was then

resuspended in 450-1,000 µL ChIP dilution buffer (0.01% SDS, 1.1% Triton-X 100, 1.2 mM EDTA, 16.7 mM Tris-HCl pH 8.1, 167 mM NaCl, 1x cOmplete, EDTA-free, Protease Inhibitor Cocktail Tablets (Roche)), and pre-cleared by adding 30 µL Protein A Dynabeads (Invitrogen) and rotated for 30 minutes at 4°C. Samples were then incubated overnight at 4 °C with 5 µg of polyclonal rabbit H3K4Me2 (milipore 07-030) (a no antibody control sample was included). 65 µL Dynabeads were then added to the samples and rotated for 2 h at 4°C. Dynabeads were then washed 2x with a low salt wash (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris pH 8.1, 150 mM NaCl); 1x with LiCl wash (0.25 M LiCl, 0.5% NP-40, 0.5% Na Deoxycholate, 1 mM EDTA, 10 mM Tris-HCl pH 8.1); and 2x with TE pH 8.0. Elution buffer was then added to the beads (1% SDS, 0.1 M $NaHCO_3$) and samples were vortexed and rotated at RT for 15 minutes and sample transferred to a new tube. This step was repeated 2x. Cross-linking was reversed by the addition of 20 µL 5 M NaCl and heating at 65°C for 4 h. 10 mM EDTA, 40 mM Tris-HCl pH 6.5, and 40 µg Proteinase K (Thermo Scientific #EO0491) were then added and samples were incubated for 1 h at 45 °C. 500 µL phenol:chloroform was then added to the samples and they were rotated overnight at 4°C. Samples were then spun and the top layer (aqueous) was placed in a new tube. An equal volume of chloroform was added and vortexed and spun and the bottom layer discarded again. 50 µg mL^{-1} of GlycoBlue (Life Technologies AM9515), 0.5 M NaOAc pH 5.2, and 2 volumes of 100% ethanol was added and samples were placed on ice for 15 minutes. Samples were then spun down and pellet was washed with 1 volume 70% EtOH and let dry. The pellets were then resuspended in TE and DNA concentrations were quantified by Qubit assay HS kit (Invitrogen Q32851).

Next Generation Sequencing/Library Generation. Libraries were prepared from 10-20 ng of IP ChIP DNA and 100 ng of input DNA according to Illumina's instructions along with the ChIP-seq DNA Sample Prep Kit (IP-102-1001). Briefly, samples were checked for quality and concentration from 150-250 bp on a bioanalyzer. DNA was end-repaired using Klenow polymerase in 58 µL of reaction buffer. For IP DNA, Klenow was diluted 1:5. Samples were incubated at 20°C for 30 minutes and subsequently purified on QIAquick PCR purification columns. A-tails were then added to the DNA with Klenow

and dATP in NEB buffer 2 at 37°C for 30 minutes and cleaned with Qiagen MiniElute PCR purification columns. Sequencing adapters were then ligated onto the DNA for 15 minutes at room temperature followed by cleaning with MiniElute columns. Samples were then run on 2% agarose gels and DNA from 216-366 bp (DNA plus adapters) were cut from the gel and purified with a Qiagen QIAquickGel Extraction kit. Concentrations were then checked on a bioanalyzer and 8 ng were PCR amplified with Phusion polymerase (Fisher) for 15 cycles (10 sec 98°C, 30 sec 65°C, 30 sec 72°C) followed by 5 minutes at 72°C. Samples were then cleaned with Ampure kits (Illumina) and washed with 80% ethanol. DNA samples were resuspended at the end of the cleanup into 17.5 µL buffer EB (Qiagen) and subjected to next generation sequencing on Illumina HiSeq platform according to manufacturers instructions.

Peak Calling and Statistical Analysis of ChIP-seq Data. 46 bp paired-end sequencing data were aligned to the reference human genome (hg19) using BWA with default parameters[13]. After alignment, duplicate reads were removed and only uniquely aligned reads were kept for further analysis. For narrow H3K4Me2 peaks, MACS2 were used for peak calling with default parameters[14]. Differential peaks between samples with two biological replicates were identified by diffReps[15]. Ensemble human genome annotations were used to identify the human genes around identified peak regions. A gene is defined to be around a peak region if the closest distance between its Transcription Start Site (TSS) and the peak region is less than 2000 bp. In total 2432 Ensemble genes were found to be around the identified peak regions. Furthermore, to compare this ChIP-seq data set to the data set generated by Kerenyi et al., where target genes around LSD1(-/-)-specific and wt-specific histone modification peaks in Gr1dim Mac1+ cells were reported[16], we translated our Ensemble gene names into official symbol gene names. In this process, microRNA and genes represented by non-standard gene names were removed. A total of 1767 genes with official symbol names were identified. Utilizing all of the human genes identified with official symbol names for normalization, we computed the overlap significance by cumulative hypergeometric distribution. 146 of the 1587 Lsd1 KO-specific genes were recovered from our data set (p-val=0.0028). As a negative control, only 17 of the wt specific genes (TSG) were

recovered (p-val=0.186). Additionally, to identify the number of tumor suppressor genes in the 146 genes identified to be in common, we used two TSG data sets. One data set used was from Vanderbilt University (http://bioinfo.mc.vanderbilt.edu/TSGene/Human_716_TSGs.txt), which contains 716 TSG genes. The other data set used was from Memorial Sloan-Kettering Cancer Center (http://cbio.mskcc.org/CancerGenes/), which contains 873 TSG genes. Utilizing all of the human genes to normalize, we utilized a cumulative hypergeometric distribution to compute the number of TSG in our data set. From the two TSG datasets, 18 and 19 of the 146 recovered genes are TSG genes, with p-val of 3.72E-7 and 1.50E-6 respectively. Combining the two dataset together to define the total TSG genes (covering 1146 distinct TSG genes in total), we identified 26 of the 146 recovered genes as TSG genes, with a p-val of 5.80E-9.

[^{3}H] Thymidine Assay. Cells were seeded in 96 well plates (Corning 3595). Cells were treated at ~70% confluency with **12d** (bizine) (>97% purity as determined by NMR) in serum-free media for 48 h. 6 hours prior to harvesting cells, 10 µL of 0.1 mCi ml^{-1} Thymidine [methyl-^{3}H] (ARC ART0178) was added to each well. The cells were then harvested (PerkinElmer) and radioactivity was measured with a liquid scintillation counter (PerkinElmer MicroBeta).

Drug Combination Experiments. The H460 cell line was exposed to drugs alone or in combination. **12d** (bizine) (>97% purity as determined by NMR) was added at three different fixed concentrations while the concentration of the other drug added was varied. After 48 h of treatment in serum-free media the [^{3}H] Thymidine Assay was performed as described above. The CPM of drug treated wells were compared to the CPM of control wells to calculate each fraction affected (FA), where FA = X means a decrease in growth of X%. Drug synergy was determined by isobologram analysis and derived from the median-effect principle of the Chou-Talalay method[17]. The combination index (CI) was calculated using CompuSyn^{TM} (ComboSyn Inc., Paramus, NJ) and the multiple drug effect equation[17] to evaluate drug interactions. A CI greater than, equal to, and less than one, respectively, indicates antagonistic activity, additivity, or synergy

between two drugs. Data are presented from one representative experiment. Each experiment was repeated at least two independent times with nearly identical results.

Accession Numbers. The GEO accession number for all unpublished ChIP-seq data reported in this paper is GSE55089.

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