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Structure–Activity Relationship Studies of α-Ketoamides as Inhibitors of the Phospholipase A and Acyltransferase Enzyme Family

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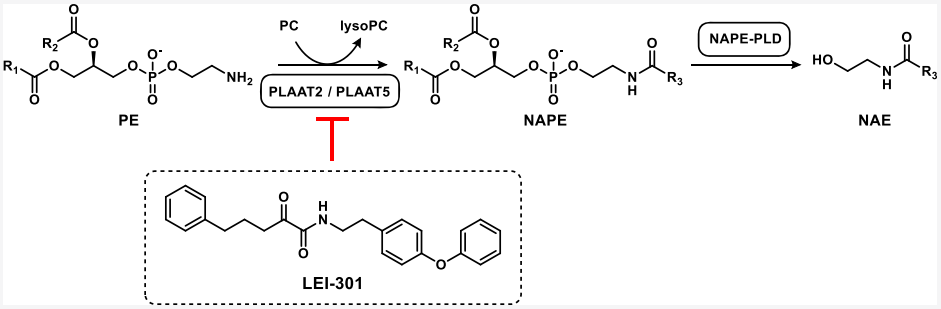

ABSTRACT: The phospholipase A and acyltransferase (PLAAT) family of cysteine hydrolases consists of five members, which are involved in the Ca^{2+}-independent production of *N*-acylphosphatidylethanolamines (NAPEs). NAPEs are lipid precursors for bioactive *N*-acylethanolamines (NAEs) that are involved in various physiological processes such as food intake, pain, inflammation, stress, and anxiety. Recently, we identified α-ketoamides as the first pan-active PLAAT inhibitor scaffold that reduced arachidonic acid levels in PLAAT3-overexpressing U2OS cells and in HepG2 cells. Here, we report the structure–activity relationships of the α-ketoamide series using activity-based protein profiling. This led to the identification of **LEI-301**, a nanomolar potent inhibitor for the PLAAT family members. **LEI-301** reduced the NAE levels, including anandamide, in cells overexpressing PLAAT2 or PLAAT5. Collectively, **LEI-301** may help to dissect the physiological role of the PLAATs.

INTRODUCTION

The subfamily of phospholipase A and acyltransferases (PLAATs) consists of five members with reported roles in tumor suppression and phospholipid metabolism.[1,2] They belong to the lecithin retinol acyltransferase (LRAT) protein family that is part of the NlpC/P60 superfamily of thiol hydrolases. The LRAT family has a conserved catalytic motif of six amino acids (NCEHFV) containing a cysteine residue that acts as the active site nucleophile.[3] A C-terminal hydrophobic tail is shared by PLAAT1–4, serving as a single-pass transmembrane anchoring domain.[4] The physiological functions of the PLAAT family members are only partly understood.[5] PLAATs are multifunctional enzymes that display varying degrees of *N*- and *O*-acyltransferase or phospholipase $A_{1/2}$ activity *in vitro*.[5−11] In addition, PLAAT4 (also known as RARRES3, HRASLS4, TIG3, or RIG1) is involved in protein deacylation of WNT and H-RAS proteins in breast cancer cells, thereby controlling cell growth.[12] Biological functions have been described for PLAAT3 (also known as PLA2G16, HRASLS3, AdPLA, or HREV107), which primarily acts as a phospholipase $A_{1/2}$ and regulates lipolysis in adipose tissue.[4,10,13] Notably, *Plaat3* knockout mice were protected against diet-induced obesity.[12] Recently, PLAAT3 was reported to be a host factor for picornaviridae by facilitating the entry of viral RNA into the cytosol from virus-containing endosomes.[14,15] As such, inhibitors of PLAAT3 hold promise as anti-obesity or anti-viral agents.

Less is known about the other PLAAT enzymes. Ueda and co-workers reported that *in vitro*, PLAAT2 (also known as HRASLS2) displays the highest *N*-acyltransferase activity of all PLAATs, followed at some distance by PLAAT5 (also known

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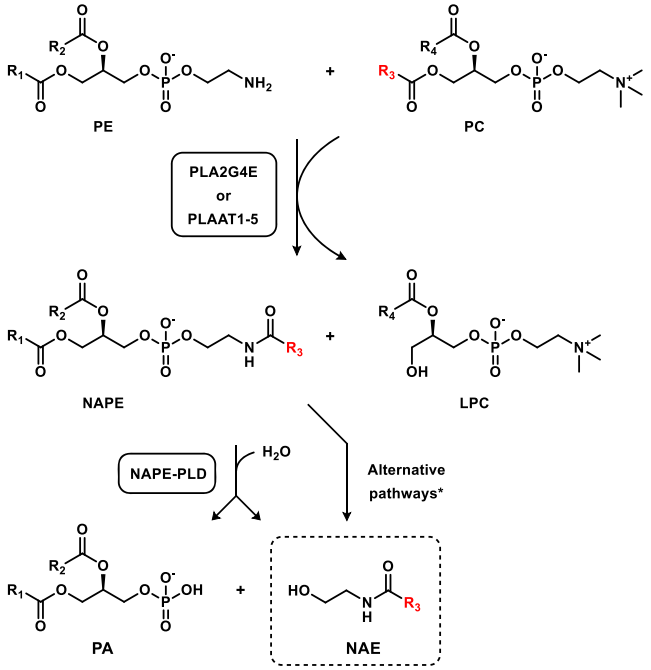
as iNAT or HRASLS5).[16] This reaction involves the transfer of an acyl group from phosphatidylcholine (PC) to phosphatidylethanolamine (PE), generating *N*-acylphosphatidylethanolamine (NAPE) and lyso-PC (LPC) (Scheme 1). First, the phospholipids that serve as precursors for *N*-acylethanolamines (NAEs), an important family of signaling molecules that includes the endocannabinoid anandamide [*N*-arachidonoylethanolamine (AEA)].[17] Through activation of the cannabinoid CB_1 receptor, anandamide is involved in physiological processes such as appetite, pain sensation, memory formation, stress, and anxiety.[18−20] The canonical enzyme responsible for NAPE biosynthesis in the brain is a Ca^{2+}-dependent *N*-acyltransferase (Ca-NAT), recently identified as PLA2G4E.[21] NAPEs are in turn converted to NAEs in one step by NAPE-phospholipase D (NAPE-PLD) as well as other multistep pathways.[5] In contrast, the PLAAT family members operate *via* a calcium-independent mechanism, providing an alternative pathway through which NAPEs and NAEs are biosynthesized.[16] PLAAT2 was reported to preferably transfer the sn-1 over the sn-2 acyl group of PC, which suggests that it mostly generates saturated or monounsaturated NAEs.[16] Furthermore, HEK293 cells stably overexpressing PLAAT2 exhibited highly increased NAPE and NAE levels. Gene expression of *PLAAT2* was found in the lung, liver, kidney, small intestine, colon, testis, and trachea.[9,22] NAEs have well-established signaling roles in the gastrointestinal system.[23] For instance, *N*-oleoylethanolamine (OEA) was found to act as a satiety factor *via* activation of peroxisome proliferator-activated receptor-α (PPAR-α).[24] This raises the possibility that PLAAT2 is involved in NAE biosynthesis in the gut. Notably, rodents lack the gene that encodes PLAAT2, thereby hindering the development of genetic models.[9]

As of yet, no physiological functions are known for PLAAT5. Of all PLAAT family members, PLAAT5 does not have a reported tumor-suppressing role.[5] High gene expression levels were found in testes of mice, rats, and humans as well as in human pancreas.[8,25] PLAAT5 activity was mainly localized to the cytosol fraction, while an inactive form of the enzyme was found to be membrane associated.[8] *In vitro*, PLAAT5 displayed higher *N*-acyltransferase than phospholipase $A_{1/2}$ activity.[16] Importantly, compared to PLAAT2, PLAAT5 showed no preference for the sn-1 or sn-2 acyl group of PC, suggesting that it could be involved in *N*-arachidonoyl-PE and thus anandamide biosynthesis.[6,8] PLAAT inhibitors would be valuable pharmacological tools to study the biological role of PLAAT2 and PLAAT5.

Scheme 1. Biosynthesis of NAPEs and NAEs[a]



[a]The sn-1 acyl group of PC is transferred to the amine of PE by the acyltransferase activity of PLA2G4E or PLAAT1−5 forming *N*-acyl-PE (NAPE) and LPC. NAPE-PLD hydrolyzes the phosphodiester bond of NAPE to form NAE and phosphatidic acid (PA). R_1, R_2, and R_3 denote saturated, mono-, or poly-unsaturated fatty acids. * For the alternative pathways see ref 5.

PLAAT enzyme forms an acyl thioester intermediate using its Cys-His-His catalytic triad, thereby expelling LPC. This is followed by the nucleophilic attack of the PE-amino group, producing the NAPE product and liberating the catalytic cysteine. NAPEs are an underexplored class of triacylated

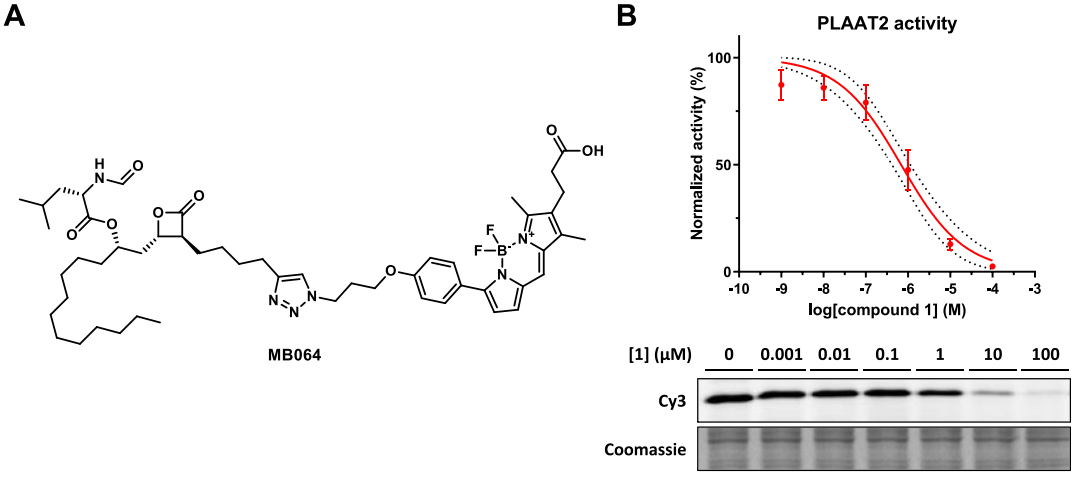


Figure 1. Evaluating PLAAT activity using competitive ABPP. (A) Structure of broad-spectrum lipase probe MB064. (B) Representative gel and apparent IC_{50} curve of a competitive ABPP experiment for PLAAT2. Labeling by MB064 and dose-dependent inhibition by 1 (apparent $pIC_{50} = 6.2 \pm 0.1$, dotted lines show 95% confidence interval). Data represent mean values ± SEM ($n = 3$). Coomassie staining was used as a protein loading control.

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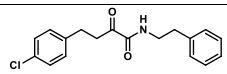
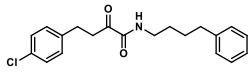
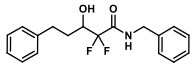
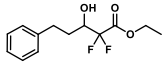
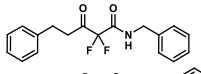
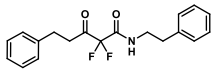
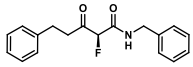
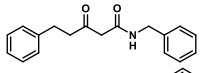
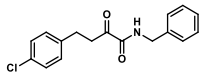
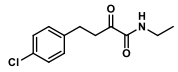
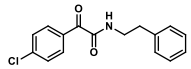
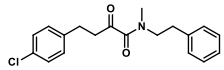
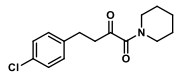
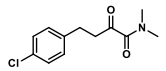
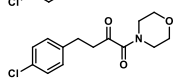
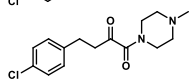
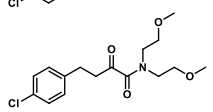
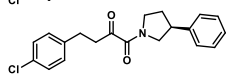
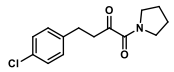
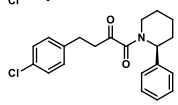
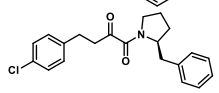
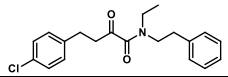
Table 1. SAR Analysis of Keto- and Hydroxy-amides 1−22

ID	Structure	apparent pIC_{50} ± SEM			
		PLAAT2	PLAAT3	PLAAT4	PLAAT5
1		6.2 ± 0.1	6.0 ± 0.1	5.9 ± 0.1	6.4 ± 0.1
2		6.2 ± 0.1	6.0 ± 0.1	5.8 ± 0.1	6.7 ± 0.1
3		< 5	< 5	< 5	< 5
4		< 5	< 5	< 5	< 5
5		< 5	< 5	< 5	< 5
6		< 5	< 5	< 5	< 5
7		< 5	< 5	< 5	< 5
8		< 5	< 5	< 5	< 5
9		5.6 ± 0.1	5.8 ± 0.1	5.6 ± 0.1	6.6 ± 0.1
10		5.6 ± 0.1	5.1 ± 0.1	5.4 ± 0.1	6.0 ± 0.1
11		< 5	< 5	< 5	< 5
12		< 5	< 5	< 5	< 5
13		< 5	< 5	< 5	< 5
14		< 5	< 5	< 5	< 5
15		< 5	< 5	< 5	< 5
16		< 5	< 5	< 5	< 5
17		< 5	< 5	< 5	< 5
18		< 5	< 5	< 5	< 5
19		< 5	< 5	< 5	< 5
20		< 5	< 5	< 5	< 5
21		< 5	< 5	< 5	< 5
22		< 5	< 5	< 5	< 5

Recently, we described the discovery of α-ketoamide **LEI-110** as the first pan-active PLAAT inhibitor using a gel-based competitive activity-based protein profiling (ABPP) assay.[26] **LEI-110** was able to reduce arachidonic acid levels in PLAAT3-overexpressing cells and in HepG2 cells. Here, we report the structure−activity relationship (SAR) of a library of α-

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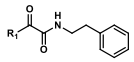
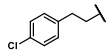
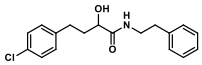
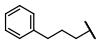
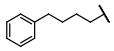
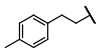
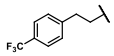
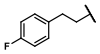
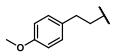
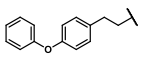
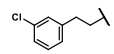
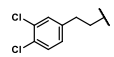
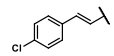
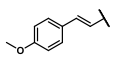
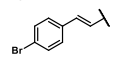
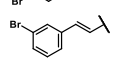
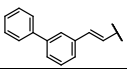
Table 2. SAR Analysis of α-Ketoamide Analogues 1 and 23−42



ID	R_1:	cLogP[a]	apparent pIC_{50} ± SEM			
			PLAAT2	PLAAT3	PLAAT4	PLAAT5
1		4.37	6.2 ± 0.1	6.0 ± 0.1	6.2 ± 0.1	6.4 ± 0.1
23		3.83	< 5	< 5	< 5	< 5
24		3.66	< 5	< 5	5.5 ± 0.1	6.0 ± 0.1
25		4.04	6.3 ± 0.1	6.4 ± 0.1	6.2 ± 0.1	7.0 ± 0.1
26		4.57	5.5 ± 0.1	6.0 ± 0.1	5.8 ± 0.1	6.8 ± 0.1
27		3.04	< 5	< 5	< 5	< 5
28		1.56	< 5	< 5	< 5	< 5
29		4.16	5.3 ± 0.1	5.7 ± 0.1	5.8 ± 0.1	5.6 ± 0.1
30		4.54	5.7 ± 0.1	5.1 ± 0.1	5.6 ± 0.1	6.5 ± 0.1
31		3.80	5.1 ± 0.1	5.3 ± 0.1	5.0 ± 0.1	5.8 ± 0.1
32		3.58	5.5 ± 0.1	5.5 ± 0.1	5.9 ± 0.1	5.9 ± 0.1
33		5.76	5.4 ± 0.1	5.4 ± 0.1	5.9 ± 0.1	6.9 ± 0.1
34		4.37	5.9 ± 0.1	5.7 ± 0.1	5.6 ± 0.1	5.8 ± 0.1
35		4.37	5.6 ± 0.1	5.8 ± 0.1	5.9 ± 0.1	5.9 ± 0.1
36		4.97	5.7 ± 0.2	7.0 ± 0.2	6.0 ± 0.1	7.0 ± 0.1
37		4.37	5.8 ± 0.1	5.6 ± 0.1	5.6 ± 0.1	6.2 ± 0.1
38		3.66	5.1 ± 0.1	5.8 ± 0.1	5.0 ± 0.1	5.2 ± 0.2
39		3.58	5.8 ± 0.1	5.3 ± 0.1	5.6 ± 0.1	6.0 ± 0.1
40		4.52	5.3 ± 0.1	5.6 ± 0.1	5.2 ± 0.1	6.2 ± 0.1
41		4.52	< 5	< 5	5.1 ± 0.1	5.5 ± 0.1
42		5.55	5.6 ± 0.1	5.5 ± 0.1	5.6 ± 0.1	6.0 ± 0.1

[a]clogP was calculated using Chemdraw 15.

ketoamides on the PLAAT family members. Next to **LEI-110**, we also identified **LEI-301** as a nanomolar potent PLAAT2 inhibitor with similar potency for other members of the PLAAT family, which was selective over the proteins of the endocannabinoid system (ECS). **LEI-301** reduced the NAE levels in PLAAT2- and PLAAT5-overexpressing cells but not in control cells. These findings show that **LEI-301** is a new pharmacological tool to study the biological role of PLAATs in cellular systems.

RESULTS AND DISCUSSION

Screening for PLAAT Inhibitors Using Competitive ABPP. A focused in-house library of lipase inhibitors was screened for PLAAT inhibition using gel-based competitive ABPP.[26] This method uses an activity-based probe (ABP) containing an electrophilic group that forms a covalent bond with the catalytic nucleophile of an enzyme.[27] ABPs are also equipped with a reporter group such as a fluorophore or biotin, which allows visualization of enzymatic activity in a native biological setting. In a competitive ABPP experiment, potential inhibitors are pretreated with a cell lysate that contains the protein of interest, followed by incubation with an ABP. After resolving the proteins by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and in-gel fluorescence scanning, the residual enzymatic activity can be determined by measuring the probe labeling intensity. Previously, MB064,[28] which incorporates a β-lactone electrophilic group, was validated as an effective ABP for the PLAAT enzyme family.[26]

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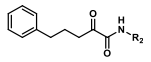
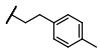
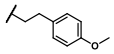
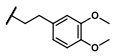
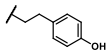
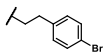
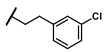
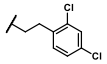
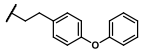
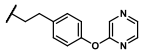
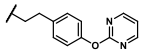
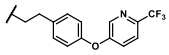
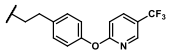
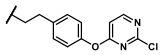
Table 3. SAR Analysis of Phenethyl Analogues 25 and 43−56



ID	R_2:	cLogP[a]	apparent pIC_{50} ± SEM			
			PLAAT2	PLAAT3	PLAAT4	PLAAT5
25		4.04	6.3 ± 0.1	6.4 ± 0.1	6.2 ± 0.1	7.0 ± 0.1
43		4.54	5.7 ± 0.1	6.9 ± 0.1	5.9 ± 0.1	7.4 ± 0.1
44		3.96	5.8 ± 0.1	6.1 ± 0.1	5.8 ± 0.1	7.1 ± 0.1
45		3.70	5.3 ± 0.1	6.0 ± 0.2	5.2 ± 0.1	6.5 ± 0.1
46		3.37	5.9 ± 0.1	5.8 ± 0.1	5.6 ± 0.1	6.8 ± 0.1
47		4.90	6.6 ± 0.1	6.8 ± 0.1	6.7 ± 0.1	8.0 ± 0.1
48		4.75	6.1 ± 0.1	6.7 ± 0.1	6.0 ± 0.1	7.3 ± 0.1
49		4.75	5.2 ± 0.1	5.9 ± 0.1	5.6 ± 0.1	6.1 ± 0.2
50		5.47	5.9 ± 0.1	6.6 ± 0.1	6.1 ± 0.1	7.2 ± 0.1
51 (LEI-301)		6.14	7.3 ± 0.1	6.6 ± 0.1	7.3 ± 0.2	7.4 ± 0.1
52		3.68	6.3 ± 0.1	6.6 ± 0.1	6.2 ± 0.1	7.2 ± 0.1
53		3.68	5.7 ± 0.1	5.7 ± 0.1	5.9 ± 0.1	6.6 ± 0.1
54		5.67	6.7 ± 0.1	6.8 ± 0.1	7.1 ± 0.1	7.1 ± 0.1
55 (LEI-110)		5.67	6.8 ± 0.1	7.0 ± 0.1	6.2 ± 0.1	7.5 ± 0.1
56		4.44	6.2 ± 0.1	6.6 ± 0.1	6.7 ± 0.1	7.1 ± 0.1

[a]cLogP was calculated using Chemdraw 15.

Here, cytosol fractions of human embryonic kidney HEK293T cells that overexpressed PLAAT2−5 were treated with potential inhibitors (10 μM, 30 min), followed by incubation with MB064 (250 nM, 20 min) (Figure 1A). The proteins were then resolved by SDS-PAGE, and the PLAAT activity was quantified by in-gel fluorescence scanning. A compound that showed residual protein labeling of ≤50% was considered to be active. For these active compounds, an IC_{50} curve was generated using a dose−response ABPP experiment (Figure 1B). Data for PLAAT2, PLAAT3, PLAAT4, and PLAAT5 are reported in Table 1 as pIC_{50} ± SEM (n = 3). Unfortunately, PLAAT1 could not be tested because of a lack of protein expression in HEK293T cells. α-Ketoamides **1** and **2** were identified as submicromolar hits, showing similar potency for all tested PLAAT members. An early SAR emerged from the structurally similar keto- and hydroxy-amides (**3**−**22**) present in this focused screening library. The α-position of the ketone next to the amide was essential for binding (compare α-ketoamides **1** and **2** with β-ketoamides **5**−**8**). β-Hydroxyamides (**3** and **4**) were inactive. Removing the alkyl spacer (**11**) was also detrimental for activity. Furthermore, the phenethylamine of **1** was preferred over benzylamine (**9**) and ethylamine (**10**). N-methylation resulted in complete loss of activity (**12**), which suggested that the N−H is potentially involved in hydrogen bond formation or that the methyl group has a steric clash with the protein. Similarly, secondary amides incorporating either (hetero)cyclic (**13**, **15**, **16**, and **18**−**21**) or acyclic (**14**, **17**, and **22**) motifs did not show any activity.

Evaluation of an α-Ketoamide Inhibitor Library Delivers Nanomolar Hit LEI-301. α-Ketoamide **1** exhibited the smallest molecular weight (MW = 316) and highest overall potency for the PLAAT enzymes; therefore, this compound was resynthesized and its activity was confirmed on all PLAAT members with a pIC_{50} ranging from 6.0 to 6.4 (Table 2). It was envisioned that the electrophilic ketone of **1** could bind with the PLAAT active site cysteine through a reversible covalent mechanism forming a hemithioketal adduct, similar to other reported α-ketoamide inhibitors.[26,29] To test this hypothesis, compound **23** was prepared, in which the ketone was replaced by an alcohol. This compound showed no activity at 10 μM (Table 2), which is in line with the hypothesis.

To systematically investigate the SAR and improve the potency of **1**, R_1-ketone and R_2-phenethyl analogues were synthesized (compounds **24**−**56**) (Tables 2 and 3). First, the effect of various substitutions on the R_1-group of **1** was evaluated with derivatives **24**−**36** (Table 2). The removal of chlorine (**24**) was detrimental for the activity for PLAAT2−4 but not PLAAT5. The length of the alkyl chain was studied in analogues **24**−**27**, showing that the propylene derivative **25** was optimal, which had increased potency for PLAAT2, PLAAT3, and PLAAT5. The 4-chloro substituent on the phenyl ring seemed to be optimal based on the inhibitory activity of compounds **29**−

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33. Electron-donating groups such as 4-methyl (29) and 4-methoxy (32) substituents decreased the potency, but a lipophilic electron-withdrawing group (*e.g.*, 4-trifluoromethyl, 30) was tolerated for PLAAT5. A small (4-fluoro, 31) substituent lowered the activity, while a large group (4-phenoxy, 33) provided a selectivity window for PLAAT5 over the other PLAATs of 10- to 30-fold. Furthermore, the substitution of the 4-chloro to the ortho or meta positions did not result in improved potency (compounds 34 and 35). The 3,4-dichloro derivative 36, however, presented increased activities for PLAAT3 and PLAAT5. Taken together, these compound series point toward the presence of a small lipophilic pocket, restricted in size, which is occupied by the alkylphenyl group.

Next, β,γ-unsaturated α-ketoamides 37−42 were evaluated to test whether conformational restriction of the alkyl linker would lead to a gain in activity (Table 2). Although unsaturation was tolerated in the alkyl chain, no or little improvement in potency was observed for these derivatives (compare compounds 1, 24, 32, and 37−39). Also, 4-bromo, 3-bromo, or 3-phenyl substitutions (40−42) did not provide the desired inhibitory activity increase. Overall, this suggests that the R_1 group is positioned toward a shallow pocket.

Next, the focus was shifted toward the optimization of the R_2-phenethyl moiety. Analogues 43−56 incorporating substituted phenethylamines were prepared in combination with the 2-oxo-5-phenylpentanoyl motif of compound 25, which demonstrated the highest PLAAT2 activity (Table 3). The compounds showed a comparable SAR on PLAAT2 and PLAAT4, as substitutions on the para position were unfavorable for methyl (43), methoxy (44), and hydroxyl (46). Substitutions on the meta and ortho positions (48, 49) or 2,4-disubstituted methoxy (45) or chloro (50) also did not afford an improvement in potency. Increasing the lipophilicity gave a 2- to 3-fold increase in activity for 4-bromo analogue 47. Further expansion with a 4-phenoxy moiety (51) improved the potency both for PLAAT2 and PLAAT4 by 10-fold compared to 25. The introduction of a 4-methyl group in compound 43 enhanced the inhibitory activity for PLAAT3 and PLAAT5. Other lipophilic groups [4-bromo (47) and 4-phenoxy (51)] gave even higher activities for PLAAT5 especially.

The addition of the phenoxy group increased the clogP of 51 with 2 log units to 6.14 (calculated with Chemdraw 15); therefore, more polar heteroaryl rings were investigated to lower the lipophilicity (52−56) (Table 3). Compound 55 (LEI-110) was identified as the most potent inhibitor of PLAAT3 and its biological characterization has previously been described in detail.[26] With regard to PLAAT2, a decrease in activity was observed for compounds 52−56 compared to 51. Therefore, being the most potent inhibitor of PLAAT2, 51 (termed LEI-301) was selected for further characterization.

***In Silico* Modeling of α-Ketoamide Inhibitors.** To explain the binding mode of the α-ketoamide inhibitors in PLAAT2, LEI-301 and 1 were docked in a PLAAT2 crystal structure (PDB: 4DPZ).[4] Residues 39−52 and 105−111 were absent from this structure; therefore, a homology model was prepared using the closely related PLAAT3 crystal structure (PDB: 4DOT)[4] from which the shape of the loop for residues 105−111 could be adopted. A second loop comprising residues 39−52 was modeled based on sequence, as it is not present in both crystal structures. Because our data suggested that the electrophilic ketone of α-ketoamides could engage with the active site cysteine through a reversible covalent mechanism,[29] LEI-301 and 1 were covalently docked to Cys113 in the enzyme (Figure 2). Both compounds revealed a hydrogen bonding network between the oxy-anion and amide carbonyl with His23

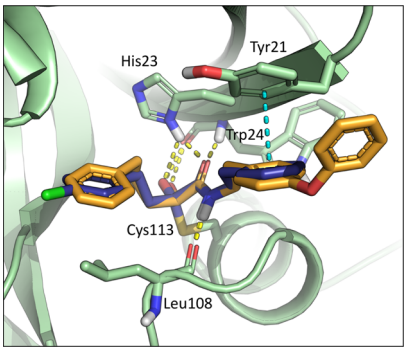

Figure 2. Docking pose of **LEI-301** and **1** with PLAAT2. Compounds **1** (blue) and **LEI-301** (orange) in complex with PLAAT2, covalently bound to Cys113. Yellow dotted lines represent a hydrogen bond, and cyan represents π-interactions.

and the Trp24 backbone amide N−H, while the backbone carbonyl of Leu108 formed a H-bond with the amide of the inhibitors. Introduction of the 4-phenoxy group in LEI-301 suggested that an additional $\pi-\pi$ stacking interaction with Tyr21 would be possible. This offered a potential reason for the observed activity increase of LEI-301.

Selectivity Profile of LEI-301 for the ECS. The affinity or activity of LEI-301 for the receptors and metabolic enzymes of the ECS was determined to assess its selectivity profile. Minimal affinity (<50%) was observed at 10 μM for the cannabinoid receptors types 1 and 2 (CB_1/CB_2) (Table 4). The enzymes

Table 4. Affinity of LEI-301 for Cannabinoid Receptors CB_1 and CB_2

radioligand displacement at 10 μM **LEI-301** (% ± SD; N = 2, n = 2)	
hCB_1	hCB_2
49 ± 8	32 ± 4

involved in NAE biosynthesis (PLA2G4E, NAPE-PLD) and degradation (FAAH) were also not inhibited at this concentration (Table 5). The enzymes involved in the metabolism of the other endocannabinoid 2-arachidonoylglycerol (2-AG), such as diacylglycerol lipase α and β (DAGLα/β), monoacylglycerol lipase (MAGL), and α,β-hydrolase domain containing 6 (ABHD6) were not inhibited.

Targeted Lipidomics Shows That LEI-301 Reduces NAEs in PLAAT2-Overexpressing Cells. Having established that LEI-301 is a potent inhibitor of PLAAT2 and selective over the other enzymes of the ECS, it was investigated whether LEI-301 is active in a cellular setting. To this end, NAE levels, which are downstream metabolites of NAPEs generated by NAPE-PLD, were measured in living cells. Human U2OS osteosarcoma cells were therefore transiently transfected with a pcDNA3.1 plasmid containing the gene for PLAAT2, PLAAT5, or an empty (mock) vector. Of note, mRNA of NAPE-PLD was detected by quantitative PCR (qPCR) in this cell line, suggesting that NAPEs can be converted to NAEs (*NAPEPLD*: quantification cycle C_q ± SEM = 27.3 ± 0.05, *RPS18* (housekeeping gene): C_q ± SEM = 17.8 ± 0.01, n = 3; the presence of reference gene mRNA in combination with a $C_q \leq 29$ for the targeted mRNA is considered sufficient[30]). Targeted lipidomics on the lipid extracts of the transfected cells allowed the quantification of 8

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Table 5. Inhibitory Activities of LEI-301 for Metabolic Enzymes of the ECS[a]

remaining enzyme activity at 10 μM **LEI-301** (% ± SD; n = 3)						
hNAPE-PLD	hPLA2G4E	mDAGLα	mDAGLβ	hMAGL	mFAAH	mABHD6
92 ± 8	95 ± 5	97 ± 10	83 ± 1	105 ± 19	108 ± 4	92 ± 5

[a]Activities were obtained from surrogate (hNAPE-PLD) or natural (hMAGL) substrate assays. hPLA2G4E, mDAGLα/β, mFAAH, and mABHD6 were determined by gel-based ABPP.

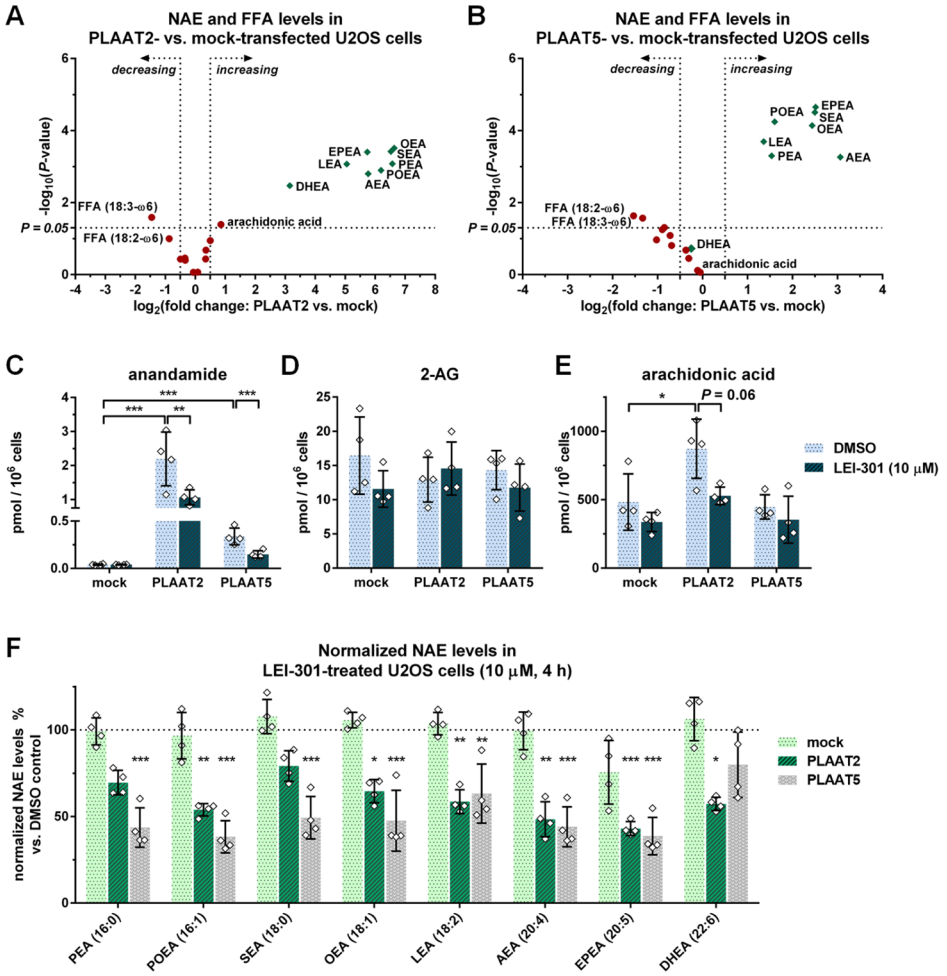

Figure 3. U2OS cells transfected with PLAAT2 or PLAAT5 exhibit highly increased NAE levels and LEI-301 can inhibit NAE formation. (A,B) Volcano plots depicting the $\log_2$(fold change) vs $-\log_{10}$(P-value) of NAEs (green diamonds) and FFAs (red circles) in (A) PLAAT2- or (B) PLAAT5- vs mock-transfected U2OS cells. (C–E) Absolute levels of (C) anandamide (AEA), (D) 2-AG and (E) arachidonic acid in mock-, PLAAT2- or PLAAT5-transfected cells treated with vehicle (DMSO) or **LEI-301** (10 μM, 4 h). (F) Normalized NAE levels of mock-, PLAAT2- or PLAAT5-transfected cells treated with **LEI-301** (10 μM, 4 h) represented as effect %. The data were normalized against mock-, PLAAT2- or PLAAT5-transfected cells treated with vehicle (DMSO). Absolute values are depicted in Figure S1. Data represent mean values ± SD for four biological replicates. *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$ by one-way ANOVA.

different NAEs and 10 free fatty acids (FFAs) by liquid chromatography–mass spectrometry (LC–MS). A striking increase of 9- to 99-fold for all NAE species was observed for the PLAAT2-overexpressing cells compared to control, including anandamide (AEA, 54-fold) (Figure 3A, Table 6). This result confirms previous findings.[16] Notably, PLAAT2 overexpression did not elevate most FFA species except for arachidonic acid (fold change ± SD = 1.81 ± 0.45, $P = 0.04$), while levels of γ-linolenic (18:3-ω6) and linoleic acid (18:2-ω6) were significantly decreased (Figure 3A, Table S1). In PLAAT5-transfected cells also a significant increase of NAE content was observed, although smaller in magnitude compared to PLAAT2 (Figure 3B, Table 6). Interestingly, *N*-docosahexaenoylethanolamine (DHEA) levels were unaffected by overexpression of PLAAT5. Furthermore, FFA levels including arachidonic acid were not elevated for this enzyme (Figure 3B, Table S1). Next, the PLAAT2/PLAAT5 or mock-transfected cells were incubated with 10 μM **LEI-301** for 4 h. A significant 2-fold reduction of anandamide was apparent in the PLAAT2 and PLAAT5 cells, which was absent in the control samples (for PLAAT2: $P = 0.006$; for PLAAT5: $P < 0.0001$) (Figure 3C,F). Other saturated, mono- and poly-unsaturated NAEs also showed significant reductions upon treatment with **LEI-301** in the PLAAT2 and PLAAT5 overexpressing cells but not in the mock cells (Figures 3F, S1). **LEI-301** did reduce arachidonic acid levels in PLAAT2-transfected cells; however, this did not meet significance ($P = 0.06$) (Figure 3E). In the case of PLAAT5, DHEA as well as arachidonic acid levels were not affected by

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Table 6. PLAAT2 and PLAAT5 Overexpression Greatly Increases the NAE Content in U2OS Cells[a,b]

	absolute NAE levels (pmol/10^6 cells ± SD)			fold change ± SD		*P*-value	
NAE	mock	PLAAT2	PLAAT5	PLAAT2/mock	PLAAT5/mock	PLAAT2	PLAAT5
PEA (16:0)	0.196 ± 0.05	18.62 ± 5.97	0.569 ± 0.10	95 ± 30	2.9 ± 0.5	0.0008	0.0005
POEA (16:1)	0.031 ± 0.01	2.247 ± 0.78	0.093 ± 0.01	73 ± 25	3.0 ± 0.4	0.0013	0.0001
SEA (18:0)	0.516 ± 0.12	47.39 ± 13.1	2.916 ± 0.42	92 ± 25	5.7 ± 0.8	0.0004	<0.0001
OEA (18:1)	0.190 ± 0.05	18.83 ± 5.02	1.027 ± 0.17	99 ± 26	5.4 ± 0.9	0.0003	0.0001
LEA (18:2)	0.047 ± 0.01	1.569 ± 0.50	0.121 ± 0.01	33 ± 10	2.6 ± 0.3	0.0009	0.0002
AEA (20:4)	0.040 ± 0.01	2.194 ± 0.79	0.337 ± 0.09	54 ± 20	8.4 ± 2.2	0.0016	0.0005
EPEA (20:5)	0.010 ± 0.01	0.555 ± 0.15	0.059 ± 0.01	53 ± 14	5.7 ± 0.8	0.0004	<0.0001
DHEA (22:6)	0.032 ± 0.01	0.286 ± 0.11	0.027 ± 0.01	8.9 ± 3.4	0.84 ± 0.1	0.0034	0.1886

[a]Data represent mean values ± SD for four biological replicates. *P*-values were determined by one-way ANOVA. [b]Abbreviations: PEA = *N*-palmitoylethanolamine, POEA = *N*-palmitoleoylethanolamine, SEA = *N*-stearoylethanolamine, OEA = *N*-oleoylethanolamine, LEA = *N*-linoleoylethanolamine, AEA = *N*-arachidonoylethanolamine, EPEA = *N*-eicosapentaenoylethanolamine, DHEA = *N*-docosahexaenoylethanolamine.

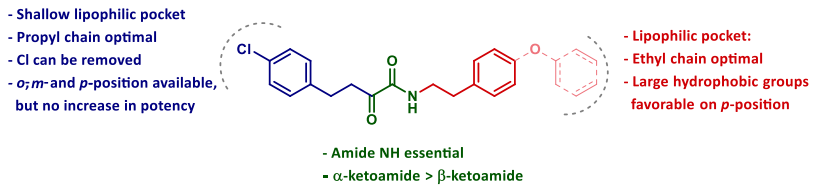
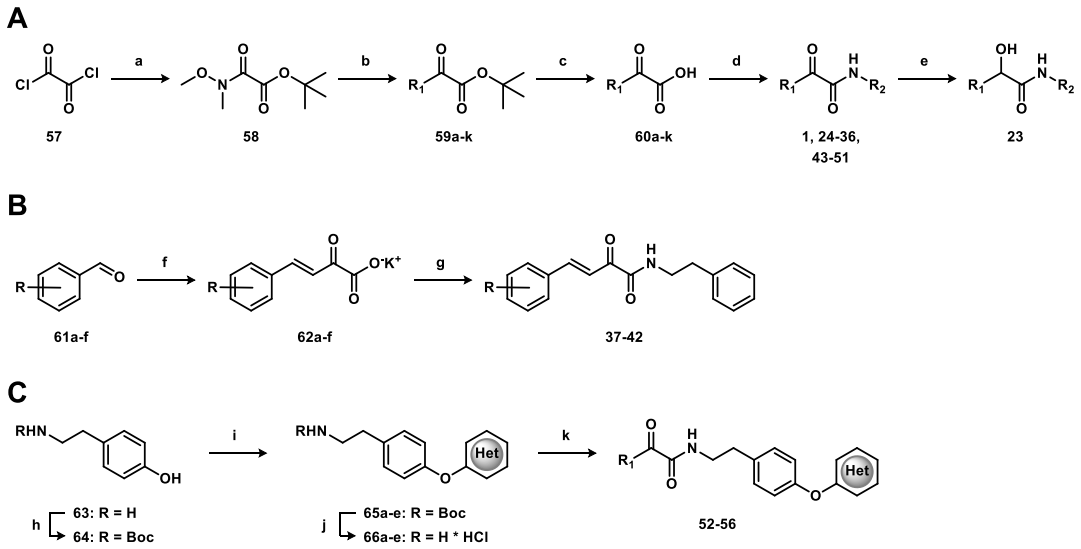


Figure 4. Structure−activity map for the PLAAT α-ketoamide inhibitor library.

Scheme 2. General Synthetic Routes for (A) α-Ketoamide 1 Analogues, (B) β,γ-Unsaturated α-Ketoamides, and (C) O-Heteroaryl Phenethylamine Derivatives[a]



[a]Reagents and conditions: (a) (i) *t*-BuOH, THF, 0 °C; (ii) *N*,*O*-dimethylhydroxylamine·HCl, Et_3N, 0 °C, 75%; (b) (i) Mg, alkylbromide, Et_2O, reflux; (ii) Weinreb amide, −78 °C, 21−83%; (c) TFA, DCM, rt, 99%; (d) HATU or HCTU, DiPEA, amine, DMF, rt, 22−80%; (e) $NaBH_4$, THF, rt, 72%. (f) Pyruvic acid or sodium pyruvate, KOH, MeOH, 0 °C to rt; (g) (i) oxalyl chloride, DCM, 0 °C to rt; (ii) phenethylamine, DCM, 0 °C to rt, 14−35% over two steps. (h) Boc_2O, $NaHCO_3$, THF, H_2O, rt, 85%; (i) heteroaryl halide, K_2CO_3, DMSO or DMF, rt or 85 °C, 63−92%; (j) HCl, dioxane, rt, 99%; (k) EDC·HCl, HOBt, ketoacid, NMM, DCM, 0 °C to rt, 15−30%.

treatment with **LEI-301** (Figure 3E,F). Notably, **LEI-301** did not alter the levels of the other endocannabinoid 2-AG in all tested conditions (Figure 3D). Previously, α-ketoamides (also termed 2-oxo-amides) were reported as inhibitors of various PLA_2 enzymes.[29,31−34] To test the selectivity of **LEI-301** in U2OS cells, we performed competitive ABPP experiments in membrane and cytosol fractions with broad-spectrum lipase probes fluorophosphonate-carboxytetramethylrhodamine (FP-TAMRA) and MB064. No inhibitory activity was observed for **LEI-301** at 10 μM for any of the probe-labeled enzymes (Figure S2A). In addition, members of the PLA2G4 family were also not inhibited by **LEI-301** (Figure S2B,C). Taken together, these results indicate that **LEI-301** can be used to study the biological roles of PLAAT2 and PLAAT5 in cellular systems.

CONCLUSIONS

In summary, we have described the discovery and optimization of an α-ketoamide inhibitor library for the PLAAT enzyme subfamily. The SAR of the R_1-ketone moiety proved to be narrow with little room for expansion (Figure 4). The R_2-phenethylamine side did allow the introduction of a large hydrophobic para-phenoxy group, which led to the identi-

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fication of **LEI-301** as a potent inhibitor for PLAAT2−5, having a 10-fold higher potency for PLAAT2 and PLAAT5 than our initial hit. Covalent docking in the PLAAT2 crystal structure provided a possible binding mode of **LEI-301**. Overexpression of PLAAT2 in U2OS cells resulted in a large increase of all measured NAE species, including the endocannabinoid anandamide, whereas no significant elevations of FFAs were observed except for arachidonic acid. Also PLAAT5 was able to increase the NAE content upon transient transfection, although this was smaller in magnitude compared to PLAAT2. These findings support the notion that PLAAT2 and PLAAT5 are involved in the biosynthetic pathways of the NAEs. Furthermore, treatment of overexpressing PLAAT2 and PLAAT5 cells with **LEI-301** gave a twofold reduction of anandamide levels, which was absent in control cells. This validates **LEI-301** as a promising tool compound to study PLAAT2 and PLAAT5 functions in biological systems. **LEI-301** allows acute blockade of these enzymes, which can be beneficial compared to genetic knockout models, where long-term compensatory effects can occur. In addition, *Plaat2* is not present in the rodent genome, which hampers the study of its biological function. Currently, it is unknown if the Ca^{2+}-independent PLAAT enzymes contribute to physiological NAPE and thus NAE biosynthesis. In contrast to the Ca^{2+}-dependent NAPE production by PLA2G4E, PLAATs may continuously produce NAPEs from their abundant PE and PC substrates. So far, PLA2G4E activity has been reported in heart, brain, and skeletal muscles.[21] Peripheral organs such as kidney, small intestine, and testis have well established NAE signaling roles and reported PLAAT2 or PLAAT5 expression.[5,18] Furthermore, these tissues show low Ca^{2+}-dependent PLA2G4E activity.[21] Therefore, these organs are prime candidates to assess the contribution of PLAAT enzymes with regard to NAPE and NAE formation using the inhibitors here disclosed.

CHEMISTRY

Oxalyl chloride (**57**) was reacted with *tert*-butanol and *N,O*-dimethylhydroxylamine·HCl, giving Weinreb amide **58**. Treatment with an *in situ* formed Grignard reagent from 4-chlorophenethyl bromide followed by *tert*-butyl deprotection gave ketoacid **60a**. Finally, amide coupling using HCTU afforded α-ketoamide **1**.

R_1-derivatives **24**−**36** and R_2-analogues **43**−**51** were synthesized *via* the general route (Scheme 2A). β,γ-Unsaturated α-ketoamides (**37**−**42**) were prepared using a two-step procedure (Scheme 2B): condensation of benzaldehyde (**61a**−**f**) with pyruvic acid, which afforded the β,γ-unsaturated α-ketoacid as the potassium salt (**62a**−**f**), followed by acid chloride formation and coupling with phenethylamine. O-Arylated 4-hydroxyphenethylamine derivatives **52**−**56** were synthesized *via* Scheme 2C. Tyramine (**63**) was Boc-protected, followed by nucleophilic aromatic substitution (S_NAr) with a heteroaryl halide. Boc deprotection and subsequent amide coupling provided the α-ketoamides **52**−**56**.

EXPERIMENTAL SECTION

Biological Procedures. *Plasmids.* Full-length cDNA of human PLAAT1−5 (obtained from prof. Ueda[8]) was cloned into mammalian expression vector pcDNA3.1 with a C-terminal FLAG-tag and containing genes for ampicillin and neomycin resistance. Plasmids were isolated from transformed XL10-Gold competent cells (prepared using Escherichia coli transformation buffer set; Zymo Research) using plasmid isolation kits following the supplier's protocol (Qiagen). All sequences were analyzed by Sanger sequencing (Macrogen) and verified (CLC Main Workbench).

Cell Culture. HEK293T and U2OS cells (ATCC) were cultured at 37 °C and 7% CO_2 in Dulbecco's modified Eagle's medium (Sigma-Aldrich, D6546) with GlutaMax (2 mM), penicillin (100 μg/mL, Duchefa), streptomycin (100 μg/mL, Duchefa), and 10% (v/v) newborn calf serum (Seradigm). The medium was refreshed every 2−3 days, and cells were passaged twice a week at 80−90% confluence. The cells were passaged twice a week by thorough pipetting (HEK293T) or trypsinization (U2OS) to appropriate confluence.

Transient Transfection. Transient transfection was performed, as described previously.[26] In brief, 10^7 HEK293T cells were seeded in 15 cm Petri dishes 1 day before transfection. Two hours before transfection, the medium was refreshed with 13 mL of the medium. Transfection was performed with polyethyleneimine (PEI, 60 μg per dish) in a ratio of 3:1 with plasmid DNA (20 μg per dish). PEI and plasmid DNA were incubated in a serum-free medium (2 mL per dish) at rt for 15 min, followed by dropwise addition to the cells. Transfection with the empty pcDNA3.1 vector was used to generate control (mock) samples. The medium was refreshed after 24 h, and cells were harvested after 48 or 72 h in cold phosphate-buffered saline (PBS). Cells were pelleted by centrifugation (5 min, 1000*g*), and the pellet was washed with PBS. The supernatant was removed and cell pellets were flash-frozen in liquid N_2 and stored at −80 °C.

Cell Lysate Preparation. Cell pellets were thawed on ice, resuspended in cold lysis buffer [50 mM Tris-HCl pH 8, 2 mM dithiothreitol (DTT), 1 mM $MgCl_2$, 2.5 U/mL benzonase], and incubated on ice for 30 min. The cytosolic fraction (supernatant) was separated from the membranes by ultra-centrifugation (100,000*g*, 45 min, 4 °C, Beckman Coulter, Ti 70.1 rotor). The pellet (membrane fraction) was resuspended in cold storage buffer (50 mM Tris-HCl pH 8, 2 mM DTT) and homogenized by thorough pipetting and passage through an insulin needle (29G). Protein concentrations were determined by a Quick Start Bradford protein assay (Bio-Rad) or Qubit protein assay (Invitrogen). The samples were flash-frozen in liquid N_2 and stored at −80 °C.

Mouse Brain Lysate Preparation. Mouse brains were thawed on ice, dounce homogenized in cold lysis buffer (20 mM HEPES pH 7.2, 2 mM DTT, 1 mM $MgCl_2$, 2.5 U/mL benzonase), and incubated on ice for 30 min. The membrane and cytosolic fractions of cell or tissue lysates were separated by ultracentrifugation (100,000*g*, 45 min, 4 °C). The supernatant was collected (cytosolic fraction) and the membrane pellet was resuspended in cold storage buffer (20 mM HEPES pH 7.2, 2 mM DTT) and homogenized by thorough pipetting and passage through an insulin needle (29G). Protein concentrations were determined using a Bradford assay (Bio-Rad). The samples were flash-frozen in liquid N_2 and stored at −80 °C.

ABPP on PLAAT2−5 Transfected HEK293T Cell Lysate. Gel-based ABPP was performed with minor changes, as described previously.[26] For ABPP assays on HEK293T cells overexpressing PLAAT2, the cytosol proteome (0.25 μg/μL, 20 μL) was preincubated with a vehicle (DMSO) or inhibitor (0.5 μL in DMSO, 30 min, rt), followed by incubation with MB064 (final concentration: 250 nM, 20 min, rt). For PLAAT3, PLAAT4, and PLAAT5, the protocols differed for the protein concentrations (0.5, 1, and 1 μg/μL, respectively) and MB064 concentrations (250, 500, and 500 nM, respectively). The final concentrations for the inhibitors are indicated in the main text and figure legends. For the dose−response experiments, only cytosol proteome was used. Proteins were denatured with 4× Laemmli buffer [5 μL, stock concentration: 240 mM Tris pH 6.8, 8% (w/v) SDS, 40% (v/v) glycerol, 5% (v/v) β-mercaptoethanol, 0.04% (v/v) bromophenol blue]. Ten μL of sample per reaction was resolved on a 10% acrylamide SDS-PAGE gel (180 V, 70 min). Gels were scanned using Cy3 and Cy5 multichannel settings (605/50 and 695/55 filters, respectively) on a ChemiDoc Imaging System (Bio-Rad). Fluorescence was normalized to Coomassie staining and quantified with Image Lab (Bio-Rad). The experiments were performed in triplicate. Dose−response IC_{50} curves were generated with GraphPad Prism 6.

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qPCR. For primer sequences used, see Table S2. RNA isolation and cDNA synthesis: total RNA from U2OS cells was extracted using a NucleoSpin RNA kit (Macherey-Nagel) according to the manufacturer's instructions. Subsequently, cDNA synthesis was carried out with a SuperScript First-Strand Synthesis System (Invitrogen) according to the manufacturer's instructions. qPCR analysis: 2.5 ng of input cDNA was analyzed using SYBRGreen qPCR master mix (Thermo Fisher) on a CFX96 optical thermal cycler (Bio-Rad). Data analysis was performed using CFX Manager software (Bio-Rad). The housekeeping gene 40S ribosomal protein S18 (*RPS18*) was used as a control. The data are expressed in quantitation cycles (C_q) ± SEM of three technical replicates.

Radioligand Displacement Assays for CB1 and CB2 Receptors. [^{3}H]CP55940 displacement assays to determine the affinity for the cannabinoid CB_1 and CB_2 receptors were performed, as previously described.[35]

NAPE-PLD Surrogate Substrate (PED6) Activity Assay. The human NAPE-PLD activity assay was performed, as previously described.[35]

Natural Substrate-Based Fluorescence Assay MAGL. The natural substrate assay for human MAGL was performed, as reported previously.[36]

ABPP for Determining mDAGLα/β, mFAAH, mABHD6, and hPLA2G4E Activities. Gel-based ABPP was performed, as previously described.[28] In brief, mouse brain membrane proteome or hPLA2G4E-overexpressing membrane lysate (9.5 μL and 2 μg/μL or 19.5 μL and 1 μg/μL, respectively) was preincubated with a vehicle or inhibitor (0.5 μL 20× or 0.5 μL 40× inhibitor stock in DMSO, respectively, 30 min, rt), followed by incubation with the activity-based probe MB064 (250 nM, 0.5 μL 20× stock in DMSO) or FP-TAMRA (500 nM, 0.5 μL 20× stock in DMSO) for mouse brain lysate (15 min, rt) or FP-TAMRA (50 nM, 0.5 μL 40× stock in DMSO) for PLA2G4E overexpressing lysate (5 min, rt). The final concentrations for the inhibitors are indicated in the main text and figure legends. Proteins were denatured with 4× Laemmli buffer (3.5 μL, stock concentrations: 240 mM Tris-HCl pH 6.8, 8% w/v SDS, 40% v/v glycerol, 5% v/v β-mercaptoethanol, 0.04% v/v bromophenol blue, 30 min, rt). The samples (10 μL per slot) were resolved by SDS-PAGE (respectively, 10 or 8% acrylamide for mouse brain or PLA2G4E lysate, 180 V, 75 min). Gels were scanned using Cy3 and Cy5 multichannel settings (605/50 and 695/55 filters, respectively) on a ChemiDoc Imaging System (Bio-Rad). Fluorescence was normalized to Coomassie staining and quantified with Image Lab (Bio-Rad).

*ABPP for Determining the Selectivity Profile of **LEI-301** in U2OS Cell Lysates.* U2OS cytosol or membrane lysate (19 μL, 2.5 μg/μL) was preincubated with a vehicle or inhibitor (0.5 μL 40× stock in DMSO, 30 min, rt), followed by incubation with the activity-based probe MB064 (2 μM, 0.5 μL 40× stock in DMSO, 15 min, rt) or FP-TAMRA (500 nM, 0.5 μL 40× stock in DMSO, 15 min, rt). The final concentrations for the inhibitors are indicated in the figure legends. Proteins were denatured with 3× Laemmli buffer (10 μL, stock concentrations: 240 mM Tris-HCl pH 6.8, 8% w/v SDS, 40% v/v glycerol, 5% v/v β-mercaptoethanol, 0.04% v/v bromophenol blue, 15 min, rt). The samples (12.5 μL per slot) were resolved by SDS-PAGE (180 V, 75 min). Gels were scanned using Cy3 and Cy5 multichannel settings (605/50 and 695/55 filters, respectively) on a ChemiDoc Imaging System (Bio-Rad). Fluorescence was normalized to Coomassie staining and quantified with Image Lab (Bio-Rad).

ABPP for Determining PLA2G4B, PLA2G4C, and PLA2G4D Activities. hPLA2G4B, hPLA2G4C, or hPLA2G4D-overexpressing membrane lysate (19 μL, 1 μg/μL) was pre-incubated with a vehicle or inhibitor (0.5 μL 40× inhibitor stock in DMSO, 30 min, rt), followed by incubation with the activity-based probe FP-TAMRA (500 nM, 0.5 μL 20× stock in DMSO, 20 min, rt). The final concentrations for the inhibitors are indicated in the figure legends. Proteins were denatured with 3× Laemmli buffer (10 μL, stock concentrations: 240 mM Tris-HCl pH 6.8, 8% w/v SDS, 40% v/v glycerol, 5% v/v β-mercaptoethanol, 0.04% v/v bromophenol blue, 30 min, rt). The samples (12.5 μL per slot) were resolved by SDS-PAGE (180 V, 75 min). Gels were scanned using Cy3 and Cy5 multichannel settings (605/50 and 695/55 filters, respectively) on a ChemiDoc Imaging System (Bio-Rad). Fluorescence was normalized to Coomassie staining and quantified with Image Lab (Bio-Rad).

ABPP for Determining the PLA2G4A Activity. Recombinant full-length human PLA2G4A (0.73 μL, 440 ng/μL, R&D systems, 6659-PL) was diluted with assay buffer (13.3 μL, 50 mM Tris HCl pH 8.0, NaCl 500 mM, $CaCl_2$ 20 mM in Milli-Q) and preincubated with a vehicle or inhibitor (0.5 μL 29× inhibitor stock in DMSO, 30 min, 37 °C). FP-alkyne was used as a positive control.[37] This was followed by incubation with FP-TAMRA (500 nM, 0.5 μL 30× inhibitor stock in DMSO, 20 min, rt). The final concentrations for the inhibitors are indicated in the figure legends. Proteins were denatured with 4× Laemmli buffer (5 μL, stock concentrations: 240 mM Tris-HCl pH 6.8, 8% w/v SDS, 40% v/v glycerol, 5% v/v β-mercaptoethanol, 0.04% v/v bromophenol blue, 30 min, 95 °C). The samples (12.5 μL, ∼200 ng per slot) were resolved by SDS-PAGE (180 V, 75 min). Gels were scanned using Cy3 and Cy5 multichannel settings (605/50 and 695/55 filters, respectively) on a ChemiDoc Imaging System (Bio-Rad). Fluorescence was normalized to Coomassie staining and quantified with Image Lab (Bio-Rad).

Targeted Lipidomics in U2OS Cells. The targeted lipidomic experiments are based on previously reported methods with small alterations as specified below.[38]

Sample Preparation. U2OS cells (2×10^6, grown at 37 °C, 7% CO_2) were seeded 1 day before transfection in 6 cm dishes. After 24 h, PLAAT2, PLAAT5, or mock plasmid DNA (2.7 μg/dish) and PEI (1 μg/μL, 8 μg/dish) were incubated in a serum-free culture medium (15 min, rt) and then added dropwise to the cells in a ratio of 1:5 (plasmid/PEI). After 24 h, the medium was aspirated and cells were washed once with serum-free medium. A new serum-free medium was added with **LEI-301** (final concentration: 10 μM, 0.1% DMSO) or DMSO as a control. After incubating for 4 h (37 °C, 7% CO_2), the medium was removed and the cells were washed with cold PBS (3×). The cells were harvested in 1.5 mL Eppendorf tubes by trypsinization, followed by centrifugation (10 min, 1500 rpm). PBS was removed, and the cell pellets were flash-frozen with liquid N_2 and stored at −80 °C. Live cell count with trypan blue was performed after compound treatment to test for cell viability and for sample normalization after lipid measurements.

Lipid Extraction. Lipid extraction was performed on ice. In brief, cell pellets with 2×10^6 cells were spiked with 10 μL each of deuterium-labeled internal standard mix for endocannabinoids [AEA-d_8, DHEA-d_4, 2-AG-d_8, *N*-stearoylethanolamine (SEA)-d_3, *N*-palmitoylethanolamine (PEA)-d_4, *N*-linoleoylethanolamine (LEA)-d_3, and *N*-oleoylethanolamine (OEA)-d_4] and negative polar lipids (FA 17:0-d_{33}), followed by the addition of ammonium acetate buffer (100 μL, 0.1 M, pH 4). After extraction with methyl *tert*-butyl ether (MTBE, 1 mL), the tubes were thoroughly mixed for 4 min using a bullet blender at medium speed (Next Advance Inc., Averill park, NY, USA), followed by a centrifugation step (5000*g*, 12 min, 4 °C). Then, 925 μL of the upper MTBE layer was transferred into clean 1.5 mL Eppendorf tubes. The samples were dried in a SpeedVac, followed by reconstitution in acetonitrile/water (50 μL, 90:10, v/v). The samples were centrifuged (14,000*g*, 3 min, 4 °C) before transferring into LC−MS vials. Each sample was injected on two different lipidomic platforms: endocannabinoids (5 μL) and negative polar lipids (8 μL).

LC−MS/MS Analysis for Endocannabinoids. A targeted analysis of endocannabinoids and related NAEs was measured using an Acquity UPLC I class binary solvent manager pump (Waters, Milford, USA) in conjugation with AB SCIEX 6500 quadrupole ion trap (QTRAP) (AB Sciex, Massachusetts, USA). Separation was performed with an Acquity HSS T3 column (1.2 × 100 mm, 1.8 μm) maintained at 40 °C. The aqueous mobile phase A consisted of 2 mM ammonium formate and 10 mM formic acid, and the organic mobile phase B was acetonitrile. The flow rate was set to 0.4 mL/min; the initial gradient conditions were 55% B held for 2 min and linearly ramped to 100% B over 6 min and held for 2 min; after 10 s, the system returned to initial conditions and held for 2 min before next injection. Electrospray ionization-MS was operated in positive mode for measurement of endocannabinoids and NAEs, and a selective multiple reaction mode was used for quantification.

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LC–MS/MS Analysis for Negative Polar Lipids. This method is measured on an Acquity UPLC binary solvent manager 8 pump (Waters) coupled to an Agilent 6530 electrospray ionization quadrupole time-of-flight (ESI-Q-TOF, Agilent, Jose, CA, USA) high-resolution mass spectrometer using reference mass correction. The chromatographic separation was achieved on an Acquity HSS T3 column (1.2 × 100 mm, 1.8 μm) maintained at 40 °C. The negative apolar lipids that constitute FFAs were separated with a flow of 0.4 mL/min over a 15 min gradient. In negative mode, the aqueous mobile phase A consisted of 5:95 (v/v) acetonitrile/H_2O with 10 mM ammonium formate, and the organic mobile phase B consisted of 99% (v/v) methanol with 10 mM ammonium formate.

Statistical Analysis. Absolute values of lipid levels were corrected using the measured live cell count numbers (cell viability was >90%). The data were tested for significance with GraphPad v6 using one-way ANOVA with Tukey correction for multiple comparisons. *P*-values < 0.05 were considered significant.

Computational Chemistry. *Ligand Preparation.* Molecular structures of **LEI-301** and **1** were drawn with specified chirality and prepared for docking using Ligprep from Schrödinger.[39] Default Ligprep settings were applied: states of heteroatoms were generated using Epik at a pH 7.[40] No tautomers were created by the program, which resulted in one standardized structure per ligand.

Protein Preparation. The X-ray structure of PLAAT2 was extracted from the PDB (PDB ID: 4DPZ).[4] The apo protein structure was prepared for docking with the Protein Preparation tool from the Schrödinger 2017-4 suite. Waters were removed, and explicit hydrogens were added. Missing side chains and loops were added with homology modeling using Prime:[41] loops 39–53 were modeled based on the protein sequence and loops 105–111 were based on the structure of PLAAT3 (PDB ID: 4DOT).[4]

Docking. The PLAAT2 binding pocket was induced using the binding pose from **1** in PLAAT3, as previously reported.[26,42] The complex of superposed **1** covalently bound to PLAAT2 was optimized using molecular dynamic simulations (10 ns). Compound **1** was removed, and the cysteine was restored to its nonbonded state. Subsequently, **LEI-301** and **1** were covalently docked to Cys113 using the Schrödinger 2017-4 suite.[42] The poses with the lowest docking scores were manually examined, and one pose per ligand was selected. Selection was based on the docking score, frequency of recurring poses, and interactions made between the ligand and the protein.

Synthetic Procedures. *General.* All chemicals (Sigma-Aldrich, Fluka, Acros, Merck) were used as received. All solvents used for reactions were of analytical grade. Tetrahydrofuran (THF), Et_2O, dimethylformamide (DMF), CH_3CN, and dichloromethane (DCM) were dried over activated 4 Å molecular sieves, and MeOH was dried over 3 Å molecular sieves. Flash chromatography was performed on silica gel (Screening Devices BV, 40–63 μm, 60 Å). The eluent EtOAc was of technical grade and distilled before use. Reactions were monitored by thin-layer chromatography (TLC) analysis using Merck aluminium sheets (Silica gel 60, F_{254}). Compounds were visualized by UV absorption (254 nm) and spraying for general compounds $KMnO_4$ (20 g/L) and K_2CO_3 (10 g/L) in water or for amines ninhydrin (0.75 g/L) and acetic acid (12.5 mL/L) in ethanol, followed by charring at ∼150 °C. ^1H and ^{13}C NMR experiments were recorded on a Bruker AV-300 (300/75 MHz), Bruker AV-400 (400/100 MHz), or Bruker DMX-400 (400/101 MHz). Chemical shifts are given in ppm (δ) relative to tetramethylsilane or $CDCl_3$ as internal standards. Multiplicity: s = singlet, br s = broad singlet, d = doublet, dd = doublet of doublet, t = triplet, q = quartet, p = pentet, m = multiplet. Coupling constants (J) are given in Hz. LC–MS measurements were performed on a Thermo Finnigan LCQ Advantage MAX ion-trap mass spectrometer (ESI^+) coupled to a Surveyor HPLC system (Thermo Finnigan) equipped with a standard C18 (Gemini, 4.6 mmD × 50 mmL, 5 μm particle size, Phenomenex) analytical column and buffers A: H_2O, B: CH_3CN, C: 0.1% aq trifluoroacetic acid (TFA). High-resolution mass spectra were recorded on a LTQ Orbitrap (Thermo Finnigan) mass spectrometer or a Synapt G2-Si high definition mass spectrometer (Waters) equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10 mL/min, capillary temperature 250 °C) with resolution R = 60,000 at m/z 400 (mass range m/z = 150–2000) and dioctylphthalate (m/z = 391.28428) as a lock mass. Preparative high-performance liquid chromatography (HPLC) was performed on a Waters Acquity Ultra Performance LC with a C18 column (Gemini, 150 × 21.2 mm, Phenomenex). All final compounds were determined to be >95% pure by integrating UV intensity recorded *via* HPLC.

General Procedure A: α-Ketoester Synthesis. Magnesium turnings were activated by stirring in a 3 M solution of HCl for 5 min. The magnesium was then washed with water and acetone and dried under reduced pressure. A round-bottom flask connected to a reflux condenser was flame-dried before the addition of activated magnesium turnings (2 equiv) under an argon atmosphere. Dry Et_2O (2 mL) and a small piece of iodine were added followed by dropwise addition of a solution of alkyl bromide (1–1.5 equiv) in dry Et_2O (1 M). The reaction was initiated with a heat gun and refluxed for 1 h. In a separate flask, a solution of the Weinreb amide **58** (1 equiv) in dry Et_2O (1 M) was prepared and cooled to −78 °C. The Grignard solution was taken up by syringe and added dropwise to the Weinreb amide solution. After stirring for 2 h at −78 °C, the reaction was quenched with sat. aq NH_4Cl and extracted with Et_2O (2×). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The crude residue was purified using silica gel column chromatography (EtOAc/pentane), affording the α-ketoester.

General Procedure B: α-Ketoester Deprotection. A round-bottom flask was charged with α-ketoester (1 equiv), DCM (0.3 M) and TFA (5–10 equiv) and stirred for 1–24 h at rt. The reaction mixture was concentrated under reduced pressure after TLC analysis showed complete consumption of the starting material, followed by coevaporation with toluene (3×). The obtained α-ketoacid was used in the next step without further purification.

General Procedure C: α-Ketoamide Synthesis. A round-bottom flask was charged with α-ketoacid (1 equiv) and DMF (0.2 M). HATU or HCTU (1–1.2 equiv), *N*,*N*-diisopropylethylamine (DiPEA) (1–2 equiv) or Et_3N (1–2 equiv), and amine (1–1.1 equiv) were added, and the mixture was stirred for 2–24 h at rt. Water was added, and the mixture was extracted with DCM (2×). The combined organic layers were washed with 1 M HCl, sat. aq $NaHCO_3$, and brine, dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography (EtOAc/pentane), affording the α-ketoamide.

General Procedure D: α-Ketoamide Synthesis. A round-bottom flask was charged with α-ketoacid (1 equiv) and THF or DCM (0.2 M) at 0 °C. *N*-(3-(Dimethylamino)propyl)-*N*′-ethylcarbodiimide (EDC)·HCl (1–1.5 equiv) and HOBt (1–1.5 equiv) were added, and the mixture was stirred for 30 min, followed by the addition of NMM (optional, 4 equiv) and the amine (1.2 equiv). The mixture was stirred for 1–4 days warming to rt. Work-up involved the addition of sat. aq $NaHCO_3$ and extraction with EtOAc (2 × 25 mL). The combined organic layers were washed with brine (1×), dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography (EtOAc/pentane) or preparative HPLC, affording the α-ketoamide.

General Procedure E: N-Boc-tyramine O-Arylation via S_NAr. A microwave vial was charged with *N*-Boc-tyramine **64** (1 equiv), heteroaryl halide (1 equiv), and K_2CO_3 (2 equiv) in DMSO or DMF (0.2–1 M) and capped. The mixture was stirred for 24–42 h at 85 °C in an oil bath until TLC showed complete conversion. The mixture was diluted with H_2O and extracted with EtOAc (3×). The combined organic layers were washed with brine (1×), dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography (EtOAc/pentane), affording the product.

General Procedure F: β,γ-Unsaturated α-Ketoamide Synthesis. A round-bottom flask was charged with pyruvic acid or sodium pyruvate (1 equiv), aldehyde (1 equiv), and MeOH (1 M) and cooled to 0 °C. A solution of KOH (2 M, 1.5 equiv) in MeOH was added dropwise while keeping the temperature below 25 °C. The reaction was stirred at rt overnight, forming a yellow precipitate. The reaction mixture was filtered, and the precipitate was washed with cold MeOH (2×), Et_2O

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(2×) and dried, affording the α-ketoacid as the potassium salt. A new round-bottom flask was charged with the potassium salt and DCM (0.5 M), and the suspension was sonicated for 20 min. This was followed by cooling to 0 °C and addition of oxalyl chloride (2 equiv). After consumption of the potassium salt, the reaction mixture was concentrated under reduced pressure and coevaporated with toluene (2×). The α-ketoacid chloride was then dissolved in DCM (0.5 M) and cooled to 0 °C, followed by the addition of phenethylamine (1 equiv) and Et_3N (2 equiv). The reaction was stirred for 2 h. Work-up involved the addition of H_2O and extraction with EtOAc. The organic layer was then washed with 1 M HCl (2×), sat. aq $NaHCO_3$ (2×), and brine (1×), dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography (EtOAc/pentane), affording the α-ketoamide.

*4-(4-Chlorophenyl)-2-oxo-N-phenethylbutanamide (**1**).* *t*-Butyl deprotection 59a: the α-ketoacid was prepared according to the general procedure B using α-ketoester 59a (0.85 g, 3.2 mmol, 1 equiv) and TFA (2.5 mL, 32 mmol, 10 equiv), affording the α-ketoacid 60a (0.68 g, 3.2 mmol, quant.). Amide coupling: the title compound was prepared according to the general procedure C using the α-ketoacid 60a (0.68 g, 3.2 mmol, 1 equiv), phenethylamine(0.15 mL, 1.2 mmol, 1.1 equiv), HATU (1.2 g, 3.2 mmol, 1 equiv), and DiPEA (0.61 mL, 3.5 mmol, 1.1 equiv) in DMF, affording the product (0.70 g, 2.2 mmol, 70%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.54−6.79 (m, 10H), 3.52 (q, *J* = 6.9 Hz, 2H), 3.21 (t, *J* = 7.4 Hz, 2H), 2.91−2.73 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 197.80, 159.82, 138.78, 138.13, 131.88, 129.71, 128.67, 128.60, 128.51, 126.67, 40.44, 38.06, 35.28, 28.35. HRMS: $[C_{18}H_{18}NClO_2 + H]^+$, 316.1099 calcd, 316.1099 found.

*4-(4-Chlorophenyl)-2-hydroxy-N-phenethylbutanamide (**23**).* A round-bottom flask was charged with α-ketoamide **1** (70 mg, 0.22 mmol, 1 equiv) and THF (1 mL). $NaBH_4$ (12 mg, 0.33 mmol, 1.5 equiv) was added, and the mixture was stirred for 15 min. The reaction was quenched with water (10 mL) and extracted with EtOAc (1 × 10 mL). The organic layer was washed with 1 M aq HCl (2 × 10 mL) and brine (1 × 10 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure. Purification by silica gel column chromatography afforded the product (50 mg, 0.16 mmol, 72%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.34−7.25 (m, 2H), 7.25−7.14 (m, 5H), 7.08 (d, *J* = 8.4 Hz, 2H), 6.61 (t, *J* = 5.4 Hz, 1H), 4.03 (dd, *J* = 7.9, 3.7 Hz, 1H), 3.66−3.40 (m, 2H), 3.24 (br s, 1H), 2.80 (t, *J* = 7.0 Hz, 2H), 2.65 (t, *J* = 7.9 Hz, 2H), 2.11−1.97 (m, 1H), 1.90−1.80 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 173.79, 139.74, 138.63, 131.87, 129.93, 128.81, 128.78, 128.65, 126.74, 71.33, 40.33, 36.38, 35.76, 30.57. HRMS: $[C_{18}H_{20}NClO_2 + H]^+$, 318.1255 calcd, 318.1252 found.

*2-Oxo-N-phenethyl-4-phenylbutanamide (**24**).* *t*-Butyl deprotection **59b**: the α-ketoacid was prepared according to the general procedure B using α-ketoester **59b** (0.50 g, 2.1 mmol, 1 equiv) and TFA (0.80 mL, 32 mmol, 5 equiv), affording the α-ketoacid **60b** (0.40 g, 2.1 mmol, quant.). Amide coupling: the title compound was prepared according to the general procedure C using the α-ketoacid **60b** (0.20 g, 1.2 mmol, 1 equiv), phenethylamine (0.15 mL, 1.2 mmol, 1.1 equiv), HCTU (0.48 g, 1.15 mmol, 1 equiv), and DiPEA (0.22 mL, 1.3 mmol, 1.1 equiv) in DMF, affording the product (80 mg, 0.28 mmol, 24%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.40−7.07 (m, 10H), 7.07−6.88 (m, 1H), 3.55 (q, *J* = 6.9 Hz, 2H), 3.26 (t, *J* = 7.5 Hz, 2H), 2.92 (t, *J* = 7.5 Hz, 2H), 2.83 (t, *J* = 7.1 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 198.25, 160.05, 140.46, 138.27, 128.85, 128.77, 128.60, 128.46, 126.85, 126.36, 40.58, 38.40, 35.51, 29.22. HRMS: $[C_{18}H_{19}NO_2 + H]^+$, 282.1489 calcd, 282.1487 found.

*2-Oxo-N-phenethyl-5-phenylpentanamide (**25**).* The title compound was prepared according to the general procedure C using the α-ketoacid **60c** (0.12 g, 0.63 mmol, 1 equiv), phenethylamine (86 μL, 0.69 mmol, 1.1 equiv), HCTU (0.26 g, 0.63 mmol, 1 equiv), and DiPEA (0.12 mL, 0.70 mmol, 1.1 equiv) in DCM, affording the product (70 mg, 0.24 mmol, 38%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.34−7.20 (m, 5H), 7.20−7.13 (m, 5H), 6.98 (br s, 1H), 3.53 (q, *J* = 7.0 Hz, 2H), 2.92 (t, *J* = 7.3 Hz, 2H), 2.83 (t, *J* = 7.1 Hz, 2H), 2.64 (t, *J* = 7.6 Hz, 2H), 1.92 (p, *J* = 7.4 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 198.97, 160.15, 141.42, 138.30, 128.83, 128.75, 128.57, 128.51, 126.83, 126.12, 40.56, 36.20, 35.52, 35.12, 24.92. HRMS: $[C_{19}H_{21}NO_2 + H]^+$, 296.1645 calcd, 296.1646 found.

*2-Oxo-N-phenethyl-6-phenylhexanamide (**26**).* *t*-Butyl deprotection **59d**: the α-ketoacid was prepared according to the general procedure B using α-ketoester **59d** (0.33 g, 1.3 mmol, 1 equiv) and TFA (1.9 mL, 25 mmol, 19 equiv), affording the α-ketoacid **60d** (0.26 g, 1.3 mmol, quant.). Amide coupling: the title compound was prepared according to the general procedure C using the α-ketoacid **60d** (0.26 g, 1.3 mmol, 1 equiv), phenethylamine (0.22 mL, 1.72 mmol, 1.3 equiv), HATU (0.59 g, 1.56 mmol, 1.2 equiv), and DiPEA (0.30 mL, 1.72 mmol, 1.3 equiv), affording the product (0.34 g, 1.11 mmol, 71%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.43−7.08 (m, 10H), 7.09−6.89 (m, 1H), 3.52 (q, *J* = 6.9 Hz, 2H), 3.01−2.86 (m, 2H), 2.81 (t, *J* = 7.1 Hz, 2H), 2.61 (t, *J* = 7.0 Hz, 2H), 1.62 (p, *J* = 3.5 Hz, 4H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 198.99, 160.11, 142.05, 138.25, 128.74, 128.68, 128.40, 128.34, 126.73, 125.81, 40.50, 36.55, 35.62, 35.42, 30.79, 22.81. HRMS: $[C_{20}H_{23}NO_2 + H]^+$, 310.1802 calcd, 310.1801 found.

*2-Oxo-N-phenethyl-2-phenylacetamide (**27**).* *t*-Butyl deprotection **59e**: the α-ketoacid was prepared according to the general procedure B using α-ketoester **59e** (0.68 g, 3.3 mmol, 1 equiv) and TFA (2.4 mL, 32 mmol, 10 equiv), affording the α-ketoacid **60e** (0.58 g, 3.2 mmol, quant.). Amide coupling: the title compound was prepared according to the general procedure C using the α-ketoacid **60e** (0.25 g, 1.7 mmol, 1 equiv), phenethylamine (0.23 mL, 1.8 mmol, 1.1 equiv), HCTU (0.69 g, 1.7 mmol, 1 equiv), and DiPEA (0.32 mL, 1.8 mmol, 1.1 equiv) in DCM, affording the product (0.22 g, 0.86 mmol, 52%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.33−8.19 (m, 2H), 7.62−7.52 (m, 1H), 7.47−7.38 (m, 2H), 7.34−7.26 (m, 2H), 7.26−7.14 (m, 4H), 3.62 (q, *J* = 7.0 Hz, 2H), 2.88 (t, *J* = 7.2 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 187.86, 161.98, 138.36, 134.36, 133.30, 131.11, 128.75, 128.72, 128.47, 126.68, 40.60, 35.46. HRMS: $[C_{16}H_{15}NO_2 + H]^+$, 254.1176 calcd, 254.1175 found.

*2-Oxo-N-phenethylpropanamide (**28**).* A round-bottom flask was charged with pyruvic acid (0.79 mL, 12 mmol, 1 equiv) and cooled to 0 °C. Thionyl chloride (0.93 mL, 13 mmol, 1.1 equiv) was added, and the mixture was stirred for 3 h at rt. The reaction mixture was concentrated under reduced pressure and coevaporated with toluene (3×). The acid chloride was dissolved in DCM (50 mL) and cooled to 0 °C. Phenethylamine (1.5 mL, 12 mmol, 1 equiv) and Et_3N (1.8 mL, 13 mmol, 1.1 equiv) were added, and the reaction was stirred for 2 h. Work-up involved the addition of H_2O and extraction with EtOAc. The organic layer was then washed with 1 M HCl (2×), sat. aq $NaHCO_3$ (2×), and brine (1×), dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography (EtOAc/pentane), affording the product (100 mg, 0.52 mmol, 4%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.43−7.15 (m, 5H), 7.13−6.82 (m, 1H), 3.57 (q, *J* = 7.0 Hz, 2H), 2.87 (t, *J* = 7.1 Hz, 2H), 2.47 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 197.16, 160.18, 138.27, 128.88, 128.78, 126.87, 40.65, 35.54, 24.58. HRMS: $[C_{11}H_{13}NO_2 + H]^+$, 192.1019 calcd, 192.1019 found.

*2-Oxo-N-phenethyl-4-(p-tolyl)butanamide (**29**).* *t*-Butyl deprotection **59f**: the α-ketoacid was prepared according to the general procedure B using α-ketoester **59f** (0.54 g, 2.2 mmol, 1 equiv) and TFA (1.6 mL, 22 mmol, 10 equiv), affording the α-ketoacid **60f** (0.42 g, 2.2 mmol, quant.). Amide coupling: the title compound was prepared according to the general procedure C using the α-ketoacid **60f** (0.42 g, 2.2 mmol, 1 equiv), phenethylamine (0.30 mL, 2.4 mmol, 1.1 equiv), HATU (0.83 g, 2.2 mmol, 1 equiv), and DiPEA (0.42 mL, 2.4 mmol, 1.1 equiv) in DCM, affording the product (0.48 g, 1.6 mmol, 74%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.32−7.23 (m, 2H), 7.23−7.18 (m, 1H), 7.18−7.11 (m, 2H), 7.11−6.97 (m, 5H), 3.51 (q, *J* = 6.9 Hz, 2H), 3.21 (t, *J* = 7.5 Hz, 2H), 2.97−2.69 (m, 4H), 2.28 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 198.18, 159.96, 138.21, 137.24, 135.61, 129.12, 128.66, 128.62, 128.18, 126.64, 40.44, 38.37, 35.33, 28.64, 20.97. HRMS: $[C_{19}H_{21}NO_2 + H]^+$, 296.1645 calcd, 296.1643 found.

*2-Oxo-N-phenethyl-4-(4-(trifluoromethyl)phenyl)butanamide (**30**).* *t*-Butyl deprotection **59g**: the α-ketoacid was prepared according to the general procedure B using α-ketoester **59g** (0.25 g, 0.83 mmol, 1 equiv) and TFA (0.62 mL, 8.3 mmol, 10 equiv), affording the α-ketoacid **60g** (0.20 g, 0.83 mmol, quant.). Amide coupling: The title

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compound was prepared according to the general procedure C using the α-ketoacid **60g** (0.20 g, 0.80 mmol, 1 equiv), phenethylamine (0.11 mL, 0.88 mmol, 1.1 equiv), HATU (0.38 g, 0.80 mmol, 1 equiv), and DiPEA (0.15 mL, 0.80 mmol, 1.1 equiv) in DMF. Column chromatography (20 → 60% EtOAc in pentane) afforded the product (0.22 g, 0.64 mmol, 80%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.57 (d, *J* = 8.1 Hz, 2H), 7.39−7.31 (m, 4H), 7.31−7.25 (m, 1H), 7.25−7.19 (m, 2H), 7.12−7.00 (m, 1H), 3.60 (q, *J* = 7.0 Hz, 2H), 3.32 (t, *J* = 7.4 Hz, 2H), 3.01 (t, *J* = 7.4 Hz, 2H), 2.88 (t, *J* = 7.1 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 197.70, 159.88, 144.56 (q, *J* = 1.3 Hz), 138.19, 128.78, 128.70, 128.48, 126.80, 125.46 (q, *J* = 3.8 Hz), 124.31 (q, *J* = 271.8 Hz), 40.55, 37.89, 35.39, 28.89. HRMS: $[C_{19}H_{18}F_3NO_2 + H]^+$, 350.1362 calcd, 350.1362 found.

*4-(4-Fluorophenyl)-2-oxo-N-phenethylbutanamide (**31**).* *t*-Butyl deprotection **59h**: the α-ketoacid was prepared according to the general procedure B using α-ketoester **59h** (0.15 g, 0.59 mmol, 1 equiv) and TFA (0.44 mL, 5.9 mmol, 10 equiv), affording the α-ketoacid **60h** (0.12 g, 0.59 mmol, quant.). Amide coupling: the title compound was prepared according to the general procedure C using the α-ketoacid **60h** (0.12 g, 0.63 mmol, 1 equiv), phenethylamine (80 μL, 0.63 mmol, 1 equiv), HCTU (0.26 g, 0.63 mmol, 1 equiv), and DiPEA (0.12 mL, 0.69 mmol, 1.1 equiv) in DMF, affording the product (0.12 g, 0.39 mmol, 62%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.35−7.21 (m, 3H), 7.21−7.08 (m, 4H), 7.08−6.99 (m, 1H), 6.99−6.88 (m, 2H), 3.54 (q, *J* = 6.9 Hz, 2H), 3.22 (t, *J* = 7.4 Hz, 2H), 2.99−2.64 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 198.05, 163.13, 159.97, 138.22, 136.05 (d, *J* = 3.2 Hz), 129.85 (d, *J* = 7.9 Hz), 128.77 (d, *J* = 6.8 Hz), 126.82, 115.32 (d, *J* = 21.2 Hz), 40.55, 38.44, 35.44, 28.39. HRMS: $[C_{18}H_{18}FNO_2 + H]^+$, 300.1394 calcd, 300.1393 found.

*4-(4-Methoxyphenyl)-2-oxo-N-phenethylbutanamide (**32**).* A round-bottom flask was charged with unsaturated α-ketoamide **39** (0.10 g, 0.32 mmol, 1 equiv) and MeOH (1 mL) and flushed with N_2. Pd/C (10 wt %, 10 mg, 9.4 μmol, 3 mol %) was added, and the flask was purged again with N_2, followed by H_2, and the reaction was stirred overnight under a H_2 atmosphere (balloon). The reaction was filtered over celite, which was washed with MeOH, and the filtrate was concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography (EtOAc/pentane), affording the product (50 mg, 0.16 mmol, 50%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.36−7.27 (m, 2H), 7.27−7.21 (m, 1H), 7.18 (d, *J* = 7.0 Hz, 2H), 7.11 (d, *J* = 8.6 Hz, 2H), 7.05−6.90 (m, 1H), 6.88−6.71 (m, 2H), 3.77 (s, 3H), 3.55 (q, *J* = 7.0 Hz, 2H), 3.22 (t, *J* = 7.5 Hz, 2H), 2.95−2.73 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 198.37, 160.05, 158.13, 138.26, 132.48, 129.41, 128.86, 128.77, 126.85, 113.99, 55.35, 40.57, 38.67, 35.51, 28.38. HRMS: $[C_{19}H_{21}NO_3 + H]^+$, 312.1594 calcd, 312.1593 found.

*2-Oxo-N-phenethyl-4-(4-phenoxyphenyl)butanamide (**33**).* A round-bottom flask was charged with unsaturated α-ketoamide **67** (0.12 g, 0.33 mmol, 1 equiv) and MeOH (1 mL) and flushed with N_2. Pd/C (10 wt %, 10 mg, 9.4 μmol, 3 mol %) was added, and the flask was purged again with N_2, followed by H_2, and the reaction was stirred overnight under a H_2 atmosphere (balloon). The reaction was filtered over celite, which was washed with MeOH, and the filtrate was concentrated under reduced pressure. The ketone was over-reduced to the alcohol according to NMR analysis; therefore, it was reoxidized. A round-bottom flask was charged with the alcohol, Dess−Martin periodinane (0.21 g, 0.49 mmol, 1.5 equiv), and DCM (5 mL) and stirred at rt. Work-up involved the addition of H_2O and extraction with EtOAc. The organic layer was washed with 1 M HCl (2×), sat. aq $NaHCO_3$ (2×), and brine (1×), dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography (EtOAc/pentane), affording the product (80 mg, 0.23 mmol, 65%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.36−7.27 (m, 4H), 7.27−7.20 (m, 1H), 7.20−7.11 (m, 4H), 7.11−7.04 (m, 1H), 7.04−6.95 (m, 3H), 6.95−6.88 (m, 2H), 3.55 (q, *J* = 7.0 Hz, 2H), 3.25 (t, *J* = 7.5 Hz, 2H), 2.90 (t, *J* = 7.5 Hz, 2H), 2.84 (t, *J* = 7.1 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 198.20, 160.01, 157.51, 155.62, 138.23, 135.36, 129.79, 129.71, 128.85, 128.76, 126.85, 123.16, 119.14, 118.74, 40.58, 38.55, 35.49, 28.49. HRMS: $[C_{24}H_{23}NO_3 + H]^+$, 374.1751 calcd, 374.1748 found.

*4-(2-Chlorophenyl)-2-oxo-N-phenethylbutanamide (**34**).* *t*-Butyl deprotection **59i**: the α-ketoacid was prepared according to the general procedure B using α-ketoester **59i** (0.23 g, 0.87 mmol, 1 equiv) and TFA (0.94 mL, 13 mmol, 15 equiv), affording the α-ketoacid **60i** (0.18 g, 0.87 mmol, quant.). Amide coupling: the title compound was prepared according to the general procedure C using the α-ketoacid **60i** (0.18 g, 0.87 mmol, 1 equiv), phenethylamine (0.12 mL, 0.95 mmol, 1.1 equiv), HATU (0.33 g, 0.87 mmol, 1 equiv), and DiPEA (0.16 mL, 0.95 mmol, 1.1 equiv) in DCM, affording the product (0.13 g, 0.42 mmol, 48%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.50−7.08 (m, 9H), 7.08−6.89 (m, 1H), 3.55 (q, *J* = 6.9 Hz, 2H), 3.27 (t, *J* = 7.5 Hz, 2H), 3.01 (t, *J* = 7.4 Hz, 2H), 2.84 (t, *J* = 7.1 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 197.96, 159.93, 138.24, 138.01, 134.04, 130.52, 129.65, 128.82, 128.74, 127.92, 126.96, 126.82, 40.56, 36.78, 35.48, 27.23. HRMS: $[C_{18}H_{18}ClNO_2 + H]^+$, 316.1099 calcd, 316.1100 found.

*4-(3-Chlorophenyl)-2-oxo-N-phenethylbutanamide (**35**).* *t*-Butyl deprotection **59j**: the α-ketoacid was prepared according to the general procedure B using α-ketoester **59j** (0.55 g, 2.0 mmol, 1 equiv) and TFA (2 mL, 26 mmol, 13 equiv), affording the α-ketoacid **60j** (0.47 g, 2.0 mmol, quant.). Amide coupling: The title compound was prepared according to the general procedure C using the α-ketoacid **60j** (0.47 g, 2.0 mmol, 1 equiv), phenethylamine (0.31 mL, 2.4 mmol, 1.2 equiv), HATU (0.84 mg, 2.2 mmol, 1.1 equiv), and DiPEA (0.42 mL, 2.4 mmol, 1.2 equiv), affording the product (0.37 g, 1.2 mmol, 53%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.73−6.73 (m, 10H), 3.58 (q, *J* = 6.9 Hz, 2H), 3.28 (t, *J* = 7.4 Hz, 2H), 3.07−2.74 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 197.71, 159.83, 142.40, 138.16, 134.13, 129.72, 128.69, 128.62, 128.53, 126.69, 126.55, 126.42, 40.47, 37.95, 35.32, 28.65. HRMS: $[C_{18}H_{18}ClNO_2 + H]^+$, 316.1099 calcd, 316.1098 found.

*4-(3,4-Dichlorophenyl)-2-oxo-N-phenethylbutanamide (**36**).* *t*-Butyl deprotection **59k**: the α-ketoacid was prepared according to the general procedure B using α-ketoester **59k** (90 mg, 0.30 mmol, 1 equiv) and TFA (0.25 mL, 32 mmol, 10 equiv), affording the α-ketoacid **60k** (74 mg, 0.30 mmol, quant.). Amide coupling: the title compound was prepared according to the general procedure C using α-ketoacid **60k** (66 mg, 0.27 mmol, 1 equiv), phenethylamine (36 μL, 0.29 mmol, 1.1 equiv), HATU (110 mg, 0.29 mmol, 1.1 equiv), and DiPEA (92 μL, 0.53 mmol, 2 equiv) in DCM, affording the product (42 mg, 0.12 mmol, 44%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.41−7.32 (m, 4H), 7.32−7.26 (m, 1H), 7.22 (d, *J* = 7.0 Hz, 2H), 7.07 (dd, *J* = 8.2, 2.0 Hz, 1H), 7.05−6.97 (m, 1H), 3.60 (q, *J* = 7.0 Hz, 2H), 3.28 (t, *J* = 7.4 Hz, 2H), 3.03−2.79 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 197.63, 159.85, 140.68, 138.17, 132.46, 130.53, 130.50, 130.39, 128.88, 128.76, 127.98, 126.90, 40.61, 37.94, 35.49, 28.29. HRMS: $[C_{18}H_{17}Cl_2NO_2 + H]^+$, 350.0709 calcd, 350.0708 found.

*(E)-4-(4-Chlorophenyl)-2-oxo-N-phenethylbut-3-enamide (**37**).* α-Ketoacid formation: the α-ketoacid salt was prepared according to the general procedure F using pyruvic acid (1.7 mL, 18 mmol, 1 equiv), 4-chlorobenzaldehyde (2.2 mL, 19 mmol, 1 equiv), and KOH (2.1 g, 38 mmol, 2 equiv) in MeOH, affording potassium 4-(4-chlorophenyl)-2-oxobut-3-enoate **62a** (2.0 g, 8.0 mmol, 42%). Amide coupling: the title compound was prepared according to the general procedure F using potassium salt **62a** (2.0 g, 8.0 mmol, 1 equiv), oxalyl chloride (1.4 mL, 16 mmol, 2 equiv), phenethylamine (1.1 mL, 8.8 mmol, 1.1 equiv), and Et_3N (2.2 mL, 16 mmol, 2 equiv) in DCM, affording the product (0.30 g, 0.96 mmol, 12%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.86 (d, *J* = 16.2 Hz, 1H), 7.73 (d, *J* = 16.1 Hz, 1H), 7.59 (d, *J* = 8.5 Hz, 2H), 7.43−7.36 (m, 2H), 7.36−7.28 (m, 2H), 7.28−7.17 (m, 4H), 3.63 (q, *J* = 7.0 Hz, 2H), 2.89 (t, *J* = 7.1 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 185.32, 161.24, 146.51, 138.34, 137.61, 132.94, 130.37, 129.48, 128.87, 128.81, 126.86, 119.09, 40.77, 35.58. HRMS: $[C_{18}H_{16}ClNO_2 + H]^+$, 314.0942 calcd, 314.0939 found.

*(E)-2-Oxo-N-phenethyl-4-phenylbut-3-enamide (**38**).* α-Ketoacid formation: the α-ketoacid salt was prepared according to the general procedure F using pyruvic acid (0.79 mL, 11 mmol, 1 equiv), benzaldehyde (1.2 g, 11 mmol, 1 equiv), and KOH (0.98 g, 17 mmol, 1.5 equiv) in MeOH, affording potassium 2-oxo-4-phenylbut-3-enoate **62b** (0.85 g, 3.9 mmol, 36%). Amide coupling: the title compound was prepared according to the general procedure C using potassium salt **62b** (0.20 g, 0.93 mmol, 1 equiv), phenethylamine (0.12 mL, 0.93 mmol, 1

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equiv), HCTU (0.39 g, 0.93 mmol, 1 equiv), and DiPEA (0.32 mL, 1.86 mmol, 2 equiv) in DMF (5 mL), affording the product (70 mg, 0.25 mmol, 27%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.93 (d, J = 16.2 Hz, 1H), 7.77 (d, J = 16.1 Hz, 1H), 7.71−7.62 (m, 2H), 7.49−7.37 (m, 3H), 7.36−7.16 (m, 6H), 3.63 (q, J = 7.0 Hz, 2H), 2.89 (t, J = 7.1 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 185.49, 161.38, 148.12, 138.40, 134.48, 131.60, 129.28, 129.15, 128.85, 128.82, 126.83, 118.67, 40.76, 35.61. HRMS: $[C_{18}H_{17}NO_2 + H]^+$, 280.1332 calcd, 280.1331 found.

*(E)-4-(4-Methoxyphenyl)-2-oxo-N-phenethylbut-3-enamide (**39**).* α-Ketoacid formation: the α-ketoacid salt was prepared according to the general procedure F using sodium pyruvate (3.0 g, 27 mmol, 1 equiv), 4-methoxybenzaldehyde (3.3 mL, 27 mmol, 1 equiv), and KOH (2.3 g, 41 mmol, 1.5 equiv) in MeOH, affording potassium 4-(4-methoxyphenyl)-2-oxobut-3-enoate **62c** (6.5 g, 27 mmol, 98%). Amide coupling: the title compound was prepared according to the general procedure F using potassium salt **62c** (1.0 g, 4.1 mmol, 1 equiv), oxalyl chloride (0.87 mL, 8.2 mmol, 2 equiv), phenethylamine (0.52 mL, 4.1 mmol, 1 equiv), and Et_3N (1.1 mL, 8.2 mmol, 2 equiv) in DCM, affording the product (1.2 g, 3.8 mmol, 93%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.89 (d, J = 16.0 Hz, 1H), 7.68−7.57 (m, 3H), 7.37−7.27 (m, 3H), 7.26−7.18 (m, 3H), 6.95−6.88 (m, 2H), 3.83 (s, 3H), 3.61 (q, J = 7.0 Hz, 2H), 2.88 (t, J = 7.2 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 185.15, 162.54, 161.66, 147.93, 138.44, 131.23, 128.76, 127.27, 126.72, 116.20, 114.59, 55.50, 40.69, 35.56. HRMS: $[C_{19}H_{19}NO_3 + H]^+$, 310.1438 calcd, 310.1435 found.

*(E)-4-(4-Bromophenyl)-2-oxo-N-phenethylbut-3-enamide (**40**).* α-Ketoacid formation: the α-ketoacid salt was prepared according to the general procedure F using pyruvic acid (1.4 mL, 16 mmol, 1 equiv), 4-bromobenzaldehyde (1.6 mL, 16 mmol, 1 equiv), and KOH (1.8 g, 32 mmol, 2 equiv) in MeOH, affording potassium 4-(4-bromophenyl)-2-oxobut-3-enoate **62d** (2.0 g, 6.8 mmol, 42%). Amide coupling: the title compound was prepared according to the general procedure F using potassium salt **62d** (2.0 g, 6.8 mmol, 1 equiv), oxalyl chloride (1.2 mL, 14 mmol, 2 equiv), phenethylamine (0.94 mL, 7.5 mmol, 1.1 equiv), and Et_3N (1.90 mL, 14 mmol, 2 equiv) in DCM, affording the product (0.84 g, 2.3 mmol, 34%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.84 (d, J = 16.2 Hz, 1H), 7.74 (d, J = 16.1 Hz, 1H), 7.64−7.42 (m, 4H), 7.38−7.15 (m, 6H), 3.62 (q, J = 7.0 Hz, 2H), 2.89 (t, J = 7.1 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 185.37, 161.22, 146.55, 138.35, 133.37, 132.45, 130.52, 128.87, 128.81, 126.86, 126.08, 119.23, 40.77, 35.60. HRMS: $[C_{18}H_{16}BrNO_2 + H]^+$, 358.0437 calcd, 358.0437 found.

*(E)-4-(3-Bromophenyl)-2-oxo-N-phenethylbut-3-enamide (**41**).* α-Ketoacid formation: the α-ketoacid salt was prepared according to the general procedure F using sodium pyruvate (1.0 g, 11 mmol, 1 equiv), 3-bromobenzaldehyde (1.3 mL, 11 mmol, 1 equiv), and KOH (0.96 g, 17 mmol, 1.5 equiv) in MeOH, affording potassium 4-(3-bromophenyl)-2-oxobut-3-enoate **62e** (0.40 g, 1.4 mmol, 12%). Amide coupling: the title compound was prepared according to the general procedure F using potassium salt **62e** (0.40 g, 1.4 mmol, 1 equiv), oxalyl chloride (0.24 mL, 2.7 mmol, 2 equiv), phenethylamine (0.19 mL, 1.5 mmol, 1.1 equiv), and Et_3N (0.38 mL, 2.7 mmol, 2 equiv) in DCM, affording the product (0.31 g, 0.87 mmol, 62%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.86−7.78 (m, 2H), 7.74 (d, J = 16.2 Hz, 1H), 7.62−7.51 (m, 2H), 7.37−7.28 (m, 3H), 7.28−7.19 (m, 4H), 3.63 (q, J = 7.0 Hz, 2H), 2.89 (t, J = 7.1 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 185.33, 161.12, 146.13, 138.34, 136.52, 134.25, 131.84, 130.63, 128.88, 128.82, 127.72, 126.88, 123.27, 119.96, 40.78, 35.59. HRMS: $[C_{18}H_{16}BrNO_2 + H]^+$, 358.0437 calcd, 358.0437 found.

*(E)-4-([1,1′-Biphenyl]-3-yl)-2-oxo-N-phenethylbut-3-enamide (**42**).* A round-bottom flask was charged with aryl bromide **41** (0.20 g, 0.56 mmol, 1 equiv) and toluene/EtOH (4:1, 3 mL) and degassed for 20 min with sonication. $Pd(PPh_3)_4$ (13 mg, 0.01 mmol, 2 mol %), phenylboronic acid (0.10 g, 0.84 mmol, 1.5 equiv), and K_2CO_3 (0.46 g, 3.4 mmol, 6 equiv) were added, and the reaction was stirred for 16 h at 80 °C. The reaction mixture was filtered over a pad of celite and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography (EtOAc/pentane), affording the product (0.15 g, 0.42 mmol, 75%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.03 (d, J = 16.2 Hz, 1H), 7.94−7.78 (m, 2H), 7.73−7.18 (m, 14H), 3.67 (q, J = 6.9 Hz, 2H), 2.93 (t, J = 7.1 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 185.46, 161.38, 148.00, 142.21, 140.23, 138.39, 134.95, 130.35, 129.57, 129.02, 128.85, 128.81, 128.04, 127.94, 127.91, 127.24, 126.82, 118.96, 40.76, 35.59. HRMS: $[C_{24}H_{21}NO_2 + H]^+$, 356.1645 calcd, 356.1641 found.

*N-(4-Methylphenethyl)-2-oxo-5-phenylpentanamide (**43**).* The title compound was prepared according to the general procedure D using α-ketoacid **60c** (57 mg, 0.29 mmol, 1 equiv), 2-(*p*-tolyl)ethan-1-amine (47 μL, 0.32 mmol, 1.1 equiv), EDC·HCl (85 mg, 0.44 mmol, 1.5 equiv), and HOBt (60 mg, 0.44 mmol, 1.5 equiv) in DCM. Column chromatography (2.5 → 20% EtOAc in pentane) afforded the product (18 mg, 58 μmol, 20%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.39−7.22 (m, 3H), 7.22−7.14 (m, 3H), 7.12 (d, J = 7.9 Hz, 2H), 7.07 (d, J = 8.0 Hz, 2H), 7.01−6.86 (m, 1H), 3.52 (q, J = 7.0 Hz, 2H), 2.93 (t, J = 7.3 Hz, 2H), 2.80 (t, J = 7.1 Hz, 2H), 2.65 (t, J = 7.6 Hz, 2H), 2.32 (s, 3H), 1.93 (p, J = 7.5 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 199.02, 160.11, 141.47, 136.44, 135.16, 129.56, 128.65, 128.61, 128.56, 126.21, 126.16, 40.68, 36.24, 35.13, 24.94, 21.18. HRMS: $[C_{20}H_{23}NO_2 + H]^+$, 310.1802 calcd, 310.1803 found.

*N-(4-Methoxyphenethyl)-2-oxo-5-phenylpentanamide (**44**).* The title compound was prepared according to the general procedure D using α-ketoacid **60c** (89 mg, 0.46 mmol, 1 equiv), 2-(4-methoxyphenyl)ethan-1-amine (75 μL, 0.51 mmol, 1.1 equiv), EDC·HCl (0.13 g, 0.69 mmol, 1.5 equiv), and HOBt (94 mg, 0.69 mmol, 1.5 equiv) in DCM. Column chromatography (2.5 → 40% EtOAc in pentane) afforded the product (13 mg, 40 μmol, 9%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.32−7.24 (m, 2H), 7.23−7.14 (m, 3H), 7.10 (d, J = 8.6 Hz, 2H), 7.04−6.90 (m, 1H), 6.85 (d, J = 8.6 Hz, 2H), 3.79 (s, 3H), 3.50 (q, J = 6.9 Hz, 2H), 2.94 (t, J = 7.3 Hz, 2H), 2.78 (t, J = 7.1 Hz, 2H), 2.65 (t, J = 7.6 Hz, 2H), 1.93 (p, J = 7.5 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 199.03, 160.10, 158.52, 141.45, 130.25, 129.75, 128.60, 128.54, 126.15, 114.27, 55.39, 40.77, 36.23, 35.14, 34.65, 24.94. HRMS: $[C_{20}H_{23}NO_3 + H]^+$, 326.1751 calcd, 326.1752 found.

*N-(3,4-Dimethoxyphenethyl)-2-oxo-5-phenylpentanamide (**45**).* The title compound was prepared according to the general procedure D using α-ketoacid **60c** (57 mg, 0.30 mmol, 1 equiv), 2-(3,4-dimethoxyphenyl)ethan-1-amine (56 μL, 0.33 mmol, 1.1 equiv), EDC·HCl (86 mg, 0.45 mmol, 1.5 equiv), and HOBt (61 mg, 0.45 mmol, 1.5 equiv) in DCM. Column chromatography (2.5 → 40% EtOAc in pentane) afforded the product (6 mg, 17 μmol, 6%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.31−7.26 (m, 2H), 7.22−7.11 (m, 3H), 7.03−6.91 (m, 1H), 6.81 (d, J = 8.1 Hz, 1H), 6.77−6.66 (m, 2H), 3.87 (s, 3H), 3.86 (s, 3H), 3.52 (q, J = 7.0 Hz, 2H), 2.94 (t, J = 7.3 Hz, 2H), 2.78 (t, J = 7.1 Hz, 2H), 2.65 (t, J = 7.6 Hz, 2H), 1.93 (p, J = 7.5 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 199.04, 160.13, 149.01, 147.96, 141.44, 130.77, 128.56, 126.17, 120.73, 111.86, 111.53, 56.02, 40.69, 36.25, 35.16, 24.95. HRMS: $[C_{21}H_{25}NO_4 + H]^+$, 356.1856 calcd, 356.1858 found.

*N-(4-Hydroxyphenethyl)-2-oxo-5-phenylpentanamide (**46**).* The title compound was prepared according to the general procedure D using α-ketoacid **60c** (98 mg, 0.51 mmol, 1 equiv), 4-(2-aminoethyl)-phenol (77 mg, 0.56 mmol, 1.1 equiv), EDC·HCl (0.15 g, 0.77 mmol, 1.5 equiv), and HOBt (0.10 g, 0.77 mmol, 1.5 equiv) in DCM. Column chromatography (10 → 60% EtOAc in pentane) afforded the product (18 mg, 58 μmol, 20%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.32−7.23 (m, 2H), 7.23−7.12 (m, 3H), 7.04 (d, J = 8.5 Hz, 2H), 7.01−6.90 (m, 1H), 6.83−6.72 (m, 2H), 5.26 (br s, 1H), 3.50 (q, J = 7.0 Hz, 2H), 2.93 (t, J = 7.3 Hz, 2H), 2.76 (t, J = 7.1 Hz, 2H), 2.64 (t, J = 7.6 Hz, 2H), 1.93 (p, J = 7.5 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 198.97, 160.18, 154.71, 141.43, 130.14, 129.92, 128.61, 128.55, 126.16, 115.75, 40.84, 36.27, 35.13, 34.65, 24.95. HRMS: $[C_{19}H_{21}NO_3 + H]^+$, 312.1594 calcd, 312.1595 found.

*N-(4-Bromophenethyl)-2-oxo-5-phenylpentanamide (**47**).* The title compound was prepared according to the general procedure D using α-ketoacid **60c** (0.96 g, 5.0 mmol, 1 equiv), 2-(4-bromophenyl)-ethan-1-amine (0.85 mL, 5.5 mmol, 1.1 equiv), EDC·HCl (1.5 g, 7.5 mmol, 1.5 equiv), HOBt (1.2 g, 7.5 mmol, 1.5 equiv), and Et_3N (1.4 mL, 10 mmol, 2.0 equiv) in DCM. Column chromatography (2.5 → 20% EtOAc in pentane) afforded the product (0.28 g, 0.74 mmol, 15%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.41 (d, J = 7.6 Hz, 2H), 7.32−7.22 (m, 2H), 7.22−7.12 (m, 3H), 7.04 (d, J = 7.8 Hz, 2H), 7.02−6.95 (m, 1H),

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3.50 (q, J = 6.8 Hz, 2H), 2.92 (t, J = 7.2 Hz, 2H), 2.78 (t, J = 7.1 Hz, 2H), 2.64 (t, J = 7.5 Hz, 2H), 1.92 (p, J = 7.3 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 198.86, 160.13, 141.35, 137.23, 131.87, 130.47, 128.55, 128.51, 126.12, 120.68, 40.31, 36.18, 35.07, 34.91, 24.87. HRMS: $[C_{19}H_{20}NBrO_2 + H]^+$, 374.0750 calcd, 374.0751 found.

*N-(3-Chlorophenethyl)-2-oxo-5-phenylpentanamide (**48**).* The title compound was prepared according to the general procedure D using α-ketoacid **60c** (72 mg, 0.37 mmol, 1 equiv), 2-(3-chlorophenyl)-ethan-1-amine (57 μL, 0.41 mmol, 1.1 equiv), EDC·HCl (0.11 g, 0.56 mmol, 1.5 equiv), and HOBt (86 mg, 0.56 mmol, 1.5 equiv) in DCM. Column chromatography (2.5 → 40% EtOAc in pentane) afforded the product (29 mg, 87 μmol, 24%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.33−7.13 (m, 8H), 7.11−7.02 (m, 1H), 7.02−6.90 (m, 1H), 3.53 (q, J = 6.8 Hz, 2H), 2.93 (t, J = 7.3 Hz, 2H), 2.82 (t, J = 7.2 Hz, 2H), 2.65 (t, J = 7.6 Hz, 2H), 1.94 (p, J = 7.5 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 198.91, 165.51, 140.31, 130.11, 128.94, 128.61, 128.56, 127.12, 126.97, 126.17, 40.35, 36.23, 35.24, 35.15, 24.95. HRMS: $[C_{19}H_{20}ClNO_2 + H]^+$, 330.1255 calcd, 330.1256 found.

*N-(2-Chlorophenethyl)-2-oxo-5-phenylpentanamide (**49**).* The title compound was prepared according to the general procedure D using α-ketoacid **60c** (81 mg, 0.42 mmol, 1 equiv), 2-(2-chlorophenyl)-ethan-1-amine (66 μL, 0.47 mmol, 1.1 equiv), EDC·HCl (0.12 g, 0.64 mmol, 1.5 equiv), and HOBt (86 mg, 0.64 mmol, 1.5 equiv) in DCM. Column chromatography (2.5 → 20% EtOAc in pentane) afforded the product (11 mg, 32 μmol, 8%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.41−7.32 (m, 1H), 7.32−7.26 (m, 2H), 7.24−7.08 (m, 6H), 7.07−6.91 (m, 1H), 3.57 (q, J = 6.9 Hz, 2H), 2.99 (t, J = 7.1 Hz, 2H), 2.93 (t, J = 7.3 Hz, 2H), 2.65 (t, J = 7.6 Hz, 2H), 1.93 (p, J = 7.4 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 198.96, 160.23, 141.45, 136.03, 134.27, 131.00, 129.90, 128.63, 128.56, 128.45, 127.23, 126.17, 39.08, 36.23, 35.15, 33.30, 24.96. HRMS: $[C_{19}H_{20}ClNO_2 + H]^+$, 330.1255 calcd, 330.1256 found.

*N-(2,4-Dichlorophenethyl)-2-oxo-5-phenylpentanamide (**50**).* The title compound was prepared according to the general procedure D using α-ketoacid **60c** (95 mg, 0.49 mmol, 1 equiv), 2-(2,4-dichlorophenyl)ethan-1-amine (89 μL, 0.54 mmol, 1.1 equiv), EDC·HCl (0.14 g, 0.74 mmol, 1.5 equiv), and HOBt (0.10 g, 0.74 mmol, 1.5 equiv) in DCM. Column chromatography (2.5 → 40% EtOAc in pentane) afforded the product (14 mg, 38 μmol, 8%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.38 (s, 1H), 7.32−7.26 (m, 2H), 7.23−7.15 (m, 4H), 7.15−7.10 (m, 1H), 7.07−6.87 (m, 1H), 3.54 (q, J = 6.8 Hz, 2H), 2.94 (q, J = 7.5 Hz, 4H), 2.65 (t, J = 7.6 Hz, 2H), 1.94 (p, J = 7.4 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 198.87, 160.25, 141.40, 134.92, 134.63, 133.46, 131.72, 129.68, 128.61, 128.56, 127.50, 126.18, 38.91, 36.21, 35.13, 32.79, 24.93. HRMS: $[C_{19}H_{19}Cl_2NO_2 + H]^+$, 364.0866 calcd, 364.0867 found.

*2-Oxo-N-(4-phenoxyphenethyl)-5-phenylpentanamide (**51**, **LEI-301**).* The title compound was prepared according to the general procedure D using α-ketoacid **60c** (0.19 g, 1.0 mmol, 1 equiv), 2-(4-phenoxyphenyl)ethan-1-amine TFA salt (0.36 g, 1.1 mmol, 1.1 equiv), EDC·HCl (0.29 g, 1.5 mmol, 1.5 equiv), HOBt (0.23 g, 1.5 mmol, 1.5 equiv), and Et_3N (0.28 mL, 2.0 mmol, 2.0 equiv) in DCM. Column chromatography (2.5 → 20% EtOAc in pentane) afforded the product (32 mg, 86 μmol, 9%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.36−7.24 (m, 4H), 7.22−7.06 (m, 6H), 7.04−6.91 (m, 5H), 3.53 (q, J = 6.7 Hz, 2H), 2.94 (t, J = 7.2 Hz, 2H), 2.81 (t, J = 7.1 Hz, 2H), 2.65 (t, J = 7.5 Hz, 2H), 1.94 (p, J = 7.4 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 199.01, 160.14, 157.33, 156.18, 141.42, 133.07, 130.04, 129.86, 128.60, 128.55, 126.16, 123.36, 119.24, 118.95, 40.68, 36.23, 35.13, 34.82, 24.93. HRMS: $[C_{25}H_{25}NO_3 + H]^+$, 388.1907 calcd, 388.1909 found.

*2-Oxo-5-phenyl-N-(4-(pyrazin-2-yloxy)phenethyl)pentanamide (**52**).* Boc-deprotection **65a**: a round-bottom flask was charged with Boc-protected amine **65a** (0.32 g, 1.0 mmol, 1 equiv) and HCl (4 M in dioxane, 4.5 mL, 18 mmol, 18 equiv). After stirring for 1 h, the mixture was concentrated under reduced pressure and coevaporated with toluene (3×), which afforded the deprotected amine **66a** as the HCl salt (0.25 g, 1.0 mmol, quant.). Amide coupling: the title compound was prepared according to the general procedure D using the α-ketoacid **60c** (38 mg, 0.20 mmol, 1 equiv), EDC·HCl (46 mg, 0.24 mmol, 1.2 equiv), HOBt (32 mg, 0.24 mmol, 1.2 equiv), NMM (87 μL, 0.80 mmol, 4 equiv), and the amine HCl salt **66a** (60 mg, 0.24 mmol, 1.2 equiv). Column chromatography (30 → 70% EtOAc in pentane) afforded the product (23 mg, 59 μmol, 30%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.43 (d, J = 1.3 Hz, 1H), 8.26 (d, J = 2.7 Hz, 1H), 8.10 (dd, J = 2.7, 1.4 Hz, 1H), 7.33−7.22 (m, 4H), 7.22−7.15 (m, 3H), 7.14−7.08 (m, 2H), 7.07−6.98 (m, 1H), 3.57 (q, J = 7.0 Hz, 2H), 2.95 (t, J = 7.3 Hz, 2H), 2.87 (t, J = 7.2 Hz, 2H), 2.66 (t, J = 7.6 Hz, 2H), 1.94 (p, J = 7.4 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 198.97, 160.17, 151.83, 141.42, 141.17, 138.62, 136.08, 135.48, 130.17, 128.60, 128.54, 126.16, 121.66, 40.54, 36.23, 35.13, 35.01, 24.92. HRMS: $[C_{23}H_{23}N_3O_3 + H]^+$, 390.1812 calcd, 390.1823 found.

*2-Oxo-5-phenyl-N-(4-(pyrimidin-2-yloxy)phenethyl)-pentanamide (**53**).* Boc-deprotection **65b**: a round-bottom flask was charged with Boc-protected amine **65b** (0.30 g, 0.94 mmol, 1 equiv) and HCl (4 M in dioxane, 4.5 mL, 18 mmol, 19 equiv). After stirring for 1 h, the mixture was concentrated under reduced pressure and coevaporated with toluene (3×), which afforded the deprotected amine **66b** as the HCl salt (0.24 g, 0.94 mmol, quant.). Amide coupling: the title compound was prepared according to the general procedure D using α-ketoacid **60c** (38 mg, 0.20 mmol, 1 equiv), EDC·HCl (46 mg, 0.24 mmol, 1.2 equiv), HOBt (32 mg, 0.24 mmol, 1.2 equiv), NMM (87 μL, 0.80 mmol, 4 equiv), and the amine HCl salt **66b** (76 mg, 0.24 mmol, 1.2 equiv). Purification by preparative HPLC (C18 reverse phase, 45−55% ACN/H_2O + 0.2% TFA, RT 10.86 min) afforded the product (12 mg, 31 μmol, 15%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.56 (d, J = 4.8 Hz, 2H), 7.33−7.22 (m, 4H), 7.22−7.12 (m, 5H), 7.04 (t, J = 4.8 Hz, 2H), 3.57 (q, J = 6.9 Hz, 2H), 2.95 (t, J = 7.3 Hz, 2H), 2.87 (t, J = 7.2 Hz, 2H), 2.66 (t, J = 7.6 Hz, 2H), 1.95 (p, J = 7.4 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 198.96, 165.46, 160.19, 159.86, 151.70, 141.43, 135.50, 130.02, 128.61, 128.54, 126.15, 122.03, 116.32, 40.54, 36.24, 35.13, 35.04, 24.92. HRMS: $[C_{23}H_{23}N_3O_3 + H]^+$, 390.1812 calcd, 390.1824 found.

*2-Oxo-5-phenyl-N-(4-((6-(trifluoromethyl)pyridin-3-yl)oxy)-phenethyl)pentanamide (**54**).* Boc-deprotection **65c**: a round-bottom flask was charged with Boc-protected amine **65c** (0.18 g, 0.48 mmol, 1 equiv) and HCl (4 M in dioxane, 3 mL, 12 mmol, 25 equiv). After stirring for 1 h, the mixture was concentrated under reduced pressure and coevaporated with toluene (3×), which afforded the deprotected amine **66c** as the HCl salt (0.15 g, 0.48 mmol, quant.). Amide coupling: the title compound was prepared according to the general procedure D using the α-ketoacid **60c** (38 mg, 0.20 mmol, 1 equiv), EDC·HCl (46 mg, 0.24 mmol, 1.2 equiv), HOBt (32 mg, 0.24 mmol, 1.2 equiv), NMM (87 μL, 0.80 mmol, 4 equiv), and the amine HCl salt **66c** (68 mg, 0.24 mmol, 1.2 equiv). Purification by preparative HPLC (C18 reverse phase, 65−75% ACN/H_2O + 0.2% TFA, RT 8.55 min) afforded the product (21 mg, 46 μmol, 23%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.46 (d, J = 2.7 Hz, 1H), 7.62 (d, J = 8.6 Hz, 1H), 7.34−7.22 (m, 5H), 7.21−7.15 (m, 3H), 7.07−6.95 (m, 3H), 3.56 (q, J = 6.9 Hz, 2H), 2.95 (t, J = 7.3 Hz, 2H), 2.87 (t, J = 7.2 Hz, 2H), 2.69−2.62 (m, 2H), 1.94 (p, J = 7.5 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 198.97, 160.15, 156.50, 153.74, 142.12 (q, J = 35.1 Hz), 141.38, 140.89, 135.45, 130.69, 128.60, 128.56, 126.18, 124.53, 124.08 (q, J = 209.2 Hz), 121.63 (q, J = 2.7 Hz), 120.28, 40.55, 36.23, 35.12, 34.92, 24.93. HRMS: $[C_{25}H_{23}F_3N_2O_3 + H]^+$, 457.1734 calcd, 457.1743 found.

*2-Oxo-5-phenyl-N-(4-((5-(trifluoromethyl)pyridin-2-yl)oxy)-phenethyl)pentanamide (**55**).* Boc-deprotection **65d**: a round-bottom flask was charged with Boc-protected amine **65d** (0.19 g, 0.50 mmol, 1 equiv) and HCl (4 M in dioxane, 4.5 mL, 18 mmol, 36 equiv). After stirring for 1 h, the mixture was concentrated under reduced pressure and coevaporated with toluene (3×), which afforded the deprotected amine **66d** as the HCl salt (0.16 g, 0.50 mmol, quant.). Amide coupling: the title compound was prepared according to the general procedure D using the α-ketoacid **60c** (38 mg, 0.20 mmol, 1 equiv), EDC·HCl (46 mg, 0.24 mmol, 1.2 equiv), HOBt (32 mg, 0.24 mmol, 1.2 equiv), NMM (87 μL, 0.80 mmol, 4 equiv), and the amine HCl salt **66d** (76 mg, 0.24 mmol, 1.2 equiv). Purification by preparative HPLC (C18 reverse phase, 55−65% ACN/H_2O + 0.2% TFA, RT 8.74 min) afforded the product (26 mg, 57 μmol, 28%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.51−8.37 (m, 1H), 7.90 (dd, J = 8.7, 2.3 Hz, 1H), 7.32−7.22 (m, 4H), 7.22−7.15 (m, 3H), 7.13−7.07 (m, 2H), 7.07−6.98 (m, 2H), 3.57 (q, J

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= 7.0 Hz, 2H), 2.95 (t, J = 7.3 Hz, 2H), 2.87 (t, J = 7.2 Hz, 2H), 2.74−2.59 (m, 2H), 1.94 (p, J = 7.5 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 198.97, 165.88, 160.18, 151.99, 145.56 (q, J = 4.3 Hz), 141.41, 136.85 (q, J = 3.2 Hz), 135.52, 130.17, 128.60, 128.55, 126.16, 123.79 (q, J = 271.4 Hz), 121.86, 121.49, 111.52, 40.54, 36.24, 35.13, 35.01, 24.93. HRMS: $[C_{25}H_{23}F_3N_2O_3 + H]^+$, 457.1734 calcd, 457.1746 found.

*N-(4-((4-Chloropyrimidin-2-yl)oxy)phenethyl)-2-oxo-5-phenyl Pentanamide (**56**).* Boc-deprotection **65e**: a round-bottom flask was charged with Boc-protected amine **65e** (0.18 g, 0.50 mmol, 1 equiv) and HCl (4 M in dioxane, 3 mL, 12 mmol, 24 equiv). After stirring for 1 h, the mixture was concentrated under reduced pressure and coevaporated with toluene (3×), which afforded the deprotected amine **66e** as the HCl salt (0.14 g, 0.50 mmol, quant.). Amide coupling: the title compound was prepared according to the general procedure D using the α-ketoacid **60c** (38 mg, 0.20 mmol, 1 equiv), EDC·HCl (46 mg, 0.24 mmol, 1.2 equiv), HOBt (32 mg, 0.24 mmol, 1.2 equiv), NMM (87 μL, 0.80 mmol, 4 equiv), and the amine HCl salt **66e** (60 mg, 0.24 mmol, 1.2 equiv). Purification by preparative HPLC (C18 reverse phase, 50% to 60% ACN/H_2O + 0.2% TFA, RT 10.44 min) afforded the product (20 mg, 47 μmol, 24%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.43 (d, J = 5.7 Hz, 1H), 7.32−7.22 (m, 4H), 7.22−7.14 (m, 3H), 7.14−7.07 (m, 2H), 7.04 (t, J = 5.7 Hz, 1H), 6.78 (d, J = 5.7 Hz, 1H), 3.57 (q, J = 6.9 Hz, 2H), 2.95 (t, J = 7.3 Hz, 2H), 2.88 (t, J = 7.2 Hz, 2H), 2.70−2.60 (m, 2H), 1.94 (p, J = 7.5 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 198.95, 170.46, 160.74, 160.38, 160.18, 150.64, 141.39, 136.40, 130.27, 128.59, 128.54, 126.16, 121.62, 106.77, 40.51, 36.23, 35.12, 35.01, 24.92. HRMS: $[C_{23}H_{22}ClN_3O_3 + Na]^+$, 446.1242 calcd, 446.1274 found.

*tert-Butyl 2-(Methoxyamino)-2-oxoacetate (**58**).* Literature procedure:[43] a round-bottom flask was charged with oxalyl chloride (13.5 mL, 158 mmol, 1 equiv) in dry THF (200 mL) under an inert atmosphere and was cooled to 0 °C. *tert*-Butanol (14.7 mL, 154 mmol, 0.975 equiv) was added in one batch, and the mixture was stirred for 1 h at 0 °C. *N*,*O*-Dimethylhydroxylamine hydrochloride (15.4 g, 158 mmol, 1 equiv) was added to the reaction mixture followed by Et_3N (66 mL, 472 mmol, 3 equiv). The reaction mixture was stirred for 2 h at 0 °C, followed by quenching with H_2O (200 mL). The aqueous layer was extracted with EtOAc (2 × 200 mL). The combined organic layers were washed with sat. aq $NaHCO_3$ (2 × 200 mL) and brine (1 × 200 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The crude residue was purified using silica gel column chromatography (0 → 20% EtOAc in pentane), affording the product (22.3 g, 117 mmol, 75%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.76 (s, 3H), 3.20 (s, 3H), 1.56 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 161.98, 161.63, 83.89, 61.82, 30.88, 27.62.

*tert-Butyl 4-(4-Chlorophenyl)-2-oxobutanoate (**59a**).* The title compound was prepared according to the general procedure A using magnesium (0.42 g, 18.3 mmol, 2.0 equiv), 1-(2-bromoethyl)-4-chlorobenzene (1.3 mL, 9.1 mmol, 1 equiv), and Weinreb amide **58** (1.7 g, 9.1 mmol, 1.0 equiv), affording the product (0.85 g, 3.2 mmol, 35%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.24 (d, J = 8.4 Hz, 2H), 7.14 (d, J = 8.4 Hz, 2H), 3.10 (t, J = 7.3 Hz, 2H), 2.90 (t, J = 7.4 Hz, 2H), 1.53 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 194.31, 160.25, 138.78, 132.05, 129.84, 128.63, 84.12, 40.57, 28.42, 27.78.

*tert-Butyl 2-Oxo-4-phenylbutanoate (**59b**).* The title compound was prepared according to the general procedure A using magnesium (72 mg, 3.0 mmol, 0.7 equiv), (2-bromoethyl)benzene (0.58 mL, 4.2 mmol, 1 equiv), and Weinreb amide **58** (0.80 g, 4.2 mmol, 1 equiv), affording the product (0.22 g, 0.95 mmol, 22%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.33−7.25 (m, 2H), 7.25−7.09 (m, 3H), 3.15−3.06 (m, 2H), 2.93 (t, J = 7.5 Hz, 2H), 1.53 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 194.74, 160.47, 140.38, 128.65, 128.49, 126.40, 84.11, 40.92, 29.20, 27.88.

*tert-Butyl 2-Oxo-5-phenylpentanoate (**59c**).* The title compound was prepared according to the general procedure A using magnesium (2.3 g, 94 mmol, 2.0 equiv), (3-bromopropyl)benzene (11 mL, 71 mmol, 1.5 equiv) and Weinreb amide **58** (8.9 g, 47 mmol, 1.0 equiv). Column chromatography (0 → 10% EtOAc in pentane) afforded the product (7.4 g, 30 mmol, 64%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.34−7.21 (m, 2H), 7.16 (t, J = 7.1 Hz, 3H), 2.76 (t, J = 7.3 Hz, 2H), 2.63 (t, J = 7.6 Hz, 2H), 1.92 (p, J = 7.4 Hz, 2H), 1.51 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 195.22, 160.57, 141.16, 128.34, 125.95, 83.65, 38.21, 34.75, 27.66, 24.56.

*tert-Butyl 4-(2-Chlorophenyl)-2-oxobutanoate (**59d**).* The title compound was prepared according to the general procedure A using magnesium (0.17 g, 7.0 mmol, 2 equiv), (4-bromobutyl)benzene (0.62 mL, 3.5 mmol, 1 equiv), and Weinreb amide **58** (0.67 g, 3.5 mmol, 1 equiv), affording the product (0.33 g, 1.3 mmol, 36%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.29−7.20 (m, 2H), 7.20−7.10 (m, 3H), 2.83−2.70 (m, 2H), 2.66−2.55 (m, 2H), 1.71−1.57 (m, 4H), 1.51 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 195.46, 160.77, 141.94, 128.36, 128.31, 125.79, 83.76, 38.89, 35.59, 30.69, 27.77, 22.64.

*tert-Butyl 2-Oxo-2-phenylacetate (**59e**).* The title compound was prepared according to the general procedure A using magnesium (0.21 g, 8.5 mmol, 2 equiv), bromobenzene (0.43 mL, 4.2 mmol, 1 equiv), and Weinreb amide **58** (0.80 g, 4.2 mmol, 1 equiv), affording the product (0.73 g, 3.5 mmol, 83%). ^{1}H NMR (400 MHz, MeOD): δ 7.97−7.87 (m, 2H), 7.58 (t, J = 7.4 Hz, 1H), 7.46 (t, J = 7.7 Hz, 2H), 1.55 (s, 9H). ^{13}C NMR (101 MHz, MeOD): δ 187.86, 164.89, 135.68, 133.15, 130.42, 129.84, 85.53, 85.32, 28.22.

*tert-Butyl 2-Oxo-4-(p-tolyl)butanoate (**59f**).* The title compound was prepared according to the general procedure A using magnesium (0.24 g, 10 mmol, 2 equiv), 1-(2-bromoethyl)-4-methylbenzene (0.77 mL, 5.0 mmol, 1 equiv), and Weinreb amide **58** (0.95 g, 5.0 mmol, 1 equiv), affording the product (0.54 g, 2.2 mmol, 44%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.09−7.03 (m, 4H), 3.10−3.01 (m, 2H), 2.86 (t, J = 7.5 Hz, 2H), 2.28 (s, 3H), 1.50 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 194.42, 160.24, 137.09, 135.51, 129.06, 128.13, 83.59, 40.81, 28.54, 27.58.

*tert-Butyl 2-Oxo-4-(4-(trifluoromethyl)phenyl)butanoate (**59g**).* The title compound was prepared according to the general procedure A using magnesium (0.18 g, 7.9 mmol, 2 equiv), 1-(2-bromoethyl)-4-(trifluoromethyl)benzene (0.67 mL, 4.0 mmol, 1 equiv), and Weinreb amide **58** (0.75 g, 4.0 mmol, 1 equiv), affording the product (0.21 g, 0.83 mmol, 21%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.53 (d, J = 8.1 Hz, 2H), 7.33 (d, J = 8.0 Hz, 2H), 3.25−3.09 (m, 2H), 2.99 (t, J = 7.3 Hz, 2H), 1.53 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 194.12, 160.18, 144.54 (q, J = 1.1 Hz), 128.86, 128.67 (q, J = 36.7 Hz), 125.45 (q, J = 3.8 Hz), 124.30 (q, J = 272.0 Hz), 84.21, 40.27, 28.83, 27.70.

*tert-Butyl 4-(4-Fluorophenyl)-2-oxobutanoate (**59h**).* The title compound was prepared according to the general procedure A using magnesium (0.24 g, 10.6 mmol, 2 equiv), 1-(2-bromoethyl)-4-fluorobenzene (0.72 mL, 5.3 mmol, 1 equiv), and Weinreb amide **59** (1.1 g, 5.3 mmol, 1 equiv), affording the product (0.28 g, 1.1 mmol, 21%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.23−7.07 (m, 2H), 7.04−6.88 (m, 2H), 3.09 (t, J = 7.5 Hz, 2H), 2.91 (t, J = 7.4 Hz, 2H), 1.53 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 194.55, 162.77, 160.37 (d, J = 5.3 Hz), 136.00 (d, J = 3.2 Hz), 129.93 (d, J = 7.8 Hz), 115.37 (d, J = 21.2 Hz), 84.18, 40.90, 28.37, 27.85.

*tert-Butyl 4-(2-Chlorophenyl)-2-oxobutanoate (**59i**).* The title compound was prepared according to the general procedure A using magnesium (0.22 g, 9.1 mmol, 2 equiv), 1-(2-bromoethyl)-2-chlorobenzene (0.69 mL, 4.6 mmol, 1 equiv), and Weinreb amide **58** (0.86 g, 4.6 mmol, 1 equiv), affording the product (0.34 g, 1.3 mmol, 28%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.32 (dd, J = 7.4, 1.8 Hz, 1H), 7.25 (dd, J = 7.2, 2.1 Hz, 1H), 7.20−7.10 (m, 2H), 3.21−3.07 (m, 2H), 3.08−2.97 (m, 2H), 1.52 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 194.24, 160.12, 137.77, 133.79, 130.64, 129.48, 127.86, 126.93, 83.90, 38.87, 27.68, 27.14.

*tert-Butyl 4-(3-Chlorophenyl)-2-oxobutanoate (**59j**).* The title compound was prepared according to the general procedure A using magnesium (0.22 g, 9.1 mmol, 2 equiv), 1-(2-bromoethyl)-3-chlorobenzene (0.67 mL, 4.6 mmol, 1 equiv), and Weinreb amide **58** (0.86 g, 4.6 mmol, 1 equiv), affording the product (0.51 g, 1.9 mmol, 42%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.24−7.11 (m, 3H), 7.11−7.03 (m, 1H), 3.10 (t, J = 7.5 Hz, 2H), 2.89 (t, J = 7.5 Hz, 2H), 1.52 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 194.02, 160.08, 142.29, 134.06, 129.73, 128.45, 126.61, 126.38, 83.92, 40.29, 28.59, 27.64.

*tert-Butyl 4-(3,4-Dichlorophenyl)-2-oxobutanoate (**59k**).* The title compound was prepared according to the general procedure A using

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magnesium (34 mg, 1.4 mmol, 1.2 equiv), 4-(2-bromoethyl)-1,2-dichlorobenzene (0.30 g, 1.2 mmol, 1 equiv), and Weinreb amide **58** (0.27 g, 1.4 mmol, 1.2 equiv), affording the product (90 mg, 0.30 mmol, 25%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.43−7.22 (m, 2H), 7.05 (dd, J = 8.2, 1.6 Hz, 1H), 3.10 (t, J = 7.4 Hz, 2H), 2.89 (t, J = 7.4 Hz, 2H), 1.54 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 194.04, 160.22, 140.61, 132.46, 130.65, 130.53, 130.52, 128.06, 84.36, 40.28, 28.23, 27.86.

*2-Oxo-5-phenylpentanoic Acid (**60c**).* The title compound was prepared according to the general procedure B using α-ketoester **59c** (7.4 g, 30 mmol, 1 equiv) and TFA (23 mL, 300 mmol, 10 equiv), affording the product (5.8 g, 30 mmol, quant.). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.33−7.12 (m, 5H), 2.91 (t, J = 7.2 Hz, 2H), 2.66 (t, J = 7.5 Hz, 2H), 1.98 (p, J = 7.4 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 195.53, 160.50, 140.94, 128.63, 128.56, 126.32, 37.15, 34.86, 24.66. HRMS: $[C_{11}H_{12}O_3 + H]^+$, 193.0859 calcd, 193.0859 found.

*N-Boc-tyramine (**64**).* A round-bottom flask was charged with tyramine (5.0 g, 36 mmol, 1.0 equiv) and dissolved in THF (160 mL). Boc_2O (8.1 g, 37 mmol, 1.0 equiv) and a solution of $NaHCO_3$ (3.4 g, 40 mmol, 1.1 equiv) in water (80 mL) were added, and the reaction was stirred vigorously overnight. The mixture was then extracted with Et_2O (3 × 50 mL), and the combined organic layers were sequentially washed with 0.1 M HCl (1 × 100 mL), water (1 × 100 mL) and brine (1 × 100 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Purification by silica gel column chromatography (10 → 40% EtOAc in pentane) afforded the product (9.1 g, 31 mmol, 85%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.65 (br s, 1H), 6.98 (d, J = 7.8 Hz, 2H), 6.80 (d, J = 8.0 Hz, 2H), 4.92−4.47 (m, 1H), 3.46−3.17 (m, 2H), 2.77−2.56 (m, 2H), 1.44 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 156.53, 155.06, 130.01, 129.81, 115.61, 79.87, 42.14, 35.22, 28.48. HRMS: $[C_{13}H_{19}NO_3 + Na]^+$, 260.1257 calcd, 260.1253 found.

*tert-Butyl (4-(Pyrazin-2-yloxy)phenethyl)carbamate (**65a**).* The title compound was prepared according to the general procedure E using *N*-Boc-tyramine **64** (0.48 g, 2.0 mmol, 1 equiv), 2-chloropyrazine (0.18 mL, 2.0 mmol, 1 equiv) and K_2CO_3 (0.55 g, 4.0 mmol, 2 equiv) in DMSO (2 mL). Column chromatography (20 → 60% EtOAc/pentane) afforded the product (0.50 g, 1.6 mmol, 79%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.40 (s, 1H), 8.23 (s, 1H), 8.08 (s, 1H), 7.25 (d, J = 8.0 Hz, 2H), 7.10 (d, J = 8.0 Hz, 2H), 5.14−4.70 (m, 1H), 3.58−3.20 (m, 2H), 2.82 (t, J = 6.8 Hz, 2H), 1.44 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 160.04, 155.80, 151.32, 140.91, 138.28, 136.15, 135.74, 130.02, 121.16, 78.97, 41.62, 35.53, 28.31. HRMS: $[C_{17}H_{21}N_3O_3 + Na]^+$, 338.1475 calcd, 338.1469 found.

*tert-Butyl(4-(pyrimidin-2-yloxy)phenethyl)carbamate (**65b**).* The title compound was prepared according to the general procedure E using *N*-Boc-tyramine **64** (0.48 g, 2 mmol, 1 equiv), 2-chloropyrimidine (0.23 g, 2 mmol, 1 equiv) and K_2CO_3 (0.55 g, 4 mmol, 2 equiv) in DMSO (2 mL). Column chromatography (40 → 70% EtOAc/pentane) afforded the product (0.52 g, 1.7 mmol, 83%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.56 (d, J = 4.8 Hz, 2H), 7.31−7.19 (m, 2H), 7.14 (d, J = 8.4 Hz, 2H), 7.04 (t, J = 4.8 Hz, 1H), 4.84−4.29 (m, 1H), 3.40 (q, J = 6.3 Hz, 2H), 2.82 (t, J = 6.9 Hz, 2H), 1.44 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 165.60, 159.82, 155.95, 151.48, 136.25, 130.10, 121.77, 116.23, 79.26, 41.74, 35.75, 28.51. HRMS: $[C_{17}H_{21}N_3O_3 + H]^+$, 316.1656 calcd, 316.1653 found.

*tert-Butyl(4-((6-(trifluoromethyl)pyridin-3-yl)oxy)phenethyl)-carbamate (**65c**).* The title compound was prepared according to the general procedure E using *N*-Boc-tyramine **64** (0.25 g, 1.05 mmol, 1.05 equiv), 2-trifluoromethyl-5-fluoropyridine (0.12 mL, 1 mmol, 1 equiv), and K_2CO_3 (0.21 g, 1.5 mmol, 1.5 equiv) in DMF (5 mL). Column chromatography (10 → 40% EtOAc/pentane) afforded the product (0.28 g, 0.73 mmol, 73%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.46 (d, J = 2.6 Hz, 1H), 7.61 (d, J = 8.7 Hz, 1H), 7.45−7.15 (m, 3H), 7.02 (d, J = 8.5 Hz, 2H), 4.89−4.35 (m, 1H), 3.39 (q, J = 6.6 Hz, 2H), 2.82 (t, J = 7.1 Hz, 2H), 1.44 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 156.60, 155.96, 153.40, 140.77, 136.36, 130.76, 124.35, 121.54, 120.10, 79.38, 41.89, 35.75, 28.48. HRMS: $[C_{19}H_{21}F_3N_2O_3 + H]^+$, 383.1577 calcd, 383.1576 found.

*tert-Butyl(4-((5-(trifluoromethyl)pyridin-2-yl)oxy)phenethyl)-carbamate (**65d**).* The title compound was prepared according to the general procedure E using *N*-Boc-tyramine **64** (0.24 g, 1.0 mmol, 1 equiv), 2-chloro-5-(trifluoromethyl)pyridine (0.18 g, 1.0 mmol, 1 equiv) and K_2CO_3 (0.28 g, 2.0 mmol, 2 equiv). Column chromatography (20 → 60% EtOAc/pentane) afforded the product (0.35 g, 0.92 mmol, 92%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.44 (s, 1H), 8.03−7.76 (m, 1H), 7.26 (d, J = 7.7 Hz, 2H), 7.09 (d, J = 8.2 Hz, 2H), 7.01 (d, J = 8.6 Hz, 1H), 4.80−4.21 (m, 1H), 3.40 (q, J = 6.0 Hz, 2H), 2.82 (t, J = 6.9 Hz, 2H), 1.45 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 165.95, 155.99, 151.80, 145.56, 136.77, 136.36, 130.28, 121.63, 111.48, 41.85, 35.81, 28.54. HRMS: $[C_{19}H_{21}F_3N_2O_3 + H]^+$, 383.1577 calcd, 383.1575 found.

*tert-Butyl (4-((2-Chloropyrimidin-4-yl)oxy)phenethyl)carbamate (**65e**).* The title compound was prepared according to the general procedure E using *N*-Boc-tyramine 64 (0.25 g, 1.05 mmol, 1.05 equiv), 2,4-dichloropyrimidine (0.15 g, 1 mmol, 1 equiv), and K_2CO_3 (0.21 g, 1.5 mmol, 1.5 equiv) in DMF (5 mL). The reaction was stirred for 19 h at rt. Column chromatography (20 → 80% EtOAc/pentane) afforded the product(0.22 g, 0.63 mmol, 63%).^{1}H NMR (300 MHz, $CDCl_3$): δ 8.42 (d, J = 5.7 Hz, 1H), 7.26 (d, J = 8.5 Hz, 2H), 7.13−7.03 (m, 2H), 6.78 (d, J = 5.7 Hz, 1H), 4.93−4.42 (m, 1H), 3.40 (q, J = 6.5 Hz, 2H), 2.83 (t, J = 7.0 Hz, 2H), 1.44 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.49, 160.66, 160.31, 155.91, 150.36, 137.23, 130.32, 121.30, 106.64, 79.32, 41.77, 35.77, 28.47. HRMS: $[C_{17}H_{20}ClN_3O_3 + Na]^+$, 372.1085 calcd, 372.1080 found.

*(E)-2-Oxo-N-phenethyl-4-(4-phenoxyphenyl)but-3-enamide (**67**).* α-Ketoacid formation: the α-ketoacid salt was prepared according to the general procedure F using sodium pyruvate (0.44 g, 5.1 mmol, 1 equiv), 4-phenoxybenzaldehyde (1.0 g, 5.1 mmol, 1 equiv), and KOH (0.42 g, 7.6 mmol, 1.5 equiv) in MeOH, affording potassium 4-(4-phenoxyphenyl)-2-oxobut-3-enoate 62f (0.51 g, 1.9 mmol, 38%). Amide coupling: the title compound was prepared according to the general procedure C using potassium salt 62f (0.20 g, 0.93 mmol, 1 equiv), phenethylamine(0.26 mL, 2.1 mmol, 1.1 equiv), HCTU (0.87 g, 2.1 mmol, 1.1 equiv), and DiPEA (0.66 mL, 3.8 mmol, 2 equiv) in DMF, affording the product (0.31 g, 0.82 mmol, 88%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.93 (d, J = 15.9 Hz, 1H), 7.82−7.53 (m, 3H), 7.50−7.16 (m, 9H), 7.16−6.91 (m, 4H), 3.76−3.48 (m, 2H), 2.91 (t, J = 6.8 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 185.23, 161.51, 160.85, 149.08, 147.45, 138.41, 131.18, 130.11, 129.07, 128.81, 126.78, 124.56, 120.11, 118.27, 117.19, 40.73, 35.59..

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.0c00522.

FFA changes in mock-, PLAAT2-, or PLAAT5-transfected U2OS cells; primer sequences used for qPCR experiments; absolute NAE levels of the lipidomic experiments as performed in Figure 3; **LEI-301** selectivity on serine hydrolases and PLA_2 enzymes as measured by gel-based ABPP; and HPLC-traces of **1** and **LEI-301** (PDF)

Molecular formula strings (CSV)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

PLAAT, phospholipase and acyltransferase; NAPE-PLD, *N*-acylphosphatidylethanolamine phospholipase D; NAPE, *N*-acylphosphatidylethanolamine; NAE, *N*-acylethanolamine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; LPC, lyso-phosphatidylcholine; PA, phosphatidic acid; FFA, free fatty acid; ECS, endocannabinoid system; PEA, *N*-palmitoylethanolamine; POEA, *N*-palmitoleoylethanolamine; SEA, *N*-stearoylethanolamine; OEA, *N*-oleoylethanolamine; LEA, *N*-linoleoylethanolamine; AEA, *N*-arachidonoylethanolamine; EPEA, *N*-eicosapentaenoylethanolamine; DHEA, *N*-docosahexaenoylethanolamine; 2-AG, 2-arachidonoylglycerol; PLA2G4E, phospholipase A_2 group IV E; $CB_{1/2}$, cannabinoid receptor 1/2; DAGL, diacylglycerol lipase; FAAH, fatty acid amide hydrolase; MAGL, monoacylglycerol lipase; ABHD, α/β-hydrolase domain containing protein; ABPP, activity-based protein profiling; ABP, activity-based probe; FP-TAMRA, fluorophosphonate-carboxytetramethylrhodamine; DiPEA, *N*,*N*-diisopropylethylamine; EDC, *N*-(3-(dimethylamino)-propyl)-N′-ethylcarbodiimide; HOBt, 1-hydroxybenzotriazole; HCTU, *O*-(6-chlorobenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; HATU, 1-[bis-(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]-pyridinium 3-oxide hexafluorophosphate

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