Universiteit
Leiden
The Netherlands

Activity-based proteomics of the endocannabinoid system

Rooden, E.J. van

Citation

Rooden, E. J. van. (2018, September 11). *Activity-based proteomics of the endocannabinoid system*. Retrieved from https://hdl.handle.net/1887/65174

Version:	Not Applicable (or Unknown)
License:	Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden
Downloaded from:	https://hdl.handle.net/1887/65174

Note: To cite this publication please use the final published version (if applicable).

Cover Page

Universiteit Leiden

The handle http://hdl.handle.net/1887/65174 holds various files of this Leiden University dissertation.

Author: Rooden, E.J. van
Title: Activity-based proteomics of the endocannabinoid system
Issue Date: 2018-09-11

Chapter 1

General introduction

The endocannabinoid system (ECS) consists of the cannabinoid receptors CB_1 and CB_2, their endogenous ligands and the enzymes that regulate the levels of these ligands.[1] The cannabinoid receptors were discovered as the targets of Δ^9-tetrahydrocannabinol (THC), the psychoactive constituent in marijuana. The main endogenous ligands of these receptors are the lipid signaling molecules 2-arachidonoylglycerol (2-AG) and anandamide (AEA). Numerous neurological processes are regulated by the ECS, including learning, memory, pain perception, feeding and reward behaviors.

The medicinal use of preparations of the plant *Cannabis sativa* has a long history. Currently, the endocannabinoid system is viewed as a promising target for the discovery of novel therapeutics.[2–5] However, the psychoactive properties of THC and other CB_1 agonists are undesirable for patients, and these as well as the addictive properties have led to their regulated use and restricted access. The adverse side-effects can potentially be avoided if the CB_1 receptor is not activated, by using antagonists or inverse agonists instead. Rimonabant was the first (and only) inverse agonist on the CB_1 receptor that was approved as an anti-obesity drug. Due to severe psychiatric side effects Rimonabant was removed from the market. The case of Rimonabant illustrates that targeting the ECS is a delicate balancing act due to its involvement in many physiological processes.[6] Therefore, an alternative approach to modulate CB_1 receptor activity is to target the endocannabinoid metabolizing enzymes. Small molecule inhibitors can block the activity of enzymes temporarily and change the levels of the receptor's endogenous ligands. This approach is promising for more controlled tuning of the ECS and finding a suitable therapeutic window.

Activity-based protein profiling (ABPP) is an excellent method for the discovery and optimization of drug candidates to target the ECS.[1] ABPP has benefitted from the tremendous developments in proteomics technology over the last twenty years.[7] ABPP can be used to map the interactions between small molecules and enzymes in living systems.[8] The fundamental biological role of the endocannabinoid enzymes can be studied by measuring when and where these proteins are active *in vivo* by using ABPP.[9] Additionally, a comparison of endocannabinoid enzyme activity can be made between healthy and diseased states.[10] Potential inhibitors can be screened for potency and selectivity simultaneously.[11] ABPP

also has the potential to be used for personalized medicine by guiding the drug and dose selection after measuring the level of enzyme activity in an individual patient.

Endocannabinoid enzymes

A variety of enzymes are involved in the biosynthesis and degradation of both 2-AG and AEA and are therefore potential targets for therapeutic intervention strategies aimed at modulating endocannabinoid levels. 2-AG is mainly generated by the hydrolysis of diacylglycerols, as catalyzed by two diacylglycerol lipases (DAGLα and DAGLβ) (**Fig. 1**).[12] Diacylglycerols are generated by phospholipases C-β (PLC-β) from phosphatidylinositol 4,5-bisphosphate (PIP2).[13,14]

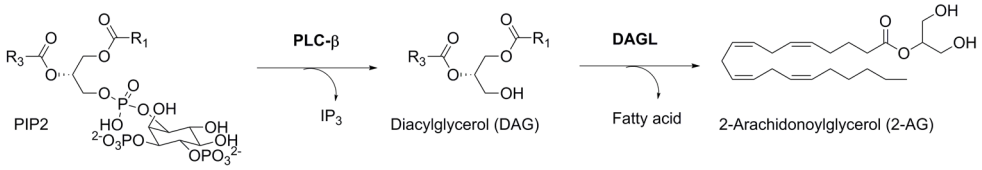


Figure 1 | Biosynthesis of 2-AG. DAGL: diacylglycerol lipase. IP3: inositol 1,4,5-trisphosphate. PIP2; phosphatidylinositol 4,5-bisphosphate. PLC: phospholipase C.

Anandamide is generated by N-acyl transferases that transfer arachidonic acid from the *sn*-1 position of membrane phospholipids to the primary amine of phosphatidylethanolamine (PE) to form N-arachidonoyl phosphatidylethanolamine (NAPE) (**Fig. 2**).[15] Several enzymes can catalyze this transfer: phospholipase A2 group IVE (PLA2G4E) is a calcium-dependent N-acyl transferase[16,17] and the phospholipase A/acyltransferase (PLA/AT) family are calcium-independent N-acyl transferases.[18] From NAPEs, several enzymatic routes can generate N-acyl ethanolamides (NAEs), including AEA.[19] The main route is hydrolysis by N-arachidonoyl phosphatidylethanolamine phospholipase D (NAPE-PLD).[20] Alternative pathways for anandamide synthesis are also proposed, such as via a phospholipase C to form phospho-anandamide, which is subsequently dephosphorylated by phosphatases SH2 domain-containing inositol 5'-phosphatase 1 (SHIP1) or protein-tyrosine phosphatase non-receptor type 22 (PTPN22).[21,22] Another possible route is via two hydrolysis steps by α/β-hydrolase domain containing protein 4 (ABHD4)[23] and one by glycerophosphodiester phosphodiesterase 1 (GDE1)[24] or GDE4.[25] A fourth proposed route goes from NAPE to NAE via secretory phospholipase A2 (sPLA2)[26] or ABHD4 and subsequent hydrolysis to NAE and phosphatidic acid by GDE4[25] or GDE7.[27]

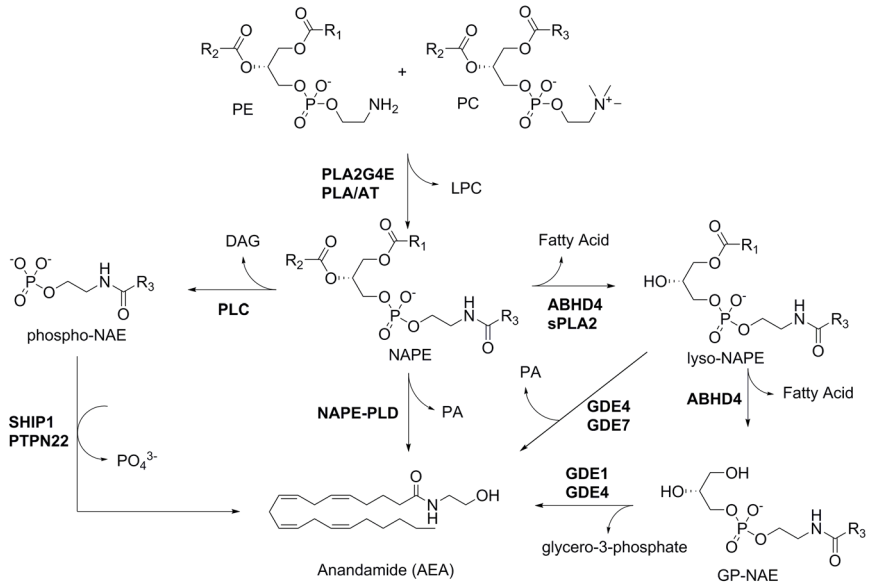


Figure 2 | Biosynthesis of anandamide. $R_{1/2}$: alkyl, R_3 = arachidonoyl. ABHD4: α/β-hydrolase domain containing protein 4. DAG: diacylglycerol. GDE: glycerophosphodiester phosphodiesterase. GP-NAE: *sn*-glycero-3-phospho-N-acylethanolamine . LPC: lysophosphatidylcholine. NAE: N-acylethanolamide. NAPE: N-arachidonoyl phosphatidylethanolamine. PA: phosphatidic acid. PC: phosphatidylcholine. PE: phosphatidylethanolamine. PLA/AT: phospholipase A/acyltransferase. PLA2G4E: phospholipase A2 group IVE. PLC: phospholipase C. PLD: phospholipase D. PTPN22: protein-tyrosine phosphatase non-receptor type 22. SHIP1: SH2 domain-containing inositol 5'-phosphatase 1. sPLA2: secretory phospholipase A2.

Both 2-AG and AEA are hydrolyzed to form arachidonic acid (AA) (**Fig. 3**). 2-AG is mainly hydrolyzed by monoacylglycerol lipase (MAGL),[28] and can also be hydrolyzed by ABHD6 and ABHD12 to form AA and glycerol.[29] Anandamide is hydrolyzed by fatty acid amide hydrolase (FAAH) to AA and ethanolamine.[30] At acidic pH, N-acylethanolamine-hydrolyzing acid amidase (NAAA) also hydrolyzes NAEs, including anandamide.[31]

Activity-based probes for the endocannabinoid system

ABPP relies on chemical probes that react with the catalytic nucleophile of target enzymes in their native biological environment.[32] These probes form a covalent and irreversible bond with the target enzyme and report on the abundance of active enzymes. Therefore, these chemical probes are called activity-based probes (ABPs). ABPP has been successfully applied to discover new enzymes involved in the ECS,[16,29,33] develop selective inhibitors for ECS enzymes[1,34–39] and compare the activity of ECS enzymes in healthy and diseased states.[10]

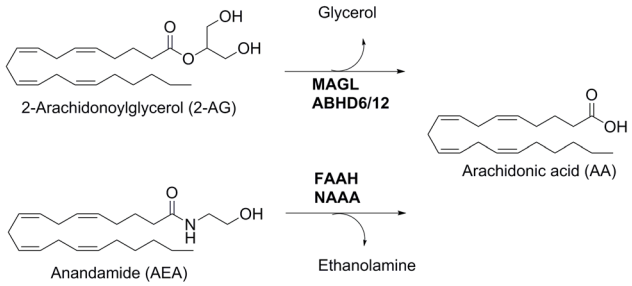


Figure 3 | Hydrolysis of 2-AG and anandamide. ABHD: α/β-hydrolase domain containing protein. FAAH: fatty acid amide hydrolase. MAGL: monoacylglycerol lipase. NAAA: N-acylethanolamine-hydrolyzing acid amidase.

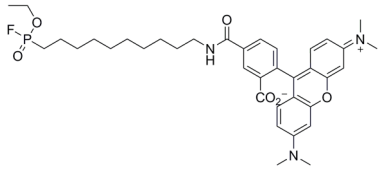
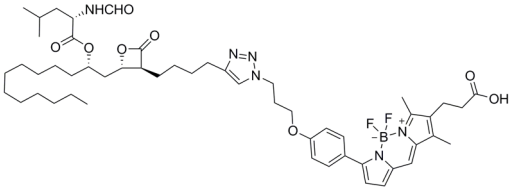
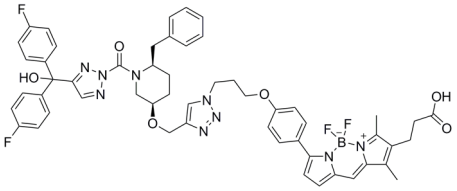
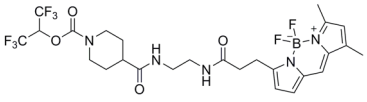
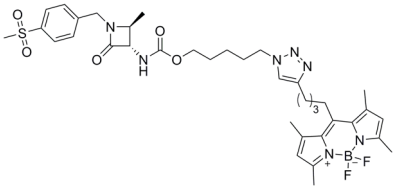
The majority of enzymes involved in the endocannabinoid system are serine hydrolases (**Table 1**). A few enzymes have a catalytic cysteine residue: the PLA/AT family and NAAA. The phosphatase PTNP22 also has a cysteine in the active site, but it is as yet unclear if this cysteine acts as a nucleophile during catalysis. Several other enzymes employ active site histidines for an acid/base mechanism with water in the active site as nucleophile (PLCβ1, PLCβ4, sPLA2[40]). GDE1, GDE4, GDE7 and NAPE-PLD are metallohydrolases and therefore these enzymes cannot be targeted with classical ABPs. The exact identity of some of the proposed ECS enzymes is still unknown (PLC).

Most of the serine hydrolases of the ECS are targeted by broad-spectrum probes with a fluorophosphonate electrophilic trap, such as FP-TAMRA (**Table 2**).[32] FP-TAMRA inhibits DAGLβ, but not DAGLα. MB064 was the first fluorescent probe reported for DAGLα. MB064 is based on the inhibitor tetrahydrolipstatin and has a β-lactone as electrophilic trap.[44] MB064 targets several other enzymes from the α,β-hydrolase fold family. The triazole urea DH379 is a more selective fluorescent probe for diacylglycerol lipases.[45] The carbamate JW912 is a dual ABHD6/MAGL probe.[41] For the cysteine hydrolase NAAA, a fluorescent probe with a β-lactam electrophilic trap is reported.[43]

Table 1 | Human endocannabinoid enzymes and reported activity-based probes.

Enzyme	Uniprot accession	Catalytic nucleophile	Probe	Route
DAGLα[12]	Q9Y4D2	S	MB064, DH379	2-AG biosynthesis
DAGLβ[12]	Q8NCG7	S	MB064, DH379	
PLCβ1[13]	Q9NQ66			
PLCβ4[14]	Q15147			
MAGL[28]	Q99685	S	FP, carbamates	2-AG hydrolysis
ABHD6[29]	Q9BV23	S	FP, carbamates,[41] MB064, DH379	
ABHD12[29]	Q8N2K0	S	FP, MB064	
NAPE-PLD[20]	Q6IQ20			Anandamide biosynthesis
PLA2G4E[16]	Q3MJ16	S	FP	
PLA/AT1-5[18]	Q9HDD0, Q9NWW9, P53816, Q9UL19, Q96KN8	C		
ABHD4[23]	Q8TB40	S	FP, MB064	
GDE1[24]	Q9NZC3			
GDE4[25]	Q8N9F7			
GDE7[27]	Q7L5L3			
PLC				
PTPN22[21]	Q9Y2R2	C?		
SHIP1[22]	Q92835			
sPLA2[26]	P04054			
FAAH[30]	O00519	S	FP	Anandamide hydrolysis
FAAH2	Q6GMR7	S	FP	
NAAA[31]	Q02083	C	β-lactam[42,43]	

Table 2 | The reported fluorescent activity-based probes for endocannabinoid metabolizing enzymes feature distinct chemotypes.

Name	Chemotype	Structure
FP-TAMRA	fluorophosphonate	
MB064	β-lactone	
DH379	triazole urea	
JW912	carbamate	
NAAA-probe	β-lactam	

Aim and outline

The aim of this thesis is to use activity-based proteomics to further our understanding of the endocannabinoid system. This thesis describes the development of a method for the label-free quantification of enzyme activity using proteomics. Furthermore, the development of new activity-based probes for the endocannabinoid enzymes DAGL and ABHD6 is described.

In **Chapter 2**, the activity-based protein profiling method is explained. An overview of available probes, analytical techniques and applications is given. ABPP is applied in **Chapter 3** to study the role of the ECS in the lysosomal storage disorder Niemann-Pick Type C. Both gel-based and mass-spectrometry-based ABPP is used to compare serine hydrolase activity between healthy and diseased brain tissue from a transgenic mouse model. In this study, both dimethyl-labeling and label-free quantitative mass spectrometry is used. This led to the development of an ABPP method with label-free quantification for which a protocol is described in **Chapter 4**. The well-characterized DAGLα inhibitor DH376, which was previously studied using dimethyl-labeling techniques,[45] was studied with this new method. **Chapters 5 and 6** describe the design and synthesis of new probes. In **Chapter 5**, the synthesis and characterization of quenched activity-based probes for DAGL and ABHD6 is described. In **Chapter 6**, the design and synthesis of new two-step probes for DAGL is described. **Chapter 7** provides a summary and points at directions for future research.

References

1. Blankman, J. L. & Cravatt, B. F. Chemical probes of endocannabinoid metabolism. *Pharmacol. Rev.* **65**, 849–71 (2013).
2. Pacher, P. The Endocannabinoid System as an Emerging Target of Pharmacotherapy. *Pharmacol. Rev.* **58**, 389–462 (2006).
3. Chiurchiù, V., van der Stelt, M., Centonze, D. & Maccarrone, M. The endocannabinoid system and its therapeutic exploitation in multiple sclerosis: Clues for other neuroinflammatory diseases. *Prog. Neurobiol.* **160**, 82–100 (2017).
4. Scotter, E. L., Abood, M. E. & Glass, M. The endocannabinoid system as a target for the treatment of neurodegenerative disease. *Br. J. Pharmacol.* **160**, 480–498 (2010).
5. Marzo, V. Di, Bifulco, M. & Petrocellis, L. De. The endocannabinoid system and its therapeutic exploitation. *Nat. Rev. Drug Discov.* **3**, 771–784 (2004).
6. Di Marzo, V. Targeting the endocannabinoid system: To enhance or reduce? *Nat. Rev. Drug Discov.* **7**, 438–455 (2008).
7. Cravatt, B. F., Wright, A. T. & Kozarich, J. W. Activity-Based Protein Profiling: From Enzyme Chemistry to Proteomic Chemistry. *Annu. Rev. Biochem.* **77**, 383–414 (2008).
8. Speers, A. E. & Cravatt, B. F. Profiling Enzyme Activities In Vivo Using Click Chemistry Methods. *Chem. Biol.* **11**, 535–546 (2004).
9. Wright, A. T. & Cravatt, B. F. Chemical Proteomic Probes for Profiling Cytochrome P450 Activities and Drug Interactions In Vivo. *Chem. Biol.* **14**, 1043–1051 (2007).
10. Nomura, D. K. *et al.* Monoacylglycerol Lipase Regulates a Fatty Acid Network that Promotes Cancer Pathogenesis. *Cell* **140**, 49–61 (2010).
11. Bachovchin, D. A., Brown, S. J., Rosen, H. & Cravatt, B. F. Identification of selective inhibitors of uncharacterized enzymes by high-throughput screening with fluorescent activity-based probes. *Nat. Biotechnol.* **27**, 387–394 (2009).
12. Bisogno, T. *et al.* Cloning of the first sn1-DAG lipases points to the spatial and temporal regulation of endocannabinoid signaling in the brain. *J. Cell Biol.* **163**, 463–468 (2003).
13. Hashimotodani, Y. *et al.* Phospholipase Cβ serves as a coincidence detector through its Ca2+dependency for triggering retrograde endocannabinoid signal. *Neuron* **45**, 257–268 (2005).
14. Maejima, T. Synaptically Driven Endocannabinoid Release Requires Ca2+-Assisted Metabotropic Glutamate Receptor Subtype 1 to Phospholipase C 4 Signaling Cascade in the Cerebellum. *J. Neurosci.* **25**, 6826–6835 (2005).
15. Rahman, I. A. S., Tsuboi, K., Uyama, T. & Ueda, N. New players in the fatty acyl ethanolamide metabolism. *Pharmacol. Res.* **86**, 1–10 (2014).
16. Ogura, Y., Parsons, W. H., Kamat, S. S. & Cravatt, B. F. A calcium-dependent acyltransferase that produces N-acyl phosphatidylethanolamines. *Nat. Chem. Biol.* **12**, 1–5 (2016).

17. Natarajan, V., Schmid, P. C., Reddy, P. V, Zuzarte-Augustin, M. Lou & Schmid, H. H. Biosynthesis of N-Acylethanolamine Phospholipids by Dog Brain Preparations. *J. Neurochem.* **41**, 1303–1312 (1983).
18. Uyama, T. *et al.* Generation of N-acylphosphatidylethanolamine by members of the phospholipase A/acyltransferase (PLA/AT) family. *J. Biol. Chem.* **287**, 31905–31919 (2012).
19. Hussain, Z., Uyama, T., Tsuboi, K. & Ueda, N. Mammalian enzymes responsible for the biosynthesis of N-acylethanolamines. *Biochim. Biophys. Acta - Mol. Cell Biol. Lipids* **1862**, 1546–1561 (2017).
20. Okamoto, Y., Morishita, J., Tsuboi, K., Tonai, T. & Ueda, N. Molecular Characterization of a Phospholipase D Generating Anandamide and Its Congeners. *J. Biol. Chem.* **279**, 5298–5305 (2004).
21. Liu, J. *et al.* A biosynthetic pathway for anandamide. *Proc. Natl. Acad. Sci.* **103**, 13345–50 (2006).
22. Liu, J. *et al.* Multiple pathways involved in the biosynthesis of anandamide. *Neuropharmacology* **54**, 1–7 (2008).
23. Simon, G. M. & Cravatt, B. F. Endocannabinoid biosynthesis proceeding through glycerophospho-N-acyl ethanolamine and a role for α/β-hydrolase 4 in this pathway. *J. Biol. Chem.* **281**, 26465–26472 (2006).
24. Simon, G. M. & Cravatt, B. F. Anandamide biosynthesis catalyzed by the phosphodiesterase GDE1 and detection of glycerophospho-N-acyl ethanolamine precursors in mouse brain. *J. Biol. Chem.* **283**, 9341–9349 (2008).
25. Tsuboi, K. *et al.* Glycerophosphodiesterase GDE4 as a novel lysophospholipase D: A possible involvement in bioactive N-acylethanolamine biosynthesis. *Biochim. Biophys. Acta - Mol. Cell Biol. Lipids* **1851**, 537–548 (2015).
26. Sun, Y.-X. *et al.* Biosynthesis of anandamide and N-palmitoylethanolamine by sequential actions of phospholipase A2 and lysophospholipase D. *Biochem. J.* **380**, 749–56 (2004).
27. Ohshima, N. *et al.* New members of the mammalian glycerophosphodiester phosphodiesterase family: GDE4 and GDE7 produce lysophosphatidic acid by lysophospholipase D activity. *J. Biol. Chem.* **290**, 4260–4271 (2015).
28. Dinh, T. P. *et al.* Brain monoglyceride lipase participating in endocannabinoid inactivation. *Proc. Natl. Acad. Sci.* **99**, 10819–10824 (2002).
29. Blankman, J. L., Simon, G. M. & Cravatt, B. F. A Comprehensive Profile of Brain Enzymes that Hydrolyze the Endocannabinoid 2-Arachidonoylglycerol. *Chem. Biol.* **14**, 1347–1356 (2007).
30. Cravatt, B. F. *et al.* Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. *Nature* **384**, 83–87 (1996).
31. Tsuboi, K. *et al.* Molecular characterization of N-acylethanolamine-hydrolyzing acid amidase, a novel member of the choloylglycine hydrolase family with structural and functional similarity to acid ceramidase. *J. Biol. Chem.* **280**, 11082–11092 (2005).
32. Liu, Y., Patricelli, M. P. & Cravatt, B. F. Activity-based protein profiling: The serine hydrolases. *Proc. Natl. Acad. Sci.* **96**, 14694–14699 (1999).
33. Barglow, K. T. & Cravatt, B. F. Activity-based protein profiling for the functional annotation of enzymes. *Nat. Methods* **4**, 822–827 (2007).
34. Ahn, K. *et al.* Discovery of a Selective Covalent Inhibitor of Lysophospholipase-like 1 (LYPLAL1) as a

Tool to Evaluate the Role of this Serine Hydrolase in Metabolism. *ACS Chem. Biol.* **11**, 2529–2540 (2016).

35. Adibekian, A. *et al.* Click-generated triazole ureas as ultrapotent in vivo-active serine hydrolase inhibitors. *Nat. Chem. Biol.* **7**, 469–478 (2011).
36. Bachovchin, D. A. *et al.* Discovery and optimization of sulfonyl acrylonitriles as selective, covalent inhibitors of protein phosphatase methylesterase-1. *J. Med. Chem.* **54**, 5229–5236 (2011).
37. Baggelaar, M. P. *et al.* A highly selective, reversible inhibitor identified by comparative chemoproteomics modulates diacylglycerol lipase activity in neurons. *J. Am. Chem. Soc.* **137**, 8851–8857 (2015).
38. Hsu, K. L. *et al.* Discovery and optimization of piperidyl-1,2,3-triazole ureas as potent, selective, and in vivo-active inhibitors of alpha/beta-hydrolase domain containing 6 (ABHD6). *J. Med. Chem.* **56**, 8270–8279 (2013).
39. Janssen, F. J. *et al.* Discovery of glycine sulfonamides as dual inhibitors of sn-1-diacylglycerol lipase a and a/b-hydrolase domain 6. *J. Med. Chem.* **57**, 6610–6622 (2014).
40. Burke, J. E. & Dennis, E. Phospholipase A2 Biochemistry. *Cardiovasc. Drugs Ther.* **23**, 49 (2009).
41. Chang, J. W., Cognetta, A. B., Niphakis, M. J. & Cravatt, B. F. Proteome-wide reactivity profiling identifies diverse carbamate chemotypes tuned for serine hydrolase inhibition. *ACS Chem. Biol.* **8**, 1590–1599 (2013).
42. Romeo, E. *et al.* Activity-based probe for N-acylethanolamine acid amidase. *ACS Chem. Biol.* **10**, 2057–2064 (2015).
43. Petracca, R. *et al.* Novel activity-based probes for N-acylethanolamine acid amidase. *Chem. Commun.* **53**, 11810–11813 (2017).
44. Baggelaar, M. P. *et al.* Development of an activity-based probe and in silico design reveal highly selective inhibitors for diacylglycerol lipase-α in brain. *Angew. Chem. Int. Ed.* **52**, 12081–12085 (2013).
45. Ogasawara, D. *et al.* Rapid and profound rewiring of brain lipid signaling networks by acute diacylglycerol lipase inhibition. *Proc. Natl. Acad. Sci.* **113**, 26–33 (2016).