Discovery of novel inhibitors to investigate diacylglycerol lipases and α/β hydrolase domain 16A

Janssen, F.J.

CHAPTER 1

General introduction

Drug discovery in industry and academia

Drug discovery is essential to improve human health and lifespan and has delivered many life-saving molecules. Yet, drug discovery is expensive, time consuming and a high risk endeavor.[1,2] Medical needs are changing due to modern way of life and a growing elderly population. As a result, society faces many disease related challenges, including, many forms of cancer, increased antimicrobial resistance and metabolic and neurodegenerative disorders (*e.g.* obesity, type-2 diabetes, Parkinson's and Alzheimer's disease). Novel therapies to prevent, or to treat these diseases are urgently required. Current market introduction rate of new drugs is low, while costs of drug development have risen substantially in the last decades,[3] due to late stage clinical failures.[4,5] Hence pharmaceutical Research & Development (R&D) is facing a productivity crisis.[6] Consequently, business models of the pharmaceutical industry are changing, which leads to mergers and acquisitions, followed by reorganizations, down-sizing of internal R&D budgets and outsourcing to lower-cost contract research organizations.[7] Nowadays, more emphasis is placed on public-private-partnerships to perform early drug discovery activities.[8] Fundamental academic research is therefore crucial for discovering novel target-lead combinations. Academia contributes to target discovery, validation and de-risking.[9] Moreover, the development of novel treatments for neglected and orphan diseases and identification of novel drug discovery methods are important fields of research for the academic drug discoverer.[9,10]

The majority of first-in-class drugs approved by the FDA between 1999 and 2013 were discovered by a target-based approach.[11] Target-based drug discovery strategies can be classified in structure-based drug design (SBDD) and ligand-based drug design (LBDD). In SBDD, knowledge of the three-dimensional structure of the target protein at a molecular level with atomic resolution is required to study the specific interactions of a ligand and its protein.[12] Usually, X-ray crystallography and/or NMR spectroscopy techniques are applied to generate three dimensional models of the protein structures. Alternatively, a homology

model can be build based on the reported structure of related proteins. The protein structure can be used to screen virtual libraries to identify novel hits and to guide the optimization of ligands.

LBDD is an important alternative drug discovery strategy when a three dimensional structure of the target is not available. High throughput screening (HTS) is the most often employed LBDD technique to discover novel ligands, especially when limited target and ligand knowledge are available. In HTS, large sets of diverse compounds are tested for their activity against purified protein or cell lysates overexpressing the target of interest, employing fast and economical multi-well activity assays most often using surrogate substrates. HTS is hampered by false positives, due to, for example, pan-assay interference compounds (PAINS)[13] or poor physico-chemical properties of the compounds. Therefore, thorough assay optimization, high assay quality and reproducibility, hit deselection and active confirmation procedures and orthogonal assays are necessary.

Another popular LBDD strategy is ligand-based pharmacophore modeling.[14] In a pharmacophore, chemical features of a series of known ligands that are deemed essential for the interaction with its target, are grouped together in a three dimensional model. The pharmacophore model can be used to mine virtual compound libraries to discover novel hits. Challenges of pharmacophore modeling include dealing with conformational flexibility and scoring and weighting of screening results.[15] Moreover, confirmation of the activity of the virtual hit in a biochemical or cellular assay is essential to identify true hits.

Serine hydrolases

Serine hydrolases are one of the largest enzyme families (>200 members) in the human genome and utilize an active site serine for substrate hydrolysis. They partake in a plethora of (patho) physiological functions, including neurotransmission, learning, pain, energy metabolism and cancer (see [16] for extensive review). Several drugs act as serine hydrolase inhibitors, such as Januvia,[17] Rivastigmine[18] and Orlistat (Tetrahydrolipstatin, marketed as Xenical and Alli).[19,20] The exact function of many serine hydrolases remains, however, unknown to date. Hence, small molecule inhibitors may help to elucidate their function in health and disease and could have tremendous untapped medical potential across this large family of proteins.

Activity-based protein profiling (ABPP) has been developed as a strategy to identify and evaluate novel inhibitors for the serine hydrolase family without having the need of knowing the endogenous substrate or developing dedicated enzymatic assays of each individual enzyme.[21] In brief, ABPP uses activity-based probes (*i.e.* inhibitors with a reporter tag, such as a fluorophore or biotin) to label endogenous activity of enzymes by mechanism-based inhibition (Figure 1A). In this manner, serine hydrolases can be investigated in complex proteomes, such as cell or tissue lysates, using for example fluorophosphonates.[21] ABPP can also be used in a competitive setting in which proteomes are pretreated with an inhibitor,

which is followed by labeling of residual enzyme activity using the activity-based probe (Figure 1B). ABPP is complementary to multi-well substrate activity assays, because it is a very powerful tool to rapidly assess inhibitor potency on target and selectivity over related enzymes.

In this Thesis, ABPP is used throughout the drug discovery process to identify and optimize inhibitors of the serine hydrolases diacylgycerol lipases (DAGLs) and α/β hydrolase domain type 16A (ABHD16A).

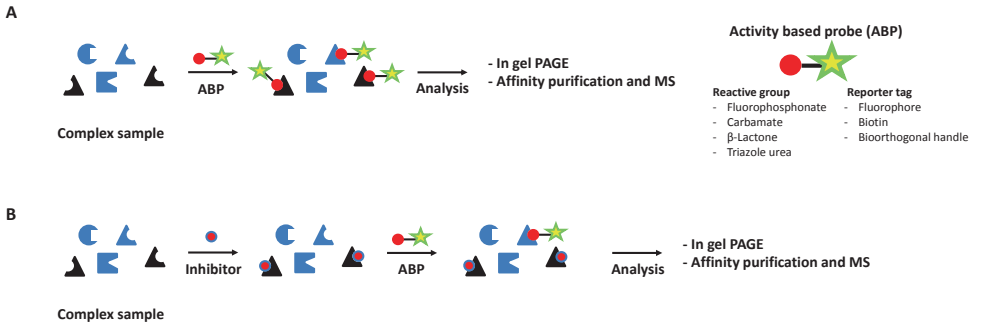

Figure 1. The concept of activity-based protein profiling (ABPP). **A)** Complex samples are incubated with an activity-based probe (ABP), which labels several proteins by mechanism-based inhibition. In case of the serine hydrolases, the active site serine is targeted with an electrophilic trap (*e.g.* fluorophosphonate, carbamate, β-lactone). In an ABP, the reactive moiety is attached to a reported tag (fluorophore or biotin). Analysis of the sample can then be performed by SDS-PAGE and standard in-gel fluorescence scanning (in case the tag is a fluorophore) or by affinity purification and mass spectrometry (MS) analysis (in case the tag is a biotin) or by Western Blot. Two-step ABPP can be performed using a biorthogonal handle as tag. **B)** In competitive ABPP, samples are pre-incubated with an inhibitor that can compete for ABP labeling. The sample is analyzed and corrected for control (vehicle).

Diacylglycerol lipases

In 1995, 2-arachidonoylglycerol (2-AG) was isolated from intestinal tissue and was characterized as the second endogenous ligand for the cannabinoid type 1 and 2 receptors (CB1R and CB2R).[22–24] The CBRs are important G-protein coupled receptors involved in a broad range of (patho)physiological functions, including addiction,[25] appetite[26–28] and memory formation.[29–31] 2-AG is considered to be an important signaling lipid. After its discovery, Stella *et al.* showed that 2-AG is highly abundant in brain and controls long term potentiation.[32] 2-AG accumulated in neurons in a Ca^{2+}-dependent manner. Combined phospholipase C (PLC) and diacylglycerol lipase (DAGL) activity were suggested as contributors to 2-AG formation (Figure 2).[32–34] In 2003, Bisogno *et al.* discovered two Ca^{2+} dependent lipases that produce 2-AG in the brain and designated them DAGLα and DAGLβ.[35] DAGLs are serine hydrolases that specifically cleave *sn*-1 fatty acyl chains of arachidonate-containing 1,2-diacylglycerols. DAGLα, a 120 kDa protein with 1042 amino acids, contains a short N-terminal sequence, followed by four transmembrane helices, an intracellular catalytic domain with a cysteine rich insert and a large C-terminal tail.[35] This tail is absent in

DAGLβ, making it a shorter homolog (672 amino acids and 70 kDa). Interestingly, DAGLα contains a PPXXF motif (Pro972-Phe976) in the C-terminus that interacts with CC-Homer proteins.[36] This interaction is required for DAGLα recruitment to the metabotropic glutamate receptor signaling complex in the plasmamembrane, but not for its enzymatic activity.[36] Both isoforms have a typical α/β hydrolase fold and a Ser-His-Asp catalytic triad typical for serine hydrolases (DAGLα: Ser472, His650, Asp524; DAGLβ: Ser443, Asp495, His: unknown). Reisenberg *et al.* postulated that both DAGLs contain a large insert of unknown structure between the 7th and 8th canonical β-sheet that may function as a regulatory loop, controlling substrate access to the catalytic site.[37]

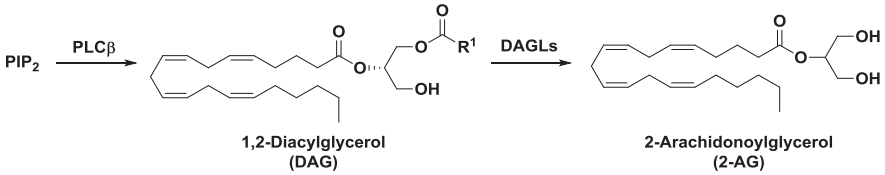

Figure 2. Phospholipase C β (PLCβ) catalyzes the formation of 1,2-diacylglycerols (DAG) from phosphatidylinositol-4,5-bisphosphate (PIP_2). *Sn*-1 specific diacylglycerol lipases (DAGLs) subsequently catalyze the formation of 2-arachidonylglycerol (2-AG) from its DAG precursor.

DAGLα and β show a marked difference in tissue expression. DAGLα is highly expressed in the central nervous system, whereas DAGLβ is predominantly located in the periphery. In line, DAGLα knockout mice show ~80% reduction in 2-AG levels in the brain and spinal cord, whereas DAGLβ knockout mice show ~50% reduction in brain.[38] In the liver of DAGLβ knockout mice 2-AG levels are reduced with ~90% , whereas for DAGLα knockout mice 2-AG levels are reduced by ~60%. A large difference was also observed in adipose tissue, where ~50% 2-AG reduction for DAGLα knockout mice was observed, while deletion of DAGLβ had no significant effect on basal 2-AG levels.[38] This indicates that specific DAGL isoforms contribute to 2-AG pools in a highly tissue dependent manner. Of note, the levels of arachidonic acid (AA), a metabolite of 2-AG, were also decreased in brain, spinal cord and liver of DAGLα knockout mice.[39] The subcellular localization of DAGLs indicate that 2-AG is produced at postsynaptic sites that are close to the presynaptically located CB receptors.[40] Gao *et al.* showed that mice lacking DAGLα have significantly reduced retrograde signaling (depolarization-induced suppression of inhibition, DSI) in brain through CB1R-dependent signaling. Moreover, both DAGLα and DAGLβ knockout mice show reduced adult neurogenesis in the hippocampus and subventricular zone. Another study performed by Tanimura *et al.* showed similar results, as both depolarization-induced suppression of excitation (DSE) and DSI were absent in DAGLα knockout mice in brain cerebellum, hippocampus, and striatum.[41] Importantly, congenital deletion of DAGLβ had no effect on retrograde suppression of synaptic transmission. This indicates that DAGLα is the main isoform responsible for the neuronal signaling pool of 2-AG.

Selective inhibitors of DAGLα may contribute to a more fundamental understanding of the physiological role of 2-AG and may serve as potential drug candidates for the treatment of obesity and neurodegenerative diseases. Currently, there are no selective inhibitors available for the study of DAGLα. The identification of selective DAGLα inhibitors is hampered by a lack of structural knowledge of the target, and lack of assays that make use of endogenous DAGLα activity in proteomes or in a multi-well format. No crystal structures are available and no homology models have been reported to aid hit identification and perform SBDD. To date, only a few DAGL inhibitors have been described, however, most of them lack selectivity, *in vivo* activity and/or pharmacokinetic properties to study the role of 2-AG in *in vivo* models of disease. Thus, there is an unmet need to identify novel chemotypes to modulate DAGL activity.

α/β Hydrolase domain type 16A

α/β Hydrolase domain type 16A (ABHD16A), has recently been identified as a key contributor to *lyso*-phosphatydylserine (*lyso*-PS) formation in mice (Figure 3).[42] *Lyso*-PS is an important signaling phospholipid involved in immune response through its interaction with Toll-like receptor 2 (TLR2).[43] In addition, *lyso*-PS has been associated with T-cell growth,[44] mast cell activation[45,46] and neurite outgrowth.[47] *Lyso*-PS is metabolized by α/β hydrolase domain type 12 (ABHD12).[48] Interestingly, absence of ABHD12 activity in humans caused by specific mutations, has been linked to the onset of multiple symptoms of the rare genetic disease polyneuropathy-hearing loss-ataxia-retinitis pigmentosa and cataract (PHARC).[49,50] As such, PHARC can be seen as a ABHD12 null model and accumulation of *lyso*-PS may be the cause rather than an effect of the disease. If this hypothesis holds true, then diminishing *lyso*-PS levels would be expected to reduce neuroinflammation, and thereby, lead to amelioration of the symptoms. To test these assumptions, *in vivo* active inhibitors of ABHD16A, which can be used in a substrate reduction therapy, are required. However, no *in vivo* active ABHD16 inhibitors have been described as of yet and their discovery is hindered by limited structural knowledge of the target, lack of multi-well activity assays and a dearth of available starting points for a hit optimization program.

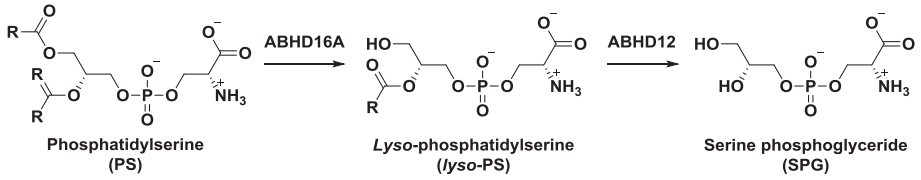

Figure 3. α/β Hydrolase domain type 16A (ABHD16A, BAT5) catalyzes the formation of *lyso*-phosphatidylserine (lyso-PS). α/β Hydrolase domain type 12 (ABDH12) subsequently hydrolases *lyso*-PS to serine phosphoglyceride (SPG).

Aim and outline of this thesis

This thesis describes the discovery and optimization of potent inhibitors for DAGLs and ABHD16A, employing several LBDD and SBDD strategies integrated with ABPP. These inhibitors can be used as tool compounds to report on the function of both targets in mammalian physiology, and may serve as leads for the development of potential drug candidates.

Chapter 2 describes the discovery of a novel reversible DAGL chemotype by *in silico* screening. The development of a ligand-based pharmacophore model, derived from a known DAGL inhibitor (Tetrahydrolipstatin), was used to screen a subset of commercially available endocannabinoid metabolism targeting inhibitors. The α-keto heterocycle LEI104 was identified as the first reversible DAGLα inhibitor.[51] To explain its molecular interactions with DAGLα, a homology model was constructed and the binding mode of LEI104 was investigated. Subsequently, the structural requirements for specific DAGLα inhibition by LEI104 were further investigated by screening a focused library of 1040 compounds in **Chapter 3**.[52] This showed that α-keto oxazolo-4*N*-pyridine was the optimal scaffold for this class of DAGL inhibitors. The comprehensive structure-activity relationships were used to validate the DAGLα homology model generated in Chapter 2, which proved to be indispensible to the discovery of LEI105, a highly potent and selective dual DAGLα/β inhibitor.[53] **Chapter 4** describes the lead optimization of LEI105 leading to LEI107, an exquisitely selective DAGL inhibitor with drug-like properties. **Chapter 5** describes the first structure-activity relationship analysis of a novel class of reversible DAGL inhibitors, the glycine sulfonamides. This class was previously identified by a HTS campaign.[54] This chapter describes the discovery and optimization of LEI106, which is the first reported reversible potent dual DAGLα/ ABHD6 inhibitor.[55] Finally, potential binding modes for the glycine sulfonamides are explored using structure-guided homology modeling. **Chapter 6** describes the discovery of several new DAGLα chemotypes by HTS and ABPP. A 300.000+ compound collection was screened for activity against DAGLα using fully automated HTS, within the European Lead Factory (ELF). The colorimetric primary assay results were followed by an orthogonal ABPP assay to assess potency and selectivity of the compounds in complex mouse brain proteome. This resulted in the discovery of sulfonyl 1,2,4-triazole ureas as a novel scaffold for serine hydrolases. Moreover eight other compound classes were discovered, including α- and β-keto amides and the previously reported glycine sulfonamides. **Chapter 7** describes the development of inhibitors for ABHD16A (BAT5) by application of ABPP in LBDD. The 1,2,4-triazole sulfonamide ureas, described in Chapter 6, were found to inhibit ABHD16A by orthogonal ABPP. Subsequent optimization and characterization led to two compounds that exhibited *in vivo* activity on brain ABHD16A in mice. **Chapter 8** provides an extensive overview of all the current DAGL inhibitors and their effect in preclinical models of neurodegenerative and metabolic disorders.[56] **Chapter 9** provides a summary of this Thesis and states future directions for additional research.

References

1. Mignani, S., Huber, S., Tomás, H., Rodrigues, J. & Majoral, J. P. Why and how have drug discovery strategies in pharma changed? What are the new mindsets? *Drug Discov. Today* **21,** 239–249 (2015).
2. Hughes, J. P., Rees, S. S., Kalindjian, S. B. & Philpott, K. L. Principles of early drug discovery. *Br. J. Pharmacol.* **162,** 1239–1249 (2011).
3. DiMasi, J. A., Hansen, R. W. & Grabowski, H. G. The price of innovation: New estimates of drug development costs. *J. Health Econ.* **22,** 151–185 (2003).
4. David, E., Tramontin, T. & Zemmel, R. Pharmaceutical R&D: the road to positive returns. *Nat. Publ. Gr.* **8,** 609–610 (2009).
5. Mervis, J. Productivity Counts — But the Definition Is Key. *Science* **309,** 726–727 (2005).
6. Pammolli, F., Magazzini, L. & Riccaboni, M. The productivity crisis in pharmaceutical R&D. *Nat. Rev. Drug Discov.* **10,** 428–438 (2011).
7. Clark, D. E. & Newton, C. G. Outsourcing lead optimisation--the quiet revolution. *Drug Discov. Today* **9,** 492–500 (2004).
8. Crommelin, D. *et al.* Pharmaceutical sciences in 2020. *Nat. Rev. Drug Discov.* **9,** 99–100 (2010).
9. Wyatt, P. G. The emerging academic drug-discovery sector. *Future Med. Chem.* **1,** 1013–1017 (2009).
10. Frye, S., Crosby, M., Edwards, T. & Juliano, R. US academic drug discovery. *Nat. Rev. Drug Discov.* **10,** 409–410 (2011).
11. Eder, J., Sedrani, R. & Wiesmann, C. The discovery of first-in-class drugs: origins and evolution. *Nat. Rev. Drug Discov.* **13,** 577–87 (2014).
12. Aparoy, P., Reddy, K. K. & Reddanna, P. Structure and ligand-based drug design strategies in the development of novel 5-LOX inhibitors. *Curr. Med. Chem.* **19,** 3763–3778 (2012).
13. Baell, J. & Walters, M. A. Chemical con artists foil drug discovery. *Nature* **513,** 481–483 (2014).
14. Acharya, C., Coop, A., Polli, J. E. & Mackerell, A. D. Recent advances in ligand-based drug design: relevance and utility of the conformationally sampled pharmacophore approach. *Curr. Comput. Aided. Drug Des.* **7,** 10–22 (2011).
15. Yang, S.-Y. Pharmacophore modeling and applications in drug discovery: challenges and recent advances. *Drug Discov. Today* **15,** 444–450 (2010).
16. Long, J. & Cravatt, B. The Metabolic Serine Hydrolases and Their Functions in Mammalian Physiology and Disease. *Chem Rev* **111,** 6022–6063 (2012).
17. Kim, D. *et al.* (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine: a potent, orally active dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes. *J. Med. Chem.* **48,** 141–151 (2005).
18. Rösler, M. *et al.* Efficacy and safety of rivastigmine in patients with Alzheimer's disease: international randomised controlled trial. *BMJ* **318,** 633–8 (1999).
19. Borgstriim, B. Mode of action of tetrahydrolipstatin: a derivative of the naturally occurring lipase inhibitor lipstatin. *Enzyme* 308–316 (1988).
20. Hadváry, P., Lengsfeld, H. & Wolfer, H. Inhibition of pancreatic lipase in vitro by the covalent inhibitor tetrahydrolipstatin. *Biochem. J.* **256,** 357–361 (1988).
21. Liu, Y., Patricelli, M. P. & Cravatt, B. F. Activity-based protein profiling: the serine hydrolases. *Proc. Natl. Acad. Sci. U.S.A.* **96,** 14694–14699 (1999).
22. Sugiura, T. *et al.* 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. *Biochem. Biophys. Res. Commun.* **215,** 89–97 (1995).
23. Mechoulam, R. *et al.* Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem. Pharmacol.* **50,** 83–90 (1995).
24. Devane, W. A. *et al.* Isolation and Structure of a Brain Constituent That binds to the Cannabinoid Receptor. *Science* **258,** 1946–1949 (1992).
25. Ledent, C. *et al.* Unresponsiveness to Cannabinoids and Reduced Addictive Effects of Opiates in CB1 receptor Knockout Mice. *Science* **283,** 401–404 (1999).
26. Colombo, G. *et al.* Appetite supression and weight loss after the cannabinoid antagonist SR 141716. *Life Sci.* **63,** 113–117 (1998).
27. Di Marzo, V. *et al.* Leptin-regulated endocannabinoids are involved in maintaining food intake. *Nature* **410,** 822–825 (2001).
28. Koch, M. *et al.* Hypothalamic POMC neurons promote cannabinoid-induced feeding. *Nature* **519,** 45–50 (2015).

29. Hampson, R. E. & Deadwyler, S. A. Role of cannabinoid receptors in memory storage. *Neurobiol. Dis.* **5,** 474–482 (1998).
30. Reibaud, M. *et al.* Enhancement of memory in cannabinoid CB1 receptor knock-out mice. *Eur. J. Pharmacol.* **379,** R1–R2 (1999).
31. Marsicano, G. *et al.* The endogenous cannabinoid system controls extinction of aversive memories. *Nature* **418,** 530–534 (2002).
32. Stella, N., Schweitzer, P. & Piomelli, D. A second endogenous cannabinoid that modulates long-term potentiation. *Nature* **388,** 773–778 (1997).
33. Prescott, S. M. & Majerus, P. W. Characterization of 1,2-diacylglycerol hydrolysis in human platelets. Demonstration of an arachidonoyl-monoacylglycerol intermediate. *J. Biol. Chem.* **258,** 764–769 (1983).
34. Farooqui, A. A., Taylor, W. A. & Horrocks, L. A. Characterizaiton and solubilization of membrane bound diacylglycerol lipases from bovine brain. **18,** 991–997 (1986).
35. 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).
36. Jung, K.-M. *et al.* A key role for diacylglycerol lipase-a in metabotropic glutamate receptor-dependent endocannabinoid mobilization. *Mol. Pharmacol.* **72,** 612–621 (2007).
37. Reisenberg, M., Singh, P. K., Williams, G. & Doherty, P. The diacylglycerol lipases: structure, regulation and roles in and beyond endocannabinoid signaling. *Philos. Trans. R. Soc. B Biol. Sci.* **367,** 3264–3275 (2012).
38. Gao, Y. *et al.* Loss of Retrograde Endocannabinoid Signaling and Reduced Adult Neurogenesis in Diacylglycerol Lipase Knock-out Mice. *J. Neurosci.* **30,** 2017–2024 (2010).
39. Nomura, D. K. *et al.* Endocannabinoid hydrolysis generates brain prostaglandins that promote neuroinflammation. *Science* **334,** 809–813 (2011).
40. Yoshida, T. Localization of diacylglycerol lipase-a around postsynaptic spine suggests close proximity between production site of an endocannabinoid, 2-arachidonoyl-glycerol, and presynaptic cannabinoid CB1 receptor. *J. Neurosci.* **26,** 4740–4751 (2006).
41. Tanimura, A. *et al.* The endocannabinoid 2-arachidonoylglycerol produced by diacylglycerol lipase a mediates retrograde suppression of synaptic transmission. *Neuron* **65,** 320–327 (2010).
42. Kamat, S. S. *et al.* Immunomodulatory lysophosphatidylserines are regulated by ABHD16A and ABHD12 interplay. *Nat. Chem. Biol.* **11,** 164–71 (2015).
43. van der Kleij, D. *et al.* A novel host-parasite lipid cross-talk. Schistosomal lyso-phosphatidylserine activates toll-like receptor 2 and affects immune polarization. *J. Biol. Chem.* **277,** 48122–48129 (2002).
44. Bellini, F. & Brunib, A. Role of a serum phospholipase A1 in the phosphatidylserine-induced T cell inhibition. *FEBS Lett.* **316,** 4–7 (1993).
45. Martin, T. W. & Lagunoff, D. Interactions of lysophospholipids and mast cells. (1979).
46. Smith, G. A., Hesketh, T. R., Plumb, R. W. & Metcalfe, J. C. The exogenous lipid requirement for histamine release from rat peritoneal mast cells stimulated by concanavalin A. *FEBS Lett.* **105,** (1979).
47. Lourenssen, S. & Blennerhassett, M. G. Lysophosphatidylserine potentiates nerve growth factor-induced differentiation of PC12 cells. *Neurosci. Lett.* **248,** 77–80 (1998).
48. Blankman, J. L., Long, J. Z., Trauger, S. a, Siuzdak, G. & Cravatt, B. F. ABHD12 controls brain lysophosphatidylserine pathways that are deregulated in a murine model of the neurodegenerative disease PHARC. *Proc. Natl. Acad. Sci. USA* **110,** 1500–1505 (2013).
49. Fiskerstrand, T. *et al.* A novel Refsum-like disorder that maps to chromosome 20. *Neurology* **72,** 20–27 (2009).
50. Fiskerstrand, T. *et al.* Mutations in ABHD12 cause the neurodegenerative disease PHARC: An inborn error of endocannabinoid metabolism. *Am. J. Hum. Genet.* **87,** 410–417 (2010).
51. Baggelaar, M. P. *et al.* Development of an activity-based probe and in silico design reveal highly selective inhibitors for diacylglycerol lipase-a in brain. *Angew. Chem. Int. Ed.* **52,** 12081–12085 (2013).
52. Janssen, F. J. *et al.* Comprehensive Analysis of Structure-Activity Relationships of α-Ketoheterocycles as sn-1-Diacylglycerol Lipase α Inhibitors. *J. Med. Chem.* **58,** 9742–9753 (2015).
53. Baggelaar, M. P. *et al.* Highly Selective, Reversible Inhibitor Identified by Comparative Chemoproteomics Modulates Diacylglycerol Lipase Activity in Neurons. *J. Am. Chem. Soc.* **137,** 8851–8857 (2015).
54. Appiah, K. K. *et al.* Identification of small molecules that selectively inhibit diacylglycerol lipase-alpha activity. *J Biomol Screen* **19,** 595–605 (2014).
55. Janssen, F. J. *et al.* Discovery of glycine sulfonamides as dual inhibitors of sn-1-diacylglycerol lipase α and α/β hydrolase domain 6. *J. Med. Chem.* **57,** 6610–6622 (2014).

56. Janssen, F. J. & van der Stelt, M. Inhibitors of diacylglycerol lipases in neurodegenerative and metabolic disorders. *Bioorg. Med. Chem. Lett.* **26,** 3831–3837 (Invited BMCL Digest) (2016).