Inhibitor selectivity: profiling and prediction

Janssen, A.P.A.

All you really need to know for the moment is that the universe is a lot more complicated than you might think, even if you start from a position of thinking it's pretty damn complicated in the first place.
Douglas Adams

1 General introduction

The drug discovery process

The discovery and development of new small molecule medicines is a long and expensive process, which requires numerous fields of research to come together. The general timeline for the discovery and development of new drugs is depicted in Figure 1.1.[1,2] Initially, in the drug discovery phase, a biological target has to be found and validated. Next, the stages of hit finding and optimization are aimed at the discovery of molecules to effectively modulate the target. The best molecule, a so-called lead, is then taken into the lead optimization phase, where typically animal models are used to optimize the pharmacokinetic, efficacy

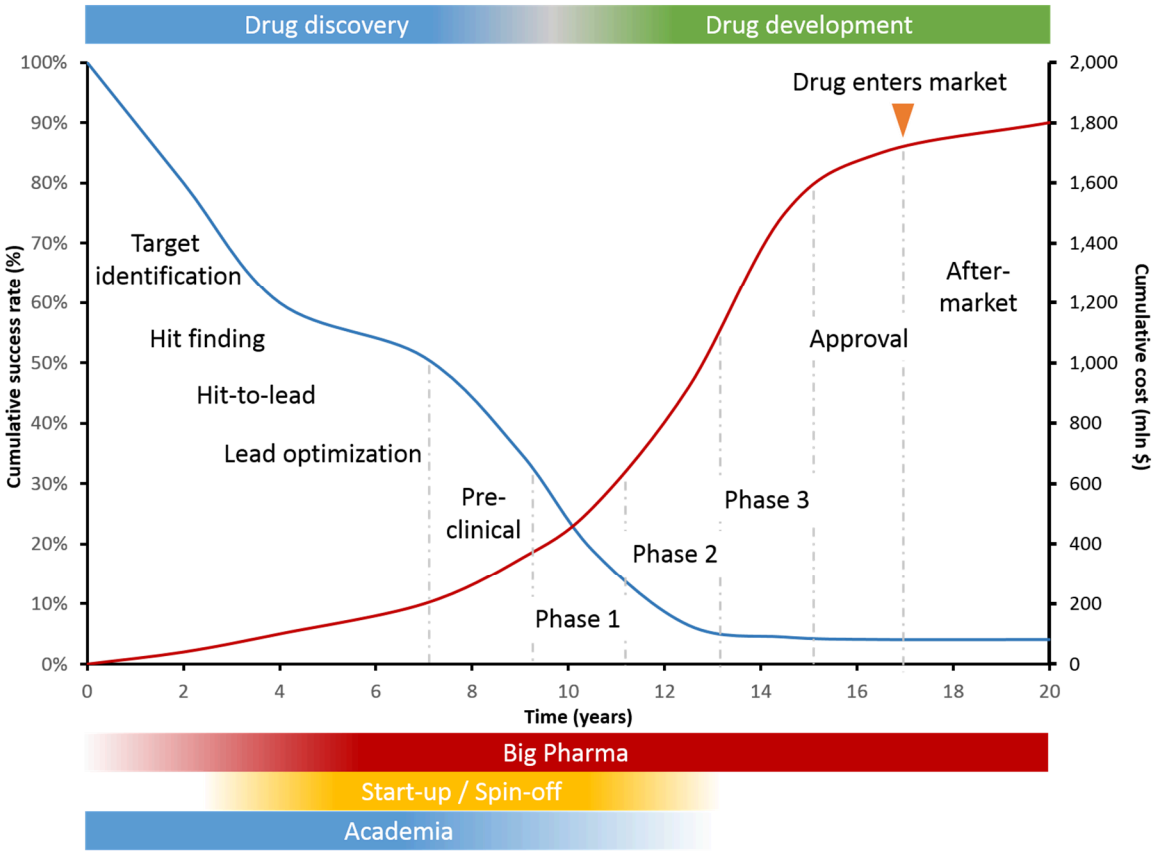

Figure 1.1 | Overview of the general stages of drug discovery and development, the timeline, and cumulative cost and success rate. Figure constructed based on Ref 1 with data from Ref 2.[1,2]

and safety profile of the leads, through iterative rounds of synthesis and testing. Once the drug candidate has been selected for drug development, extensive optimisation of the pharmaceutical formulation and pre-clinical toxicological profiling is performed, before the compound is tested in humans. The next 3 stages are referred to as the clinical trials. Phase 1 typically utilizes healthy volunteers mainly to assess the pharmacokinetic and safety profile of the drug candidate. Phase 2 has an emphasis on finding an efficacious dose in a small cohort of patients. If successful, phase 3 enrols a large patient cohort to determine the efficacy and safety of the experimental drug. It can then be submitted for approval by the authorities. Even after the drug enters the market, data is gathered to assess safety in the larger population, a process often referred to as phase 4. This stringent process of clinical trials is associated with high attrition rates, meaning that less than 1 in 10 devised therapies actually make it to the patient. This is exemplified in Figure 1.1 where the cumulative cost and success rate of drug discovery and development is tracked over time.[1] Failure in the clinical stage is mostly due to toxicity or to lack of efficacy. The high cost and slow progress in drug development is a major concern, as this inevitably pushes up drug prices.

Target-based drug discovery

As discussed above, modern drug discovery projects typically start from either a well-established biological target, or by validating a novel target. The biological target can be

anything from a co-factor to a protein-complex and generally performs a function which is, in the given condition, unwanted. Currently, four major classes of drug targets can be discerned: transporters, ion channels, receptors (nuclear or G-protein coupled) and enzymes, together accounting for just under 90% of the FDA approved drugs.[3] This thesis will focus primarily on enzymes. Pharmaceutical intervention in the functioning of enzymes, barring a few exceptions, always aims at inhibiting the reaction they catalyze. This is typically achieved by compounds that block the binding site of the natural substrate of the enzyme, called competitive inhibition.[4] To find the molecules that have the required interactions to efficiently block the binding site of a given protein, a plethora of techniques have been developed which found their way to the drug industry's toolbox.

The most important approach to find new inhibitors is, although conceptually simple, technically very challenging. So-called high-throughput screening campaigns typically use some biochemical or cellular assay capable of measuring the protein activity in a highly controlled sample and run this assay for thousands or even millions of compounds. This kind of screening is performed by specialized, highly expensive robots and are mostly restricted to the pharmaceutical industry. Smaller screens can be performed with less sophisticated robots or even by hand.

With the dawn of computers, it did not take long before they were put to use in the drug discovery world.[5] This field of research often utilizes virtual screenings, which are enabled by the availability of many 3D structures of proteins, either from crystal structures, NMR-structures, or homology models.[6] In virtual screenings, large numbers of molecules are tried, or docked, inside the (proposed) binding site of a protein, making it essentially the digital mimic of above-mentioned high-throughput screenings. The field of computational drug discovery comprises a broad set of other applications, which are not necessarily structure-based. Quantitative structure activity relations (QSAR) or machine learning approaches also strongly contribute, and the field, together with the number of developed techniques, is still growing. With the advent of machine learning and artificial intelligence this growth is likely to persist.

Off-target activity

The key of target-based drug discovery is that a specific enzyme is targeted to be inhibited. This inhibition is known or predicted to have a designated effect on physiology, which is supposed to be beneficial for the therapeutic indication at hand. With an estimated number of around 20,000 translated genes, it seems inevitable that the binding site of some proteins will be highly similar.[7] This is especially true for enzymes one step preceding or following the targeted enzyme in an enzyme cascade, as the product and substrate of these are identical. It is also the case for protein families within (large) protein families, with high overall similarity, such as the kinases or serine hydrolases. The high similarity of binding sites between proteins can lead to small molecules unintendedly inhibiting other proteins. These are referred to as off-targets.

Interfering with a number of proteins simultaneously can have additive beneficial effects and is referred to as polypharmacology.[8] Some drugs are actively tuned to inhibit multiple targets, which can be a challenging undertaking.[9] Usually however, small molecule

inhibitors are carefully optimized to minimize the cross-reactivity with off-targets, to minimize the chance of unwanted side effects or toxicity.[10]

The endocannabinoid system

The endocannabinoid system (ECS) influences many physiological processes in the human body, including food intake, energy balance, motor coordination, pain sensation, memory formation and anxiety.[11,12] The ECS has, therefore, been under active investigation for therapeutic exploitation in which its receptors and several metabolic enzymes serve as possible drug targets.[13,14] There are two main cannabinoid receptors, CB_1R and CB_2R, which belong to the family of GPCRs. They are activated by two endogenous ligands, *i.e.* anandamide (AEA) and 2-arachidonoyl glycerol (2-AG).[15,16] The production and degradation of these endocannabinoids is mainly performed by serine hydrolases (Figure 1.2). Diacylglycerol lipase-α and -β (DAGL-α and -β) are the main enzymes responsible for the biosynthesis of 2-AG through the *sn*-1-specific hydrolysis of diacylglycerol (DAG).[17–19] The DAGL-α isoform is expressed mainly in the brain, whereas the DAGL-β isoform is predominantly found in the periphery, and is highly abundant in macrophages.[20,21] Monoacylglycerol lipase (MAGL) and α/β-hydrolase-domain containing protein 6 and 12 (ABHD6 and ABHD12) together account for 99% of the 2-AG hydrolysis to arachidonic acid (AA) and glycerol in the brain.[22,23] The Ca^{2+}-dependent biosynthesis of endogenous AEA is mediated by the subsequent actions of PLA2G4E[24] and *N*-acylphosphatidylethanolamine-phospholipase D (NAPE-PLD) or ABHD4[25], although other biosynthetic pathways have also been uncovered.[13,14,26] Fatty acid amide hydrolase (FAAH) is the key enzyme for the hydrolysis of AEA to AA.[27,28] Several drugs targeting the ECS have already entered the market: Δ^9-tetrahydrocannabinol (THC, marketed as Marinol®), cannabidiol (CBD, marketed as Epidiolex®), a combination of THC and CBD (marketed as Sativex®) and Rimonabant®. The latter was withdrawn from the market after the discovery of psychological side effects. Several FAAH and MAGL inhibitors have entered clinical trials, but they have not (yet) reached the market.[29–34]

The exact function and tissue specific roles of the ECS are still poorly understood.[14] Inhibitors of the metabolic enzymes are thus crucial to investigate the biological role of the hydrolases and may serve as drug candidates to modulate the endocannabinoid levels in human disease. With its central role in the production of the main ECS signaling lipid 2-AG, modulation of DAGL activity holds large therapeutic promise. Specifically, DAGL modulation might aid in the alleviation of symptoms in neuroinflammatory conditions, such as observed in Parkinson's and Alzheimer's disease.[35,36]

Activity-based protein profiling

The DAGLs, like most of the synthetic and degradative enzymes in the ECS, belong to the superfamily of the serine hydrolases (SH).[37] This enzyme family has a conserved mechanism of action where a nucleophilic serine is used to hydrolyze ester or amide bonds. In the process, the serine forms a covalent bond with the substrate. This covalent intermediate is exploited in mechanism-based, covalent inhibitors, as well as in activity-based protein profiling (ABPP).[38] The enzymes of the endocannabinoid system have been

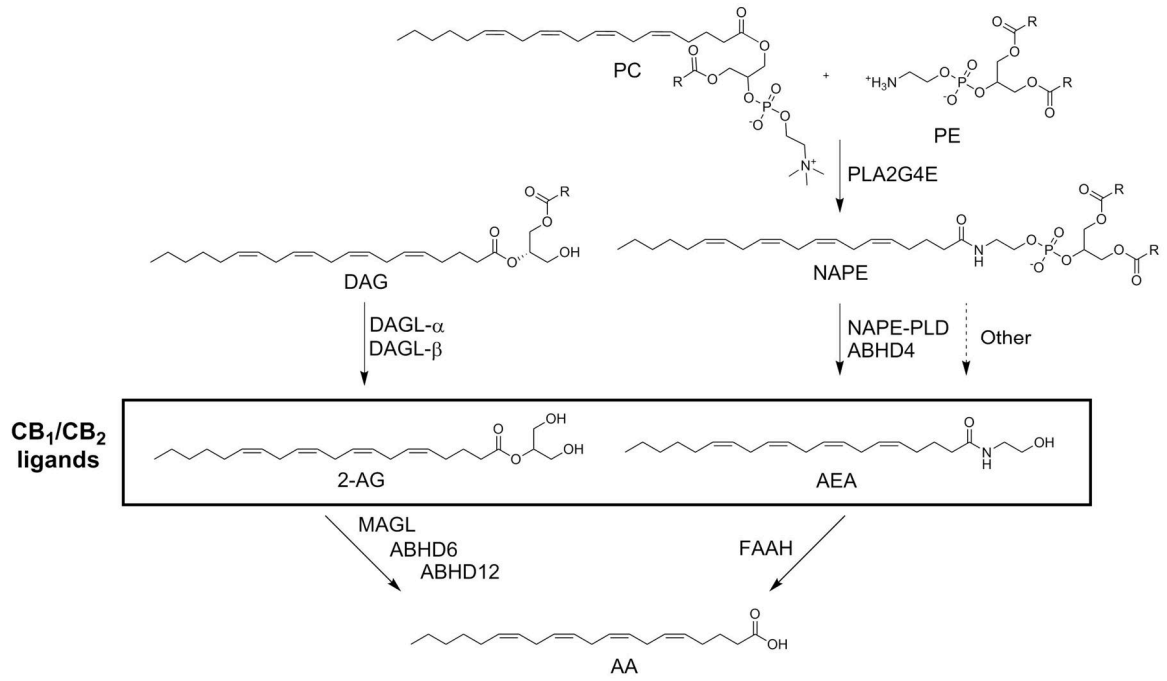

Figure 1.2 | Schematic overview of the main biosynthetic pathways within the endocannabinoid system. All enzymes except NAPE-PLD belong to the serine hydrolase protein family. PC: phosphatidylcholine; PE: phosphatidylethanolamine; DAG: diacylglycerol; NAPE: *N*-acylphosphatidylethanolamine; AA: arachidonic acid; PLA2G4E: phospholipase A2 group IV E; DAGL: diacylglycerol lipase; NAPE-PLD: *N*-acylphosphatidylethanolamine phospholipase D; MAGL: monoacylglycerol lipase; ABHD: α/β-hydrolase-domain containing protein; FAAH: fatty acid amide hydrolase.

extensively investigated using ABPP.[24,39–45] Specifically the development of the tailored activity-based probe (ABP) MB064 to study the DAGLs was instrumental in the further development of several inhibitor classes.[39,42,43]

Activity-based protein profiling (ABPP) for serine hydrolases was introduced in the late ´90s (Figure 1.3).[46] In ABPP a chemical probe, typically consisting of a reactive 'warhead' and a reporter tag, reacts with the catalytically active nucleophilic serine of a serine hydrolase. The reporter tag can be either a fluorophore to visualize the probe-protein adduct by SDS-PAGE and fluorescence scanning,[47] or a biotin-group to enrich proteins from proteomes for identification by high resolution LC-MS/MS[48] or visualization by western blotting.[46] As the labeling of enzymes is typically activity dependent, inhibitor function can be studied using so-called competitive ABPP. This technique is used in drug discovery to efficiently profile activity of inhibitors in a wide variety of proteomes. Importantly, the selectivity of inhibitors over a protein family can also be investigated in native biological samples. Other advances in chemical biology, such as photoaffinity labeling, efficient bio-orthogonal chemistry, and improvements in analysis techniques such as proteomics, have broadened the scope of applications of this protein profiling technique significantly. The flexibility in potential protein sources and robust application to more complex samples make ABPP a powerful technique in all stages of drug discovery.

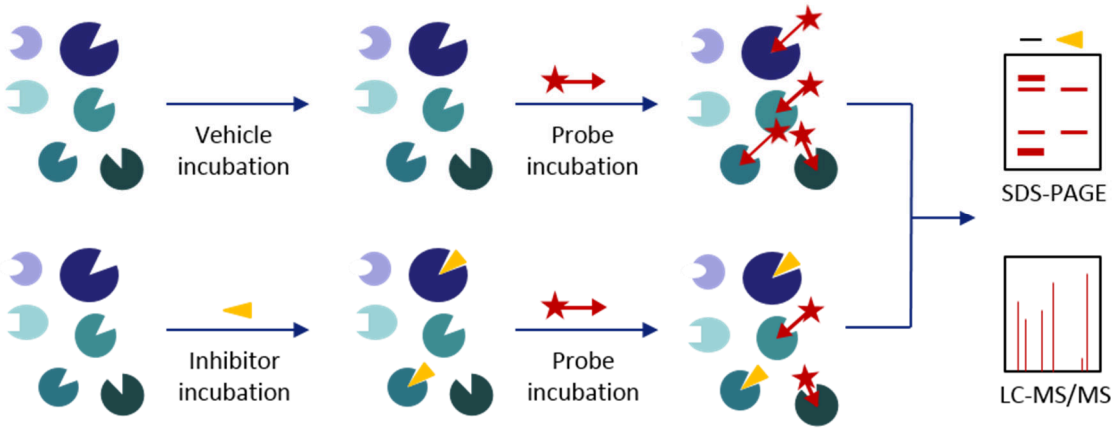

Figure 1.3 | General scheme of activity-based protein profiling. Probe binds specifically to active enzymes, which enables competitive ABPP (bottom). Probe labeling can be visualized using SDS-PAGE or LC-MS/MS analysis.

Aim and outline of this thesis

The aim of the research presented in this thesis is to develop methods to assess or predict the selectivity of (endocannabinoid related) inhibitors, and to use those methods in the discovery of better inhibitors of diacylglycerol lipase. The outline of this thesis is as follows:

Chapter 2 discusses the activity- and selectivity-driven optimization of a new scaffold of DAGL inhibitors found in a previously reported high-throughput screening campaign.[49] The structure activity relations are studied in detail and the optimized inhibitor is fully profiled biochemically. This is then further tested in cultures of murine neuroblastoma cells, before finally being administered to mice in a proof of target engagement study.

Chapter 3 combines the structural insights of Chapter 2 with those from literature[42] to enhance the physicochemical properties of the inhibitors from Chapter 2. The dual aim is to generate more drug-like compounds by enhancing pharmacokinetic properties, combined with increasing the topological polar surface area to restrict brain access. In this way, the more peripherally expressed DAGL-β isoform could be targeted selectively, reducing the risk of side effects mediated centrally. A small library of piperazine derivatives is synthesized and profiled biochemically, before testing the most promising compounds *in situ*.

Chapter 4 focusses more on the fundamental aspects of binding of covalent inhibitors. DH376 derivatives, wherein the number and positioning of the nitrogen atoms in the heterocyclic ring vary, are synthesized.[50] These are tested in an adapted surrogate substrate assay to determine the kinetics of enzyme inhibition and to study the role of affinity (K_i) and reactivity (k_{inact}). Surprisingly, the heterocycle is found to be more important in the former than in the latter. Insight in the specific binding kinetics is expected to aid in the design of inhibitors that are more potent and more selective, by increasing K_i and reducing k_{inact}, respectively.

Chapter 5 presents the work on the selectivity profiling of the experimental drug BIA 10-2474, which was designed as a FAAH inhibitor. This drug made headlines worldwide when, during a phase 1 first-in-human clinical trial in January 2016, a healthy volunteer passed away and four others were hospitalized with severe neurological symptoms.[51,52] Off-

target activity was quickly hypothesized as a possible cause of the observed toxicity. This hypothesis was investigated using activity-based protein profiling techniques, the results of which are disclosed in this chapter. BIA 10-2474, an important metabolite and three alkynylated derivatives are synthesized and extensively tested. It is shown that BIA 10-2474 is an a-specific and covalent inhibitor that severely disrupts neural lipid metabolism *in situ* through the inhibition of several serine hydrolase lipases.

Chapter 6 extends the chemical toolbox to study the endocannabinoid serine hydrolases. A new fluorophosphonate (FP) activity-based probe is synthesized and characterized. The profound influence on enzyme labeling efficiency due to the change in fluorophore is investigated in detail. The FP probe is found to label FAAH and MAGL at low-nanomolar concentrations, allowing it to be combined with MB064 to create an efficient probe cocktail capable of labeling most of the ECS-related serine hydrolases in one experiment. This cocktail is validated and used to profile the inhibition of two covalent MAGL inhibitors.

Chapter 7 attempts to take a prospective approach to target selectivity. By employing a relatively new machine learning algorithm, t-distributed Stochastic Neighbour Embedding (t-SNE), molecular similarity is shown for a large set of clinically relevant substances.[53] The same approach is able to visualize similarity in the protein sequences of the serine hydrolase superfamily, recapitulating phylogenetic information. A workflow is envisioned wherein bio-activity data spanning large amounts of compounds and targets are used to predict interaction profiles for serine hydrolase inhibitors.

Chapter 8 builds on the concepts explored in Chapter 7 for serine hydrolases and applies it to the more extensively studied kinase family. Using the Published Kinase Inhibitor Set, a model is trained and validated capable of predicting interaction profiles across the kinome.[54,55] The validated model is used to find new leads for the oncogene FLT3[56], which are validated in parallel using high-throughput methods. Two hits are resynthesized and profiled *in vitro* and *in situ* against acute myeloid leukaemia derived cells. The presented model is completely open source, and released as a readily usable executable.

Chapter 9 summarizes the work presented in this thesis, and shows future directions for the disclosed research.

References

1. Blass, B. E. Drug discovery and development. in *Basic principles of drug discovery and development* 1–27 (Academic Press, 2015).
2. Scannell, J. W., Blanckley, A., Boldon, H. & Warrington, B. Diagnosing the decline in pharmaceutical R&D efficiency. *Nat. Rev. Drug Discov.* **11**, 191–200 (2012).
3. Gaulton, A. *et al.* ChEMBL: a large-scale bioactivity database for drug discovery. *Nucleic Acids Res.* **40**, D1100–D1107 (2012).
4. Kenakin, T. P. Enzymes as Drug Targets. in *Pharmacology in Drug Discovery* 105–124 (Elsevier, 2012). doi:10.1016/B978-0-12-384856-7.00006-9
5. Hol, W. G. J. Protein Crystallography and Computer Graphics—toward Rational Drug Design. *Angew. Chemie Int. Ed. English* **25**, 767–778 (1986).
6. Lavecchia, A. & Di Giovanni, C. Virtual screening strategies in drug discovery: a critical review. *Curr. Med. Chem.* **20**, 2839–60 (2013).
7. Ezkurdia, I. *et al.* Multiple evidence strands suggest that there may be as few as 19 000 human protein-coding genes. *Hum. Mol. Genet.* **23**, 5866–5878 (2014).
8. Reddy, A. S. & Zhang, S. Polypharmacology: drug discovery for the future. *Expert Rev. Clin. Pharmacol.* **6**, 41–47 (2013).
9. Rodig, S. J. & Shapiro, G. I. Crizotinib, a small-molecule dual inhibitor of the c-Met and ALK receptor tyrosine kinases. *Curr. Opin. Investig. Drugs* **11**, 1477–90 (2010).
10. Bowes, J. *et al.* Reducing safety-related drug attrition: the use of in vitro pharmacological profiling. *Nat. Rev. Drug Discov.* **11**, 909–922 (2012).
11. Mechoulam, R. & Parker, L. A. The Endocannabinoid System and the Brain. *Annu. Rev. Psychol.* **64**, 21–47 (2013).
12. Soethoudt, M. *et al.* Selective Photoaffinity Probe That Enables Assessment of Cannabinoid CB 2 Receptor Expression and Ligand Engagement in Human Cells. *J. Am. Chem. Soc.* **140**, 6067–6075 (2018).
13. Donvito, G. *et al.* The Endogenous Cannabinoid System: A Budding Source of Targets for Treating Inflammatory and Neuropathic Pain. *Neuropsychopharmacology* **43**, 52–79 (2018).
14. Di Marzo, V. Targeting the endocannabinoid system: to enhance or reduce? *Nat. Rev. Drug Discov.* **7**, 438–55 (2008).
15. Devane, W. A. *et al.* Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* **258**, 1946–1949 (1992).
16. 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).
17. 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).
18. Tanimura, A. *et al.* The Endocannabinoid 2-Arachidonoylglycerol Produced by Diacylglycerol Lipase α Mediates Retrograde Suppression of Synaptic Transmission. *Neuron* **65**, 320–327 (2010).
19. Reisenberg, M., Singh, P. K., Williams, G. & Doherty, P. The diacylglycerol lipases: structure, regulation and roles in and beyond endocannabinoid signalling. *Philos. Trans. R. Soc. B Biol. Sci.* **367**, 3264–3275 (2012).
20. 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).
21. Murataeva, N., Straiker, A. & Mackie, K. Parsing the players: 2-arachidonoylglycerol synthesis and degradation in the CNS. *Br. J. Pharmacol.* **171**, 1379–1391 (2014).
22. Savinainen, J. R., Saario, S. M. & Laitinen, J. T. The serine hydrolases MAGL, ABHD6 and ABHD12 as guardians of 2-arachidonoylglycerol signalling through cannabinoid receptors. *Acta Physiol.* **204**, 267–276 (2012).
23. Long, J. Z. *et al.* Dual blockade of FAAH and MAGL identifies behavioral processes regulated by endocannabinoid crosstalk in vivo. *Proc. Natl. Acad. Sci.* **106**, 20270–20275 (2009).
24. Ogura, Y., Parsons, W. H., Kamat, S. S. & Cravatt, B. F. A calcium-dependent acyltransferase that produces N-acyl phosphatidylethanolamines. *Nat. Chem. Biol.* **12**, 669–671 (2016).
25. Simon, G. M. & Cravatt, B. F. Endocannabinoid biosynthesis proceeding through glycerophospho-N-acyl ethanolamine and a role for alpha/beta-hydrolase 4 in this pathway. *J. Biol. Chem.* **281**, 26465–72 (2006).
26. Liu, J. *et al.* Multiple pathways involved in the biosynthesis of anandamide. *Neuropharmacology* **54**, 1–7 (2008).
27. Cravatt, B. F. *et al.* Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. *Nature* **384**, 83–87 (1996).
28. Patricelli, M. P., Lovato, M. A. & Cravatt, B. F. Chemical and mutagenic investigations of fatty acid amide hydrolase: Evidence for a family of serine hydrolases with distinct catalytic properties. *Biochemistry* **38**, 9804–9812 (1999).

29. Huggins, J. P., Smart, T. S., Langman, S., Taylor, L. & Young, T. An efficient randomised, placebo-controlled clinical trial with the irreversible fatty acid amide hydrolase-1 inhibitor PF-04457845, which modulates endocannabinoids but fails to induce effective analgesia in patients with pain due to osteoarthritis of th. *Pain* **153**, 1837–1846 (2012).
30. Fraser, I. *et al.* Preclinical characterization and first-in-human administration of a selective monoacylglycerol lipase inhibitor, ABX-1431. in *Front. Pharmacol. Conference Abstract: EUFEMED 2017* (2017). doi:10.3389/conf.fphar.2017.62.00011
31. Cisar, J. S. *et al.* Identification of ABX-1431, a Selective Inhibitor of Monoacylglycerol Lipase and Clinical Candidate for Treatment of Neurological Disorders. *J. Med. Chem.* **61**, 9062–9084 (2018).
32. Li, G. L. *et al.* Assessment of the pharmacology and tolerability of PF-04457845, an irreversible inhibitor of fatty acid amide hydrolase-1, in healthy subjects. *Br. J. Clin. Pharmacol.* **73**, 706–716 (2012).
33. Postnov, A. *et al.* Fatty Acid Amide Hydrolase Inhibition by JNJ-42165279: A Multiple-Ascending Dose and a Positron Emission Tomography Study in Healthy Volunteers. *Clin. Transl. Sci.* **11**, 397–404 (2018).
34. Kiss, L. E. *et al.* Discovery of a Potent, Long-Acting, and CNS-Active Inhibitor (BIA 10-2474) of Fatty Acid Amide Hydrolase. *ChemMedChem* **13**, 2177–2188 (2018).
35. Janssen, F. J. & van der Stelt, M. Inhibitors of diacylglycerol lipases in neurodegenerative and metabolic disorders. *Bioorg. Med. Chem. Lett.* **26**, 3831–3837 (2016).
36. Baggelaar, M. P., Maccarrone, M. & van der Stelt, M. 2-Arachidonoylglycerol: A signaling lipid with manifold actions in the brain. *Prog. Lipid Res.* **71**, 1–17 (2018).
37. Long, J. Z. & Cravatt, B. F. The Metabolic Serine Hydrolases and Their Functions in Mammalian Physiology and Disease. *Chem. Rev.* **111**, 6022–6063 (2011).
38. Kidd, D., Liu, Y. & Cravatt, B. F. Profiling serine hydrolase activities in complex proteomes. *Biochemistry* **40**, 4005–4015 (2001).
39. 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).
40. van Rooden, E. J. *et al.* Mapping in vivo target interaction profiles of covalent inhibitors using chemical proteomics with label-free quantification. *Nat. Protoc.* **13**, 752–767 (2018).
41. Baggelaar, M. P. *et al.* Chemical Proteomics Maps Brain Region Specific Activity of Endocannabinoid Hydrolases. *ACS Chem. Biol.* **12**, 852–861 (2017).
42. 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).
43. 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. Chemie Int. Ed.* **52**, 12081–12085 (2013).
44. Johnson, D. S. *et al.* Discovery of PF-04457845: A Highly Potent, Orally Bioavailable, and Selective Urea FAAH Inhibitor. *ACS Med. Chem. Lett.* **2**, 91–96 (2011).
45. Adibekian, A. *et al. Optimization and characterization of a triazole urea inhibitor for alpha/beta hydrolase domain-containing protein 11 (ABHD11): anti-probe for LYPLA1/LYPLA2 dual inhibitor ML211*. *Probe Reports from the NIH Molecular Libraries Program* (National Center for Biotechnology Information (US), 2010).
46. Liu, Y., Patricelli, M. P. & Cravatt, B. F. Activity-based protein profiling: The serine hydrolases. *Proc. Natl. Acad. Sci.* **96**, 14694–14699 (1999).
47. Patricelli, M. P., Giang, D. K., Stamp, L. M. & Burbaum, J. J. Direct visualization of serine hydrolase activities in complex proteomes using fluorescent active site-directed probes. *Proteomics* **1**, 1067–1071 (2001).
48. Jessani, N. *et al.* A streamlined platform for high-content functional proteomics of primary human specimens. *Nat. Methods* **2**, 691–697 (2005).
49. Janssen, F. J. Discovery of sulfonyl-1,2,4-triazole ureas as DAGLα inhibitors by HTS-ABPP. in *Discovery of novel inhibitors to investigate diacylglycerol lipases and α/β-hydrolase domain 16A* 109–139 (2016).
50. Deng, H. *et al.* Triazole Ureas Act as Diacylglycerol Lipase Inhibitors and Prevent Fasting-Induced Refeeding. *J. Med. Chem.* **60**, 428–440 (2017).
51. Butler, D. & Callaway, E. Scientists in the dark after French clinical trial proves fatal. *Nature* **529**, 263–264 (2016).
52. Kerbrat, A. *et al.* Acute Neurologic Disorder from an Inhibitor of Fatty Acid Amide Hydrolase. *N. Engl. J. Med.* **375**, 1717–1725 (2016).
53. Van Der Maaten, L. & Hinton, G. Visualizing Data using t-SNE. *J. Mach. Learn. Res.* **9**, 2579–2605 (2008).
54. Drewry, D. H., Willson, T. M. & Zuercher, W. J. Seeding collaborations to advance kinase science with the GSK Published Kinase Inhibitor Set (PKIS). *Curr. Top. Med. Chem.* **14**, 340–2 (2014).
55. Elkins, J. M. *et al.* Comprehensive characterization of the Published Kinase Inhibitor Set. *Nat. Biotechnol.* **34**, 95–103 (2015).
56. Larrosa-Garcia, M. & Baer, M. R. FLT3 Inhibitors in Acute Myeloid Leukemia: Current Status and Future Directions. *Mol. Cancer Ther.* **16**, 991–1001 (2017).