Universiteit Leiden
The Netherlands

Inhibitor discovery of phospholipases and N-acyltransferases

Zhou, J.

Citation

Zhou, J. (2020, November 11). *Inhibitor discovery of phospholipases and N-acyltransferases*. Retrieved from https://hdl.handle.net/1887/138014

Version:	Publisher's Version
License:	Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden
Downloaded from:	https://hdl.handle.net/1887/138014

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

Cover Page

The handle http://hdl.handle.net/1887/138014 holds various files of this Leiden University dissertation.

Author: Zhou, J.
Title: Inhibitor discovery of phospholipases and N-acyltransferases
Issue date: 2020-11-11

1

General introduction

1.1 Introduction

The plant *Cannabis sativa* and its extracts, such as marijuana and hashish, have been used for recreational and medicinal purposes for a long time.[1] Marijuana has both psychological and physical effects. It modulates neurotransmission in the brain, resulting in changes in mood, appetite, memory, motor coordination and other behavioral responses.[2] In 1964, Δ^9-tetrahydrocannabinol (Δ^9-THC) (Figure 1) was purified and characterized as the major psychoactive component of *Cannabis sativa*.[3] The cannabinoid receptor type 1 (CB1R) was identified as a target protein of THC in 1990.[4] The CB1R is a member of the G-protein-coupled receptor family and is expressed in the peripheral nervous system and central nervous system.[5] In 1993, a second THC-responsive protein, the cannabinoid receptor type 2 (CB2R), was discovered.[6] CBR2 is expressed predominantly in peripheral immune cells.[7, 8]

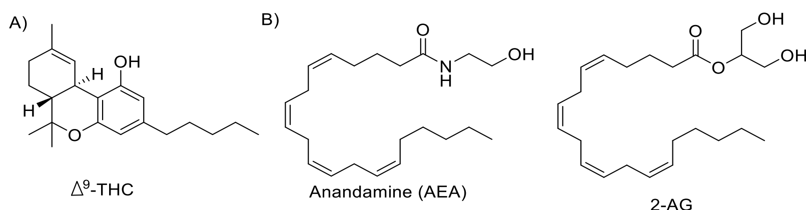

Figure 1. Chemical structures of (A) the plant natural product, Δ^9-THC and (B) the endocannabinoids, 2-arachidonoylglycerol (2-AG) and *N*-arachidonoylethanolamine (also known as anandamide, AEA).

The finding that Δ^9-THC, a plant natural product, interacts with mammalian receptor proteins, suggested the existence of endogenous cannabinoid receptor ligands. Indeed, in 1992, two years after the discovery of the CB1R, anandamide (or *N*-arachidonoylethanolamine, AEA, Figure 1B) was isolated from pig brain as the first endogenous cannabinoid ligand.[9] In 1995, 2-arachidonoylglycerol (2-AG, Figure 1B), previously known as a common intermediate in the metabolisms of glycerophospholipids and triglyceride, was reported as a second endogenous ligand for both cannabinoid receptors.[10]

In the past decades several *N*-acylethanolamines (NAEs) structurally related to AEA have been discovered (Figure 2B).[11, 12] These NAEs are involved in various physiological processes, such as anti-inflammation,[13] neuroprotection,[14] anorexic effects[15] and anti-proliferative effects.[16-19] Unlike AEA, these NAEs do not bind to cannabinoid receptors,[20] but exert their biological activities via binding to other receptors pathways.[19]

Palmitoylethanolamide (PEA) was discovered in 1957.[21] Accumulation of PEA has been observed during inflammation.[13, 22] The nuclear receptor peroxisome proliferator-activated receptor-alpha (PPAR-alpha) has been identified as the main target through which PEA exerts its anti-inflammatory effect.[23] PEA agonizes PRAP-alfa and alters the expression of a number of targets genes. It is this signaling process that is thought to be behind the observed neuroprotective

proterty of PEA.[24] Besides PPAR-alpha, PEA can also bind to cannabinoid-like G-coupled receptors GPR55 and GPR119.[22]

Oleoylethanolamide (OEA) is an endogenous agonist for PPAR-alpha and stimulates lipolysis.[25] OEA may also function as an endogenous ligand for GPR119.[26, 27] OEA regulates feeding and body weight in mice[14, 28] and pythons.[29] It was reported that OEA could promote longevity of the life span in *Caenorhabditis elegans* probably via binding to nuclear receptor NHR-80.[30]

Stearoylethanolamide (SEA) is a saturated analogue of OEA. In LPS-induced pulmonary inflammation, SEA shows an anti-inflammatory effect, because it inhibited the translocation of NF-κB, a critical transcription factor for the expression of many cytokines, into the nucleus of rat peritoneal macrophages.[31] Zhukov and coworkers found that in an inflammatory rat model, the administration of SEA could accelerate the healing process of skin burn.[32] In addition, SEA was shown to restore the morphine-induced alterations of brain phospholipid composition and the restoration of brain phospholipid composition was associated with a decline in morphine dependence.[33, 34] Recently, it was shown that SEA protects the brain from inflammation and improves memory in mice.[35]

Docosahexaenoylethanolamide (DHEA) is a conjugate of docosahexaenoic acid and ethanolamine. Like other members of NAEs, DHEA also has anti-inflammatory properties.[36-38] DHEA displayed anti-proliferative activity in LNCaP and PC3 prostate cancer cells[16] and promoted neurite growth and synaptogenesis.[39]

1.2 Biosynthetic pathways of NAEs

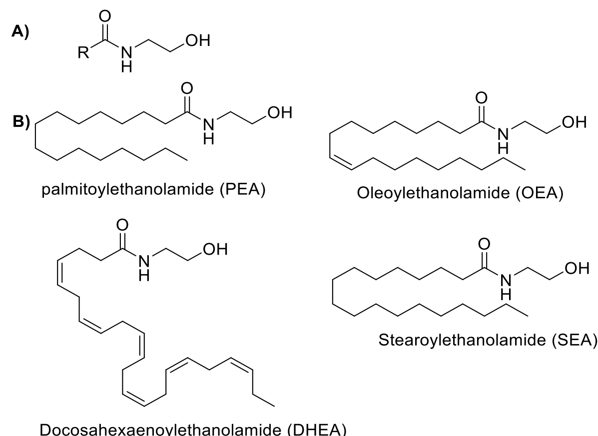

Figure 2. (A) General chemical structures of NAEs. R represents various acyl groups. (B) Chemical structures of PEA, OEA, DHEA and SEA.

NAEs are derived from phospholipids in a couple of chemical transformations as shown in figure 3.[12, 20, 40, 41] First, *N*-acyltransferases (NAT) acylate the amine of phosphatidylethanolamine (PE) to produce *N*-acyl-phosphatidylethanolamine (NAPE). There are two classes of NATs: Ca^{2+}-dependent NAT (Ca-NAT) and Ca^{2+}-independent NATs (PLAAT1-5).[42]

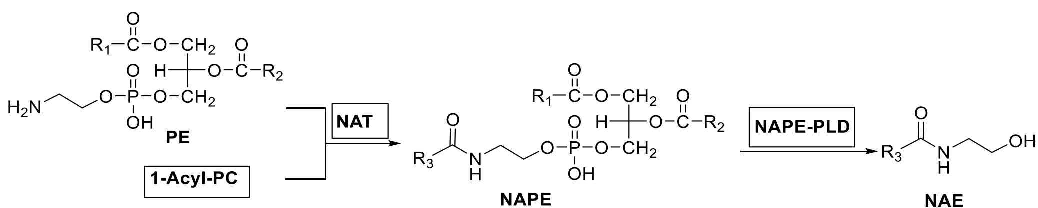

Figure 3. The NAPE-PLD-dependent pathway for the biosynthesis of NAEs.

The phosphodiester bond in NAPEs, generated by PLAATs or PLA2G4E, is subsequently hydrolyzed by a NAPE-selective phospholipase D (NAPE-PLD) yielding various NAEs.[43-45] NAEs can also be generated from NAPE via NAPE-PLD-independent pathways, which were first proposed in 1984.[46] Studies on mice genetically lacking NAPE-PLD further confirmed the existence of other pathways.[47-49] For example, NAPE can be deacylated by ABHD4 to yield glycerophospho-*N*-acyl-ethanolamine (GP-NAE), which is a substrate for GDE1 or GDE4 (Figure 4, route 1).[50-52] In a second pathway, cytosolic phospholipase A2 ($cPLA_2$) hydrolyzes NAPE to LysoNAPE, which is directly cleaved by GDE4 or GDE7 to produce NAEs.[53] In a third pathway, NAPE is hydrolyzed to phospho-NAE by phospholipase C (PLC) and NAEs are liberated via SHIP1 or PTPN22.[42, 54, 55]

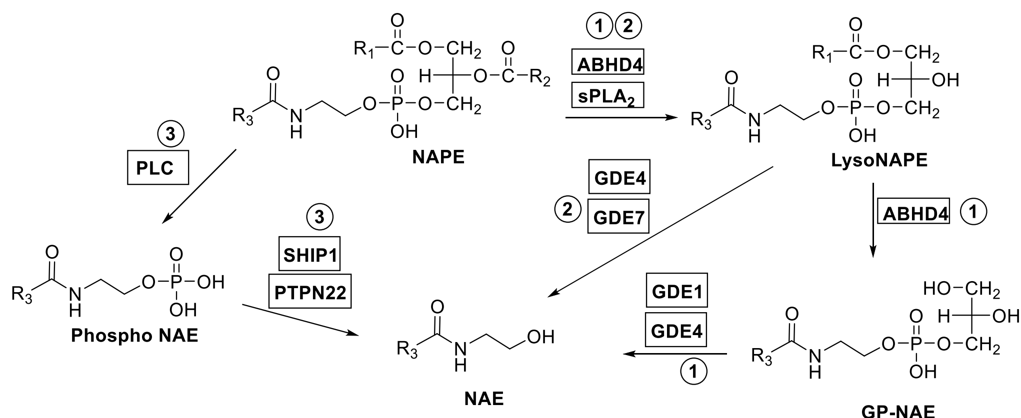

Figure 4. The NAPE-PLD-dependent pathways for the biosynthesis of NAEs.

Alternatively, NAEs can also be generated from another type of NAPE, the plasmalogen-NAPE (pNAPE) (Figure 5).[42, 48] pNAPE serve as a substrate in both NAPE-PLD-dependent (Figure 5, route 1) and NAPE-PLD-independent pathways (Figure 5, route 2). The formation of lyso-pNAPE is catalyzed by ABHD4 or $sPLA_2$ and GDE1, 4, and 7 may hydrolyze Lyso-pNAPE yielding AEA.[51, 56]

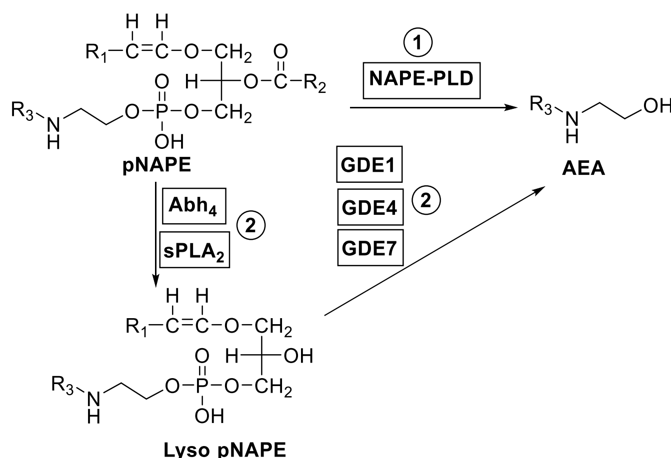

Figure 5. The biosynthetic pathway of NAEs from pNPPE.

The enzyme responsible for the Ca^{2+}-dependent formation of NAPEs remained elusive until Cravatt and colleagues showed that PLA2G4E (also known as $cPLA_2\varepsilon$) was able to transfer an acyl chain from the *sn*-1 position of phosphatidylcholine (PC) to the amine of phosphatidylethanolamine (PE), thereby producing NAPEs.[57] PLA2G4E was previously discovered in a comprehensive homology search against murine genome and EST data bases and annotated as a phospholipase (PLA).[58] It belongs to the cytosolic phospholipase A2 group IV (PLA2G4) proteins, a subfamily from the PLA2 proteins, which catalyzes the hydrolysis of the

sn-2 acyl bond of phospholipids, thereby releasing fatty acids. This leads to a signaling cascade initiated by lipid second messengers, which regulates a wide variety of physiological responses and plays an important role in diseases, such as cancer.[59] There are six members in this protein family, namely PLA2G4A, PLA2G4B, PLA2G4C, PLA2G4D, PLA2G4E and PLA2G4F, which are structurally similar. They contain a N-terminal C2 domain and a C-terminal catalytic domain. The C2 domain has a binding site for intracellular Ca^{2+}. Upon binding of calcium, the protein is transported from the cytosol to the Golgi membrane, which is needed for catalytic activity.[60] The catalytic pocket contains a Ser/Asp dyad, which is located in the α/β hydrolase domain.[60, 61] In contrast to the other family members, PLA2G4E has a strong preference for catalyzing the *N*-acyltransferase reactions over phospholipid hydrolysis.[57]

The human phospholipase A/acyl transferase (PLAAT1-5) family consists of five members (namely, PLA/AT 1-5).[62, 63] They are the products of the *Hrasls* genes.[64] PLAATs possess Ca^{2+}-independent phospholipase activity *in vitro* with both phosphatidylcholine (PC) and phosphatidylethanolamine (PE) acting as substrates. All members also exhibit O-acyl transferase activity with preference for the *sn*-1 position of lysophosphatidylcholine (lyso PC) as well as *N*-acyltransferase activity with the ability to produce N-acylphosphatidylethanolamines (NAPEs) through transfer of the acyl chain from the *sn*-1 position of glycerophospholipids to the amine function of PE.[63] All enzymes, except PLAAT3, show a preference for PLA1 activity over PLA2 activity.

PLAAT3 (also known as PLA2G16) is the most studied PLAAT protein. The protein is mostly expressed in white adipose tissue and to a lesser extent in brown adipose tissue.[65] The enzyme exhibits predominant PLA activity over O-acyltransferase or N-acyltransferase activities. It is in fact responsible for the majority of phospholipase activity in adipocytes.[63] The *in vivo* relevance of PLAAT3 has not been studied extensively, but a mouse model constitutively lacking the *Hrasls3* gene has been generated.[65] Ablation of PLAAT3 prevented obesity caused by high fat diet or leptin deficiency, thus establishing PLAAT3 as a potential target for the treatment of obesity. The PLAAT3 deficient mice exhibited a higher rate of lipolysis, due to decreased levels of prostaglandin E2 (PGE2) that were most likely caused by a decrease in arachidonic acid levels. Increased fatty acid oxidation in adipose tissue was also reported.

1.3 Activity-based protein profiling (ABPP)

Activity-based protein profiling is a powerful chemical biological technique that uses chemical probes which can covalently binds to catalytic amino acid of the target protein.[66] It allows efficient lead discovery studies by assessing inhibitor activity and selectivity in complex proteomes.[67] The activity-based probe (ABP) contains a warhead, a recognition part and a linker part, which is conjugated to a fluorophore or biotin reporter tag for fluorescent- or mass-spectrometry-based detection, respectively. The main advantage of ABPP is that the activity-based probe monitors the abundance of active proteins, thereby taking into account post-translational modifications and protein-protein-interactions. In comparative ABPP, the abundance and diversity of active enzymes in various biological samples are compared. In this setting, new enzyme activities can also be discovered or the presence of unexpected ones can be revealed. When a competitive ABPP is performed, the method can also be applied to screen the inhibitors for specific enzyme or to identify the bind targets of small-molecular inhibitor. In the field of serine hydrolases, various ABPs have been developed to decipher the physiological functions of these enzymes, discover new serine hydrolases or screen inhibitors.[68] MB064 is a β-lactone-based probe (structure shown in Figure 5), which has been developed and applied as a broad-spectrum serine hydrolase probe for the identification of highly potent and selective diacylglycerol lipase inhibitors.[69, 70] In addition, MB064 was instrumental in the discovery of the off-target profile of the fatty acid amide hydrolase inhibitor BIA 10-2474 that caused the death of a volunteer in a clinical phase 1 study.[71] The β-lactone warhead covalently reacts with the catalytic serine in many serine hydrolases, forming an acyl-enzyme intermediate. Interestingly, MB064 has also been reported to form thioester bonds with the catalytic cysteine of various enzymes.[72] In this thesis, MB064 is also used to develop assays to screen and modify inhibitors for PLAATs and PLA2G4E.

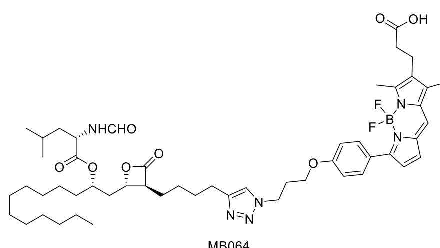

Figure 5. Chemical structure of probe MB064.

1.4 Aim and outline of this thesis

The aim of this thesis is to develop an activity-based protein profiling method to monitor the activity of PLAAT3 and the Ca^{2+}-dependent N-acyltransferase that catalyzes the first step in NAE production and to identify inhibitors for these enzymes. In **Chapter 2**, the fluorescent activity-based probe MB064 is shown to label recombinant and endogenously expressed PLAAT3. Competitive activity-based protein profiling (ABPP) using MB064 enabled the discovery of α-ketoamides as the first PLAAT3 inhibitors. **Chapter 3** presents the systematic optimization of the initially discovered α-ketoamide hit. In the end, LEI110 is identified as the most potent and selective inhibitor that is available for PLAAT3. In **Chapter 4**, the biological activity of LEI110 is investigated. Gel-based ABPP and chemical proteomics showed that LEI110 is a selective pan-inhibitor of the PLAAT family of thiol hydrolases (i.e., PLAAT1, PLAAT2, PLAAT4 and PLAAT5). Molecular dynamic simulations of LEI110 in the reported crystal structure of PLAAT3 provided insight in the potential ligand−protein interactions to explain its binding mode. **Chapter 5** reports the development of an ABPP assay to study the Ca^{2+}-dependent NAT, PLA2G4E, again using probe MB064. After optimizing the labeling conditions, a compound library is screened for PLA2G4E inhibitors. Several inhibitors for PLA2G4E were identified. **Chapter 6** provides a summary of the research described in this thesis and provides some future prospects.

1.5 References

1. Ligresti, A.; De Petrocellis, L.; Di Marzo, V., From Phytocannabinoids to Cannabinoid Receptors and Endocannabinoids: Pleiotropic Physiological and Pathological Roles Through Complex Pharmacology. *Physiol. Rev.* **2016,** *96* (4), 1593-659.

2. Rinder, I. D., The effects of marijuana: a social psychological interpretation. *Psychiatry* **1978,** *41* (2), 202-6.

3. Gaoni, Y.; Mechoulam, R., Isolation, structure, and partial synthesis of an active constituent of Hashish. *J. Am. Chem. Soc.* **1964,** *86* (8), 1646-1647.

4. Matsuda, L. A.; Lolait, S. J.; Brownstein, M. J.; Young, A. C.; Bonner, T. I., Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature* **1990,** *346* (6284), 561-4.

5. Van Waes, V.; Beverley, J. A.; Siman, H.; Tseng, K. Y.; Steiner, H., CB1 Cannabinoid receptor expression in the striatum: association with corticostriatal circuits and developmental regulation. *Front. Pharmacol.* **2012,** *3*, 21.

6. Munro, S.; Thomas, K. L.; Abu-Shaar, M., Molecular characterization of a peripheral receptor for cannabinoids. *Nature* **1993,** *365* (6441), 61-5.

7. Galiegue, S.; Mary, S.; Marchand, J.; Dussossoy, D.; Carriere, D.; Carayon, P.; Bouaboula, M.; Shire, D.; Le Fur, G.; Casellas, P., Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. *Eur. J. Biochem.* **1995,** *232* (1), 54-61.

8. Ashton, J. C.; Friberg, D.; Darlington, C. L.; Smith, P. F., Expression of the cannabinoid CB2 receptor in the rat cerebellum: an immunohistochemical study. *Neurosci. Lett.* **2006,** *396* (2), 113-6.

9. Devane, W. A.; Hanus, L.; Breuer, A.; Pertwee, R. G.; Stevenson, L. A.; Griffin, G.; Gibson, D.; Mandelbaum, A.; Etinger, A.; Mechoulam, R., Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* **1992,** *258* (5090), 1946-9.

10. Mechoulam, R.; Ben-Shabat, S.; Hanus, L.; Ligumsky, M.; Kaminski, N. E.; Schatz, A. R.; Gopher, A.; Almog, S.; Martin, B. R.; Compton, D. R.; et al., Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem. Pharmacol.* **1995,** *50* (1), 83-90.

11. Schmid, H. H. O.; Schmid, P. C.; Natarajan, V., N-Acylated glycerophospholipids and their derivatives. *Prog. Llipid Res.* **1990,** *29* (1), 1-43.

12. Hansen, H. S.; Moesgaard, B.; Hansen, H. H.; Petersen, G., N-Acylethanolamines and precursor phospholipids — relation to cell injury. *Chem. Phys. Lipids* **2000,** *108* (1), 135-150.

13. Didier, M. L.; Severine, V.; Kent-Olov, J.; Christopher, J. F., The palmitoylethanolamide family: a new class of anti-inflammatory agents ? *Curr. Med. Chem.* **2002,** *9* (6), 663-674.

14. Lo Verme, J.; Gaetani, S.; Fu, J.; Oveisi, F.; Burton, K.; Piomelli, D., Regulation of food intake by oleoylethanolamide. *Cell. Mol. Life Sci.* **2005,** *62* (6), 708.

15. Rodríguez de Fonseca, F.; Navarro, M.; Gómez, R.; Escuredo, L.; Nava, F.; Fu, J.; Murillo-Rodríguez, E.; Giuffrida, A.; LoVerme, J.; Gaetani, S.; Kathuria, S.; Gall, C.; Piomelli, D., An anorexic lipid mediator regulated by feeding. *Nature* **2001,** *414* (6860), 209-212.

16. Brown, I.; Cascio, M. G.; Wahle, K. W. J.; Smoum, R.; Mechoulam, R.; Ross, R. A.; Pertwee, R. G.; Heys, S. D., Cannabinoid receptor-dependent and -independent anti-proliferative effects of omega-3 ethanolamides in androgen receptor-positive and -negative prostate cancer cell lines. *Carcinogenesis* **2010,** *31* (9), 1584-1591.

17. Berdyshev, E. V.; Schmid, P. C.; Krebsbach, R. J.; Kuwae, T.; Huang, C.; Ma, W. Y.; Dong, Z.; Schmid, H. H., Role of N-acylethanolamines in cell signaling. *World Rev. Nutr. Diet.* **2001,** *88*, 207-14.

18. Coulon, D.; Faure, L.; Salmon, M.; Wattelet, V.; Bessoule, J. J., N-Acylethanolamines and related compounds: aspects of metabolism and functions. *Plant Sci.* **2012,** *184*, 129-40.

19. Tsuboi, K.; Uyama, T.; Okamoto, Y.; Ueda, N., Endocannabinoids and related N-acylethanolamines: biological activities and metabolism. *Inflamm. Regen.* **2018,** *38*, 28.

20. Schmid, H. H. O.; Berdyshev, E. V., Cannabinoid receptor-inactive N-acylethanolamines and other fatty acid amides: metabolism and function. *Prostag. Leukotr. Ess.* **2002,** *66* (2), 363-376.

21. Petrosino, S.; Iuvone, T.; Di Marzo, V., N-palmitoyl-ethanolamine: biochemistry and new therapeutic opportunities. *Biochimie* **2010,** *92* (6), 724-727.

22. Godlewski, G.; Offertáler, L.; Wagner, J. A.; Kunos, G., Receptors for acylethanolamides—GPR55 and GPR119. *Prostag. Oth. Lipid M.* **2009,** *89* (3), 105-111.

23. Lo Verme, J.; Fu, J.; Astarita, G.; La Rana, G.; Russo, R.; Calignano, A.; Piomelli, D., The nuclear receptor peroxisome proliferator-activated receptor-alpha mediates the anti-inflammatory actions of palmitoylethanolamide. *Mol. Pharmacol.* **2005,** *67* (1), 15-9.

24. Fidaleo, M.; Fanelli, F.; Ceru, M. P.; Moreno, S., Neuroprotective properties of peroxisome proliferator-activated receptor alpha (PPARalpha) and its lipid ligands. *Curr Med Chem* **2014,** *21* (24), 2803-21.

25. Gaetani, S.; Kaye, W. H.; Cuomo, V.; Piomelli, D., Role of endocannabinoids and their analogues in obesity and eating disorders. *Eat. Weight Disord.* **2008,** *13* (3), e42-8.

26. Overton, H. A.; Babbs, A. J.; Doel, S. M.; Fyfe, M. C. T.; Gardner, L. S.; Griffin, G.; Jackson, H. C.; Procter, M. J.; Rasamison, C. M.; Tang-Christensen, M.; Widdowson, P. S.; Williams, G. M.; Reynet, C., Deorphanization of a G protein-coupled receptor for oleoylethanolamide and its use in the discovery of small-molecule hypophagic agents. *Cell Metab.* **2006,** *3* (3), 167-175.

27. Brown, A. J., Novel cannabinoid receptors. *Br. J. Pharmacol.* **2007,** *152* (5), 567-75.

28. Gaetani, S.; Oveisi, F.; Piomelli, D., Modulation of meal pattern in the rat by the anorexic lipid mediator oleoylethanolamide. *Neuropsychopharmacology* **2003,** *28* (7), 1311-1316.
29. Astarita, G.; Rourke, B. C.; Andersen, J. B.; Fu, J.; Kim, J. H.; Bennett, A. F.; Hicks, J. W.; Piomelli, D., Postprandial increase of oleoylethanolamide mobilization in small intestine of the Burmese python (Python molurus). *Am. J. Physiol.-Reg. I.* **2006,** *290* (5), R1407-R1412.
30. Folick, A.; Oakley, H. D.; Yu, Y.; Armstrong, E. H.; Kumari, M.; Sanor, L.; Moore, D. D.; Ortlund, E. A.; Zechner, R.; Wang, M. C., Lysosomal signaling molecules regulate longevity in Caenorhabditis elegans. *Science* **2015,** *347* (6217), 83-86.
31. Berdyshev, A. G.; Kosiakova, H. V.; Onopchenko, O. V.; Panchuk, R. R.; Stoika, R. S.; Hula, N. M., N-Stearoylethanolamine suppresses the pro-inflammatory cytokines production by inhibition of NF-κB translocation. *Prostag. Oth. Lipid M.* **2015,** *121*, 91-96.
32. A. D. Zhukov, A. G. B., G. V. Kosiakova, V. M. Klimashevskiy, T. M. Gorid’ko, O. F. Meged, N. M. Hula, N-stearoylethanolamine effect on the level of 11-hydroxycorticosteroids, cytokines IL-1β, IL-6 and TNFα in rats with nonspecific inflammation caused by thermal burn of skin. *Ukr. Biochem. J.* **2014,** *86* (3), 88-97.
33. Hula, N. M.; Hulii, M. F.; Kharchenko, N. K.; Horid'ko, T. M.; Marhitych, V. M., Neuroprotective effect of N-acylethanolamines in chronic morphine dependence. III. Influence on the content of neurotransmitters in the rat brain. *Ukr. Biokhim. Zh.* **2005,** *77* (1), 47-51.
34. Hula, N. M.; Marhitych, V. M.; Artamonov, M. V.; Zhukov, O. D.; Horid'ko, T. M.; Klimashevs'kyi, V. M., Neuroprotective effect of N-acylethanolamines in chronic morphine dependence. I. Rat brain phospholipids as a target of their action. *Ukr. Biokhim. Zh.* **2004,** *76* (5), 123-31.
35. Lykhmus, O.; Uspenska, K.; Koval, L.; Lytovchenko, D.; Voytenko, L.; Horid'ko, T.; Kosiakova, H.; Gula, N.; Komisarenko, S.; Skok, M., N-Stearoylethanolamine protects the brain and improves memory of mice treated with lipopolysaccharide or immunized with the extracellular domain of α7 nicotinic acetylcholine receptor. *Int. Immunopharmacol.* **2017,** *52*, 290-296.
36. Balvers, M. G.; Verhoeckx, K. C.; Meijerink, J.; Bijlsma, S.; Rubingh, C. M.; Wortelboer, H. M.; Witkamp, R. F., Time-dependent effect of in vivo inflammation on eicosanoid and endocannabinoid levels in plasma, liver, ileum and adipose tissue in C57BL/6 mice fed a fish-oil diet. *Int Immunopharmacol* **2012,** *13* (2), 204-14.
37. Balvers, M. G.; Verhoeckx, K. C.; Plastina, P.; Wortelboer, H. M.; Meijerink, J.; Witkamp, R. F., Docosahexaenoic acid and eicosapentaenoic acid are converted by 3T3-L1 adipocytes to N-acyl ethanolamines with anti-inflammatory properties. *Biochim. Biophys. Acta* **2010,** *1801* (10), 1107-14.

38. Meijerink, J.; Plastina, P.; Vincken, J. P.; Poland, M.; Attya, M.; Balvers, M.; Gruppen, H.; Gabriele, B.;Witkamp, R. F., The ethanolamide metabolite of DHA, docosahexaenoylethanolamine, shows immunomodulating effects in mouse peritoneal and RAW264.7 macrophages: evidence for a new link between fish oil and inflammation. *Br. J. Nutr.* **2011,** *105* (12), 1798-807.

39. Kim, H.-Y.; Spector, A. A.; Xiong, Z.-M., A synaptogenic amide N-docosahexaenoylethanolamide promotes hippocampal development. *Prostag. Oth. Lipid M.* **2011,** *96* (1), 114-120.

40. Sugiura, T.; Kobayashi, Y.; Oka, S.; Waku, K., Biosynthesis and degradation of anandamide and 2-arachidonoylglycerol and their possible physiological significance. *Prostagl. Leukotr. Ess.* **2002,** *66* (2), 173-192.

41. Schmid, H. H. O., Pathways and mechanisms of N-acylethanolamine biosynthesis: can anandamide be generated selectively? *Chem. Phys. Lipids* **2000,** *108* (1), 71-87.

42. Hussain, Z.; Uyama, T.; Tsuboi, K.; Ueda, N., Mammalian enzymes responsible for the biosynthesis of N-acylethanolamines. *BBA-Mol. Cell Biol. L.* **2017,** *1862* (12), 1546-1561.

43. Magotti, P.; Bauer, I.; Igarashi, M.; Babagoli, M.; Marotta, R.; Piomelli, D.; Garau, G., Structure of human N-acylphosphatidylethanolamine-hydrolyzing phospholipase D: regulation of fatty acid ethanolamide biosynthesis by bile acids. *Structure* **2015,** *23* (3), 598-604.

44. Okamoto, Y.; Morishita, J.; Tsuboi, K.; Tonai, T.; Ueda, N., Molecular characterization of a phospholipase D generating anandamide and its congeners. *J. Biol. Chem.* **2004,** *279* (7), 5298-305.

45. Wang, J.; Ueda, N., Biology of endocannabinoid synthesis system. *Prostag. Oth. Lipid M.* **2009,** *89* (3), 112-119.

46. Natarajan, V.; Schmid, P. C.; Reddy, P. V.; Schmid, H. H. O., Catabolism of N-acylethanolamine phospholipids by dog brain preparations. *J. Neurochem.* **1984,** *42* (6), 1613-1619.

47. Leung, D.; Saghatelian, A.; Simon, G. M.; Cravatt, B. F., Inactivation of N-acyl phosphatidylethanolamine phospholipase D reveals multiple mechanisms for the biosynthesis of endocannabinoids. *Biochemistry* **2006,** *45* (15), 4720-4726.

48. Tsuboi, K.; Okamoto, Y.; Ikematsu, N.; Inoue, M.; Shimizu, Y.; Uyama, T.; Wang, J.; Deutsch, D. G.; Burns, M. P.; Ulloa, N. M.; Tokumura, A.; Ueda, N., Enzymatic formation of N-acylethanolamines from N-acylethanolamine plasmalogen through N-acylphosphatidylethanolamine-hydrolyzing phospholipase D-dependent and -independent pathways. *BBA-Mol. Cell Biol. L.* **2011,** *1811* (10), 565-577.

49. Leishman, E.; Mackie, K.; Luquet, S.; Bradshaw, H. B., Lipidomics profile of a NAPE-PLD KO mouse provides evidence of a broader role of this enzyme in lipid metabolism in the brain. *BBA-Mol. Cell Biol. L.* **2016,** *1861* (6), 491-500.

50. 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.* **2006,** *281* (36), 26465-72.

51. Rahman, I. A. S.; Tsuboi, K.; Hussain, Z.; Yamashita, R.; Okamoto, Y.; Uyama, T.; Yamazaki, N.; Tanaka, T.; Tokumura, A.; Ueda, N., Calcium-dependent generation of N-acylethanolamines and lysophosphatidic acids by glycerophosphodiesterase GDE7. *BBA-Mol. Cell Biol. L.* **2016,** *1861* (12, Part A), 1881-1892.

52. 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.* **2008,** *283* (14), 9341-9.

53. Sun, Y. X.; Tsuboi, K.; Okamoto, Y.; Tonai, T.; Murakami, M.; Kudo, I.; Ueda, N., Biosynthesis of anandamide and N-palmitoylethanolamine by sequential actions of phospholipase A2 and lysophospholipase D. *Biochem. J.* **2004,** *380* (Pt 3), 749-56.

54. Liu, J.; Wang, L.; Harvey-White, J.; Osei-Hyiaman, D.; Razdan, R.; Gong, Q.; Chan, A. C.; Zhou, Z.; Huang, B. X.; Kim, H.-Y.; Kunos, G., A biosynthetic pathway for anandamide. *Proc. Natl. Acad. Sci. U. S. A.* **2006,** *103* (36), 13345-13350.

55. Liu, J.; Wang, L.; Harvey-White, J.; Huang, B. X.; Kim, H. Y.; Luquet, S.; Palmiter, R. D.; Krystal, G.; Rai, R.; Mahadevan, A.; Razdan, R. K.; Kunos, G., Multiple pathways involved in the biosynthesis of anandamide. *Neuropharmacology* **2008,** *54* (1), 1-7.

56. Tsuboi, K.; Okamoto, Y.; Rahman, I. A. S.; Uyama, T.; Inoue, T.; Tokumura, A.; Ueda, N., Glycerophosphodiesterase GDE4 as a novel lysophospholipase D: a possible involvement in bioactive N-acylethanolamine biosynthesis. *BBA-Mol. Cell Biol. L.* **2015,** *1851* (5), 537-548.

57. Ogura, Y.; Parsons, W. H.; Kamat, S. S.; Cravatt, B. F., A calcium-dependent acyltransferase that produces N-acyl phosphatidylethanolamines. *Nat. Chem. Biol.* **2016,** *12* (9), 669-71.

58. Ohto, T.; Uozumi, N.; Hirabayashi, T.; Shimizu, T., Identification of novel cytosolic phospholipase A2s, murine cPLA2δ, ϵ, and ζ, Which form a gene cluster with cPLA2β. *J. Biol.* **2005,** *280* (26), 24576-24583.

59. Lucas, K. K.; Dennis, E. A., Distinguishing phospholipase A2 types in biological samples by employing group-specific assays in the presence of inhibitors. *Prostag. Oth. Lipid M.* **2005,** *77* (1-4), 235-48.

60. Dennis, E. A.; Cao, J.; Hsu, Y. H.; Magrioti, V.; Kokotos, G., Phospholipase A2 enzymes: physical structure, biological function, disease implication, chemical inhibition, and therapeutic intervention. *Chem. Rev.* **2011,** *111* (10), 6130-85.

61. Ghosh, M.; Tucker, D. E.; Burchett, S. A.; Leslie, C. C., Properties of the Group IV phospholipase A2 family. *Prog. Lipid Res.* **2006,** *45* (6), 487-510.

62. Jin, X.-H.; Uyama, T.; Wang, J.; Okamoto, Y.; Tonai, T.; Ueda, N., cDNA cloning and characterization of human and mouse Ca^{2+}-independent phosphatidylethanolamine N-acyltransferases. *Biochimica et BBA-Mol. Cell Biol. L.* **2009,** *1791* (1), 32-38.

63. Uyama, T.; Ikematsu, N.; Inoue, M.; Shinohara, N.; Jin, X. H.; Tsuboi, K.; Tonai, T.; Tokumura, A.; Ueda, N., Generation of N-acylphosphatidylethanolamine by members of the phospholipase A/acyltransferase (PLA/AT) family. *J. Biol. Chem.* **2012,** *287* (38), 31905-19.

64. Mardian, E. B.; Bradley, R. M.; Duncan, R. E., The HRASLS (PLA/AT) subfamily of enzymes. *J. Biomed. Sci.* **2015,** *22* (1), 99.

65. Jaworski, K.; Ahmadian, M.; Duncan, R. E.; Sarkadi-Nagy, E.; Varady, K. A.; Hellerstein, M. K.; Lee, H.-Y.; Samuel, V. T.; Shulman, G. I.; Kim, K.-H.; de Val, S.; Kang, C.; Sul, H. S., AdPLA ablation increases lipolysis and prevents obesity induced by high-fat feeding or leptin deficiency. *Nat. Med.* **2009,** *15*, 159.

66. Liu, Y. S.; Patricelli, M. P.; Cravatt, B. F., Activity-based protein profiling: the serine hydrolases. *Proc. Natl. Acad. Sci. U. S. A.* **1999,** *96* (26), 14694-14699.

67. Niphakis, M. J.; Cravatt, B. F., Enzyme inhibitor discovery by activity-based protein profiling. *Annu. Rev. Biochem.* **2014**; *83*, 341-377.

68. Simon, G. M.; Cravatt, B. F., Activity-based proteomics of enzyme superfamilies: serine hydrolases as a case study. *J. Biol. Chem.* **2010,** *285* (15), 11051-5.

69. Baggelaar, M. P.; Janssen, F. J.; van Esbroeck, A. C. M.; den Dulk, H.; Allara, M.; Hoogendoorn, S.; McGuire, R.; Florea, B. I.; Meeuwenoord, N.; van den Elst, H.; van der Marel, G. A.; Brouwer, J.; Di Marzo, V.; Overkleeft, H. S.; van der Stelt, M., Development of an activity-based probe and in silico design reveal highly selective inhibitors for diacylglycerol lipase-alpha in brain. *Angew. Chem.-Int. Edit.* **2013,** *52* (46), 12081-12085.

70. Baggelaar, M. P.; Chameau, P. J. P.; Kantae, V.; Hummel, J.; Hsu, K. L.; Janssen, F.; van der Wel, T.; Soethoudt, M.; Deng, H.; den Dulk, H.; Allara, M.; Florea, B. I.; Di Marzo, V.; Wadman, W. J.; Kruse, C. G.; Overkleeft, H. S.; Hankemeier, T.; Werkman, T. R.; Cravatt, B. F.; van der Stelt, M., Highly selective, reversible inhibitor identified by comparative chemoproteomics modulates diacylglycerol lipase activity in neurons. *J. Am. Chem. Soc.* **2015,** *137* (27), 8851-8857.

71. van Esbroeck, A. C. M.; Janssen, A. P. A.; Cognetta, A. B.; Ogasawara, D.; Shpak, G.; van der Kroeg, M.; Kantae, V.; Baggelaar, M. P.; de Vrij, F. M. S.; Deng, H.; Allara, M.; Fezza, F.; Lin, Z.; van der Wel, T.; Soethoudt, M.; Mock, E. D.; den Dulk, H.; Baak, I. L.; Florea, B. I.; Hendriks, G.; de Petrocellis, L.; Overkleeft, H. S.; Hankemeier, T.; De Zeeuw, C. I.; Di Marzo, V.; Maccarrone, M.; Cravatt, B. F.; Kushner, S. A.; van der Stelt, M., Activity-based protein profiling reveals off-target proteins of the FAAH inhibitor BIA 10-2474. *Science* **2017,** *356* (6342), 1084-1087.

72. Yang, P. Y.; Liu, K.; Ngai, M. H.; Lear, M. J.; Wenk, M. R.; Yao, S. Q., Activity-based proteome profiling of potential cellular targets of Orlistat - an FDA-approved drug with anti-tumor activities. *J. Am. Chem. Soc.* **2010,** *132* (2), 656-666.