doi:10.2533/chimia.2024.499 Chimia 78 (2024) 499–512 © U. Grether *et al.*

Enhancing Drug Discovery and Development through the Integration of Medicinal Chemistry, Chemical Biology, and Academia-Industry Partnerships: Insights from Roche's Endocannabinoid System Projects

Johannes Aebi[a], Kenneth Atz[a], Simon M. Ametamey[b], Jörg Benz[a], Julie Blaising[a], Stefania Butini[c], Giuseppe Campiani[c], Erick M. Carreira[d], Ludovic Collin[a], Eva de Lago[e], Thais Gazzi[a], Jürg Gertsch[f], Luca Gobbi[a], Wolfgang Guba[a], Javier Fernández-Ruiz[e], Jürgen Fingerle[a], Achi Haider[a], Yingfang He[b], Laura H. Heitman[g], Michael Honer[a], Daniel Hunziker[a], Bernd Kuhn[a], Mauro Maccarrone[h], Hans Peter Märki[a], Rainer E. Martin[a], Peter Mohr[a], Linjing Mu[b], Marc Nazaré[i], David F. Nippa[a], Sergio Oddi[j], Fionn O'Hara[a], Pal Pacher[k], Julian Romero[l], Stephan Röver[a], Arne C. Rufer[a], Roger Schibli[b], Gisbert Schneider[m], Antonia F. Stepan[a], David A. Sykes[n], Christoph Ullmer[a], Mario van der Stelt[o], Dmitry B. Veprintsev[n], Matthias B. Wittwer[a], and Uwe Grether[*§a]

[§]Industrial Science Award 2023

Abstract: The endocannabinoid system (ECS) is a critical regulatory network composed of endogenous cannabinoids (eCBs), their synthesizing and degrading enzymes, and associated receptors. It is integral to maintaining homeostasis and orchestrating key functions within the central nervous and immune systems. Given its therapeutic significance, we have launched a series of drug discovery endeavors aimed at ECS targets, including peroxisome proliferator-activated receptors (PPARs), cannabinoid receptors types 1 (CB_1R) and 2 (CB_2R), and monoacylglycerol lipase (MAGL), addressing a wide array of medical needs. The pursuit of new therapeutic agents has been enhanced by the creation of specialized labeled chemical probes, which aid in target localization, mechanistic studies, assay development, and the establishment of biomarkers for target engagement. By fusing medicinal chemistry with chemical biology in a comprehensive, translational end-to-end drug discovery strategy, we have expedited the development of novel therapeutics. Additionally, this strategy promises to foster highly productive partnerships between industry and academia, as will be illustrated through various examples.

Keywords: Academia-industry collaboration · CB_1R · CB_2R · Endocannabinoid system · Labeled chemical probe · MAGL · PPAR

**Correspondence:* Dr. U. Grether[a], E-mail: uwe.grether@roche.com

[a]Pharma Research and Early Development (pRED), Roche Innovation Center Basel, F. Hoffmann-La Roche Ltd, Grenzacherstrasse 124, CH-4070 Basel, Switzerland; [b]Center for Radiopharmaceutical Sciences ETH, PSI and USZ, Institute of Pharmaceutical Sciences, ETH Zurich, Vladimir-Prelog-Weg 4, CH-8093 Zurich, Switzerland; [c]Department of Biotechnology, Chemistry and Pharmacy, University of Siena, Via Aldo Moro 2, 53100 Siena, Italy; [d]Laboratorium für Organische Chemie, ETH Zurich, Vladimir-Prelog-Weg 3, CH-8093 Zurich, Switzerland; [e]Instituto Universitario de Investigación en Neuroquímica, Departamento de Bioquímica y Biología Molecular, Facultad de Medicina, Universidad Complutense, Madrid, Spain & Centro de Investigación Biomédica en Red de Enfermedades Neurodegenerativas (CIBERNED), Madrid, Spain & Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Madrid, Spain; [f]Institute of Biochemistry and Molecular Medicine, University of Bern, CH-3012 Bern, Switzerland; [g]Division of Drug Discovery and Safety, Leiden University & Oncode Institute, Leiden, Netherlands; [h]Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila, Italy & European Center for Brain Research, Santa Lucia Foundation, 00128 Rome, Italy; [i]Leibniz-Forschungsinstitut für Molekulare Pharmakologie FMP, Campus Berlin-Buch, 13125 Berlin, Germany; [j]Department of Veterinary Medicine, University of Teramo, 64100 Teramo, Italy & European, Center for Brain Research (CERC)/Santa Lucia Foundation, 00179 Rome, Italy; [k]National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Rockville, MD 20852, USA; [l]Faculty of Experimental Sciences, Universidad Francisco de Vitoria, Pozuelo de Alarcón, 28223, Madrid, Spain; [m]ETH Zurich, Department of Biosystems Science and Engineering, Klingelbergstrasse 48, CH-4056 Basel; [n]Faculty of Medicine & Health Sciences, University of Nottingham, Nottingham NG7 2UH, UK & United Centre of Membrane Proteins and Receptors (COMPARE), University of Birmingham and University of Nottingham, Midlands, UK; [o]Department of Molecular Physiology, Leiden Institute of Chemistry, 2333 CC Leiden University, Leiden, Netherlands.

Dr. Uwe Grether completed his PhD in organic chemistry under the mentorship of Prof. Herbert Waldmann in 2000 at the University of Karlsruhe, Germany. Following his doctoral studies, he pursued postdoctoral research with Prof. James D. White at Oregon State University. In 2001, Dr. Grether commenced his career at the Pharma Research and Early Development division of F. Hoffmann-La Roche Ltd. in Basel, Switzerland. With extensive professional experience in drug discovery and development, he has consistently emphasized comprehensive strategies aimed at achieving clinical objectives, successfully advancing multiple new molecular entities up to phase 3 clinical trials. Dr. Grether's research interests span medicinal chemistry, the application of late-stage functionalization techniques, and the expanding field of chemical biology.

1. Introduction

1.1 *The Endocannabinoid System*

The endocannabinoid system (ECS) is a complex lipid signaling network ubiquitously expressed in mammalian tissues.[1] It plays a crucial role in maintaining body homeostasis and regulates several vital bodily functions, including sleep, body temperature, appetite, learning, memory, and emotional processing. The ECS comprises endogenous signaling molecules known as endocannabinoids (eCBs), their receptors, enzymes, and transporters (Fig. 1).[1b,1c,2] The ECS components facilitate and regulate the diverse actions of eCBs in both the central nervous system[3] and peripheral tissues.[4] The most active and well-studied eCBs are 2-arachidonoylglycerol (2-AG)[5] and anandamide (*N*-arachidonoylethanolamine, AEA).[6] These eCBs activate various receptors located on the plasma membrane and within the nucleus. Key eCB-binding receptors include the G protein-coupled receptors cannabinoid receptor type-1 (CB_1R) and type-2 (CB_2R), which are also the molecular targets of the phytocannabinoid (–)-trans-Δ^9-tetrahydrocannabinol (THC).[7] Additionally, nuclear peroxisome proliferator-activated receptors (PPARs) α,[8] γ,[9] and δ,[10] which are transcription factors regulating gene expression, are integral components of the ECS.[11]

The receptor-mediated activities of eCBs are tightly controlled spatiotemporally, with their cellular concentrations being regulated by a balance between synthesis and degradation *via* various biosynthetic and hydrolytic enzymes.[1b] 2-AG is synthesized by diacylglycerol lipases (DAGL) α and β,[12] and its signaling activity is primarily terminated by monoacylglycerol lipase (MAGL).[13] *N*-Acyl phosphatidylethanolamines-specific phospholipase D (NAPE-PLD)[14] catalyzes the release of AEA from phospholipid precursors, while fatty acid amide hydrolase (FAAH)[15] hydrolyzes AEA to arachidonic acid and ethanolamine.

The metabolic control of eCB tone is further modulated by transporters that facilitate eCB movement across the plasma membrane *via* a purported eCB membrane transporter (EMT)[16] and intracellularly by different proteins, *e.g.* fatty acid binding proteins (FABP) 5 and 7.[17] Dysregulation of the ECS and changes in eCB concentrations have been linked to various pathological conditions, including cancer, osteoporosis, neuromotor, neuropsychological, and neurodegenerative diseases, respiratory diseases such as asthma, cardiovascular diseases such as stroke, atherosclerosis, myocardial infarction, metabolic disorders, arrhythmias, and hypertension.[18]

Given its extensive role in physiological regulation, the ECS holds significant therapeutic potential. Multiple small molecules targeting the ECS have either been approved or are in advanced clinical stages.[18]

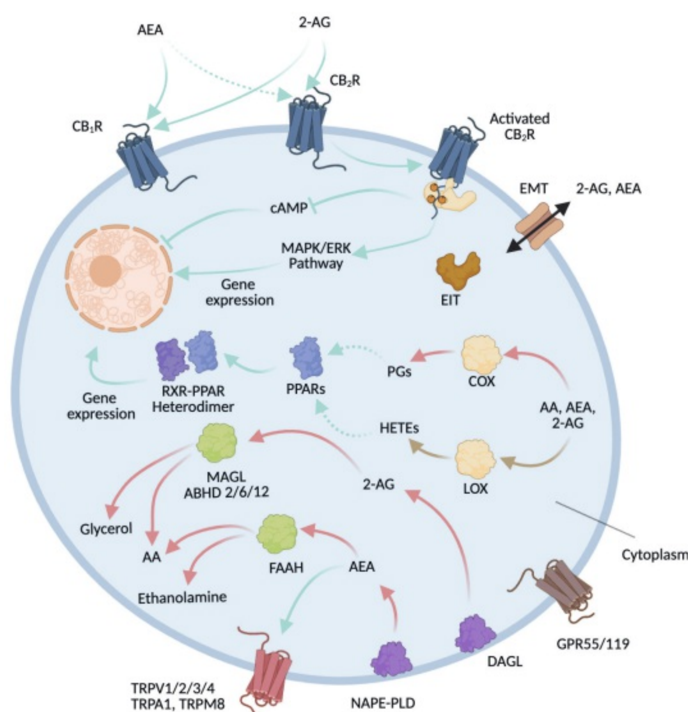

Fig. 1. Schematic illustration of the essential elements of the endocannabinoid system (ECS). 2-Arachidonoylglycerol (2-AG) and *N*-arachidonoylethanolamine (AEA) extracellularly activate G protein-coupled receptors (GPCRs) such as cannabinoid receptor type-1 (CB_1R) and type-2 (CB_2R). Intracellularly, they interact with transient receptor potential (TRP) channels and, either directly or after metabolism, with nuclear hormone receptors such as peroxisome proliferator-activated receptors (PPARs) in the nucleus. 2-AG is released from membrane lipids through the action of diacylglycerol lipase (DAGL) and can be hydrolyzed to glycerol and arachidonic acid (AA) by cytosolic monoacylglycerol lipase (MAGL). AEA is primarily synthesized by *N*-acylphosphatidylethanolamine-specific phospholipase D (NAPE-PLD) and degraded by fatty acid amide hydrolase (FAAH) to form arachidonic acid (AA) and ethanolamine. A specific endocannabinoid membrane transporter (EMT) facilitates the transmembrane exchange of eCBs. Intracellular traffic is facilitated by eCB intracellular transporters (EIT). Oxidative metabolism of eCBs involves the addition of oxygen to the fatty acid moiety by cyclooxygenase-2 (COX-2) to generate prostaglandins (PGs) and by lipoxygenases (LOXs) to form hydroxyeicosatetraenoic acids (HETEs). ABHD = α/β-hydrolase domain; cAMP = cyclic adenosine monophosphate; ERK = extracellular signal-regulated kinase; MAPK = mitogen-activated protein kinases; RXR = retinoid X receptor.

1.2 *The ECS and Roche*

Due to the enormous therapeutic potential of the ECS, Roche embarked on small molecule drug discovery programs targeting specific ECS components as early as the late 1990s. Initially, the primary focus was on metabolic diseases. Subsequently, our attention shifted to disorders of the central nervous system, and later expanded to include ophthalmic and peripheral inflammatory diseases.

To maximize the chances of delivering effective medicines to patients, we adopt an end-to-end approach across the entire discovery and development value chain. This approach is informed by frameworks such as the 'Three Pillars of Survival',[19] the 'Four Pillars of Target Validation',[20] both introduced by Pfizer researchers, and the '5Rs'.[21]

In support of our drug discovery efforts, we have generated labeled chemical probes to facilitate compound profiling, target localization, mechanism of action studies, target engagement, selectivity assessments, and biomarker studies throughout the value chain.[22]

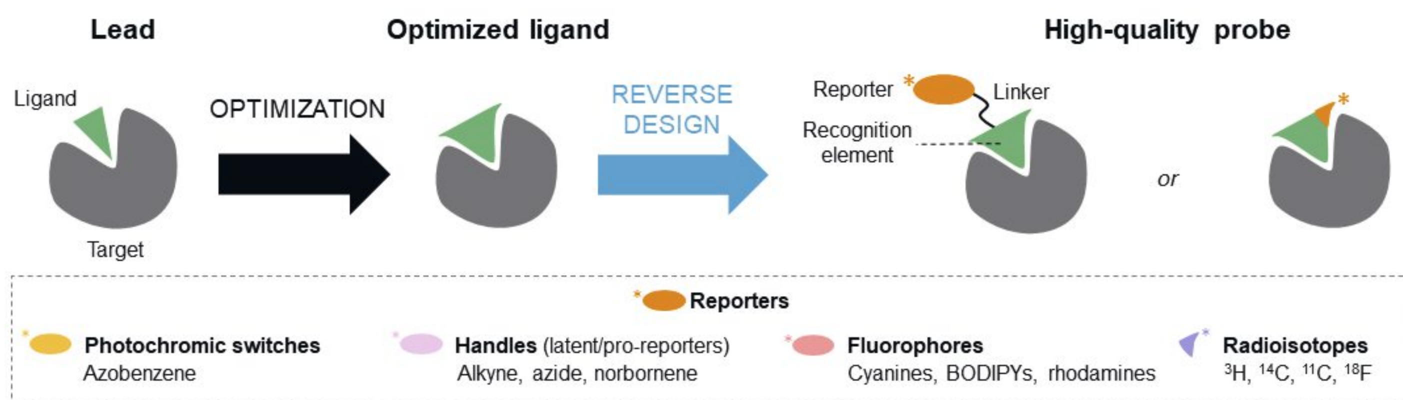

Fig. 2. Reverse-design strategy for labeled chemical probes and examples of reporter units. Reproduced from ref. [23], CCBY.

1.3 *Labeled Chemical Probes and Reverse Design*

A labeled chemical probe is a small molecule that acts as a ligand for a specific target and includes a reporter unit that enables the characterization of ligand-target interactions, optionally connected by a linker (Fig. 2). To develop effective and reliable probes, we employed a reverse-design approach, leveraging prior optimization performed by medicinal chemists.[23]

By initiating probe generation from optimized ligands and utilizing existing structure-activity relationship (SAR) knowledge, we expedite the incorporation of reporter units. This approach ensures that the affinity for the biological target is maintained, while also preserving selectivity against off-targets and suitable absorption distribution metabolism excretion and toxicology (ADMET) characteristics, such as cellular permeability for targeting intracellular proteins.

2. Agonists and Labeled Chemical Probes Targeting Peroxisome Proliferator-activated Receptors

The peroxisome proliferator-activated receptors (PPARs) α, γ, and δ are ligand-dependent nuclear transcription factors that belong to the nuclear hormone receptor superfamily. PPARs form heterodimers with members of the retinoid X receptor (RXR) family,[24] which transactivate PPAR-responsive elements (PPREs) of target genes in the presence of specific cofactors. These target genes are involved in insulin signaling, lipid/glucose metabolism, immune response, cell cycle, and differentiation of epithelial or mesenchymal cells.[25] PPARs are selectively activated by natural ligands, such as endocannabinoids (eCBs) and their metabolites, *e.g.* prostaglandins (PGs), or by synthetic PPAR agonists.[26] Multiple PPAR ligands with diverse subtype selectivity have been introduced to the market or reached advanced clinical stages, primarily aimed at combating metabolic syndrome.[26,27]

The link between PPAR activation and metabolic syndrome was identified in the early 1970s. Patients with type 2 diabetes taking clofibrate, the first of the fibric acid derivatives developed for the treatment of hypertriglyceridemia, showed a reduction in fasting blood glucose concentrations.[28] Subsequent studies revealed that PPARα is mainly expressed in skeletal muscle, liver, and heart.[29] Its activation is largely associated with lipid metabolism, inflammation reduction, and stabilization of atherosclerotic plaques. In contrast, PPARγ, which has three human isoforms (PPARγ1, γ2, and γ3), is widely distributed throughout the body, including muscle and fat cells. PPARδ (also known as PPARβ) is ubiquitously expressed and is particularly important for regulating fatty acid uptake, transport, and oxidation, as well as insulin secretion and sensitivity.[30]

PPARs are highly druggable targets, and the generation of novel ligands for all three subtypes has been supported by structure-based drug design. Generally, PPAR ligands consist of three key elements (Fig. 3): an acidic element that forms up to four pivotal hydrogen bonds with hydrophilic amino acid residues of the respective protein, which is indispensable for obtaining potent agonists, an aromatic center, and a partly solvent-exposed cyclic tail.[31] These three elements are interconnected by linker moieties, which can optionally be branched to access additional subpockets in the receptor.

Fig. 3. Simplified structure of typical synthetic PPAR agonists. The linkers may be branched to reach additional subpockets within the receptor.

The fibrates, such as fenofibrate, a prodrug of the PPARα agonist fenofibric acid, are clinically used to treat dyslipidemia and its associated cardiovascular risk (Fig. 4).[32] New-generation PPARα agonists with more sophisticated acidic head groups, such as NS-220, exhibit enhanced potency and subtype selectivity.[33] We licensed NS-220 (R-1593) and initiated phase 1 clinical trials for the potential treatment of lipid metabolism disorders. In parallel, we have also developed in-house ligands with high potency for PPARα, including pyridine **1**,[34] and dual-acting PPARα/δ co-agonists, such as pyrimidine **2**.[35]

The thiazolidinediones (TZDs) are PPARγ agonists that have been shown to improve insulin sensitivity and glucose homeostasis,[38] in addition to exhibiting anti-inflammatory[39] and antihypertensive effects.[40] A common feature of these compounds is their thiazolidinedione moiety, as seen in edaglitazone,[41] which provides selectivity over the PPARα subtype. Edaglitazone, developed by Chugai, was Roche's first advanced PPAR ligand and was investigated in phase 3 clinical trials for the treatment of type 2 diabetes.[42] To set-up a PPARγ scintillation proximity binding assay, we tritiated the molecule at the benzylic position between the thiazolidinedione and benzthiophene moiety.[43] For functional assays, a homogeneous time-resolved fluorescence resonance energy transfer (TR-FRET) cofactor recruitment assay proved to be highly beneficial.[44] Furthermore, we also discovered a partial non-TZD based PPARγ agonist.[45]

To harness the beneficial effects of both PPARα and PPARγ agonism within a single molecule, glitazars have been developed.[46] These dual PPARα/γ co-agonists, such as aleglitazar,[31] phenylpropionic acid **3**,[46b] and indole **4**,[47] contain an α-alkoxy acid moiety as a common structural motif. Additionally, the discovery of selective PPARδ ligands, such as L-165041,[48] and PPARδ ligands with concomitant PPARα activity, such as

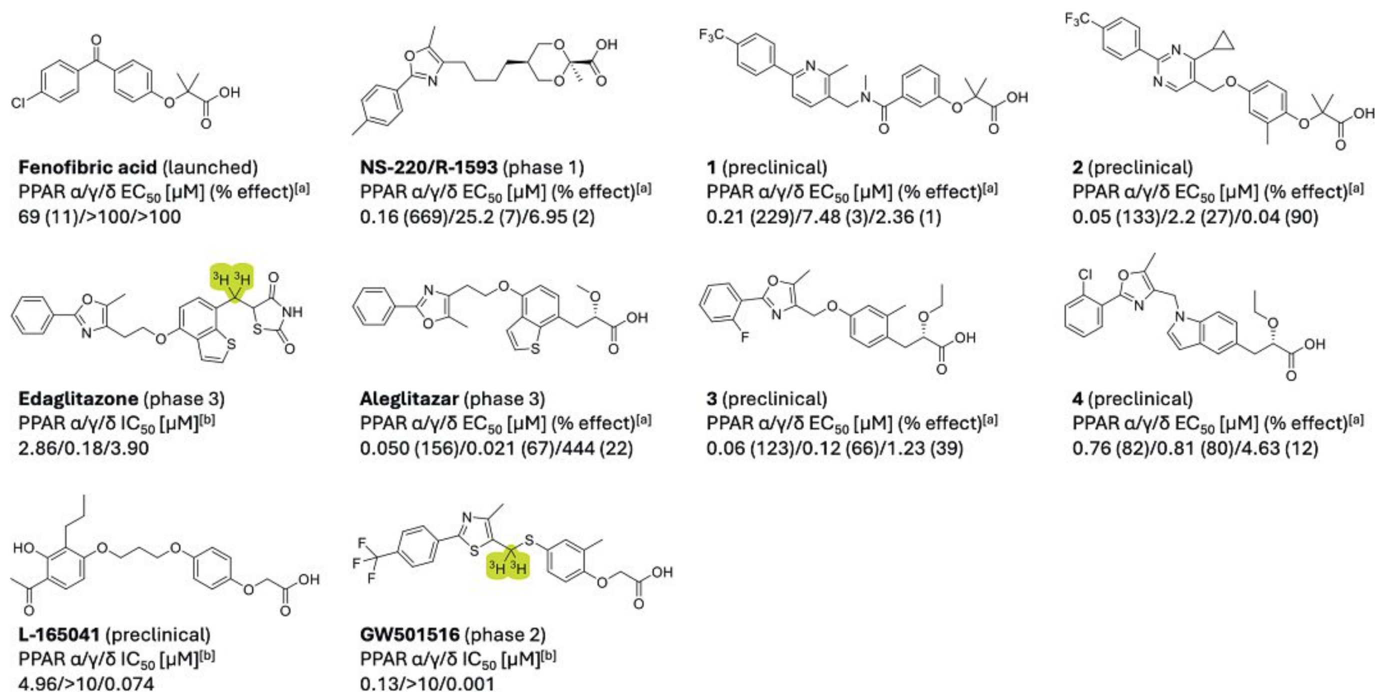

Fig. 4. Chemical structures, EC_{50} and IC_{50} values, and highest development phase of reference and Roche PPARα, γ and δ agonists. The tritium labeling positions for the radiolabeled ligands [^{3}H]edaglitazone and [^{3}H]GW501516 have been specified. [a]Effects are reported in relation to reference compounds whose activity was set to 100%: farglitazar (GW262570)[36] for PPARα; edaglitazone for PPARγ; GW501516 for PPARδ. [b]PPARα, γ, and δ radioligand binding and functional transactivation (luciferase transcriptional reporter gene) assays were performed as described in Binggeli *et al*.[37] Radioisotopes are highlighted in green.

GW501516,[49] has been reported. The latter was tritiated to set up a PPARδ radioligand binding assay.[43]

More recently, PPAR pan agonists, which activate all three subtypes in a balanced manner, have been proposed for improved management of the global cardiovascular risk in type 2 diabetes mellitus patients. This pan activity could potentially lower the risk of adverse side effects, such as obesity, fluid retention, and edema.[27] A recent publication using prospective *de novo* drug design with deep interactome-based learning for ligand- and structure-based generation of drug-like molecules focused on the discovery of novel PPAR ligands, demonstrating the continued interest in this target class.[50]

3. Cannabinoid Receptor Type-1 Ligands

The G-protein coupled receptor (GPCR) cannabinoid receptor 1 (CB_1R) was identified as the specific brain receptor for THC (Fig. 5), the psychoactive constituent of marijuana,[51] by the Howlett group in 1988.[52] CB_1R is highly abundant in the central nervous system (CNS) and many peripheral tissues and organs.[53] It plays a critical role in regulating mood and appetite, pain perception, learning and memory, as well as motor control.[54]

CB_1R has been recognized as a target for pharmacotherapeutic development based on extensive preclinical data (for reviews see Mackie, 2008;[55] Pertwee, 2008,[56] 2012;[57] Tsang, 2016;[58] Lu, 2017;[59] Amin, 2019;[60] Schurman, 2020;[61] Wilkerson, 2021[62]). However, the development of CB_1R agonists and antagonists has faced challenges related to selectivity due to the widespread distribution of CB_1R throughout the brain, including expression by both neuronal and non-neuronal cells. This broad distribution increases the likelihood of unwanted side effects accompanying therapeutic benefits.

The only FDA-approved CB_1R agonists are THC itself (synthesized as dronabinol) and its dimethylheptyl analog nabilone (LY-109514), specifically for treating cancer chemotherapy-induced nausea and vomiting. These medicines remain within the US Pharmacopeia.[63] The European Medicines Agency (EMA) has approved a mixture of THC and CBD (cannabidiol) extracted and purified from cannabis (nabiximols) for the treatment of spasticity and pain in multiple sclerosis (MS). Dronabinol, nabilone, and nabiximols exhibit agonist activity at both CB_1R and CB_2R, with therapeutic responses and side effects attributed to one or both CB receptors, as determined by pharmacological characterization in *in vivo* or *in vitro* models.

Despite these challenges, targeting CB_1R for unmet therapeutic needs has continued to evolve based on preclinical investigations.[18] Several experimental studies have shown that THC and 2-AG stimulate appetite and food intake in animal models.[64] CB_1R knock-out mice exhibit markedly lower daily food intake and body weight, even when fed a high-fat diet (HFD).[65] This led to the search for CB_1R inverse agonists for controlling appetite, food intake, and ultimately body weight, which commenced in the late 1990s.[66]

Target-based drug discovery has progressed and delivered many different series of CB_1-receptor antagonists.[67] Through the application of the evolutionary fragment-based *de novo* design tool TOPology Assigning System (TOPAS), starting from a known CB_1R ligand and followed by further optimization, we identified a novel benzodioxole CB_1R inverse agonist series.[68] Extensive multidimensional optimization yielded the promising lead compound **5**, which showed reduced body-weight gain and fat mass in diet-induced obese Sprague-Dawley rats.

The most advanced CB_1R antagonist, rimonabant (SR141716A), received marketing authorization in the European Union as an anti-obesity medication but was later withdrawn due to an increased incidence of anxiety, depression, and suicidality.[69] These psychiatric side effects appear to preclude the use of CB_1 receptor antagonists for non-life-threatening diseases or chronic treatment. However, it has become apparent that the CB_1 receptor has peripheral functions, suggesting that peripherally active and selective agents may have therapeutic potential with reduced psychiatric side effects.

Roche preclinical research identified 6-alkoxy-5-aryl-3-pyridinecarboxamides (*cf.* compound **6**) as potent CB_1 receptor

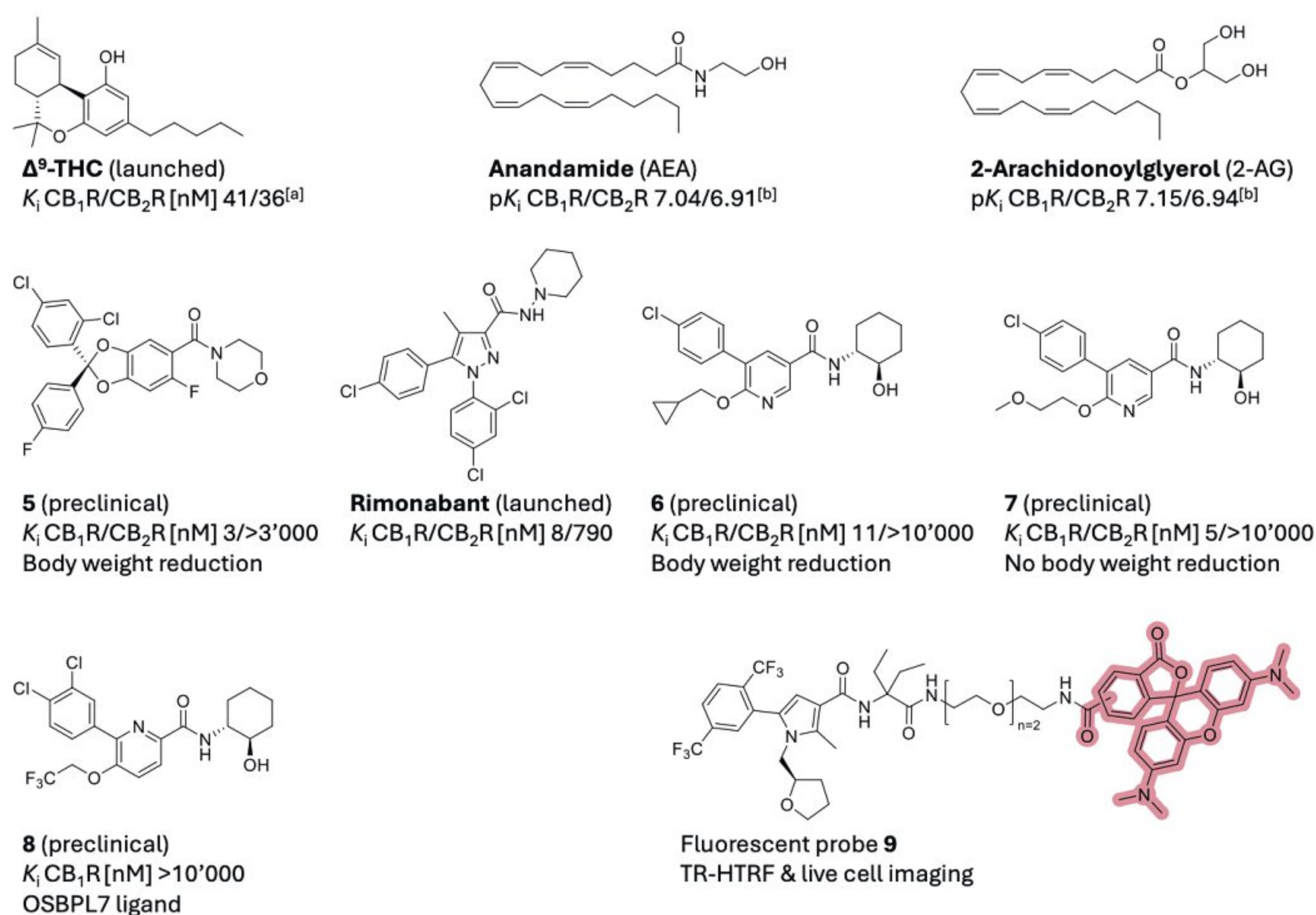

Fig. 5. Chemical structures, *in vitro* pharmacology data and highest development phase of reference and Roche CB_1R ligands. [a]Binding affinity on human cannabinoid receptors.[73] [b]Consensus data for human CBRs from a collaborative multicentric profiling effort between multiple independent academic laboratories and industry.[74] Fluorophore is highlighted in red.

antagonists with high selectivity over CB_2 receptors.[70] The series was optimized to reduce lipophilicity, achieving peripherally active molecules with minimal central effects. This led to the development of a highly potent and selective CB_1R inverse agonist, pyridine **7**, with markedly reduced brain exposure compared to rimonabant. Despite high peripheral exposures, the ligand had no significant effect on body weight and only a marginal effect on body fat reduction in high-fat-fed rats. These data, consistent with reported effects in tissue-specific CB_1 receptor KO mice, suggest that the metabolic benefits of CB_1 receptor antagonists are primarily centrally mediated.

Interestingly, ligands from the 5-arylnicotinamide series became important seed structures for further drug discovery programs. Compound **6** was found to stimulate cholesterol efflux to extracellular apoAI in a phenotypic screening assay using cholesterol-loaded human macrophages (THP1 cells).[71] Oxysterol Binding Protein Like 7 (OSBPL7) was identified as the molecular target of this ATP-binding cassette transporter (ABCA1)-mediated cholesterol efflux. Compound **8**, devoid of CB_1R activity, was highly efficacious in improving renal function in mouse models reflecting two diverse etiologies of progressive renal disease. These compounds may offer a potential new therapy for renal disease by targeting cellular cholesterol metabolism through a new mode of action, addressing the significant unmet need in chronic kidney disease (CKD).

Furthermore, specifically decorated 5-arylnicotinamides were found to be selective for CB_2R and devoid of CB_1R activation, becoming a lead series for a Roche CB_2R program focusing on the treatment of diseases with an inflammatory background. In contrast, a pyrrole carboxamide CB_1R ligand became the seed structure for the generation of reverse design-approach-based fluorescently labeled CB_1R probes in collaboration with the Nazaré group.[72] A modular design concept with a diethyl glycine-based building block as the centerpiece, supported by computational docking studies, allowed for the generation of pyrrole-based CB_1R fluorescent probes such as tetramethyl-6-carboxyrhodamine (TAMRA) derivative **9**. These probes were successfully applied in time-resolved fluorescence resonance target-engagement studies (TR-HTRF) and CB_1R live cell imaging studies, holding great potential for deepening the understanding of mechanistic aspects of CB_1R localization, trafficking, and activation essential for the function and role of this receptor in pathological conditions.

4. Cannabinoid Receptor Type-2 Ligands

CB_2R was discovered in 1993.[75] Like CB_1R, it belongs to the class A (rhodopsin-like) GPCRs. CB_2R and CB_1R share a high degree of homology, with 44% overall sequence identity and 68% homology in the ligand-binding domain. Remarkably, no natural ligands have been identified that exhibit exclusive selectivity for either of the two cannabinoid receptors. Selectivity, if any, seems to be regulated by the varying expression of receptors and endocannabinoid metabolizing enzymes in different cell types and tissues.[76]

CB_2R is highly abundant in immune tissues such as the spleen, lymph nodes, and bone marrow, where it is found in immune cells including macrophages, T and B cells, natural killer cells, monocytes, mast cells and polymorphonuclear neutrophils.[53b,77] CB_2R has also been identified in other peripheral tissues such as the liver,[78] kidney,[79] spleen,[77b] gastrointestinal tract,[80] and bone,[80,81] predominantly in immune-derived cells (*e.g.* Kupffer

cells, osteoblasts, and osteoclasts, among others). Furthermore, CB_2R mRNA has been found in microglia, particularly during neuroinflammation, where microglia are activated and CB_2R levels are upregulated.[82] Apart from immune cells, CB_2R expression levels are generally very low, making it challenging to definitively establish its presence.[82,83]

CB_2R has been reported to be expressed both at the cell surface and intracellularly.[84] Inflammation and tissue injury can trigger rapid elevations in local endocannabinoid (eCB) levels, with likely contributions from both infiltrating immune cells and damaged parenchymal cells. This is often accompanied by an increase in CB_2R expression levels, most likely originating from the infiltration of immune cells and the activation of resident ones. Such changes in eCB levels and CB_2R expression have been reported for a plethora of diseases affecting humans.[85] These eCB level changes modulate CB_2R-mediated signaling responses in immune and other cells.

At the molecular level, CB_2R couples to $G\alpha i/o$ protein and inhibits cyclic adenosine monophosphate (cAMP) production *via* adenylate cyclase, which in turn stimulates downstream signaling cascades. Internalization and ERK phosphorylation (pERK) are additional important signaling pathways. The signaling fingerprint may vary among structurally diverse orthosteric binders, illustrating the phenomenon of biased signaling.[74] This variability gives rise to distinct cellular responses, ultimately opening the door to the potential development of tailored CB_2R therapeutics.[86]

Generally, CB_2R activation in immune or immune-derived cells mediates immunosuppressive effects; proliferation is inhibited, apoptosis is induced, and cytokine and chemokine production as well as migration of stimulated immune cells is suppressed.[87] Furthermore, regulatory T cells are induced, leading to an attenuation of autoimmune inflammatory responses.[88] In summary, critical functions are modulated, leading to the prevention, attenuation, and repair of inflicted damage, thereby limiting tissue injury in a variety of pathological conditions such as cardiovascular,[89] gastrointestinal/inflammatory bowel,[90] liver,[91] kidney,[79,92] lung,[53b] and neurodegenerative diseases,[93] systemic sclerosis and scleroderma,[94] diabetes and its complications (retinopathy, nephropathy, and cardiomyopathy),[95] and pain,[96] especially in those associated with sterile inflammatory responses. In various forms of tissue injury or inflammation, the activation of CB_2R is generally protective. However, in certain disease states or stages, the immunosuppressive effects of CB_2R activation may context-dependently exacerbate tissue damage.[85] In such cases, CB_2R inverse agonists and antagonists might offer therapeutic benefits.

The immense therapeutic potential of CB_2R modulation has garnered recognition from both academic and industry institutions, resulting in the discovery of numerous ligands and chemical probes designed to target CB_2R.[73, 97] The first manuscripts and patents appeared in 1991[98] and 1996,[99] respectively. Since then, more than 1130 basic patent applications have been filed until the date of submission of this article. The reported structures cover a vast, structurally diverse chemical space and can be divided into endogenous cannabinoids and related fatty acid derivatives, marijuana-derived cannabinoids, and synthetic cannabinoids.

In recent years, there has been a notable shift in the focus of CB_2R ligand development, with an emphasis on agonists exhibiting a high degree of selectivity over CB_1R and specificity towards CB_2R. This shift aligns with an increased interest in indications associated with inflammation or tissue injury. Concurrently, more than 20 CB_2R agonists have undergone human studies.[97h]

Starting activities in kidney diseases and recognizing the therapeutic potential of CB_2R agonism, we began generating CB_2R agonists to explore their potential for reducing chemotaxis, oxidative stress, and tissue protection. Initially, we focused on damage resulting from ischemia-reperfusion injury and fibrosis, as these are closely linked to both acute kidney injury (AKI) and chronic kidney disease (CKD), for which there is a high unmet medical need. To identify hits, we employed a three-pronged approach: a high-throughput screening campaign, an information-based approach leveraging our CB_1R inverse agonist program, and a licensing deal for HU-910[91c] from the Mechoulam lab (Fig. 6). All approaches were successful and provided highly potent and very selective CB_2R agonists that matched our target compound profile. High selectivity over CB_1R was particularly important, as CB_1R agonism in the brain and its associated psychotropic effects were considered a significant drawback for indications such as diabetic nephropathy.

HU-910 belongs to the group of non-classical cannabinoids, in which the pyranyl ring of classical cannabinoids such as THC is not present. It exhibits a favorable overall profile, including high binding and functional selectivity against CB_1R and a representative set of further off-targets. Therefore, HU-910 has been recommended as a selective CB_2R agonist to study the role of CB_2R in biological and disease processes.[74] HU-910 demonstrated a sustained beneficial effect in liver post-ischemia paradigms[91c] and limited albuminuria and renal injury in mice with type 2 diabetic nephropathy (10 mg/kg *p.o.* for 11 weeks) to a similar extent as the clinically approved angiotensin-converting enzyme (ACE) inhibitor lisinopril, thereby suggesting that CB_2R agonism has the potential to become a treatment for type 2 diabetic nephropathy.[92a] In a mouse *in vivo* model of endotoxin-induced uveitis (EIU), HU-910 showed beneficial immunomodulating actions in the eye by decreasing leukocyte adhesion through inhibition of resident ocular immune cells.[100]

Highly selective and potent bicyclic (het)aryl-derived CB_2R agonists were developed from a high-throughput screening (HTS) hit.[101] Hit-to-lead work culminated in the discovery of triazolopyrimidine RO6871304, which demonstrated equal potency toward the human and mouse CB_2 receptor isoforms in the subnanomolar range (human CB_2R cAMP EC_{50} = 1 nM, mouse CB_2R cAMP EC_{50} = 0.5 nM). The selectivity in binding versus CB_1R was exceptionally high (41'190-fold). Average free plasma concentrations were high after both intravenous (*i.v.*) and oral (*p.o.*) administration, translating into efficacy in a kidney ischemia–reperfusion model and protective properties in a model of renal fibrosis. Furthermore, RO6871304 effectively reduced leukocyte–neutrophil activity in an *in vivo* mouse model of endotoxin-induced uveitis (EIU) after topical administration.[100] *In vitro*, triazolopyrimidine RO6871304 decreased neutrophil migration of wild-type (WT) neutrophils but not neutrophils from CB_2R-/- mice, and attenuated adhesion of adoptively transferred leukocytes in EIU.

The selective 2,4,5-trisubstituted pyridine-derived CB_2R agonist RO6871085, which was derived from CB_1R starting points, also showed decreased leukocyte-endothelial adhesion in the same mouse model of EIU.[100] This ligand is a full agonist for human and mouse CB_2R with cAMP EC_{50} values of eight and three nM, respectively.[102] Due to its favorable overall ADME profile, the molecule achieves high bioavailability in mice (F = 68% at 3 mg/kg *p.o.*).

To gain a better understanding of CB_2R, we also generated highly potent and selective CB_2R inverse agonists such as RO6851228.[100] Importantly, this structurally close analogue of RO6871085 was able to antagonize the therapeutically beneficial decrease in leukocyte-endothelial adhesion observed with the agonist RO6871085. As leukocyte adhesion and inflammation are key pathological features of diabetic retinopathy (DR), the rodent *in vivo* efficacy data generated with structurally diverse, highly

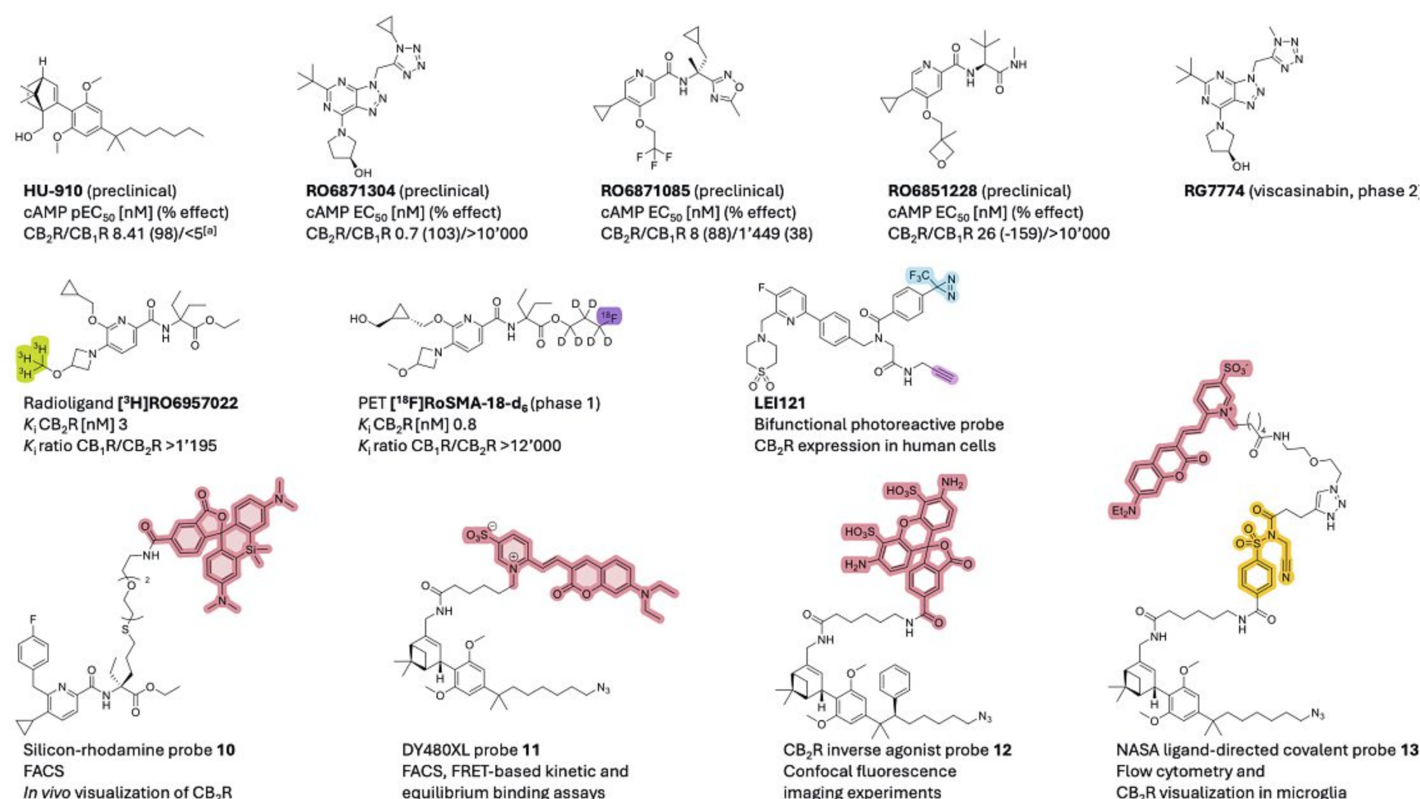

Fig. 6. Chemical structures, *in vitro* pharmacology data and highest development phase of reference and Roche CB_2R ligands. [a]Consensus data for human CBRs from a collaborative multicentric profiling effort between multiple independent academic laboratories and industry. The effect of agonists is normalized to the effect of 10 µM CP55'940.[74] Radioisotopes are highlighted in green (^3H) and purple (^{18}F). Photoactivatable group is highlighted in cyan. Ligation handle is highlighted in pink. Fluorophores are highlighted in red. Covalent labeling moiety is highlighted in orange.

potent, and selective CB_2R agonists enabled the repurposing of the program toward the treatment of DR.

Lead optimization work culminated in the discovery of CB_2R agonist RG7774, a highly potent, selective, and orally bioavailable ligand with a favorable systemic and ocular pharmacokinetic profile.[103] Oral administration of RG7774 (vicasinabin) demonstrated beneficial effects on vascular permeability, leukocyte adhesion, and ocular inflammation – key pathological features of DR – in animal models of retinal disease. Due to its drug-like properties, the CB_2R agonist showed a favorable outcome in the single ascending dose (SAD) and multiple ascending dose (MAD) phase 1 studies. However, due to a lack of efficacy, vicasinabin was discontinued in the phase 2 DR trial.[104]

Since activation of CB_2R holds potential for the treatment of neuroinflammatory diseases, we explored therapeutic options for our ligands beyond retinal diseases.[102,105] To support these activities and enhance the understanding of CB_2R expression and its molecular and cellular mechanisms of action, we embarked on the generation of labeled chemical probes for translational imaging, thus overcoming specificity issues of existing anti-CB_2R antibodies.[83] Such translational imaging, including the generation of therapeutically effective occupancy levels, is critically important for designing clinical trials.

To this end, we aimed to develop positron emission tomography (PET) probes in collaboration with the group of Simon Ametamey at ETH Zurich. Starting from the pyridine-derived lead series, reverse design led to the discovery of the PET imaging agent [^{11}C]RSR-056, which demonstrated specific binding to CB_2R-positive spleen tissue of rats and mice *in vitro* and *in vivo*.[106] A tritiated version of this highly potent and selective CB_2R inverse agonist, [^{3}H]RO6957022, was successfully employed together with the Heitman group to study drug-target binding kinetics for a wide range of CB_2R reference ligands, spanning full, partial, and inverse agonists.[107]

In the search for a fluorine-18 labeled CB_2R PET tracer, we commenced from [^{11}C]RSR-056 with the aim of finding a suitable analogue for radiofluorination and further improving the pharmacological properties. Fluorine atoms were incorporated at all three exit vectors and the pyridine core,[108] resulting in the discovery of [^{18}F]RoSMA-18-d_6.[109] The CB_2R PET tracer exhibits a sub-nanomolar affinity (K_i for CB_2R = 0.8 nM) and a remarkable selectivity factor of >12'000 over CB_1R. [^{18}F]RoSMA-18-d_6 showed exceptional CB_2R attributes as demonstrated by *in vitro* autoradiography, *ex vivo* biodistribution, and *in vivo* PET studies across species, and thus progressed into clinical trials to monitor neuroinflammatory processes in amyotrophic lateral sclerosis (ALS) patients.[110]

In collaboration with the groups of Mario van der Stelt, Erick Carreira, and Marc Nazaré, we developed highly specific CB_2R fluorescent probes, which allow for *ex vivo* target occupancy studies and were successfully applied for live cell imaging of CB_2R-positive cell populations and even the *in vivo* visualization of CB_2R. LEI121 is a CB_2R-specific bifunctional photoreactive probe.[111] It allows for monitoring endogenous GPCR expression and engagement in human cells using tandem photoclick chemistry. It covalently captures CB_2R upon photoactivation of the diazirine moiety, while the incorporated alkyne serves as a ligation handle for the introduction of reporter groups such as fluorescent dyes and biotin.

Together with the groups of Marc Nazaré and Erick Carreira, the reverse design concept was applied for the generation of reversible CB_2R fluorescent probes. Key to success was the structure-based design of core linker tag constructs, which aimed to place the fluorophore toward the extracellular space so that the

large fluorescence dye would not compromise binding affinity, an issue found in early attempts where the reporter dye was incorporated within the binding cavity. Silicon-rhodamine labeled CB_2R agonist **10** is cell-permeable, allowing the labeling of extra- and intracellular CB_2R pools.[112] It preserves interspecies affinity and selectivity for both mouse and human CB_2R and was applied for CB_2R detection in endogenously expressing living cells and zebrafish larvae.

DY480XL labeled CB_2R agonist **11**, based on a HU-308 probe platform,[113] is also a highly versatile tool for studying CB_2R and was successfully applied for setting up FRET-based CB_2R kinetic and equilibrium binding assays.[114] Rational design enabled the conversion of functionality and allowed for the design of fluorescently labeled CB_2R inverse agonists (*cf.* compound **12**), thus complementing the toolbox for an in-depth understanding of CB_2 receptor pharmacology.[115]

Furthermore, a sophisticated tool for visualizing and studying CB_2R while maintaining its ability to bind other ligands at the orthosteric site was generated using ligand-directed covalent (LDC) labeling of CB_2R.[116] Covalent labeling of a peripheral CB_2R lysine residue by exploiting fluorescent *O*-nitrobenzoxadiazole (*O*-NBD), *N*-sulfonyl pyridone (*N*-SP), and *N*-acyl-*N*-alkyl sulfonamide (NASA) (*cf.* probe **13**) LDC functionalized probes was applied to specifically visualize CB_2R in conventional and imaging flow cytometry as well as in confocal fluorescence microscopy using overexpressing and endogenously expressing microglial live cells.

These and other recently developed high-quality labeled CB_2R fluorescent and PET probes have significantly contributed to a better understanding of CB_2R expression and function. However, numerous unanswered questions remain regarding the receptor's mechanism of action and how this translates to clinical benefit. Biased signaling induced by different ligands, resulting from the existence of different ligand-dependent CB_2R conformations, is yet poorly understood but highly relevant as it results in distinct pharmacological responses.[74,117] Additionally, determining binding kinetics, *i.e.* the association rates as well as residence time of a ligand, may provide important insights and rationale for *in vivo* efficacy and might even influence signaling bias.[107] Association equilibria of CB_2R receptors homo- and heterodimers might translate into different functional properties and thus provide yet another mode of therapeutic intervention. Lastly, improving the understanding of translatability from promising preclinical *in vivo* efficacy studies to humans is key. A feedback loop and back-translation of clinical data and knowledge from patients or human systems to preclinical research will tremendously increase the chance of success.

5. Monoacylglycerol Lipase Inhibitors

The eCB 2-AG is a full agonist for both CB_1R and CB_2R.[118] 2-AG is the most relevant eCB in the brain.[119] MAGL is the key enzyme that controls the lifespan of 2-AG and, consequently, its biological activity. In the mouse CNS, MAGL activity is responsible for degrading approximately 85% of 2-AG.[120] MAGL is a cytosolic membrane-bound serine hydrolase with two known isoforms of ~33 and ~35 kDa in mice and humans. The hydrolysis of 2-AG by MAGL leads to the formation of glycerol and most of the AA produced in the brain, which is then available for conversion to pro-inflammatory eicosanoids, including prostaglandins by the cyclooxygenases COX-1 and COX-2.[121]

Acute pharmacological blockade or genetic deletion of MAGL in wild-type mice increases the levels of 2-AG and achieves a rapid and sustained reduction in AA levels. For example, brain 2-AG levels increase by approximately 10-fold in MAGL-/- mice, reflecting a 90% decrease in the rate of 2-AG degradation.[122] Due to their pivotal involvement in eCB and eicosanoid signaling, MAGL inhibitors have emerged as promising therapeutic options for a range of disorders, including (neuro-)inflammatory and neuropsychiatric diseases, as well as conditions involving acute tissue injury and cancer.[123]

CNS-penetrant MAGL inhibitors have shown efficacy in multiple preclinical models of neuropathic pain, encompassing models of traumatic nerve injury, chemotherapy-induced peripheral neuropathy, and multiple sclerosis *via* cannabinoid CB_1 and CB_2 receptor mechanisms.[124] First-generation MAGL inhibitors[125] act irreversibly by forming a covalent bond with the catalytic Ser122 residue of MAGL.[126] However, prolonged exposure to irreversible MAGL inhibitors can lead to unwanted CNS-mediated side effects. Additionally, irreversible hydrolase inhibitors often lack selectivity, leading to potential adverse effects that can be fatal in some cases.[127] Therefore, the development of highly selective reversible MAGL inhibitors was proposed to improve the pharmacological control of enzyme activity and reduce the risk for off-target and adverse effects.

To identify novel reversible MAGL inhibitors with high potency and selectivity for the target, as well as ADME properties, we employed various hit-finding approaches. Through optimization of a DNA-encoded library (DEL) screening hit, we arrived at the bicyclopiperazine inhibitor MAGLi 432 (Fig. 7).[128] This ligand potently and reversibly inhibits MAGL across species and was found to be highly selective against other hydrolases by competitive Activity-Based Protein Profiling (ABPP). The brain-penetrant ligand significantly ameliorated experimental autoimmune encephalomyelitis (EAE) clinical disability and striatal inflammatory synaptopathy through potent anti-inflammatory effects in C57/BL6 female mice with EAE.[129] These findings provide new mechanistic insights into the neuroprotective role of the ECS during neuroinflammation and highlight the therapeutic potential of MAGLi-based drugs in mitigating MS-related inflammatory and neurodegenerative brain damage.

By means of a focused screening approach, we were also able to develop oxazinone-based reversible MAGL inhibitors exhibiting picomolar potencies, such as azetidine urea **14**.[130] In collaboration with the Butini and Campiani groups, covalent brain-penetrant azetidin-2-one-derived MAGL inhibitors, such as **15**, showing potent *in vivo* target engagement, have been elaborated.[131] Considering the ubiquitous and high expression of MAGL in the CNS and periphery, we explored the brain-periphery crosstalk by developing peripherally preferred, highly potent, and selective reversible MAGL inhibitors such as triazole **16**.[132]

Together with the van der Stelt group, we recently reported on the discovery of the peripherally preferred covalent MAGL inhibitor LEI-515, which suppressed chemotherapy-induced neuropathic nociception in mice without inducing cardinal signs of CB_1R activation. This highlights peripheral MAGL inhibition as a promising therapeutic strategy for developing safe and effective anti-inflammatory and analgesic agents.[123c]

To support our drug discovery activities, we embarked on the generation of labeled chemical probes using the reverse design concept. An initial probe was the tritiated analogue of the covalent PET tracer [^{11}C]MA-PB-1.[133] This ligand enables specific *ex vivo* autoradiography target binding in mouse and rat brain slices. In MAGL knockout (*k.o.*) brain slices and after pre-treatment with 10 µM of MAGLi 432, no specific binding of the tracer was observed.[134] These data served as the starting point for the generation of MAGL PET tracers to exploit this technique for non-invasively measuring target occupancy in clinical studies. Quantification and kinetic modeling of such interactions require reversible PET tracers.[135] Thus, we embarked on the search for ^{11}C and ^{18}F-labeled reversible MAGL ligands in collaboration with the Mu and Schibli groups.

Multiparameter optimization scores guided the selection and synthesis of two morpholine-3-one-derived reversible MAGL

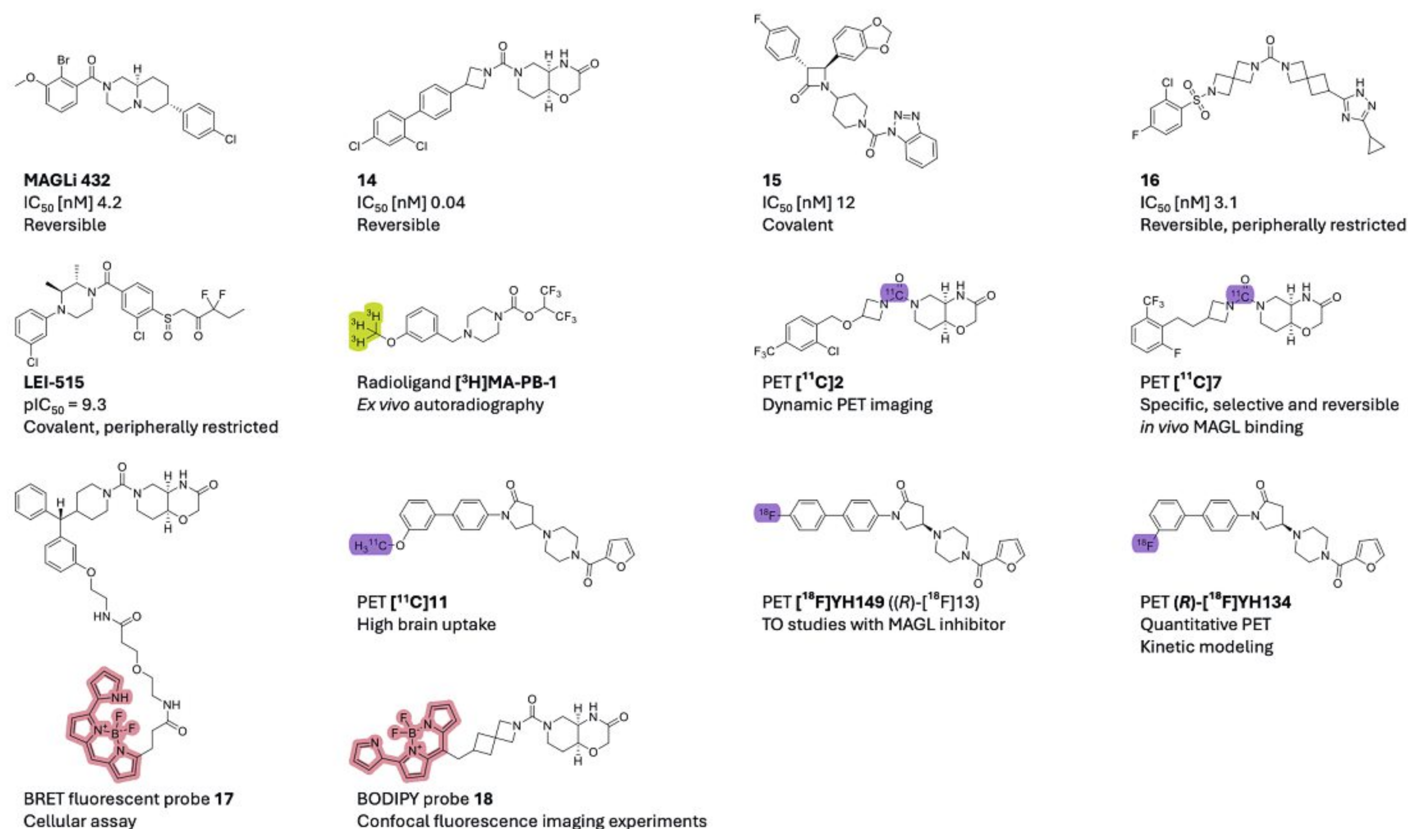

Fig. 7. Chemical structures, *in vitro* pharmacology data and applications of reference and Roche MAGL ligands. Radioisotopes are highlighted in green (^{3}H) and purple (^{11}C and ^{18}F). Fluorophores are highlighted in red.

PET tracer candidates.[136] [^{11}C]**1** and [^{11}C]**2** were radiolabeled by direct [^{11}C]CO_2 fixation. Dynamic PET imaging using MAGL *k.o.* and wild-type mice confirmed the *in vivo* specificity of [^{11}C]**2**. Further structural optimization, particularly based on MAGL inhibitory potency, *in vitro* metabolic stability, and surface plasmon resonance assays, led to the discovery of [^{11}C]**7**, which demonstrated an improved kinetic profile compared to the lead structure.[136]

To develop an ^{18}F-labeled PET tracer with a longer half-life of 109.8 minutes compared to ^{11}C labeled probes (half-life = 20.4 minutes), we also worked on a piperazinyl pyrrolidin-2-one scaffold that builds on work from Takeda.[137] (*R*)-[^{18}F]YH149 ((*R*)-[^{18}F]**13**) was identified through the preliminary evaluation of two carbon-11-labeled racemic structures [^{11}C]**11** and [^{11}C]**16**.[138] High brain uptake and brain-to-blood ratio were achieved by (*R*)-[^{18}F]**13** in comparison with previously reported reversible MAGL PET radiotracers. Target occupancy (TO) studies with a therapeutic MAGL inhibitor revealed a dose-dependent reduction of (*R*)-[^{18}F]**13** accumulation in the mouse brain. However, subsequent work detected a radiometabolite in the mouse brain, complicating quantitative PET and kinetic modeling.

Thus, we investigated a novel compound, (*R*)-[^{18}F]YH134, the *meta*-fluorine–substituted analog of (*R*)-[^{18}F]YH149, which turned out to be a highly specific and selective PET tracer with favorable kinetic properties for imaging MAGL in rodent brain[132b] and non-human primates.[139]

To support various other translational investigations, such as setting up a bioluminescence resonance energy transfer (BRET)-based cellular assay, cell-free fluorescence polarization assays, fluorescence-activated cell sorting analysis, and confocal fluorescence microscopy in live cells and organoids, we collaborated with the Nazaré group on the generation of fluorescent MAGL probes. This resulted in BRET fluorescent probe **17**[140] and miniaturized probes such as the boron-dipyrromethene (BODIPY) ligand **18**, exhibiting a 0.77 nanomolar potency (IC_{50}) for human MAGL.[141]

6. Conclusions

The endocannabinoid lipid signaling network plays a crucial role in many human health and disease states, making the ECS a significant focus for drug discovery activities. Among the various components of the ECS, Roche has embarked on medicinal chemistry programs targeting PPARα, γ, and δ, CB_1R, CB_2R, and MAGL to develop therapeutic drugs for human diseases. To conduct this science of drug discovery with the highest possible quality and maximize the chance of success, we have continuously invested in exploiting synergies between chemical biology and medicinal chemistry. This includes the application of best practices in developing and using chemical probes to explore cellular pathways, identify and validate therapeutic targets, and generate and optimize hits.[22]

In particular, we employed labeled chemical probes consisting of a target recognition element and a reporter group, optionally interconnected by a linker, to achieve these aims.[23] The reverse-design approach leverages the generation of chemical probes from leads already optimized by medicinal chemists, thereby maximizing the chances for successful outcomes. Furthermore, research towards the discovery of labeled chemical probes and chemical biology in general often allows for collaborations with academic partners.[142] This collaboration bridges industry activities, which are highly goal-oriented and often do not allow for following up on promising side discoveries, with academic research, which can explore these interesting scientific avenues in more depth.

On the example of the ECS, we successfully showcased that this approach can be mutually rewarding for both parties. Our holistic drug discovery and development approach, fostering an end-to-end thinking philosophy, has organically grown. While

the PPAR programs were accompanied by limited chemical biology activities mainly concentrating on radiolabeled ligands, activities around CB_1R involved 'clickable' ligands allowing for the identification of OSBPL7 as a novel attractive target and, more recently, the generation of fluorescently labeled CB_1R inverse agonists. The reverse-design-based generation of multiple labeled chemical probes accompanied and supported the CB_2R drug discovery program, culminating in the phase 2 ligand RG7774 (vicasinabin), a thorough understanding of target localization, mechanism of action, and a phase 1 PET tracer for non-invasively measuring therapeutically relevant target engagement levels.

In the course of the MAGL inhibitor program, the concept of intertwining medicinal chemistry and chemical biology has fully matured, allowing the progression of RG6182 as an innovative treatment for MS towards clinical trials within only four years.[143] Multiple labeled chemical probes enabled the setup of cellular assays, target localization and expression studies, selectivity and target occupancy assessment,[144] thus highlighting the enormous potential of this symbiotic end-to-end approach by linking medicinal chemistry and chemical biology to translational drug discovery. We are convinced that this approach can serve as a blueprint for further applications in drug discovery and development.

Acknowledgements

We would like to extend our heartfelt thanks to our numerous colleagues at F. Hoffmann-La Roche and in academia for their invaluable contributions, insightful discussions, and exceptional collaborative spirit. Their efforts were instrumental in advancing our common ECS activities and in the development and application of novel concepts and technologies.

Author Contributions

JA, SMA, SB, GC, EMC, LC, EL, JG, LG, JFR, JF, LHH, BK, MM, HPM, PM, LM, MN, FO, PP, JR, SR, RS, GS, AFS, CU, MS, DBV, and UG contributed to the conceptualization of the research. KA, JB, JB, TG, WG, AH, YH, MH, REM, DFN, SO, AR, DAS, and MBW developed the methodology and protocols. UG drafted the initial manuscript. All authors participated in data analysis, result interpretation, and manuscript review and editing.

Received: June 9, 2024

[1] a) L. Cristino, T. Bisogno, V. Di Marzo, *Nat. Rev. Neurol*. **2020**, *16*, 9, https://doi.org/10.1038/s41582-019-0284-z; b) M. Maccarrone, *Trends Mol. Med*. **2020**, *26*, 263, https://doi.org/10.1016/j.molmed.2019.11.002; c) 'New Tools To Interrogate Endocannabinoid Signalling: From Natural Compounds To Synthetic Drugs. [In: RSC Drug Discovery Ser., **2021**; 76]', Royal Society of Chemistry, 2021.

[2] K. Atz, W. Guba, U. Grether, G. Schneider, *Methods Mol. Biol*. **2023**, *2576*, 477, https://doi.org/10.1007/978-1-0716-2728-0_39.

[3] a) F. A. Iannotti, V. Di Marzo, S. Petrosino, *Prog. Lipid Res*. **2016**, *62*, 107, https://doi.org/10.1016/j.plipres.2016.02.002; b) M. P. Baggelaar, M. Maccarrone, M. van der Stelt, *Prog. Lipid Res*. **2018**, *71*, 1, https://doi.org/10.1016/j.plipres.2018.05.002.

[4] M. Maccarrone, I. Bab, T. Biro, G. A. Cabral, S. K. Dey, V. Di Marzo, J. C. Konje, G. Kunos, R. Mechoulam, P. Pacher, K. A. Sharkey, A. Zimmer, *Trends Pharmacol. Sci*. **2015**, *36*, 277, https://doi.org/10.1016/j.tips.2015.02.008.

[5] a) R. Mechoulam, S. Ben-Shabat, L. Hanus, M. Ligumsky, N. E. Kaminski, A. R. Schatz, A. Gopher, S. Almog, B. R. Martin, *Biochem. Pharmacol*. **1995**, *50*, 83, https://doi.org/10.1016/0006-2952(95)00109-d; b) T. Sugiura, S. Kondo, A. Sukagawa, S. Nakane, A. Shinoda, K. Itoh, A. Yamashita, K. Waku, *Biochem. Biophys. Res. Commun*. **1995**, *215*, 89, https://doi.org/10.1006/bbrc.1995.2437.

[6] W. A. Devane, L. Hanus, A. Breuer, R. G. Pertwee, L. A. Stevenson, G. Griffin, D. Gibson, A. Mandelbaum, A. Etinger, R. Mechoulam, *Science* **1992**, *258*, 1946, https://doi.org/10.1126/science.1470919.

[7] R. G. Pertwee, *Br. J. Pharmacol*. **2008**, *153*, 199, https://doi.org/10.1038/sj.bjp.0707442.

[8] M. Guzman, J. Lo Verme, J. Fu, F. Oveisi, C. Blazquez, D. Piomelli, *J. Biol. Chem*. **2004**, *279*, 27849, https://doi.org/10.1074/jbc.m404087200.

[9] C. E. Rockwell, N. T. Snider, J. T. Thompson, J. P. Vanden Heuvel, N. E. Kaminski, *Mol. Pharmacol*. **2006**, *70*, 101, https://doi.org/10.1124/mol.105.019117.

[10] I. Paterniti, D. Impellizzeri, R. Crupi, R. Morabito, M. Campolo, E. Esposito, S. Cuzzocrea, *J. Neuroinflammation* **2013**, *10*, 20, https://doi.org/10.1186/1742-2094-10-20.

[11] F. A. Iannotti, R. M. Vitale, *Cells* **2021**, *10*, 586, https://doi.org/10.3390/cells10030586.

[12] T. Bisogno, F. Howell, G. Williams, A. Minassi, M. Grazia Cascio, A. Ligresti, I. Matias, A. Schiano-Moriello, P. Paul, E.-J. Williams, U. Gangadharan, C. Hobbs, V. Di Marzo, P. Doherty, *J. Cell Biol*. **2003**, *163*, 463, https://doi.org/10.1083/jcb.200305129.

[13] T. P. Dinh, D. Carpenter, F. M. Leslie, T. F. Freund, I. Katona, S. L. Sensi, S. Kathuria, D. Piomelli, *Proc. Natl. Acad. Sci. U. S. A*. **2002**, *99*, 10819, https://doi.org/10.1073/pnas.152334899.

[14] Y. Okamoto, J. Morishita, K. Tsuboi, T. Tonai, N. Ueda, *J. Biol. Chem*. **2004**, *279*, 5298, https://doi.org/10.1074/jbc.m306642200.

[15] D. K. Giang, B. F. Cravatt, *Proc. Natl. Acad. Sci. U. S. A*. **1997**, *94*, 2238, https://doi.org/10.1073/pnas.94.6.2238.

[16] A. Chicca, J. Marazzi, S. Nicolussi, J. Gertsch, *J. Biol. Chem*. **2012**, *287*, 34660, https://doi.org/10.1074/jbc.m112.373241.

[17] M. Kaczocha, S. T. Glaser, D. G. Deutsch, *Proc. Natl. Acad. Sci. U. S. A*. **2009**, *106*, 6375, https://doi.org/10.1073/pnas.0901515106.

[18] M. Maccarrone, V. Di Marzo, J. Gertsch, U. Grether, A. C. Howlett, T. Hua, A. Makriyannis, D. Piomelli, N. Ueda, M. van der Stelt, *Pharmacol. Rev*. **2023**, *75*, 885, https://doi.org/10.1124/pharmrev.122.000600.

[19] P. Morgan, P. H. Van Der Graaf, J. Arrowsmith, D. E. Feltner, K. S. Drummond, C. D. Wegner, S. D. A. Street, *Drug Discovery Today* **2012**, *17*, 419, https://doi.org/10.1016/j.drudis.2011.12.020.

[20] M. E. Bunnage, E. L. P. Chekler, L. H. Jones, *Nat. Chem. Biol*. **2013**, *9*, 195, https://doi.org/10.1038/nchembio.1197.

[21] D. Cook, D. Brown, R. Alexander, R. March, P. Morgan, G. Satterthwaite, M. N. Pangalos, *Nat. Rev. Drug Discovery* **2014**, *13*, 419, https://doi.org/10.1038/nrd4309.

[22] Y. P. Auberson, P. B. Arimondo, M. Duca, S. Essig, U. Grether, A. C. Rufer, G. Sbardella, U. Schopfer, A. Torrens, M. van der Stelt, B. Vauzeilles, O. Vazquez, A. X. Zhang, *ChemBioChem* **2023**, *24*, e202200690, https://doi.org/10.1002/cbic.202200690.

[23] M. Guberman, M. Kosar, A. Omran, E. M. Carreira, M. Nazare, U. Grether, *CHIMIA* **2022**, *76*, 425, https://doi.org/10.2533/chimia.2022.425.

[24] Y. Kodera, K.-I. Takeyama, A. Murayama, M. Suzawa, Y. Masuhiro, S. Kato, *J. Biol. Chem*. **2000**, *275*, 33201, https://doi.org/10.1074/jbc.c000517200.

[25] a) J. Berger, D. E. Moller, Annu. Rev. Med. 2002, 53, 409, https://doi.org/10.1146/annurev.med.53.082901.104018; b) M. Botta, M. Audano, A. Sahebkar, C. R. Sirtori, N. Mitro, M. Ruscica, *Int. J. Mol. Sci*. **2018**, *19*, 1197/1, https://doi.org/10.3390/ijms19041197.

[26] a) R. S. Savkur, A. R. Miller, *Expert Opin. Invest. Drugs* **2006**, *15*, 763, https://doi.org/10.1517/13543784.15.7.763; b) W. W. Cheatham, *Am. J. Clin. Nutr*. **2010**, *91*, 262S, https://doi.org/10.3945/ajcn.2009.28449d.

[27] A. Chandra, P. Kaur, S. K. Sahu, A. Mittal, *Chem. Biol. Drug Des*. **2022**, *100*, 947, https://doi.org/10.1111/cbdd.14020.

[28] D. Barnett, J. G. Craig, D. S. Robinson, M. Perenna Rogers, *Br. J. Clin. Pharmacol*. **1977**, *4*, 455, https://doi.org/10.1111/j.1365-2125.1977.tb00761.x.

[29] L. Michalik, J. Auwerx, J. P. Berger, V. K. Chatterjee, C. K. Glass, F. J. Gonzalez, P. A. Grimaldi, T. Kadowaki, M. A. Lazar, S. O'Rahilly, C. N. A. Palmer, J. Plutzky, J. K. Reddy, B. M. Spiegelman, B. Staels, W. Wahli, *Pharmacol. Rev*. **2006**, *58*, 726, https://doi.org/10.1124/pr.58.4.5.

[30] Y. Liu, J. K. Colby, X. Zuo, J. Jaoude, D. Wei, I. Shureiqi, *Int. J. Mol. Sci*. **2018**, *19*, 3339/1, https://doi.org/10.3390/ijms19113339.

[31] A. Benardeau, J. Benz, A. Binggeli, D. Blum, M. Boehringer, U. Grether, H. Hilpert, B. Kuhn, H. P. Maerki, M. Meyer, K. Puentener, S. Raab, A. Ruf, D. Schlatter, P. Mohr, *Bioorg. Med. Chem. Lett*. **2009**, *19*, 2468, https://doi.org/10.1016/j.bmcl.2009.03.036.

[32] a) A. Keech, R. J. Simes, P. Barter, J. Best, R. Scott, M. R. Taskinen, P. Forder, A. Pillai, T. Davis, P. Glasziou, P. Drury, Y. A. Kesäniemi, D. Sullivan, D. Hunt, P. Colman, M. d'Emden, M. Whiting, C. Ehnholm, M. Laakso, *Lancet* **2005**, *366*, 1849, https://doi.org/10.1016/s0140-6736(05)67667-2; b) M. Jun, C. Foote, J. Lv, B. Neal, A. Patel, S. J. Nicholls, D. E. Grobbee, A. Cass, J. Chalmers, V. Perkovic, *Lancet* **2010**, *375*, 1875, https://doi.org/10.1016/s0140-6736(10)60656-3.

[33] T. Asaki, T. Aoki, T. Hamamoto, Y. Sugiyama, S. Ohmachi, K. Kuwabara, K. Murakami, M. Todo, *Bioorg. Med. Chem*. **2008**, *16*, 981, https://doi.org/10.1016/j.bmc.2007.10.007.

[34] M. Dietz, P. Mohr, B. Kuhn, H. P. Maerki, P. Hartman, A. Ruf, J. Benz, U. Grether, M. B. Wright, *ChemMedChem* **2012**, *7*, 1101, https://doi.org/10.1002/cmdc.201100598.

[35] J. Ackermann, J. Aebi, A. Binggeli, U. Grether, G. Hirth, B. Kuhn, H.-P. Maerki, M. Meyer, P. Mohr, M. B. Wright, US20050096337, **2005**.
[36] L. A. Sorbera, P. A. Leeson, L. Martin, J. Castaner, *Drugs Future* **2001**, *26*, 354, https://doi.org/10.1358/dof.2001.026.04.617323.
[37] A. Binggeli, M. Boehringer, U. Grether, H. Hilpert, H.-P. Maerki, M. Meyer, P. Mohr, F. Ricklin, WO2002092084, **2002**.
[38] S. E. Kahn, S. M. Haffner, M. A. Heise, W. H. Herman, R. R. Holman, N. P. Jones, B. G. Kravitz, J. M. Lachin, C. O'Neill, B. Zinman, G. Viberti, *N. Engl. J. Med*. **2006**, *355*, 2427, https://doi.org/10.1056/nejmoa066224.
[39] G. Pascual, A. L. Sullivan, S. Ogawa, A. Gamliel, V. Perissi, M. G. Rosenfeld, C. K. Glass, *Novartis Found. Symp*. **2007**, *286*, 183.
[40] a) A. S. Kelly, A. J. Bank, *J. Diabetes Complications* **2007**, *21*, 326, https://doi.org/10.1016/j.jdiacomp.2006.03.006; b) V. T. Chetty, A. M. Sharma, *Vasc. Pharmacol*. **2006**, *45*, 46, https://doi.org/10.1016/j.vph.2005.11.010; c) C. M. Halabi, C. D. Sigmund, *Am. J. Cardiovasc. Drugs* **2005**, *5*, 389, https://doi.org/10.2165/00129784-200505060-00006.
[41] C. Furnsinn, B. Brunmair, M. Meyer, S. Neschen, R. Furtmuller, M. Roden, H. F. Kuhnle, P. Nowotny, B. Schneider, W. Waldhausl, *Br. J. Pharmacol*. **1999**, *128*, 1141, https://doi.org/10.1038/sj.bjp.0702886.
[42] Citeline data base, https://clinicalintelligence.citeline.com/trials/results?qId=66b9d5af-6419-48ed-b31f-883924bb505c, accessed May 23, 2024.
[43] U. Grether, J. Benz, T. Hartung, D. Roth, *Methods Mol. Biol*. **2023**, *2576*, 145, https://doi.org/10.1007/978-1-0716-2728-0_11.
[44] D. Roth, J. Benz, U. Grether, M. Dietz, *Methods Mol. Biol*. **2023**, *2576*, 155, https://doi.org/10.1007/978-1-0716-2728-0_12.
[45] E. Burgermeister, A. Schnoebelen, A. Flament, J. Benz, M. Stihle, B. Gsell, A. Rufer, A. Ruf, B. Kuhn, H. P. Marki, J. Mizrahi, E. Sebokova, E. Niesor, M. Meyer, *Mol. Endocrinol*. **2006**, *20*, 809, https://doi.org/10.1210/me.2005-0171.
[46] a) B. Pourcet, J.-C. Fruchart, B. Staels, C. Glineur, *Expert Opin. Emerging Drugs* **2006**, *11*, 379, https://doi.org/10.1517/14728214.11.3.379; b) U. Grether, A. Benardeau, J. Benz, A. Binggeli, D. Blum, H. Hilpert, B. Kuhn, H. P. Marki, M. Meyer, P. Mohr, K. Puntener, S. Raab, A. Ruf, D. Schlatter, *ChemMedChem* **2009**, *4*, 951, https://doi.org/10.1002/cmdc.200800425.
[47] B. Kuhn, H. Hilpert, J. Benz, A. Binggeli, U. Grether, R. Humm, H. P. Maerki, M. Meyer, P. Mohr, *Bioorg. Med. Chem. Lett*. **2006**, *16*, 4016, https://doi.org/10.1016/j.bmcl.2006.05.007.
[48] J. Berger, M. D. Leibowitz, T. W. Doebber, A. Elbrecht, B. Zhang, G. Zhou, C. Biswas, C. A. Cullinan, N. S. Hayes, Y. Li, M. Tanen, J. Ventre, M. S. Wu, G. D. Berger, R. Mosley, R. Marquis, C. Santini, S. P. Sahoo, R. L. Tolman, R. G. Smith, D. E. Moller, *J. Biol. Chem*. **1999**, *274*, 6718, https://doi.org/10.1074/jbc.274.10.6718.
[49] M. L. Sznaidman, C. D. Haffner, P. R. Maloney, A. Fivush, E. Chao, D. Goreham, M. L. Sierra, C. LeGrumelec, H. E. Xu, V. G. Montana, M. H. Lambert, T. M. Willson, W. R. Oliver, D. D. Sternbach, *Bioorg. Med. Chem. Lett*. **2003**, *13*, 1517, https://doi.org/10.1016/s0960-894x(03)00207-5.
[50] K. Atz, L. Cotos, C. Isert, M. Hakansson, D. Focht, M. Hilleke, D. F. Nippa, M. Iff, J. Ledergerber, C. C. G. Schiebroek, V. Romeo, J. A. Hiss, D. Merk, P. Schneider, B. Kuhn, U. Grether, G. Schneider, *Nat. Commun*. **2024**, *15*, 3408, https://doi.org/10.1038/s41467-024-47613-w.
[51] L. Iversen, *Brain* **2003**, *126*, 1252, https://doi.org/10.1093/brain/awg143.
[52] W. A. Devane, F. A. Dysarz, III, M. R. Johnson, L. S. Melvin, A. C. Howlett, *Mol. Pharmacol*. **1988**, *34*, 605.
[53] a) A. C. Howlett, F. Barth, T. I. Bonner, G. Cabral, P. Casellas, W. A. Devane, C. C. Felder, M. Herkenham, K. Mackie, B. R. Martin, R. Mechoulam, R. G. Pertwee, *Pharmacol. Rev*. **2002**, *54*, 161, https://doi.org/10.1124/pr.54.2.161; b) P. Pacher, S. Batkai, G. Kunos, *Pharmacol. Rev*. **2006**, *58*, 389, https://doi.org/10.1124/pr.58.3.2.
[54] a) G. Marsicano, B. Lutz, *J. Endocrinol. Invest.* **2006**, *29*, 27; b) M. Kano, T. Ohno-Shosaku, Y. Hashimotodani, M. Uchigashima, M. Watanabe, *Physiol. Rev*. **2009**, *89*, 309, https://doi.org/10.1152/physrev.00019.2008; c) A. De Laurentiis, H. A. Araujo, V. Rettori, *Curr. Pharm. Des*. **2014**, *20*, 4697, https://doi.org/10.2174/1381612820666140130212957.
[55] K. Mackie, *Mol. Cell. Endocrinol*. **2008**, *286*, S60, https://doi.org/10.1016/j.mce.2008.01.022.
[56] R. G. Pertwee, *Addict. Biol*. **2008**, *13*, 147, https://doi.org/10.1111/j.1369-1600.2008.00108.x.
[57] R. G. Pertwee, *Philos. Trans. R. Soc., B* **2012**, *367*, 3353, https://doi.org/10.1098/rstb.2011.0381.
[58] C. C. Tsang, M. G. Giudice, *Pharmacotherapy* **2016**, *36*, 273, https://doi.org/10.1002/phar.1709.
[59] Y. Lu, H. D. Anderson, *Can. J. Physiol. Pharmacol*. **2017**, *95*, 311, https://doi.org/10.1139/cjpp-2016-0346.
[60] R. M. Amin, D. W. Ali, *Adv. Exp. Med. Biol*. **2019**, *1162*, 151, https://doi.org/10.1007/978-3-030-21737-2_8.
[61] L. D. Schurman, D. Lu, D. A. Kendall, A. C. Howlett, A. H. Lichtman, *Handb. Exp. Pharmacol*. **2020**, *258*, 323, https://doi.org/10.1007/164_2019_298.
[62] J. L. Wilkerson, J. A. Bilbrey, J. S. Felix, A. Makriyannis, L. R. McMahon, *Pharmacol., Biochem. Behav*. **2021**, *206*, 173192, https://doi.org/10.1016/j.pbb.2021.173192.
[63] a) Clarivate Cortellis data base, drug report on dronabinol, available from: access.clarivate.com/login?app=cortellis, accessed May 20, 2024; b) Adis Insight web page, nabilone drug profile, https://adisinsight.springer.com/drugs/800025856, accessed May 19, 2024.
[64] a) W. C. Milano, K. D. Wild, Y. Hui, C. L. Hubbell, L. D. Reid, *Pharmacol. Biochem. Behav*. **1988**, *31*, 893, https://doi.org/10.1016/0091-3057(88)90401-7; b) T. C. Kirkham, C. M. Williams, F. Fezza, V. Di Marzo, *Br. J. Pharmacol.* **2002**, *136*, 550, https://doi.org/10.1038/sj.bjp.0704767.
[65] C. Ravinet Trillou, C. Delgorge, C. Menet, M. Arnone, P. Soubrie, *Int. J. Obes*. **2004**, *28*, 640, https://doi.org/10.1038/sj.ijo.0802583.
[66] R. G. Pertwee, *Int. J. Obes*. **2006**, *30*, S13, https://doi.org/10.1038/sj.ijo.0803272.
[67] L. F. Van Gaal, A. M. Rissanen, A. J. Scheen, O. Ziegler, S. Roessner, *Lancet* **2005**, *365*, 1389, https://doi.org/10.1016/s0140-6736(05)66374-x.
[68] L. Alig, J. Alsenz, M. Andjelkovic, S. Bendels, A. Benardeau, K. Bleicher, A. Bourson, P. David-Pierson, W. Guba, S. Hildbrand, D. Kube, T. Luebbers, A. V. Mayweg, R. Narquizian, W. Neidhart, M. Nettekoven, J.-M. Plancher, C. Rocha, M. Rogers-Evans, S. Roever, G. Schneider, S. Taylor, P. Waldmeier, *J. Med. Chem*. **2008**, *51*, 2115, https://doi.org/10.1021/jm701487t.
[69] A. H. Sam, V. Salem, M. A. Ghatei, *J. Obes*. **2011**, https://doi.org/10.1155/2011/432607.
[70] S. Rover, M. Andjelkovic, A. Benardeau, E. Chaput, W. Guba, P. Hebeisen, S. Mohr, M. Nettekoven, U. Obst, W. F. Richter, C. Ullmer, P. Waldmeier, M. B. Wright, *J. Med. Chem*. **2013**, *56*, 9874, https://doi.org/10.1021/jm4010708.
[71] M. B. Wright, J. Varona Santos, C. Kemmer, C. Maugeais, J.-P. Carralot, S. Roever, J. Molina, G. M. Ducasa, A. Mitrofanova, A. Sloan, A. Ahmad, C. Pedigo, M. Ge, J. Pressly, L. Barisoni, A. Mendez, J. Sgrignani, A. Cavalli, S. Merscher, M. Prunotto, A. Fornoni, *Nat. Commun*. **2021**, *12*, 4662, https://doi.org/10.1038/s41467-021-24890-3.
[72] L. Mach, A. Omran, J. Bouma, S. Radetzki, D. A. Sykes, W. Guba, X. Li, C. Hoeffelmeyer, A. Hentsch, T. Gazzi, Y. Mostinski, M. Wasinska-Kalwa, F. de Molnier, C. van der Horst, J. P. von Kries, M. Vendrell, T. Hua, D. B. Veprintsev, L. H. Heitman, U. Grether, M. Nazare, *ChemRxiv* **2024**, *1*, https://doi.org/10.26434/chemrxiv-2024-xg4x8.
[73] S. Han, J. Thatte, D. J. Buzard, R. M. Jones, *J. Med. Chem*. **2013**, *56*, 8224, https://doi.org/10.1021/jm4005626.
[74] M. Soethoudt, U. Grether, J. Fingerle, T. W. Grim, F. Fezza, L. de Petrocellis, C. Ullmer, B. Rothenhaeusler, C. Perret, N. van Gils, D. Finlay, C. MacDonald, A. Chicca, M. D. Gens, J. Stuart, H. de Vries, N. Mastrangelo, L. Xia, G. Alachouzos, M. P. Baggelaar, A. Martella, E. D. Mock, H. Deng, L. H. Heitman, M. Connor, V. Di Marzo, J. Gertsch, A. H. Lichtman, M. Maccarrone, P. Pacher, M. Glass, M. van der Stelt, *Nat. Commun*. **2017**, *8*, 13958, https://doi.org/10.1038/ncomms13958.
[75] S. Munro, K. L. Thomas, M. Abu-Shaar, *Nature* **1993**, *365*, 61, https://doi.org/10.1038/365061a0.
[76] V. Di Marzo, *Rev. Physiol., Biochem. Pharmacol*. **2008**, *160*, 1, https://doi.org/10.1007/112_0505.
[77] a) M. Bouaboula, M. Rinaldi, P. Carayon, C. Carillon, B. Delpech, D. Shire, G. Le Fur, P. Casellas, *Eur. J. Biochem*. **1993**, *214*, 173, https://doi.org/10.1111/j.1432-1033.1993.tb17910.x; b) S. Galiegue, S. Mary, J. Marchand, D. Dussossoy, D. Carriere, P. Carayon, M. Bouaboula, D. Shire, G. Le Fur, P. Casellas, *Eur. J. Biochem*. **1995**, *232*, 54, https://doi.org/10.1111/j.1432-1033.1995.tb20780.x.
[78] a) B. K. Atwood, K. Mackie, *Br. J. Pharmacol*. **2010**, *160*, 467, https://doi.org/10.1111/j.1476-5381.2010.00729.x; b) A. Mallat, F. Teixeira-Clerc, S. Lotersztajn, *J. Hepatol*. **2013**, *59*, 891, https://doi.org/10.1016/j.jhep.2013.03.032.
[79] a) P. Mukhopadhyay, M. Rajesh, H. Pan, V. Patel, B. Mukhopadhyay, S. Batkai, B. Gao, G. Hasko, P. Pacher, *Free Radical Biol. Med.* **2010**, *48*, 457, https://doi.org/10.1016/j.freeradbiomed.2009.11.022; b) P. Mukhopadhyay, H. Pan, M. Rajesh, S. Batkai, V. Patel, J. Harvey-White, B. Mukhopadhyay, G. Hasko, B. Gao, K. Mackie, P. Pacher, *Br. J. Pharmacol*. **2010**, *160*, 657, https://doi.org/10.1111/j.1476-5381.2010.00769.x.
[80] B. K. Atwood, J. Wager-Miller, C. Haskins, A. Straiker, K. Mackie, *Mol. Pharmacol*. **2012**, *81*, 250, https://doi.org/10.1124/mol.111.074013.
[81] O. Ofek, M. Karsak, N. Leclerc, M. Fogel, B. Frenkel, K. Wright, J. Tam, M. Attar-Namdar, V. Kram, E. Shohami, R. Mechoulam, A. Zimmer, I. Bab, *Proc. Natl. Acad. Sci. U. S. A*. **2006**, *103*, 696, https://doi.org/10.1073/pnas.0504187103.
[82] G. A. Cabral, E. S. Raborn, L. Griffin, J. Dennis, F. Marciano-Cabral, *Br. J. Pharmacol*. **2008**, *153*, 240, https://doi.org/10.1038/sj.bjp.0707584.
[83] Y. Marchalant, P. W. Brownjohn, A. Bonnet, T. Kleffmann, J. C. Ashton, *J. Histochem. Cytochem*. **2014**, *62*, 395, https://doi.org/10.1369/0022155414530995.
[84] a) J. Kleyer, S. Nicolussi, P. Taylor, D. Simonelli, E. Furger, P. Anderle, J. Gertsch, *Biochem. Pharmacol*. **2012**, *83*, 1393, https://doi.org/10.1016/j.bcp.2012.02.014; b) G. C. Brailoiu, E. Deliu, J. Marcu, N. E. Hoffman, L. Console-Bram, P. Zhao, M.

Madesh, M. E. Abood, E. Brailoiu, *Biochemistry* **2014**, *53*, 4990, https://doi.org/10.1021/bi500632a; c) J. T. Castaneda, A. Harui, M. D. Roth, *J. Neuroimmune Pharmacol*. **2017**, *12*, 544, https://doi.org/10.1007/s11481-017-9744-7.

[85] P. Pacher, R. Mechoulam, *Prog. Lipid Res*. **2011**, *50*, 193, https://doi.org/10.1016/j.plipres.2011.01.001.

[86] C. R. M. Oyagawa, S. M. De La Harpe, Y. Saroz, M. Glass, A. J. Vernall, N. L. Grimsey, *Front. Pharmacol*. **2018**, *9*, 1202, https://doi.org/10.3389/fphar.2018.01202.

[87] a) S. Kishimoto, M. Muramatsu, M. Gokoh, S. Oka, K. Waku, T. Sugiura, *J. Biochem*. **2005**, *137*, 217, https://doi.org/10.1093/jb/mvi021; b) D. Ziring, B. Wei, P. Velazquez, M. Schrage, N. E. Buckley, J. Braun, *Immunogenetics* **2006**, *58*, 714, https://doi.org/10.1007/s00251-006-0138-x; c) C. Boerner, M. Smida, V. Hoellt, B. Schraven, J. Kraus, *J. Biol. Chem*. **2009**, *284*, 35450, https://doi.org/10.1074/jbc.m109.006338; d) M. T. Cencioni, V. Chiurchiu, G. Catanzaro, G. Borsellino, G. Bernardi, L. Battistini, M. Maccarrone, *PLoS One* **2010**, *5*, https://doi.org/10.1371/journal.pone.0008688; e) F. Correa, M. Hernangomez-Herrero, L. Mestre, F. Loria, F. Docagne, C. Guaza, *Brain, Behav., Immun*. **2011**, *25*, 736, https://doi.org/10.1016/j.bbi.2011.01.020.

[88] V. L. Hegde, S. Hegde, B. F. Cravatt, L. J. Hofseth, M. Nagarkatti, P. S. Nagarkatti, *Mol. Pharmacol*. **2008**, *74*, 20, https://doi.org/10.1124/mol.108.047035.

[89] a) P. Pacher, P. Mukhopadhyay, R. Mohanraj, G. Godlewski, S. Batkai, G. Kunos, *Hypertension* **2008**, *52*, 601, https://doi.org/10.1161/hypertensionaha.105.063651; b) P. Pacher, S. Steffens, *Semin. Immunopathol*. **2009**, *31*, 63, https://doi.org/10.1007/s00281-009-0145-8.

[90] a) K. Wright, N. Rooney, M. Feeney, J. Tate, D. Robertson, M. Welham, S. Ward, *Gastroenterology* **2005**, *129*, 437, https://doi.org/10.1016/j.gastro.2005.05.026; b) K. L. Wright, M. Duncan, K. A. Sharkey, *Br. J. Pharmacol*. **2008**, *153*, 263, https://doi.org/10.1038/sj.bjp.0707486; c) A. A. Izzo, M. Camilleri, *Pharmacol. Res*. **2009**, *60*, 117, https://doi.org/10.1016/j.phrs.2009.03.008; d) A. A. Izzo, K. A. Sharkey, *Pharmacol. Ther*. **2010**, *126*, 21, https://doi.org/10.1016/j.pharmthera.2009.12.005.

[91] a) A. Mallat, S. Lotersztajn, *Am. J. Physiol*. **2008**, *294*, G9, https://doi.org/10.1152/ajpgi.00467.2007; b) P. Pacher, B. Gao, *Am. J. Physiol*. **2008**, *294*, G850, https://doi.org/10.1152/ajpgi.00523.2007; c) B. Horvath, L. Magid, P. Mukhopadhyay, S. Batkai, M. Rajesh, O. Park, G. Tanchian, R. Y. Gao, C. E. Goodfellow, M. Glass, R. Mechoulam, P. Pacher, *Br. J. Pharmacol.* **2012**, *165*, 2462, https://doi.org/10.1111/j.1476-5381.2011.01381.x; d) C. Matyas, K. Erdelyi, E. Trojnar, S. Zhao, Z. V. Varga, J. Paloczi, P. Mukhopadhyay, B. T. Nemeth, G. Hasko, R. Cinar, R. M. Rodrigues, Y. Ait Ahmed, B. Gao, P. Pacher, *Hepatology* **2020**, *71*, 1391, https://doi.org/10.1002/hep.30916.

[92] a) C. Zoja, M. Locatelli, D. Corna, S. Villa, D. Rottoli, V. Nava, R. Verde, F. Piscitelli, V. Di Marzo, J. Fingerle, J.-M. Adam, B. Rothenhaeusler, G. Ottaviani, A. Benardeau, M. Abbate, G. Remuzzi, A. Benigni, *Nephron*. **2016**, *132*, 59, https://doi.org/10.1159/000442679; b) E. Trojnar, K. Erdelyi, C. Matyas, S. Zhao, J. Paloczi, P. Mukhopadhyay, Z. V. Varga, G. Hasko, P. Pacher, *Free Radical Biol. Med*. **2020**, *152*, 540, https://doi.org/10.1016/j.freeradbiomed.2019.11.027.

[93] a) D. Centonze, A. Finazzi-Agro, G. Bernardi, M. Maccarrone, *Trends Pharmacol. Sci*. **2007**, *28*, 180, https://doi.org/10.1016/j.tips.2007.02.004; b) J. Fernandez-Ruiz, J. Romero, G. Velasco, R. M. Tolon, J. A. Ramos, M. Guzman, *Trends Pharmacol. Sci.* **2007**, *28*, 39, https://doi.org/10.1016/j.tips.2006.11.001; c) J. Fernandez-Ruiz, *Br. J. Pharmacol*. **2009**, *156*, 1029, https://doi.org/10.1111/j.1476-5381.2008.00088.x.

[94] a) A. Garcia-Martin, M. Garrido-Rodriguez, C. Navarrete, D. Caprioglio, B. Palomares, J. DeMesa, A. Rollland, G. Appendino, E. Munoz, *Biochem. Pharmacol.* **2019**, *163*, 321, https://doi.org/10.1016/j.bcp.2019.02.029; b) X. Jiang, S. Chen, Q. Zhang, C. Yi, J. He, X. Ye, M. Liu, W. Lu, *Phytomedicine* **2020**, *67*, 153160, https://doi.org/10.1016/j.phymed.2019.153160; c) B.-E. Jiang, X. Jiang, Q. Zhang, Q. Liang, Z.-L. Qiu, X.-B. Sun, J.-J. Yang, S. Chen, C. Yi, X. Chai, M. Liu, L.-F. Yu, W. Lu, H.-K. Zhang, *J. Med. Chem*. **2021**, *64*, 385, https://doi.org/10.1021/acs.jmedchem.0c01023; d) R. Spiera, M. Kuwana, D. Khanna, L. Hummers, T. M. Frech, W. Stevens, M. Matucci-Cerinic, S. Kafaja, O. Distler, J.-B. Jun, Y. Levy, P. Leszcyznski, J. Gordon, V. Steen, E. B. Lee, T. Jankowski, I. Litinsky, L. Chung, V. Hsu, M. Mayes, N. Sandorfi, R. W. Simms, S. Finzel, J. de Vries-Bouwstra, S. Constantine, N. Dgetluck, Q. Dinh, B. J. Bloom, D. E. Furst, B. White, C. P. Denton, *Arthritis Rheumatol*. **2023**, *75*, 1608, https://doi.org/10.1002/art.42510.

[95] a) F. Barutta, A. Corbelli, R. Mastrocola, R. Gambino, V. Di Marzo, S. Pinach, M. P. Rastaldi, P. C. Perin, G. Gruden, *Diabetes* **2010**, *59*, 1046, https://doi.org/10.2337/db09-1336; b) F. Barutta, F. Piscitelli, S. Pinach, G. Bruno, R. Gambino, M. P. Rastaldi, G. Salvidio, V. Di Marzo, P. C. Perin, G. Gruden, *Diabetes* **2011**, *60*, 2386, https://doi.org/10.2337/db10-1809; c) A. B. El-Remessy, M. Rajesh, P. Mukhopadhyay, B. Horvath, V. Patel, M. M. H. Al-Gayyar, B. A. Pillai, P. Pacher, *Diabetologia* **2011**, *54*, 1567, https://doi.org/10.1007/s00125-011-2061-4; d) M. Rajesh, S. Batkai, M. Kechrid, P. Mukhopadhyay, W.-S. Lee, B. Horvath, E. Holovac, R. Cinar, L. Liaudet, K. Mackie, G. Hasko, P. Pacher, *Diabetes* **2012**, *61*, 716, https://doi.org/10.2337/db11-0477; e) T. Jourdan, G. Szanda, A. Z. Rosenberg, J. Tam, B. J. Earley, G. Godlewski, R. Cinar, Z. Liu, J. Liu, C. Ju, P. Pacher, G. Kunos, *Proc. Natl. Acad. Sci. U. S. A*. **2014**, *111*, E5420, https://doi.org/10.1073/pnas.1419901111; f) G. Gruden, F. Barutta, G. Kunos, P. Pacher, *Br. J. Pharmacol*. **2016**, *173*, 1116, https://doi.org/10.1111/bph.13226; g) M. Rajesh, P. Mukhopadhyay, S. Batkai, M. Arif, Z. V. Varga, C. Matyas, J. Paloczi, A. Lehocki, G. Hasko, P. Pacher, *GeroScience* **2022**, *44*, 1727, https://doi.org/10.1007/s11357-022-00565-9; h) D. Spyridakos, N. Mastrodimou, K. Vemuri, T. C. Ho, S. P. Nikas, A. Makriyannis, K. Thermos, *Int. J. Mol. Sci*. **2023**, *24*, 240, https://doi.org/10.3390/ijms24010240.

[96] a) J. Guindon, A. G. Hohmann, *Br. J. Pharmacol*. **2008**, *153*, 319, https://doi.org/10.1038/sj.bjp.0707531; b) P. Anand, G. Whiteside, C. J. Fowler, A. G. Hohmann, *Brain Res. Rev*. **2009**, *60*, 255, https://doi.org/10.1016/j.brainresrev.2008.12.003.

[97] a) G. A. Thakur, R. Tichkule, S. Bajaj, A. Makriyannis, *Expert Opin. Ther. Pat*. **2009**, *19*, 1647, https://doi.org/10.1517/13543770903436505; b) D. Riether, *Expert Opin. Ther. Pat*. **2012**, *22*, 495, https://doi.org/10.1517/13543776.2012.682570; c) S. Han, J.-J. Chen, J.-Z. Chen, *Mini-Rev. Med. Chem*. **2014**, *14*, 426, https://doi.org/10.2174/1389557514666140428105753; d) C. Manera, C. Arena, A. Chicca, *Recent Pat. CNS Drug Discovery* **2015**, *10*, 142, https://doi.org/10.2174/1574889810666160519113853; e) P. Morales, L. Hernandez-Folgado, P. Goya, N. Jagerovic, *Expert Opin. Ther. Pat*. **2016**, *26*, 843, https://doi.org/10.1080/13543776.2016.1193157; f) M. Aghazadeh Tabrizi, P. G. Baraldi, P. A. Borea, K. Varani, *Chem. Rev.* **2016**, *116*, 519, https://doi.org/10.1021/acs.chemrev.5b00411; g) A. Cooper, S. Singh, S. Hook, J. D. A. Tyndall, A. J. Vernall, S. P. H. Alexander, *Pharmacol. Rev*. **2017**, *69*, 316, https://doi.org/10.1124/pr.116.013243; h) B. Brennecke, T. Gazzi, K. Atz, J. Fingerle, P. Kuner, T. Schindler, G. d. Weck, M. Nazare, U. Grether, *Pharm. Pat. Anal*. **2021**, *10*, 111, https://doi.org/10.4155/ppa-2021-0002; i) W. Guba, M. Nazare, U. Grether, *RSC Drug Discovery Ser*. **2021**, *76*, 89, https://doi.org/10.1039/9781839160752-00089.

[98] M. R. Bell, T. E. D'Ambra, V. Kumar, M. A. Eissenstat, J. L. Herrmann, Jr., J. R. Wetzel, D. Rosi, R. E. Philion, S. J. Daum, *J. Med. Chem*. **1991**, *34*, 1099, https://doi.org/10.1021/jm00107a034.

[99] M. Rinaldi, F. Barth, P. Casellas, C. Congy, D. Oustric, M. R. Bell, T. E. D'Ambra, R. E. Philion, FR2735774, **1996**.

[100] R. F. Porter, A.-M. Szczesniak, J. T. Toguri, S. Gebremeskel, B. Johnston, C. Lehmann, J. Fingerle, B. Rothenhausler, C. Perret, M. Rogers-Evans, A. Kimbara, M. Nettekoven, W. Guba, U. Grether, C. Ullmer, M. E. M. Kelly, *Molecules* **2019**, *24*, 3338, https://doi.org/10.3390/molecules24183338.

[101] M. Nettekoven, J.-M. Adam, S. Bendels, C. Bissantz, J. Fingerle, U. Grether, S. Gruener, W. Guba, A. Kimbara, G. Ottaviani, B. Puellmann, M. Rogers-Evans, S. Roever, B. Rothenhaeusler, S. Schmitt, F. Schuler, T. Schulz-Gasch, C. Ullmer, *ChemMedChem* **2016**, *11*, 179, https://doi.org/10.1002/cmdc.201500218.

[102] N. Ouali Alami, C. Schurr, F. Olde Heuvel, L. Tang, Q. Li, A. Tasdogan, A. Kimbara, M. Nettekoven, G. Ottaviani, C. Raposo, S. Roever, M. Rogers-Evans, B. Rothenhaeusler, C. Ullmer, J. Fingerle, U. Grether, I. Knuesel, T. M. Boeckers, A. Ludolph, T. Wirth, F. Roselli, B. Baumann, *EMBO J*. **2018**, *37*, https://doi.org/10.15252/embj.201798697.

[103] U. Grether, 'First Disclosure of Cannabinoid Receptor Type 2 Agonist RG7774 – An Innovative Oral Treatment For Diabetic Retinopathy, XXVII EFMC International Symposium on Medicinal Chemistry', Nice, France, **2022**.

[104] ClinicalTrials.gov, NCT04265261, https://www.clinicaltrials.gov/study/NCT04265261, accessed May 18, 2024.

[105] a) S. R. de Martin Esteban, I. Benito-Cuesta, I. Terradillos, A. M. Martinez-Relimpio, M. A. Arnanz, G. Ruiz-Perez, C. Korn, C. Raposo, R. C. Sarott, M. V. Westphal, I. Elezgarai, E. M. Carreira, C. J. Hillard, U. Grether, P. Grandes, M. T. Grande, J. Romero, *Front. Pharmacol*. **2022**, *13*, 841766, https://doi.org/10.3389/fphar.2022.841766; b) C. Gonzalo-Consuegra, I. Santos-Garcia, L. Garcia-Toscano, R. Martin-Baquero, C. Rodriguez-Cueto, M. B. Wittwer, P. Dzygiel, U. Grether, E. de Lago, J. Fernandez-Ruiz, *Biomed. Pharmacother*. **2024**, *174*, 116473, https://doi.org/10.1016/j.biopha.2024.116473.

[106] R. Slavik, U. Grether, A. Muller Herde, L. Gobbi, J. Fingerle, C. Ullmer, S. D. Kramer, R. Schibli, L. Mu, S. M. Ametamey, *J. Med. Chem*. **2015**, *58*, 4266, https://doi.org/10.1021/acs.jmedchem.5b00283.
[107] A. Martella, H. Sijben, A. C. Rufer, U. Grether, J. Fingerle, C. Ullmer, T. Hartung, A. P. Ijzerman, M. van der Stelt, L. H. Heitman, *Mol. Pharmacol*. **2017**, *92*, 389, https://doi.org/10.1124/mol.117.108605.
[108] A. Haider, J. Kretz, L. Gobbi, H. Ahmed, K. Atz, M. Burkler, C. Bartelmus, J. Fingerle, W. Guba, C. Ullmer, M. Honer, I. Knuesel, M. Weber, A. Brink, A. M. Herde, C. Keller, R. Schibli, L. Mu, U. Grether, S. M. Ametamey, *J. Med. Chem*. **2019**, *62*, 11165, https://doi.org/10.1021/acs.jmedchem.9b01280.
[109] A. Haider, L. Gobbi, J. Kretz, C. Ullmer, A. Brink, M. Honer, T. J. Woltering, D. Muri, H. Iding, M. Burkler, M. Binder, C. Bartelmus, I. Knuesel, P. Pacher, A. M. Herde, F. Spinelli, H. Ahmed, K. Atz, C. Keller, M. Weber, R. Schibli, L. Mu, U. Grether, S. M. Ametamey, *J. Med. Chem*. **2020**, *63*, 10287, https://doi.org/10.1021/acs.jmedchem.0c00778.
[110] ClinicalTrials.gov, NCT05880563, https://classic.clinicaltrials.gov/ct2/show/NCT05880563, accessed May 15, 2024.
[111] M. Soethoudt, S. C. Stolze, M. V. Westphal, L. van Stralen, A. Martella, E. J. van Rooden, W. Guba, Z. V. Varga, H. Deng, S. I. van Kasteren, U. Grether, A. P. Ijzerman, P. Pacher, E. M. Carreira, H. S. Overkleeft, A. Ioan-Facsinay, L. H. Heitman, M. van der Stelt, *J. Am. Chem. Soc*. **2018**, *140*, 6067, https://doi.org/10.1021/jacs.7b11281.
[112] T. Gazzi, B. Brennecke, K. Atz, C. Korn, D. Sykes, G. Forn-Cuni, P. Pfaff, R. C. Sarott, M. V. Westphal, Y. Mostinski, L. Mach, M. Wasinska-Kalwa, M. Weise, B. L. Hoare, T. Miljus, M. Mexi, N. Roth, E. J. Koers, W. Guba, A. Alker, A. C. Rufer, E. A. Kusznir, S. Huber, C. Raposo, E. A. Zirwes, A. Osterwald, A. Pavlovic, S. Moes, J. Beck, M. Nettekoven, I. Benito-Cuesta, T. Grande, F. Drawnel, G. Widmer, D. Holzer, T. van der Wel, H. Mandhair, M. Honer, J. Fingerle, J. Scheffel, J. Broichhagen, K. Gawrisch, J. Romero, C. J. Hillard, Z. V. Varga, M. van der Stelt, P. Pacher, J. Gertsch, C. Ullmer, P. J. McCormick, S. Oddi, H. P. Spaink, M. Maccarrone, D. B. Veprintsev, E. M. Carreira, U. Grether, M. Nazare, *Chem. Sci*. **2022**, *13*, 5539, https://doi.org/10.1039/d1sc06659e.
[113] M. V. Westphal, R. C. Sarott, E. A. Zirwes, A. Osterwald, W. Guba, C. Ullmer, U. Grether, E. M. Carreira, *Chem. Eur. J*. **2020**, *26*, 1380, https://doi.org/10.1002/chem.201904584.
[114] R. C. Sarott, M. V. Westphal, P. Pfaff, C. Korn, D. A. Sykes, T. Gazzi, B. Brennecke, K. Atz, M. Weise, Y. Mostinski, P. Hompluem, E. Koers, T. Miljus, N. J. Roth, H. Asmelash, M. C. Vong, J. Piovesan, W. Guba, A. C. Rufer, E. A. Kusznir, S. Huber, C. Raposo, E. A. Zirwes, A. Osterwald, A. Pavlovic, S. Moes, J. Beck, I. Benito-Cuesta, T. Grande, S. Ruiz de Martin Esteban, A. Yeliseev, F. Drawnel, G. Widmer, D. Holzer, T. van der Wel, H. Mandhair, C.-Y. Yuan, W. R. Drobyski, Y. Saroz, N. Grimsey, M. Honer, J. Fingerle, K. Gawrisch, J. Romero, C. J. Hillard, Z. V. Varga, M. van der Stelt, P. Pacher, J. Gertsch, P. J. McCormick, C. Ullmer, S. Oddi, M. Maccarrone, D. B. Veprintsev, M. Nazare, U. Grether, E. M. Carreira, *J. Am. Chem. Soc*. **2020**, *142*, 16953, https://doi.org/10.1021/jacs.0c05587.
[115] M. Kosar, R. C. Sarott, D. A. Sykes, A. E. G. Viray, R. M. Vitale, N. Tomasevic, X. Li, R. L. Z. Ganzoni, B. Kicin, L. Reichert, K. J. Patej, U. Gomez-Bouzo, W. Guba, P. J. McCormick, T. Hua, C. W. Gruber, D. B. Veprintsev, J. A. Frank, U. Grether, E. M. Carreira, *ACS Cent. Sci*. **2024**, *10*, 956, https://doi.org/10.1021/acscentsci.3c01461.
[116] M. Kosar, D. A. Sykes, A. E. G. Viray, R. M. Vitale, R. C. Sarott, R. L. Ganzoni, D. Onion, J. M. Tobias, P. Leippe, C. Ullmer, E. A. Zirwes, W. Guba, U. Grether, J. A. Frank, D. B. Veprintsev, E. M. Carreira, *J. Am. Chem. Soc*. **2023**, *145*, 15094, https://doi.org/10.1021/jacs.2c13629.
[117] P. Morales, P. Goya, N. Jagerovic, *Biochem. Pharmacol*. **2018**, *157*, 8, https://doi.org/10.1016/j.bcp.2018.07.031.
[118] A. C. Howlett, M. Bidaut-Russell, W. A. Devane, L. S. Melvin, M. R. Johnson, M. Herkenham, *Trends Neurosci*. **1990**, *13*, 420, https://doi.org/10.1016/0166-2236(90)90124-s.
[119] N. Stella, P. Schweitzer, D. Piomelli, *Nature* **1997**, *388*, 773, https://doi.org/10.1038/42015.
[120] J. Z. Long, D. K. Nomura, B. F. Cravatt, *Chem. Biol*. **2009**, *16*, 744, https://doi.org/10.1016/j.chembiol.2009.05.009.
[121] a) C. N. Serhan, N. Chiang, T. E. Van Dyke, *Nat. Rev. Immunol*. **2008**, *8*, 349, https://doi.org/10.1038/nri2294; b) M. M. Mulvihill, D. K. Nomura, *Life Sci*. **2013**, *92*, 492, https://doi.org/10.1016/j.lfs.2012.10.025.
[122] A. Viader, J. L. Blankman, P. Zhong, X. Liu, J. E. Schlosburg, C. M. Joslyn, Q.-S. Liu, A. J. Tomarchio, A. H. Lichtman, D. E. Selley, L. J. Sim-Selley, B. F. Cravatt, *Cell Rep*. **2015**, *12*, 798, https://doi.org/10.1016/j.celrep.2015.06.075.
[123] a) D. K. Nomura, J. Z. Long, S. Niessen, H. S. Hoover, S.-W. Ng, B. F. Cravatt, *Cell* **2010**, *140*, 49, https://doi.org/10.1016/j.cell.2009.11.027; b) E. Lomazzo, L. Bindila, F. Remmers, R. Lerner, C. Schwitter, U. Hoheisel, B. Lutz, *Neuropsychopharmacology* **2015**, *40*, 488, https://doi.org/10.1038/npp.2014.198; c) M. Jiang, M. C. W. Huizenga, J. L. Wirt, J. Paloczi, A. Amedi, R. J. B. H. N. van den Berg, J. Benz, L. Collin, H. Deng, X. Di, W. F. Driever, B. I. Florea, U. Grether, A. P. A. Janssen, T. Hankemeier, L. H. Heitman, T.-W. Lam, F. Mohr, A. Pavlovic, I. Ruf, H. van den Hurk, A. F. Stevens, D. van der Vliet, T. van der Wel, M. B. Wittwer, C. A. A. van Boeckel, P. Pacher, A. G. Hohmann, M. van der Stelt, *Nat. Commun*. **2023**, *14*, 8039, https://doi.org/10.1038/s41467-023-43606-3.
[124] a) S. G. Kinsey, J. Z. Long, S. T. O'Neal, R. A. Abdullah, J. L. Poklis, D. L. Boger, B. F. Cravatt, A. H. Lichtman, *J. Pharmacol. Exp. Ther.* **2009**, *330*, 902, https://doi.org/10.1124/jpet.109.155465; b) M. Brindisi, S. Maramai, S. Gemma, S. Brogi, A. Grillo, L. Di Cesare Mannelli, E. Gabellieri, S. Lamponi, S. Saponara, B. Gorelli, D. Tedesco, T. Bonfiglio, C. Landry, K.-M. Jung, A. Armirotti, L. Luongo, A. Ligresti, F. Piscitelli, C. Bertucci, M.-P. Dehouck, G. Campiani, S. Maione, C. Ghelardini, A. Pittaluga, D. Piomelli, V. Di Marzo, S. Butini, *J. Med. Chem*. **2016**, *59*, 2612, https://doi.org/10.1021/acs.jmedchem.5b01812.
[125] a) D. M. Kerr, B. Harhen, B. N. Okine, L. J. Egan, D. P. Finn, M. Roche, *Br. J. Pharmacol*. **2013**, *169*, 808, https://doi.org/10.1111/j.1476-5381.2012.02237.x; b) H. Deng, W. Li, *Acta Pharm. Sin. B* **2020**, *10*, 582, https://doi.org/10.1016/j.apsb.2019.10.006; c) A. F. League, B. L. Gorman, D. J. Hermes, C. T. Johnson, I. R. Jacobs, B. J. Yadav-Samudrala, J. L. Poklis, M. J. Niphakis, B. F. Cravatt, A. H. Lichtman, B. M. Ignatowska-Jankowska, S. Fitting, *Front. Neurol*. **2021**, *12*, 651272, https://doi.org/10.3389/fneur.2021.651272.
[126] A. Zanfirescu, A. Ungurianu, D. P. Mihai, D. Radulescu, G. M. Nitulescu, *Molecules* **2021**, *26*, 5668, https://doi.org/10.3390/molecules26185668.
[127] A. C. M. van Esbroeck, A. P. A. Janssen, A. B. Cognetta, III, D. Ogasawara, G. Shpak, M. van der Kroeg, V. Kantae, M. P. Baggelaar, F. M. S. de Vrij, H. Deng, M. Allara, F. Fezza, Z. Lin, T. van der Wel, M. Soethoudt, E. D. Mock, H. den Dulk, I. L. Baak, B. I. Florea, G. Hendriks, L. De Petrocellis, H. S. Overkleeft, T. Hankemeier, C. I. De Zeeuw, V. Di Marzo, M. Maccarrone, B. F. Cravatt, S. A. Kushner, M. van der Stelt, *Science* **2017**, *356*, 1084, https://doi.org/10.1126/science.aaf7497.
[128] A. M. Kemble, B. Hornsperger, I. Ruf, H. Richter, J. Benz, B. Kuhn, D. Heer, M. Wittwer, B. Engelhardt, U. Grether, L. Collin, *PLoS One* **2022**, *17*, e0268590, https://doi.org/10.1371/journal.pone.0268590.
[129] L. Guadalupi, G. Mandolesi, V. Vanni, S. Balletta, S. Caioli, A. Pavlovic, F. De Vito, D. Fresegna, K. Sanna, L. Vitiello, M. Nencini, A. Tartacca, F. Mariani, V. Rovella, S. Schippling, I. Ruf, L. Collin, D. Centonze, A. Musella, *Neuropharmacology* **2024**, *252*, 109940, https://doi.org/10.1016/j.neuropharm.2024.109940.
[130] R. W. Sabnis, *ACS Med. Chem. Lett*. **2021**, *12*, 312, https://doi.org/10.1021/acsmedchemlett.1c00055.
[131] S. Butini, U. Grether, K.-M. Jung, A. Ligresti, M. Allara, A. G. J. Postmus, S. Maramai, S. Brogi, A. Papa, G. Carullo, D. Sykes, D. Veprintsev, S. Federico, A. Grillo, B. Di Guglielmo, A. Ramunno, A. F. Stevens, D. Heer, S. Lamponi, S. Gemma, J. Benz, V. Di Marzo, M. van der Stelt, D. Piomelli, G. Campiani, *J. Med. Chem*. **2024**, *67*, 1758, https://doi.org/10.1021/acs.jmedchem.3c01278.
[132] a) M. Amoussa, J. Benz, J. E. F. Blaising, J. J. Denizot, K. Friston, R. L. Z. Ganzoni, M. Giroud, U. Grether, B. Hornsperger, I. Kaufmann, B. Kuhn, C. J. Leake, J. Margarini, R. E. Martin, F. S. O'Hara, B. Puellmann, M. Ritter, D. Rombach, V. Runtz-Schmitt, P. C. Schmid, M. B. Wittwer, WO2023144160, **2023**; b) Y. He, S. D. Kramer, U. Grether, M. B. Wittwer, L. Collin, B. Kuhn, A. Topp, D. Heer, F. O'Hara, M. Honer, A. Pavlovic, H. Richter, M. Ritter, D. Rombach, C. Keller, L. Gobbi, L. Mu, *J. Nucl. Med*. **2024**, *65*, 300, https://doi.org/10.2967/jnumed.123.266426.
[133] M. Ahamed, B. Attili, D. van Veghel, M. Ooms, P. Berben, S. Celen, M. Koole, L. Declercq, J. R. Savinainen, J. T. Laitinen, A. Verbruggen, G. Bormans, *Eur. J. Med. Chem*. **2017**, *136*, 104, https://doi.org/10.1016/j.ejmech.2017.04.066.
[134] M. Honer, L. Gobbi, L. Collin, U. Grether, unpublished results, **2024**.
[135] L. Hou, J. Rong, A. Haider, D. Ogasawara, C. Varlow, M. A. Schafroth, L. Mu, J. Gan, H. Xu, C. J. Fowler, M.-R. Zhang, N. Vasdev, S. Ametamey, B. F. Cravatt, L. Wang, S. H. Liang, *J. Med. Chem*. **2021**, *64*, 123, https://doi.org/10.1021/acs.jmedchem.0c01459.
[136] Y. He, U. Grether, M. F. Taddio, C. Meier, C. Keller, M. R. Edelmann, M. Honer, S. Huber, M. B. Wittwer, D. Heer, H. Richter, L. Collin, M. N. Hug, M. Hilbert, A. G. J. Postmus, A. F. Stevens, M. van der Stelt, S. D. Kramer, R. Schibli, L. Mu, L. C. Gobbi, *Eur. J. Med. Chem*. **2022**, *243*, 114750, https://doi.org/10.1016/j.ejmech.2022.114750.
[137] Y. Hattori, K. Aoyama, J. Maeda, N. Arimura, Y. Takahashi, M. Sasaki, M. Fujinaga, C. Seki, Y. Nagai, K. Kawamura, T. Yamasaki, M.-R. Zhang, M. Higuchi, T. Koike, *J. Med. Chem*. **2019**, *62*, 2362, https://doi.org/10.1021/acs.jmedchem.8b01576.
[138] a) Y. He, M. Schild, U. Grether, J. Benz, L. Leibrock, D. Heer, A. Topp, L. Collin, B. Kuhn, M. Wittwer, C. Keller, L. C. Gobbi, R. Schibli, L. Mu, *J. Med. Chem*. **2022**, *65*, 2191, https://doi.org/10.1021/acs.jmedchem.1c01706; b) Y. He, A. Delparente, C. V. M. L. Jie, C. Keller, R. Humm, D. Heer, L. Collin, R. Schibli, L. Gobbi, U. Grether, L. Mu, *ChemBioChem* **2024**, *25*, e202300819, https://doi.org/10.1002/cbic.202300819.

[139] Y. He, M. Zheng, J. Gu, L. Reichert, J. Trimborn, H. Zhang, C. Keller, M. Crosby, L. Collin, D. Heer, A. Pavlovic, A. Topp, M. Wittwer, U. Grether, L. Gobbi, R. Schibli, H. Huang, L. Mu, unpublished results, **2024**.
[140] J. Benz, T. Gazzi, L. Gobbi, U. Grether, B. Hornsperger, C. Kroll, B. Kuhn, Y. Mostinski, M. Nazare, F. O'Hara, H. Richter, WO2021058443, **2021**.
[141] J. Benz, M. Giroud, U. Grether, M. Guberman, A. Hentsch, B. Kuhn, R. E. Martin, M. Nazare, F. S. O'Hara, J. Paul, B. Puellmann, M. Ritter, WO2023247666, **2023**.
[142] Y. P. Auberson, U. Grether, B. Vauzeilles, O. Vazquez, *ChemBioChem* **2023**, *24*, e202300121, https://doi.org/10.1002/cbic.202300121.
[143] Roche pipeline, RG6182, https://www.roche.com/solutions/pipeline, accessed May 25, 2024.
[144] U. Grether, 'ABPP and its impact on drug discovery - successful examples from the monoacylglycerol lipase (MAGL) program, EFMC International Symposium on Chemical Biology', Basel, Switzerland, **2023**.

License and Terms

This is an Open Access article under the terms of the Creative Commons Attribution License CC BY 4.0. The material may not be used for commercial purposes.

The license is subject to the CHIMIA terms and conditions: (https://chimia.ch/chimia/about).

The definitive version of this article is the electronic one that can be found at https://doi.org/10.2533/chimia.2024.499