It's about time: novel drug discovery concepts for the molecular pharmacological characterization fo the cannabinoid CB2 receptor

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Chapter 1

General introduction

1.1 G protein-coupled receptors

G protein-coupled receptors (GPCRs) comprise the largest family of membrane-bound proteins in the human body and consist of at least 800 family members. These receptors play a crucial role in the regulation of a plethora of physiological processes due to their activation by hormones, neurotransmitters, ions, lipids, and other stimuli[1,2]. Initially, GPCRs were named according to the A-F system (class A-F and O), which covers on top of human GPCRs also the receptors in vertebrates and invertebrates[3]. An alternative classification system was employed for human GPCRs only, which subdivided them into five families following the GRAFS classification: *glutamate* (corresponding to class C), *rhodopsin* (class A), *adhesion*, *frizzled/taste2* and *secretin* (class B)[4]. Of these, the rhodopsin/class A family is the largest, consisting of over 700 receptors, and most diverse[5]. Although different in their sequences, structure and binding partners, GPCRs share a similar overall structure that is characterized by an extracellular N-terminus (N-term), seven transmembrane (TM) helices that are connected by extra- and intracellular loops (ECLs and ICLs, respectively) and an intracellular C-terminus (C-term) (**Figure 1.1**)[2]. On top of this, unique patterns of conserved amino acids or motifs have been described for this family[6]. Specifically, these include the DRY, CWxP and NPxxY motifs, where the letters refer to the amino acid codes and x indicates variable amino acids[2,6]. These conserved motifs are pivotal for stabilization and activation of GPCRs[2]. Furthermore, conserved amino acids in each of the TM helices are used to assign the Ballesteros-Weinstein numbering[7]. To this end, the helix number, 1-7, is combined with residue numbers based on the most conserved residue being defined as number 50 and the other residues counted directly within the protein sequence[7,8]. This strategy provides the opportunity to consistently describe class A GPCRs and compare structural features across receptor subtypes, species or receptor subfamilies, as well as mutation effects and ligand interactions.

GPCRs may undergo various conformational changes upon binding of endogenous or exogenous agonists, and consequently activate downstream signaling pathways[5,9]. The transduction of extracellular stimuli to intracellular effects is primarily mediated by coupling or recruitment of proteins to the receptor[9–11]. Three classes of signal transducers, i.e., G proteins, G protein-coupled receptor kinases (GRKs) and ß-arrestins, specifically engage with activated GPCRs and induce different cellular effects (**Figure 1.1**)[10].

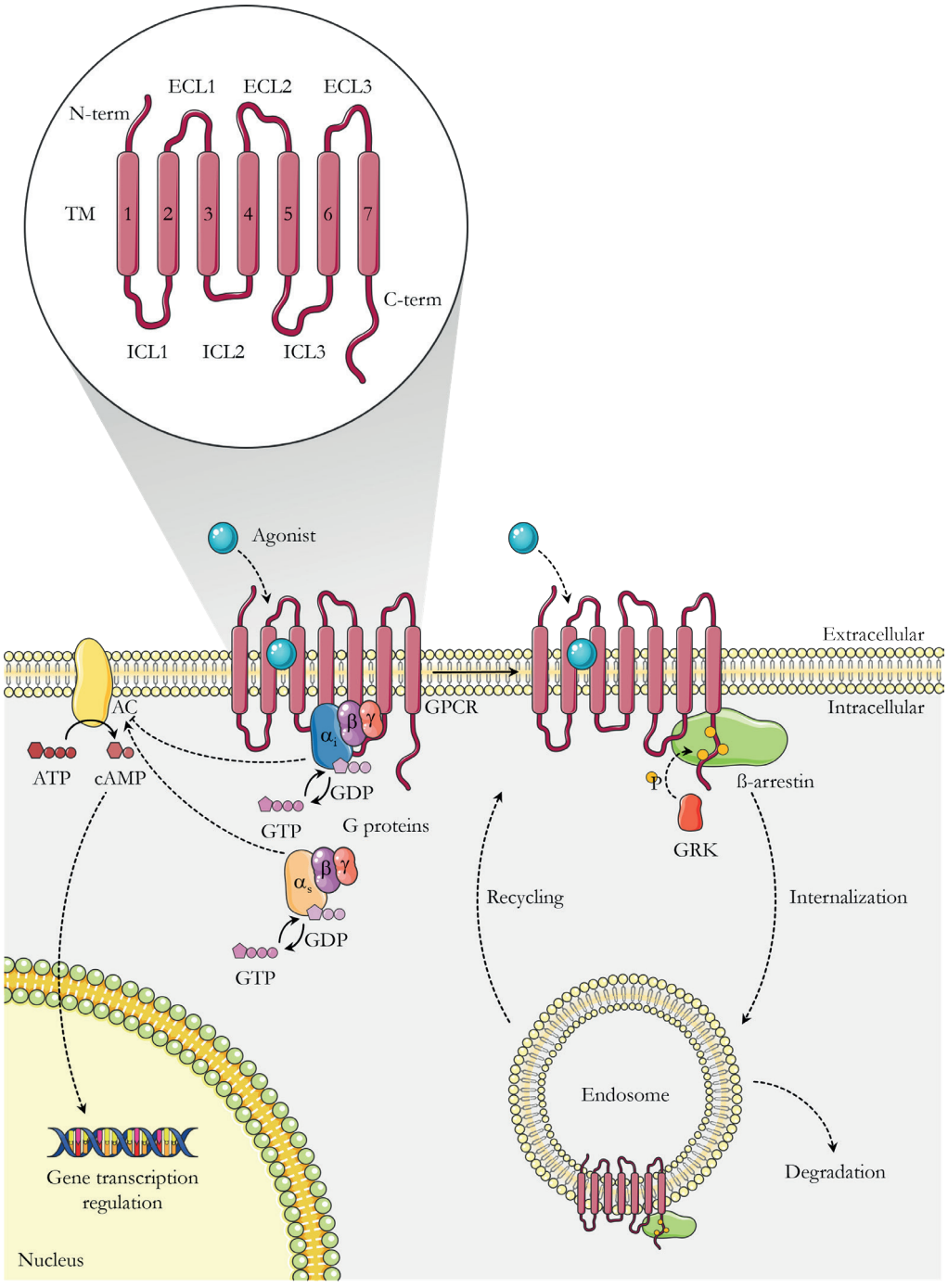
G protein signaling is considered the canonical pathway after GPCR activation, which follows a general initial mechanism (**Figure 1.1**). The heterotrimeric G proteins, which are composed of α, β and γ subunits, are bound by a guanosine diphosphate (GDP) at the Gα subunit in their inactive state. Upon binding of an extracellular stimulus to the GPCR, GDP is exchanged for guanosine triphosphate (GTP), which promotes conformational changes in the subunits and as a consequence, the Gα and Gβγ subunits dissociate to modulate effector proteins[12,13]. There are various α, β, and γ subunit types, which provides a large assortment of heterotrimeric G protein compositions[13]. The Gα proteins can be divided into four major subfamilies ($G\alpha_s$, $G\alpha_i$, $G\alpha_{q/11}$ and $G\alpha_{12/13}$), which each have distinct activation profiles via different effector proteins[14]. Conversion of adenosine triphosphate (ATP) into cyclic adenosine monophosphate (cAMP) by the adenylate cyclase (AC) can be stimulated via the $G\alpha_s$ subfamily and inhibited via $G\alpha_i$ proteins. On the other hand,

the $G\alpha_{q/11}$ subfamily activates phospholipase C to increase calcium levels, while $G\alpha_{12/13}$ family members activate Rho GTPases[13,15]. Gβγ heterodimeric subunits, which can further be subdivided into five Gβ and thirteen Gγ subunits, may induce the modulation of ion channels, while also acting as scaffolds for other effector proteins[14,16]. Ultimately, effector proteins can regulate downstream signaling processes including, but not limited to, kinase activation, gene transcription, motility and contractility[14,17]. Noteworthy, not all class A GPCRs show detectable coupling with G proteins, evident for the atypical chemokine receptors, which signal solely via recruitment of ß-arrestins[18].

Recruitment of arrestins to an activated GPCR is the consequence of phosphorylation of its ICLs and C-terminus by GRKs (**Figure 1.1**)[19]. There are seven GRK subtypes and four arrestins of which GRK3, 5 and 6 and arrestin isoforms 2 and 3 (ß-arrestin-1 and ß-arrestin-2, respectively) are widely expressed in the human body, while other subtypes are restricted to specific cellular or tissue compartments[19]. The recruitment of ß-arrestins to activated and phosphorylated GPCRs serves various multifaceted functions. First, binding of ß-arrestins to an activated GPCR prevents further coupling of a G protein by steric hindrance and as such leads to the termination of G protein signaling, often referred to as desensitization[20]. Secondly, and probably best known, is the internalization of the active receptor from the membrane to clathrin-coated pits. Subsequently, GPCRs are trafficked to endosomes from where they could be either recycled back to the plasma membrane or degraded (**Figure 1.1**)[21]. Thirdly, ß-arrestins act as scaffolds and regulators for over 100 intracellular proteins, which lead to the activation of various other pathways, including mitogen-activated protein kinase (MAPK) and extracellular signal-related kinase 1 and 2 (ERK1/2) signaling[22–24]. In the past, ß-arrestin signaling has often been called the G protein-independent pathway, however over the years this concept has been challenged. Various studies now report that ß-arrestin recruitment and signaling requires initial G protein coupling[25,26]. Altogether, this underlines the extraordinarily complex nature of GPCR activation and downstream signaling events.

Nevertheless, GPCRs provide great opportunities for pharmacological targeting due to their involvement in the regulation of many physiological processes by binding of a plethora of extracellular ligands. In 2019, at least 36% of the marketed pharmaceutical drugs already targeted GPCRs[27,28]. However, there is a high attrition rate of ligands in clinical development due to efficacy and safety issues, making this an expensive and tedious process[29]. To this end, it has been hypothesized that novel perspectives and drug discovery concepts may aid in selecting better drug candidates for clinical development[30,31]. In this chapter, specifically drug-target binding kinetics, allosteric modulation and biased signaling will be further described.

→ Figure 1.1 Simplified overview of GPCR structure, activation and downstream signaling.
General GPCR structure with N-terminus (N-term), seven transmembrane (TM) helices connected by extracellular and intracellular loops (ECL, ICL) and an intracellular C-terminus (C-term). Upon activation of a GPCR by an agonist, G proteins exchange GDP for GTP, which causes dissociation of the Gα and Gβγ subunits. $G\alpha_i$ and $G\alpha_s$ inhibit and stimulate the adenylate cyclase (AC), respectively, and subsequently the conversion of ATP into cAMP. In turn, this can regulate downstream signaling processes (not shown in figure), ultimately leading to regulation of gene transcription. Binding of an agonist may also induce phosphorylation by G protein-coupled receptor kinases (GRKs) of the C-terminus and recruitment of ß-arrestin. This could initiate internalization to endosomes and either recycling to the cell membrane or degradation of the receptor. Additionally, activation of downstream signaling processes may occur via ß-arrestin (not shown). For simplification, $G\alpha_{q/11}$, $G\alpha_{12/13}$ and Gβγ signaling are not included into the figure. This figure incorporates drawings from Servier Medical Art (smart.servier.com).


ECL1
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Agonist
Extracellular
Intracellular
AC
GPCR
ATP
cAMP
GDP
GTP
G proteins
ß-arrestin
P
GRK
Recycling
Internalization
Endosome
Degradation
Gene transcription
regulation
Nucleus

1.2 Drug-target binding kinetics

In 2006, Copeland and colleagues presented the drug-target binding kinetics model, which they suggested would present a better prediction of drug efficacy and safety *in vivo* by focusing on the dynamic interactions between a drug and target[32]. Up until that time, drug discovery focused on the measurement of so-called equilibrium or end-point values, such as target affinity in terms of half-maximal inhibitory concentration (IC_{50}) and inhibition constant (K_i), or functional potency (pEC_{50}) and efficacy (E_{max}). These parameters are often determined in *in vitro* assays under equilibrium conditions, where drug and target concentrations remain constant over time[33]. However, these conditions do not capture the complexity of an open system, such as the human body, in which ligand concentrations vary over time due to pharmacokinetic processes such as absorption, distribution, metabolism and excretion (ADME)[33]. Therefore, it was proposed that the period of time for which a drug is bound to the target and can exert its pharmacological action is more predictive for drug efficacy *in vivo*[32]. The formation of the drug-target, or ligand-receptor, complex is described by two processes; the association of the ligand to the target, defined by the association rate constant (k_{on}), and thereafter the dissociation from the target, defined the by dissociation rate constant (k_{off}) (**Figure 1.2**). Subsequently, the ratio between the k_{off} and the k_{on} values can be defined as the kinetic affinity (K_D)[33]. These rate constants can be adequately determined in *in vitro* assays and the residence time (RT), as a description of the time a ligand is bound to the receptor, can be defined as the reciprocal of the dissociation rate constant[33].

The primary focus since the introduction of the concept has been on the investigation and optimization of RT since a drug is only effective when bound to its target. An increased RT is generally hypothesized to explain the longer duration of *in vivo* efficacy. However, it is dependent on the disease whether a short or long RT ligand is preferred[34,35]. A prolonged duration of action, as a consequence of a long-acting agonist, may be sustained long after

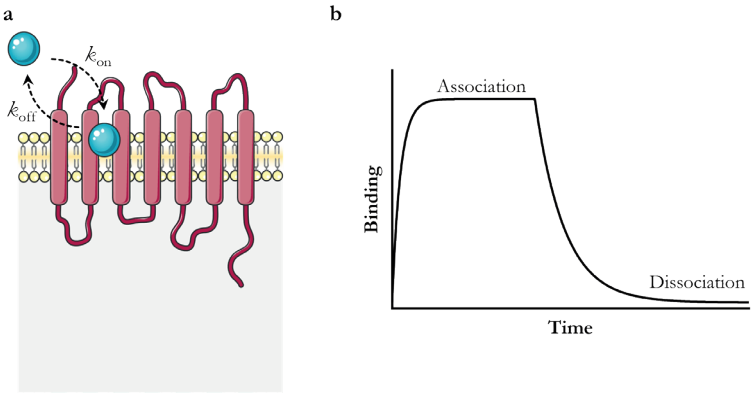

Figure 1.2 Graphical representation of target binding kinetics.
(a) The dynamic process of ligand binding and unbinding to and from a GPCR is characterized by the association rate constant (k_{on}) and the dissociation rate constant (k_{off}) of the ligand. (b) Binding of a (radio)ligand can be measured over time until equilibrium (plateau) is reached. In the absence of free ligand, the ligand can dissociate from the target over time. This figure incorporates drawings from Servier Medical Art (smart.servier.com).

the drug is cleared from the bloodstream, i.e., a long RT could challenge the pharmacokinetic parameters of a ligand[36]. On the other hand, slowly dissociating antagonists, known as insurmountable antagonists, inhibit or attenuate endogenous receptor signaling by sustained receptor blockade for a certain amount of time and as such regulate the native response[35]. The duration of receptor blockade can be increased with the use of irreversible, covalent, binders[37]. Moreover, kinetic selectivity of ligands, characterized by an increased RT for the target of interest, while having a shorter RT for off-target proteins, could contribute to a high target selectivity *in vivo* even in the absence of selectivity in equilibrium assays[38]. Nevertheless, the lifetime of the protein target may limit the utility of long RT ligands, as long RT ligands will be degraded along with the protein in the case of a rapid turnover of the target *in vivo*[36].

Initially, the association rate constant was thought to be diffusion controlled and as such would remain equal to the diffusion rate limit (~10^8 - 10^9 $M^{-1}s^{-1}$)[39]. Nevertheless, various studies have reported different association rate constants, which rejects this hypothesis and emphasizes that the k_{on} value is a ligand-specific parameter[40]. Consequently, the determination of k_{on} values is becoming increasingly more important. However, opposed to the k_{off} value, the k_{on} value is physicochemically and pharmacologically constrained and highly depends on the ligand concentration[32]. This suggests that increasing the ligand concentration, i.e., the dose, can compensate for a low k_{on} value. On the other hand, a high k_{on} value can increase the local concentration of ligand, which in turn will increase the chances of rebinding. Ultimately, this provides the possibility of extending the intracellular RT and as such increase the duration of the pharmacological effect[36,41,42]. In the case of a RT shorter than the pharmacokinetic parameters, increasing the association rate constant may provide an alternative strategy to enhance the target occupation[43]. Furthermore, a high k_{on} value may allow for more rapid therapeutic action, which could be favorable dependent on the disease type[34].

Importantly, investigation of the drug-target binding kinetics of ligands has been shown, albeit retrospectively, to contribute to the success of several marketed drugs on GPCRs[44]. In the case of the muscarinic M_3 receptor antagonist tiotropium, the sustained bronchodilation in chronic obstructive pulmonary disease (COPD) patients is attributed to the slow dissociation rate (RT 27 h) while fast dissociating antagonists with similar affinities and potencies provided less bronchoprotection[45]. Furthermore, tiotropium has a kinetic subtype selectivity for the muscarinic M_3 receptor over the muscarinic M_2 receptor, despite similar affinities for both receptors[45]. This highlights the importance of investigation of drug-target binding kinetics in early drug discovery programs for a more rational selection of hit candidates.

1.3 Allosteric modulation

Another approach of targeting GPCRs is by allosteric modulation of the receptor opposed to more traditional orthosteric binding. Orthosteric ligands bind to the same site as the endogenous ligand(s), i.e., the orthosteric binding site, whereas allosteric ligands target a topographically distinct binding site[46]. While orthosteric binding sites are under strong

evolutionary selection and as such highly conserved among receptor families, allosteric binding sites share a lower sequence homology and are thus generally less conserved between receptor subtypes which might be driven by a need for specificity[47,48]. Consequently, allosteric ligands can provide a greater subtype selectivity[46,49]. While orthosteric binding sites of membrane receptors are usually found at the extracellular site, the locations of allosteric binding sites are diverse and span the entire receptor surface[50]. Allosteric modulators are usually devoid of intrinsic agonistic properties, but upon simultaneous binding with an endogenous or orthosteric agonist they can alter the affinity and efficacy of the orthosteric ligand (**Figure 1.3**)[49,51]. Allosteric modulators that negatively affect affinity and/or efficacy of an orthosteric ligand are called negative allosteric modulators (NAMs), while positive allosteric modulators (PAMs) enhance these parameters (**Figure 1.3a**,**b**). Finally, neutral allosteric ligands (NALs) occupy the allosteric binding pocket without affecting the affinity and/or efficacy of the orthosteric ligand but they prevent further binding of PAMs or NAMs (**Figure 1.3c**)[46,52]. On top of the increased selectivity, allosteric modulators provide more beneficial properties. In the presence of high concentrations of endogenous ligand, allosteric modulators can still decrease the affinity and/or efficacy of the endogenous ligand. Particularly, in disease conditions with increased concentrations of endogenous ligands

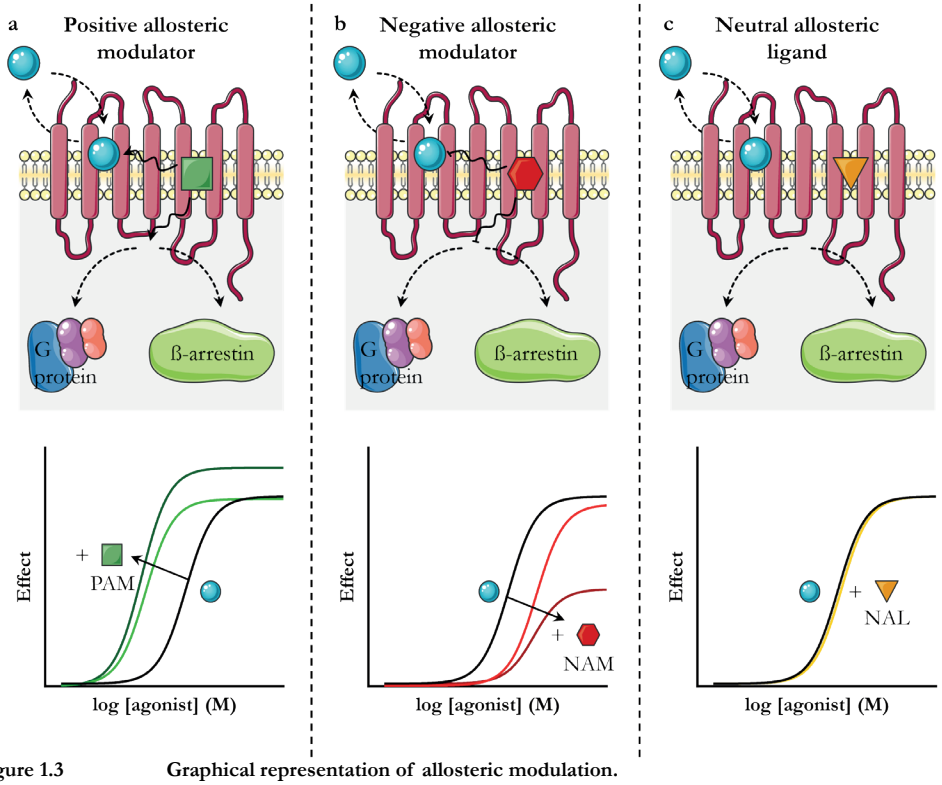

Figure 1.3 **Graphical representation of allosteric modulation.**
Allosteric modulators bind to a binding site topographically distinct from the orthosteric binding site and can affect the binding and functional effect of orthosteric ligands. (a) Positive allosteric modulators (PAMs) enhance the affinity and efficacy of the orthosteric ligand, while (b) negative allosteric modulators (NAMs) inhibit the affinity and efficacy of the orthosteric ligand. (c) Neutral allosteric ligands (NALs) occupy the allosteric binding pocket without affecting the affinity or efficacy of the orthosteric ligand but prevent further binding of PAMs or NAMs. This figure incorporates drawings from Servier Medical Art (smart.servier.com).

this insurmountability of allosteric modulators may play an important role[53]. Moreover, allosteric modulators have a 'ceiling effect', i.e., there is a limit to the pharmacological effect that can be mediated via allosteric binding due to saturation of the effect once the allosteric site is fully occupied[51,53].

Despite these advantages of allosteric over orthosteric targeting, very few allosteric modulators of GPCRs have made it to the market[54]. The first GPCR allosteric modulator that was approved by the United States Food and Drug Administration (FDA) for clinical use was cinacalcet[55,56]. Cinacalcet is a PAM for the calcium-sensing receptor (CaR), a class C GPCR, and is used for treating parathyroid cancer and secondary hyperparathyroidism. Moreover, advanced clinical trials are ongoing with several allosteric modulators for class A GPCRs, such as PAM Mevidalen (LY3154207), which enhances the affinity of dopamine for the dopamine D_1 receptor and is currently investigated for the symptomatic treatment of patients with Parkinson disease[57,58].

1.4 Biased signaling

Biased signaling, also known as ligand bias, biased agonism or functional selectivity, reflects the ability of a ligand to preferentially activate one pathway over another (**Figure 1.4**)[51,59,60]. This concept may become important if a specific signaling pathway is associated with efficacy and the other one with inducing side effects[59]. The rationale behind biased signaling is that different agonists can stabilize different active conformations of the receptor and consequently affect the coupling efficiency to transducers.

Biased signaling has already been described for GPCR families with multiple endogenous agonists, such as the chemokine and opioid receptors[61,62]. Moreover, exogenous biased agonists have been designed and studied, and even biased allosteric modulators (BAMs) are emerging[63]. For GPCR agonists, bias is most often studied between G protein coupling and ß-arrestin recruitment. However, bias may also occur within the G protein or ß-arrestin families[64,65]. Nevertheless, studying ligand bias is very complex and many factors may confound conclusions drawn about bias[31,59]. This can relate to the cellular background, referred to as system bias, by different concentrations and stoichiometry of receptor, transducers and effectors[59]. Alternatively, the experimental setup could introduce observational bias, which may be due to an artificially high level of signal amplification or the choice of specific time points[59,66].

Clinical relevance of biased agonists has only very recently been acknowledged, evident by the FDA approval of the first biased agonist oliceridine in 2020 to adults experiencing moderate to severe acute pain[67]. Oliceridine, a μ-opioid receptor agonist, was at the time described to be biased towards G protein activation over ß-arrestin recruitment. However, its therapeutic efficacy due to a biased profile is currently disputed and may relate to its partial agonism in one pathway[68]. Retrospectively more biased ligands are already on the market but were previously not described as such. An example is carvedilol, a commonly used ß-blocker, which is a functional antagonist for G protein-mediated signaling but an agonist for ß-arrestin-mediated signaling[69].

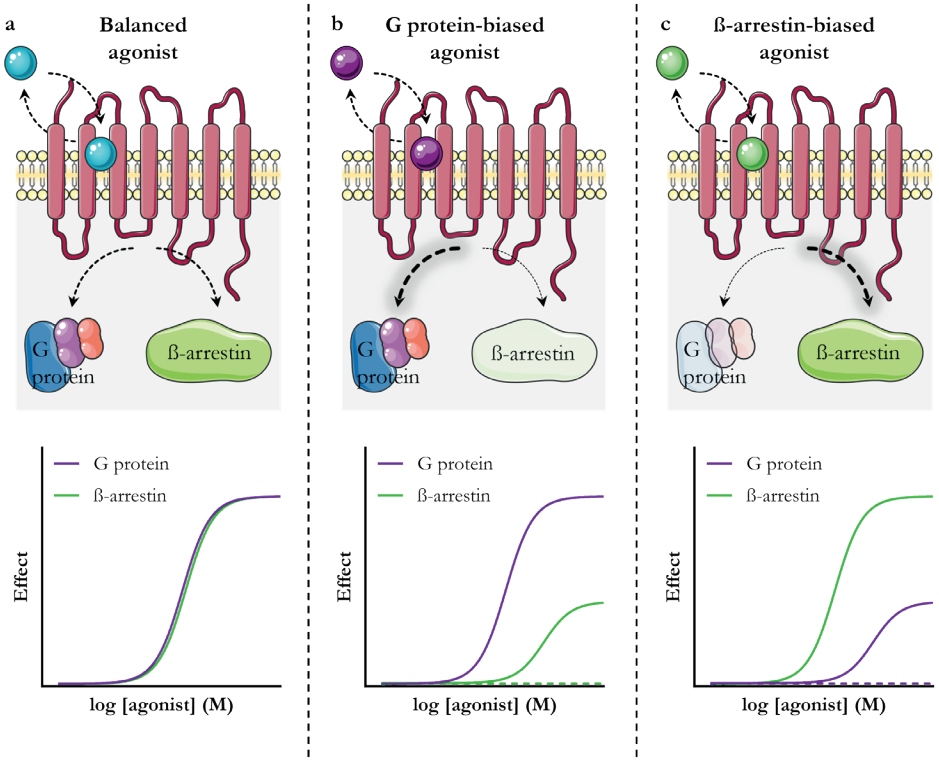

Figure 1.4 **Graphical representation of biased signaling.**
(a) A balanced agonist equally activates two pathways, such as G protein and ß-arrestin signaling. (b) A G protein-biased agonist preferentially activates G protein signaling, while ß-arrestin signaling is reduced or absent. (c) A ß-arrestin-biased agonist preferentially activates ß-arrestin signaling, while G protein activation is reduced or absent. This figure incorporates drawings from Servier Medical Art (smart.servier.com).

1.5 Endocannabinoid system

A family of class A GPCRs are the cannabinoid receptors (CBRs). The CBRs are part of the endocannabinoid system (ECS) in the human body along with their endogenous ligands, *N*-arachidonoylethanolamide (anandamide or AEA) and 2-arachidonoylglycerol (2-AG), and their respective metabolizing enzymes[70–72]. Specifically, *N*-acylphosphatidylethanolamine-specific phospholipase D (NAPE-PLD) and diacylglycerol lipase (DAGL) are involved in the biosynthesis of AEA and 2-AG, respectively, but formation may also occur via parallel routes and proteins. The degradation of AEA and 2-AG is primarily mediated by fatty-acid amide hydrolases (FAAH) and monoacylglycerol lipase (MAGL), respectively[70]. The endocannabinoids (eCBs) AEA and 2-AG activate two types of CBRs, the cannabinoid CB_1 and CB_2 receptors (CB_1R and CB_2R)[72]. The receptors derive their name from the discovery that active components from *Cannabis sativa*, such as the main psychoactive constituent Δ^9-tetrahydrocannabinol (Δ^9-THC), bound and activated these GPCRs. CB_1R and CB_2R share an overall homology of 44% and an even larger homology of 68% in their seven transmembrane domains which includes their ligand binding domains[73]. However, the receptors display a distinct tissue expression. CB_1R is highly expressed in the central

nervous system and is responsible for the psychotropic effects of Δ^9-THC. It is involved in the regulation of various physiological functions, including memory, learning and appetite. Although CB_2R expression in brain regions is heavily debated, it is evident that this receptor is predominantly expressed on immune cells and lymphatic organs. Consequently, activation of CB_2Rs plays a significant role in the regulation of several inflammatory processes[74,75]. After activation both CB_1R and CB_2R couple to $G\alpha_{i/o}$ proteins, which in turn inhibits cAMP production in cells. Furthermore, activation can lead to activation of pERK and G protein-coupled Inward Rectifying K^+-channels (GIRKs) as well as recruitment of ß-arrestin-1 and 2[65,76,77]. CB_1R can additionally bind $G\alpha_{12/13}$ proteins and activate their corresponding transduction pathways[65], which has not been shown for CB_2R.

Currently, several drugs are on the market that rely on components from *Cannabis sativa* or synthetic analogs thereof[75]. Dronabinol, synthetic Δ^9-THC, is prescribed to patients suffering from anorexia, cachexia and chemotherapy-induced emesis[78]. Similarly, a synthetic Δ^9-THC analog, Nabilone, is also approved for its antiemetic effects and specifically used for the treatment of chemotherapy-induced nausea and vomiting (CINV)[75]. Cannabidiol (CBD), marketed as Epidiolex, is prescribed to patients over 1 year old with severe forms of epilepsy such as the Lennox-Gastaut and Dravet syndrome[79]. Furthermore, CBD-containing oils and infused beverages are sold over the counter to the general public[80]. Finally, mixtures of Δ^9-THC and CBD, e.g., Sativex® (1:1 ratio) are approved for the treatment of multiple sclerosis-associated spasticity[81]. Nevertheless, Dronabinol and Nabilone bind and activate both CB_1R and CB_2R, whereas CBD exerts its effects via various additional proteins[75,82]. As activation of CB_1R is associated with the psychoactive effects of cannabinoid ligands, selective activation of CB_2R may provide a therapeutically interesting treatment strategy without inducing psychotropic effects[83].

1.6 Therapeutic potential for CB_2R

The protective effect of CB_2R activation has been indicated for neuroinflammatory and neurodegenerative disorders, including severe diseases as Alzheimer's disease (AD), Parkinsons's disease (PD), multiple sclerosis (MS) and amyotrophic lateral sclerosis (ALS), via dampening the inflammatory responses[84,85]. Furthermore, a reduction in the inflammatory response, by inhibition of leukocyte proliferation and reduced secretion of pro-inflammatory cytokines, may be beneficial in autoimmune diseases such as arthritis, scleroderma and inflammatory bowel disease (IBS)[86]. CB_2R agonists could also provide antinociceptive effects in various pain conditions, both in acute and persistent pain as well as for neuropathic pain[87]. Moreover, increased CB_2R levels have been reported in various cancer types, and agonists are described to have antitumor effects on top of the current palliative use of cannabinoid-based treatments[88–91].

To date, a large diversity of selective CB_2R agonists has been developed for preclinical investigation, which increases our understanding of targeting CB_2R[83]. Multiple CB_2R agonists have progressed to clinical development since 2010, but the majority has been discontinued due to a variety of reasons including a narrow safety margin, lack of pharmacological effect, or development has been halted due to practical reasons such as the closure of the

company[75]. Challenges in the poor preclinical to clinical translation of CB_2R agonists have been hypothesized to include, but are not limited to, a lack of translational animal models for proper biological evaluation or the potential of differential signaling bias at the receptor level although disease relevant pathways have not yet been demonstrated[75,83,92].

1.7 Aim and outline of this thesis

The potential for incorporating novel concepts in early phases of drug discovery to provide a more accurate translational perspective has been receiving increasing attention. However, limited number of studies are available for CB_2R agonist binding kinetics[93,94], allosteric modulation[95] or biased signaling[76,77,96–101] and they are generally considered as individual concepts. Therefore, *it's about time* that we further investigate and connect these novel concepts on CB_2R to improve our molecular pharmacological understanding of targeting the receptor. In this thesis, the target-binding kinetics and biased signaling of CB_2R agonists are explored, as well as allosteric modulation of the receptor by small molecules. To this end, state-of-the-art assays are used in conjunction with the design of novel methodologies. Central to the investigation of CB_2R pharmacology is providing an overall kinetic view on drug discovery.

Chapter 2 provides a step-by-step protocol for the quick and straightforward investigation of ß-arrestin-2 recruitment to stimulated CB_2R and CB_1R by agonists and inverse agonists, which is further applied in **Chapter 5**. **Chapter 3** reports a novel assay to simultaneously and kinetically detect cAMP signaling and ß-arrestin-2 recruitment after CB_2R stimulation in one cellular system. This multiplex assay is applied to a set of clinically relevant CB_2R agonists and the time-dependency of biased signaling is explored. Functional and binding kinetics are combined to obtain a holistic overview of the kinetic context of agonist-mediated CB_2R. **Chapter 4** describes the extensive profiling of a novel hydrophilic CB_2R-selective ligand, LEI-102, by the use of structural, *in vitro* and *in vivo* experimentation. Combining mutagenesis data and target binding kinetics suggests a distinct entry pathway for lipophilic agonists. In **Chapter 5**, allosteric modulation of CB_2R by small molecules is explored. CBD-DMH emerged from a newly adapted radioligand dissociation assay and is further screened on allosteric and orthosteric behavior in *in vitro* assays, including the methodology described in **Chapter 2**. In **Chapter 6,** a translation to the patient is made by investigation of the effect of cancer-associated mutations in CB_2R on receptor activation and ligand binding. Finally, **Chapter 7** provides an overall conclusion of the novel findings described in this thesis and new perspectives and opportunities for drug discovery on CB_2R and other GPCRs.

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