2023 Julius Axelrod Symposium: **Plant-derived molecules acting on GPCRs**

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ABSTRACT

Plant extracts have played a significant role in traditional medicine for centuries, contributing to improved health and the treatment of various human illnesses. G protein-coupled receptors (GPCRs) are crucial in numerous physiological functions, and there is growing evidence suggesting their involvement in the therapeutic effects of many plant extracts. In recent years, scientists have identified an expanding number of isolated molecules responsible for the biological activity of these extracts, with many believed to act on GPCRs. This article critically reviews the evidence supporting the modulation of GPCR function by these plant-derived molecules through direct binding. Structural information is now available for some of these molecules, allowing for a comparison of their binding mode with that of endogenous GPCR ligands. The final section explores future trends and challenges, focusing on the identification of new plant-derived molecules with both orthosteric and allosteric binding modes, as well as innovative strategies for designing GPCR ligands inspired by these plant-derived compounds. In conclusion, plant-derived molecules are anticipated to play an increasingly vital role as therapeutic drugs and serve as templates for drug design.

**Keywords:** Plant extract, GPCR, natural compounds, allosteric ligands, bioactive compounds

**Significance Statement**

This minireview summarizes the most pertinent publications on isolated plant-derived molecules interacting with GPCRs and comments on available structural information on GPCR/plant-derived ligand pairs. Future challenges and trends for the isolation and characterization of plant-derived molecules and drug design are discussed.

*Word count: 41*
Introduction

More than 80% of the world’s population is estimated to use traditional medicine for their primary healthcare needs according to the statistics of the World Health Organization (WHO) in 2023 (WHO-plants, 2023). Plants and plant extracts are the primary agents used in traditional medicine for more than 6000 years. In many countries plant extracts and botanically “defined mixtures” are available either over-the-counter or with prescription (Li and Weng, 2017; Lundstrom et al., 2017). According to the concept of poly-pharmacology, the effectiveness of plants and plant extracts is suspected to rely on two types of synergistic actions, the coexistence of different molecules in plant extracts that synergistically contribute to the therapeutic effect and the notion that many plant-derived molecules often have several molecular targets. In several occasions the most active molecules have been identified. Some of them have been used for a long time including opium, digitalis, quinine (Alamgir, 2017) and the acetylated derivative of the plant-derived salicylic acid commercialized as aspirin (Mahdi et al., 2006). Overall about 80% of these bioactive molecules show a positive correlation between their modern therapeutic uses and the traditional uses (Oteng Mintah et al., 2019). Plant-derived molecules are not only an important part of the currently available medication dispensed by physicians but constitute also original source of novel chemical scaffolds for the synthesis of new derivatives by the pharmaceutical industry.

Many medicinal plants act on functions of the central nervous system (CNS) which are mainly under the control of ion channels and G protein-coupled receptors (GPCRs). GPCRs constitute a super-family of membrane proteins with seven membrane-spanning helixes and approximately 800 members in humans (Fredriksson et al., 2003). They are highly druggable and are targeted by approximately 30% of the currently marketed drugs (Hauser et al., 2017).
For several plant-derived molecules, a direct action on GPCRs has been demonstrated. These molecules may span the full range of GPCR ligands including competitive ones binding to the orthosteric ligand binding site, molecules with functional selectivity and molecules with allosteric action mechanisms (Zhang et al., 2020).

This review will focus on the currently available literature of plant-derived molecules directly binding to GPCRs. We will not address effects of plant extracts or mixtures of plant-derived molecules or molecules modifying GPCR function more indirectly such as by acting on GPCR-associated signaling pathways or modifying receptor expression. The first section of the article will critically review the state-of-the-art of plant-derived molecules proposed to bind directly to GPCRs. Classical plant-derived molecules such as Δ9-tetrahydrocannabinol (Δ9-THC), caffeine, theophylline, morphine etc. are not included in this section as they have been extensively reviewed elsewhere (for recent expert review see (Serrano-Marín et al., 2020)).

The second section will give an overview of the currently available atomic GPCR structures solved in the presence of plant-derived molecules or its derivatives and compare them to the binding mode of endogenous or synthetic ligands. The third section will discuss future directions, in particular new approaches to identify and isolate new plant-derived GPCR ligands, to determine allosteric binding sites and allosteric modulators and to use plant-derived molecules as a basis for the design of synthetic GPCR ligands.

### Binding modes of plant-derived molecules to GPCRs

**Table 1** provides a non-exhaustive selection of 72 articles reporting in vitro evidence for the direct binding of isolated plant-derived molecules to GPCRs. Major chemical classes studied are plant secondary metabolites such as polyphenols, alkaloids, terpenes, flavonoids (in order of number of studies) but also bitter taste molecules, plant-derived lipids and peptides
such as cyclotides or peptides with motifs common to GPCR peptide ligands. These chemically diverse molecules are reported to act on 30 different classes of GPCRs (classes defined according to the IUPHAR classification (Alexander et al., 2019)).

**GPCR ligand binding assays and plant-derived molecules**

Several lines of evidence demonstrate or indicate direct binding of isolated plant-derived molecules to GPCRs. Among the different techniques, radioligand competition binding (CB) experiments constitute one of the most powerful and specific assays that are widely applied. Earlier studies tested the capacity of plant-derived molecules to compete the binding of radiolabeled GPCR ligands in tissues expressing endogenous GPCRs (Zhu et al., 1997). Later on, tissues were replaced by cells transfected with plasmids coding for given GPCRs (Butterweck et al., 2002; Simmen et al., 1999; Simmen et al., 2001). Although these campaigns provided a range of interesting results, they can be only considered a starting point for further studies due to some limitations. Typically, molecules were only tested at a single concentration providing only limited information on the degree of competition (partial or full) and the potency ($K_i$). Furthermore, despite the high specificity of radioligand competition binding assays, false positives cannot be ruled out when studying a large range of natural molecules as they might interfere indirectly with the radioligand binding, i.e. by destabilizing the receptor or by modifying the membrane environment. CB remains the most popular assay to address direct binding of molecules to GPCRs (used in 31/72 articles in Table 1). Other binding techniques such as the label-free surface plasmon resonance (SPR) technique have been employed recently to confirm hits from virtual screens as shown for the bisbenzylisoquinoline alkaloid neferine and orexin receptors (He et al., 2023). Recent progress in the expression and purification of recombinant GPCRs helped to determine the
binding properties or to purify plant-derived molecules by affinity chromatography on immobilized GPCRs (AT1, ETA, M3 receptors) (Fan et al., 2023; Liang et al., 2020; Liang et al., 2021; Liu et al., 2022; Shayiranbieke et al., 2022; Zhang et al., 2023).

**GPCR-associated signaling pathways and plant-derived molecules**

Monitoring the modulation of GPCR-associated signaling pathways is another commonly used approach to identify and characterize the action of plant-derived molecules on GPCRs. These assays allow to determine the agonistic or antagonistic properties of plant-derived molecules. As specified in Table 1, intracellular cAMP levels are determined as read-out for \( G_s \) and \( G_{i/o} \)-coupled GPCRs and intracellular Ca\(^{2+}\) or inositol phosphate levels for \( G_{q/11} \)-coupled receptors. Further downstream signaling events such as ERK1/2 and Akt activation are also used as readout by monitoring their phosphorylation state. When screening plant extracts or large libraries of plant-derived molecules it is mandatory to verify the specificity of positive hits for the studied receptor as a large number of molecules are likely to act on other receptors endogenously expressed in the chosen cell type or other components of the signaling pathway investigated. When working on transfected cell lines, positive hits should be tested in the parental, non-transfected, cell line. When working on endogenous receptors, the effect should be blocked by receptor-specific antagonists or siRNA molecules. Many plant extracts are colorful which constitutes an additional concern at high concentrations when using signaling assays based on fluorescent-, luminescent- or energy transfer-based readouts.

Activation of GPCRs by plant-derived molecules can be also monitored at the level of G protein activation by the \([^{35}S]\)-GTP\(\gamma\)S binding assay. Recruitment of \(\beta\)-arrestins, either as a
primary screening assay or to test individual plant-derived compounds (Table 1), is increasingly used because of the availability of commercially available and standardized kits.

*Signaling bias of plant-derived molecules*

Several plant-derived molecules were claimed to have biased signaling properties. For the apelin receptor, the flavonoid (-)-epicatechin (EC) behaves as a full agonist for β-arrestin recruitment (EC\textsubscript{50}=1.7 pM, an effect which is blocked by the apelin receptor antagonists ML221) (Portilla-Martínez et al., 2022). At the same time EC does not inhibit forskolin-stimulated cAMP production. Unfortunately, the effect of apelin-13, the reference ligand, was not determined in this assay. The functional importance of β-arrestin recruitment was further studied in C2C12 cells in which the apelin-13- and EC-induced Akt activation was blocked by the β-arrestin interaction inhibitor barbadin (Portilla-Martínez et al., 2022). Whether this effect is also blocked by the G\textsubscript{i/o} protein inhibitor, pertussis toxin, to block the second known pathway connecting the apelin receptor to Akt, was not tested thus making it difficult to rule out any contribution of G\textsubscript{i/o} proteins to the Akt signal. Molecular docking and simulation studies are presented to support the biased behavior hypothesis of EC. Taken together, this study provides interesting but still preliminary evidence for the biased properties of EC on the apelin receptor that needs further experimental validation.

For the 5-HT\textsubscript{2C} receptor the aporphine alkaloid (R)-asimilobine was shown to stimulate G\textsubscript{q/11}-dependent Ca\textsuperscript{2+} signaling but not recruitment of β-arrestin (Zhang et al., 2020). These data may indicate that (R)-asimilobine is biased for Ca\textsuperscript{2+} signaling, however, the fact that the Ca\textsuperscript{2+} assay is about a 30- to 100-fold more sensitive than the β-arrestin assay precludes such a conclusion at the current state. Molecular docking suggested binding of (R)-asimilobine to the orthosteric binding site of 5-HT\textsubscript{2C}, which was confirmed by mutagenesis studies. Of note,
the proposed binding mode is slightly different from the reference ligand lorcaserin as illustrated by the differential effect of several receptor mutants. (R)-asimilobine showed in vivo activity as it reduced food intake and body weight in the diet-induced obesity mouse model as would be predicted for a 5-HT$_{2C}$ agonist (Zhang et al., 2020).

By characterizing seven phyto-cannabinoids (Δ9-THC, Δ9-THCa, cannabidiol (CBD), CBDa, CBDV, CBG, CBC) from cannabis on CB1 and CB2 receptors, Zagzoog et al. revealed that several of them are agonists for the G$_i$/cAMP pathway but are poorly active or inactive for β-arrestin recruitment (Zagzoog et al., 2020). Similar to the work of Zhang et al. on the 5-HT$_{2C}$ receptor, the β-arrestin assay used was about 100 times less sensitive than the G protein assay for the reference compound making it difficult to discriminate between the biased behavior and an issue of assay sensitivity to interpret the experimental data. Competition curves of [³H]-CP55,940 binding to CB1 and CB2 by most of these phyto-cannabinoids showed a partial inhibition indicating a mixed ortho/allostERIC or full allostERIC binding mode in particular for CB1.

More indirect indication for the biased behavior of plant-derived molecules on the CB1 receptor comes from another study with several terpenes isolated from cannabis (LaVigne et al., 2021). None of them showed any significant agonistic property on G$_i$/cAMP signaling and β-arrestin recruitment but one, geraniol, behaved as a negative allosteric modulator of the reference agonists, WIN55,212-2 on β-arrestin recruitment. Geraniol was also the only terpene with a clear competition of [³H]-CP55,940 binding to CB1. Interestingly, terpenes showed cannabinoid tetrad behaviors in mice (antinociception, hypolocomotion, catalepsy, hypothermia). This effect was potentiated in the presence of WIN55,212 (LaVigne et al., 2021). These interesting in vivo data justify more research on the allosteric properties of geraniol in the future.
An indication of G protein bias was also proposed for cyclotides, cyclic plant-derived disulfide-rich peptides (Muratspahić et al., 2019). Several cyclotides are agonists for the $G_i/cAMP$ pathway of the $\kappa$-opioid receptor ($\kappa$-OR) (Muratspahić et al., 2021b). One of them, [T20K]kalata B1 from *Oldenlandia affinis*, activates the $G_i/cAMP$ pathway but does not recruit $\beta$-arrestin (Muratspahić et al., 2021b). Once again differences in the sensitivity of the assays with the $\beta$-arrestin assay being at least 30 times less sensitive, are likely to be a relevant confounding factor to preclude any solid conclusion on the biased behavior of this compound. Taken together, existing experimental data on the biased behavior of some plant-derived compounds is interesting but remains suggestive. Confounding parameters as differences in assay sensitivity can often not be excluded, in particular, for low affinity compounds. It is important to point out that $\beta$-arrestin assays used measure recruitment and NOT signaling or activation whereas G protein assays typically measure indeed signaling or activation. In addition, untangling $\beta$-arrestin-dependent from G protein-dependent events is not trivial as both are often interdependent. Standard methods to properly address signal bias are found in several articles (Griffin et al., 2007; Kenakin et al., 2012; Tran et al., 2009).

*Integrative cellular assays and plant-derived molecules*

Activation of luciferase reporter genes under the control of different response elements is another way to capture GPCR-derived signaling events. The serum response element (SRE) is a proxy for $G_{i/o}$, $G_{12/13}$, and/or $G_{q/11}$ activation, the serum response factor-response element (SRFRE) for $G_{12/13}$ activation, the nuclear factor of activated T-cell response element (NFAT-RE) for $G_{q/11}$ activation and the cAMP response element (CRE) for $G_s$ and $G_{i/o}$ activation. All have been extensively applied to the characterization of plant-derived molecules (Dong et al., 2013b; Fahradpour et al., 2017; Harada et al., 2022a; Harada et al., 2022b; Liu et al.,
The same general specificity concerns of discussed above apply to reporter gene assays as plant-derived compounds often have several molecular targets that might activate the reporter gene in a GPCR-independent manner. This concern may be particularly relevant for reporter gene assays as long incubation times with the test compound are typically needed (6-24h) as compared to second messenger or kinase phosphorylation assays (5-30 minutes).

Dynamic mass redistribution (DMR) is another technique to monitor integrative, phenotypic changes that Hou et al. used to study the effect of plant-derived molecules in HEK293 cells expressing the μOR (Hou et al., 2021). Out of the 82 molecules selected based on structural similarity to known OR ligands, seven were μOR antagonists against loperamide with micromolar potency (IC$_{50}$=10-300µM). Similar responses were observed in cells expressing δOR, κOR, and NOPR, although will lower potency. Screening of some of these positive hits on HEK293 cells expressing 8 other GPCRs individually revealed the existence of some off-target effects. Further studies will be necessary to sort out the profile of these compounds for various GPCRs.

Complementary in silico and in vivo approaches and plant-derived molecules

Some of the in vitro studies summarized in Table 1 were complemented by in silico and/or in vivo studies to provide either further insights on the binding mode (molecular docking and simulations) or physiological/therapeutic relevance (animal models). Several studies used receptor specific antagonists or knockout mouse models to demonstrate that the in vivo effects are indeed dependent on the same receptor for which in vitro data were obtained (Jiang et al., 2020; Lacher et al., 2007; Nishi et al., 2012; Yang et al., 2001). Some additional
studies solely based on in vivo or in silico studies are shown in Supplemental Table 1 and Supplemental Table 2, respectively.

**Proposed action mode and potencies of plant-derived molecules**

Determination of the binding site of plant-derived molecules is of fundamental importance to understand their action and to perform structure-activity relationship (SAR) analysis. As detailed above most plant-derived molecules have been identified in CB experiments implying that they are binding to the orthosteric binding site. In several cases this conclusion has been clearly confirmed, in particular when the competition was complete and when structural evidence exists, i.e. for caffeine and theophylline binding to the adenosine A<sub>2A</sub> receptor and morphine binding to the µOR (see Figure 1). However, for many plant-derived molecules, the binding mode is less clear and the competition only partial suggesting either a partial overlap with the orthosteric binding site (mixed ortho-/allosteric binding mode) or binding to a fully independent allosteric site. Although this possibility has been suggested for some molecules an extensive pharmacological demonstration is generally missing (Herrera-Hernández et al., 2017; LaVigne et al., 2021; Muratspahić et al., 2021a; Ohbuchi et al., 2016; Zagzoog et al., 2020). Definite proof is expected to come from future structural studies as has been show now for a series of synthetic allosteric ligands binding to a variety of allosteric binding sites (Thal et al., 2018) (see also below the perspective section).

The affinities of plant-derived ligands for GPCRs vary widely over 5 orders of magnitude with K<sub>i</sub> values of low nM to 100 µM. Whereas high-affinity (nM) binding affinities are always a hint for good specificity (see morphine and the µOR), physiological relevance of low-affinity (µM) binding affinities can be equally important as demonstrated for Δ9-THC and the CB1 receptor and caffeine and theophylline and the A<sub>2A</sub> receptor. Most of the described plant-derived
molecules fall into the low-affinity (µM) category and only few in the high-affinity (nM) category (compounds described in 13/72 articles of Table 1).

To reach a physiological effect in vivo, additional properties have to be taken into consideration. This includes typically the pharmacokinetic properties, solubility, uptake and passage over the blood-brain-barrier etc. (see Abdel-Tawab, 2021) for review). When consuming plant-derived products or plant extracts instead of isolated molecules, the actual concentration of the molecule has also to be taking into account to reach a physiological effect. Plant-derived molecules tend to be promiscuous by acting on several molecular targets at the same time. Prominent examples are curcumin and quercetin, which show inhibitory effects in the µM range (same as for GPCRs) on cyclooxygenases 1 (COX-1 (IC50 25–50 µM), 5-lipoxygenases (IC50 0.7 µM), microsomal prostaglandin E2 synthase-1 (IC50 0.3 µM) and on Janus kinase (JAK)3 (IC50 2 µM), the RaF/MEK1/ERK (IC50 1–10 µM) and NF-kB (IC50 4–11 µM) signaling pathways, respectively (Abdel-Tawab, 2021). Therefore, the in vivo effect might be the sum of several individual synergistic/antagonistic effects.

**GPCR structures with plant-derived molecules**

The structure of several GPCRs has been solved in the presence of plant-derived molecules or its derivatives binding to the orthosteric ligand binding site.

*Adenosine A2A receptor bound to caffeine, theophylline or XAC*

Here, we compared the binding pose of adenosine, the endogenous ligand of the A2A receptor in the intermediate state between the inactive and the fully active conformation (Lebon et al., 2011) with the pose of three plant derived non-selective A2A antagonist in the inactive state: caffeine from coffee beans (*Rubiaceae plant family*), theophylline, a
methylxanthine chemically related to caffeine, from tea leaves (Camellia sinensis) and xanthines amin congener (XAC), a synthetic derivative of natural xanthines (Doré et al., 2011). The overlayed structures within the orthosteric binding pocket of the A\textsubscript{2A} receptor show that the core of theophylline and caffeine superimposes almost exactly with the adenine moiety of adenosine (Figure 1A, B) while XAC shows more extended interactions (Figure 1C). Thus, the hydrogen bond between the exocyclic adenosine N6 with Asn253\textsuperscript{6.55} (Ballesteros-Weinstein numbering in superscripts (Ballesteros JA and H., 1995)) in TM6 is similar to the hydrogen bond between caffeine and the carboxamide of Asn253\textsuperscript{6.55} (Figure 1B). A similar hydrogen bond is seen for theophylline and XAC (Figure 1A, C). Caffeine sits in a hydrophobic pocket formed by Phe168\textsuperscript{ECL2}, Ile274\textsuperscript{7.39}, Leu249\textsuperscript{6.51} similarly to adenosine with an additional interaction with Val84\textsuperscript{3.32} for theophylline and Met270\textsuperscript{7.35} and Val84\textsuperscript{3.32} for XAC (Supplemental Figure 1A). Taken together, the binding mode of the core part of the plant-derived A\textsubscript{2A} antagonists is very similar to the adenosine agonist. As the ligand binding site of the A\textsubscript{2A} receptor shows a significant contraction upon agonist binding (Doré et al., 2011), some of the additional interactions of the antagonists might be due to more extended binding pocket of the inactive form.

Cannabinoid CB1 receptor bound to AM815 and AM11542

The active structure CB1 has been solved in the CB1-G\textsubscript{i} complex bound to AMG315, a synthetic agonist inspired by endocannabinoids (Krishna Kumar et al., 2023). AM815 and AM11542 are two CB1 agonists inspired by the chemical structure of Δ9-THC, a major bioactive component of cannabis (Cannabis Sativa) (Hua et al., 2017). Two CB1 structures bound to these two agonists are available in the intermediate receptor activation state (in the absence of the G protein). The overlay of these three structures shows that AMG315...
adopts an L-shape conformation defining the orthosteric binding pocket whereas AM841 and AM11542 occupy only part of this pocket (Figure 1D,E). The alkyl chain of AMG315 overlays well with AM841 and AM11542 within the deep part of the binding pocket. The alkyl chain of AMG315 extends into the long channel formed by TM3, TM5 and TM6 undergoing hydrophobic interactions with Leu193$^{3.29}$. The same bonds are made between Leu193$^{3.29}$ and the core of AM841 and AM11542. Common hydrophobic bonds with Tyr275$^{5.39}$ and Leu359$^{6.51}$ are made by AMG315 and AM11542 through their alkyl chain (Supplemental Figure 1B). The only hydrogen bond with Ile267$^{ECL2}$ is common between AMG315 and AM841 and involve their amine and hydroxylic moiety, respectively (Figure 1D). Additional hydrogen bonds are formed between His178$^{2.65}$ and Ser505$^{7.39}$ and the hydroxylic groups of AMG315 and AM841, respectively (Figure 1D). The tricyclic tetrahydrocannabinol ring system of AM11542 forms π–π interactions with Phe268$^{ECL2}$, Phe379$^{7.35}$ and Phe177$^{2.64}$, and the phenolic hydroxyl at position C1 forms a hydrogen bond with Ser383$^{7.39}$ (not shown in Figure 1E for clarity, see Supplemental Figure 1B). Taken together, all three ligands are positioned similarly in the orthosteric ligand binding pocket. The good overlay of the alkyl chain moiety of the ligands indicates a similar shape of the ligand binding pocket in the intermediate and fully active receptor conformation.

Muscarinic acetylcholine M2 receptor bound to NMS

N-methyl scopolamine (NMS) is a methylated derivative of the alkaloid scopolamine found in the Solanaceae (nightshade) plant family. NMS is a non-selective inverse agonist of the muscarinic acetylcholine M2 receptor for which structural data are available (Suno et al., 2018). In the absence of structural data on acetylcholine binding to M2, we overlayed the inactive NMS-M2 structure with the active M2 structure obtained in the presence of iperoxo,
a high-affinity synthetic M2 agonist, and a G protein mimetic camelid antibody fragment 
(Kruse et al., 2013). Both ligands adopt a bent conformation in the orthosteric ligand binding 
pocket (Figure 1F). Of note, TM6 participates in the ligand binding pocket and shows a 
significant inward movement of 2Å between the inactive and active receptor state. The α-
carbon of Asn404$^{6.52}$ forms a hydrogen bond between its side chain and the iperoxo 
isoazoline oxygen (Kruse et al., 2013), which is analogue to the hydrogen bond between 
this residue and the NMS’ carbonyl moiety but in the inactive receptor state (Figure 1F). 
Despite these activation-related structural changes, polar contacts between the agonist 
iperoxo and the receptor resemble those with NMS bound to the inactive M2 receptor. In 
particular, the conserved Asp103$^{3.32}$ serves as a counter-ion to the ligand amine in both 
cases (Figure 1F). Cation-p interactions with Tyr104$^{3.33}$, Tyr403$^{6.51}$ and Tyr426$^{7.39}$ form an 
aromatic lid over the ligand amine of iperoxo while Tyr426$^{7.39}$ is the only residue also 
forming an interaction with NMS (Supplemental Figure 1C). The isoxazoline ring of iperoxo 
and the ring of NMS from $\pi-\pi$ interactions with Ala194$^{5.46}$ while the amine group of both 
ligands interacts with Ser107$^{3.36}$ through van-der-walls and hydrogen bonds, respectively 
(Supplemental Figure 1C).

**GPR120 bound to oleic and linoleic acid**

Oleic acid (OA) and linoleic acid (LA) are two fatty acids found in olive oil (Hernandez et al., 
2021) but also in cotton seed, corn, soybean, safflower, and sunflower. These two plant-
derived molecules are agonists for GPR120 for which structural data are available in the 
active (G protein-bound) receptor state (Mao et al., 2023). The same study reported also the 
structure of GPR120 in the presence of its endogenous ligand, 9-hydroxystearic acid (9-HSA). 
All three ligands overlayed very well within the orthosteric binding pocket of GPR120 (Figure
assumed an overall “L” configuration and were fully buried inside the 7TM domain of the receptor (Mao et al., 2023). The C-terminus of 9-HSA as well as LA and OA form a charge interaction with the main chain carbonyl of specific amino acids such as Asp208\(^5.37\) (Supplemental Figure 1D). In addition, LA forms a charge interaction with Glu204\(^5.35\) (Supplemental Figure 1D). The hydrophobic residues of GPR120, such as Ile126\(^3.40\), contribute to interaction with the convex side of 9-HAS, OA and LA (Supplemental Figure 1D).

5-HT\(_{2B}\) receptor bound to LSD

Lysergic diethylamide acid (LSD) is a synthetic derivative of LSA (d-lysergic acid amide), an ergoline alkaloid that is present in plants from the Convolvulaceae family (Nowak et al., 2016). LSD binds most biogenic amine GPCRs and exhibits high affinities for nearly every one of the 14 distinct serotonin receptors (Cao et al., 2022). This molecule is a potent hallucinogen and has the potential to cause drug-induced valvular heart disease in human through the activation of 5-HT\(_{2B}\) in the case of chronical administration even with micro dosing (Kuypers et al., 2019; Roth, 2007). In the absence of any 5-HT\(_{2B}\) receptor structure bound to the endogenous ligand serotonin or any ligand with a serotonin-like structure we were unable to perform an overlay with the LSD-bound structure. LSD binding to the 5-HT\(_{2B}\) receptor in the intermediate activation state was observed at the typical orthosteric ligand binding pocket of amine GPCRs (Figure 1I). LSD through its core forms a carbon hydrogen bond with Thr140\(^3.37\) and a π – π interaction with Phe341\(^6.52\) (Supplemental Figure 1E). Further hydrophobic interactions are formed with Leu132\(^3.29\), Trp131\(^3.28\) through the ligand amine of LSD (Supplemental Figure 1E).
κ-opioid receptor bound to salvarine A

Here we compared the binding pose of dynorphin-(1-8), an endogenous ligand of the κOR (Livingston and Traynor, 2018) with momSalB, a semi-synthetic analogue of salvarine A (SalA), a plant-derived ligand from Salvia divinorum that displays similar in vivo pharmacology to SalA (Han et al., 2023). MomSalB is a selective agonist at the κOR. The superposition of the two active (G protein-bound) structures shows that the 8 amino acids of dynorphin-(1-8) are organized vertically into the orthosteric binding site (Figure 1J).

MomSalB binds to the κOR at the bottom part of the ligand binding pocket that is occupied by the N-terminal YGGF motif of dynorphin-(1-8) (Figure 1J). The methoxymethyl of momSalB forms a carbon hydrogen bond with the carboxyl side chain of Asp138 in the binding pocket while the pyrolidine nitrogen of dynorphin interacts with the carboxyl side chain of Asp138 forming an essential salt bridge for the binding of the endogenous ligand to κOR (Figure 1J, Supplemental Figure 1F) (Han et al., 2023). Unlike momSalB, the C-terminal LRRI motif of dynorphin-(1-8) makes additional interactions with the top region of the κOR orthosteric binding site, with its two positively charged residues R6 and R7 forming direct salt bridges with Glu209 and Glu297 (Figure 1J). Another major differences between momSalB and dynorphin-(1-8) is that momSalB mainly forms hydrophobic interactions with residues that specifically contribute to the high potency of momSalB, such as Val134, Val230 and Ile316 (Supplemental Figure 1F).

μ-opioid receptor bound to morphine

Morphine is a plant-derived molecule found in Papaver somniferum and Papaver album. Morphine is a full agonist at the μOR, the major analgesic opioid receptor (Schumacher et al., 2017). Structural information is available for the binding of morphine (Zhuang et al.,
2022) and endomorphin-1 to the κOR in its active (G protein-bound) state (Wang et al., 2023). Morphine adopts an elliptical “O” configuration, interacting with hydrophobic residues from TM3, TM6, and TM7 (Figure 1K). The amine group of endomorphin-1 forms salt bridge with the carboxylate group of Asp$^{3.32}$, which is a universal interaction of ligands with opioid and bioamine receptors (Che et al., 2018) (Supplemental Figure 1G). The overlayed structures show that morphine superimposes with the two N-terminal residues of the YPWF tetrapeptide of endomorphin-1 (Figure 1K). An additional hydrophobic pocket between TM2 and TM3 is occupied by residue W3 of endomorphin-1 and a hydrophobic pocket composed of residues His$^{7.48}$ and Ile$^{7.39}$ by residue F4 of endomorphin-1 (Wang et al., 2023). We also compared the binding pose of endomorphin-1 and mitragynine pseudoindoxyl (MP) to the active form of the κOR. MP is an alkaloid synthesized from mitragynine, which is the principal indole alkaloid isolated from the leaves of *Mitragyna speciosa Korth* (Yamamoto et al., 1999). The overlayed structures show that MP superimposes with the two N-terminal residues of the YPWF tetrapeptide of endomorphin-1 and adopts a deeper position into the central pocket (Qu et al., 2023) (Figure 1L) forming extensive contacts with Met$^{3.26}$, Ile$^{6.51}$, Ile$^{7.39}$, Tyr$^{7.43}$ (Supplemental Figure 1G).

**Perspectives and future directions**

**Identification of new plant-derived GPCR ligands**

Plants will continue to be a rich source of biologically active molecules with therapeutic potential. Thus, the isolation of new molecules with biological activity is likely to remain an important objective in clinical pharmacology. The classical way insists in the stepwise
enrichment of the biologically active molecule until purity by iterative cycles of fractionation monitored by cellular in vitro assays. Unfortunately, this procedure is not only expensive but also time-consuming (Wang et al., 2010). Due to significant advances in separation techniques, high-quality fractionation samples are rapidly obtained and tested in in vitro assays for biological activity. For example, Dong et al. combined an ultra-performance liquid chromatography/quadrupole TOF-MS (UPLC/Q-TOF-MS) separation approach with the luciferase reporter assay and identified the active components of Chuanbeipipa dropping pills, a traditional Chinese medicinal preparation used to treat chronic obstructive lung disease and cough involving the inflammatory response and the ß2-adrenergic receptor (Dong et al., 2013b). Similarly, extraction of roots of Carapichea ipecacuanha followed by MALDI-TOF/TOF MS analysis and a cell-based luciferase reporter assay identified cyclotides with biological activity on corticotropin-releasing factor 1 receptor (Fahradpour et al., 2017). The combination of chromatographic methods associated with affinity columns of purified and immobilized GPCRs have been also successfully used to isolate bioactive molecules from complex natural sources in a single step. The major challenge of this technique was the immobilization of functionally active GPCRs. This was achieved by a one-step immobilization of the purified receptor fused at its C-terminus with the kinase domain of the EGF receptor to microspheres via a linker. This technique has been successfully applied to the β1-AR (Shayiranbieke et al., 2022), the ETA receptor (Ji et al., 2023) and the M3 muscarinic receptor (Fan et al., 2023) to screen candidate ligands and to identify receptor binding molecules in herbal extracts. An alternative, two-point immobilization protocol has been also described for the AT1 receptor (Liu et al., 2022). In this study, the authors utilized two single-stranded DNA strands to immobilize a selective AT1 aptamer and the angiotensin II (3-8) peptide separately to the microsphere surface. The two-point immobilized AT1R exhibited
enhanced ligand-binding activity and stability of up to one month in comparison to the one-point immobilized receptor which was stable for 3 weeks. The strategy based on immobilized GPCRs is high-throughput screening compatible, owing to the reusability, good specificity and improved stability of the affinity columns. The increasing number of GPCRs that are now available as purified preparations is likely to contribute to the expansion of this method in the future. Some limitations exist such as the selection of molecules recognizing only one specific receptor conformation, and difficulties to detect less abundant molecules and/or molecules with low affinity for the target. Immobilization of plant-derived molecules has been also used to identify new molecular targets from tissue homogenates. Successfully examples are the identification of the G protein-coupled estrogen receptor (Moreno-Ulloa et al., 2015), the pregnane X receptor a nuclear receptor (Ortiz-Flores et al., 2020), and the apelin receptor as binders of the flavonoid (-)-epicatechin (Portilla-Martínez et al., 2022).

Taken together, these new techniques, which are based on ultra-performance liquid chromatography separation approaches and the immobilization of purified GPCRs are likely to accelerate the identification process of plant-derived molecules acting on GPCRs.

**Determination of allosteric binding sites**

Identification of plant-derived molecules has been biased towards molecules competing with orthosteric GPCR ligands. However, some of these molecules showed only a partial competition which would be compatible with an allosteric binding mode and others were shown be non-competitive. Monelin, a protein isolated from *Dioscoreophyllum cumminsii*, was identified as the first naturally-derived allosteric agonist of metabotropic glutamate receptor 5 binding to its large extracellular domain (Chen et al., 2020). An allosteric effect
was also reported on the AT1 receptor for Ginsenoside Rg1, an active component extracted from *Salviae Miltiorrhizae Radix et Rhizoma*, which is suggested to bind to an allosteric site in the extracellular loop 2 (Liu et al., 2022). Similarly, Muratspahic et al., showed that T20K, a plant-derived cyclotide in clinical development for the treatment of multiple sclerosis, is a positive allosteric modulator of the κ-OR by using binding and functional assays (Muratspahić et al., 2021b). Using different functional assays, molecular docking and *in vivo* mouse model, it has been demonstrated that osthole a natural coumarin present in the fruit of *Cnidium monnieri* is not a competitive inhibitor but rather an allosteric inhibitor that induces a structural change in the MRGPRX2 receptor (Callahan et al., 2020). Similarly, genistein is also suggested to be a negative allosteric modulator on MRGPRX2 (Kumar et al., 2020). Maybe quercetin is one of the best-characterized plant-derived allosteric modulators acting on rhodopsin bound to 9-cis-retinal (Herrera-Hernández et al., 2017). We speculate that many plant-derived molecules with allosteric action mode remain still to be discovered. A recent report on the effect of different plant extracts on the melatonin MT1 receptor strongly indicated the presence of an active principle active principle potentiating effect of melatonin on the Gí/o-dependent signaling but not on β-arrestin2 recruitment (Labani et al., 2023). This activity was extensively characterized in Pistacia vera extracts but seem to exist also in several other extracts. Methods such as affinity columns with immobilized GPCRs are likely to identify an increasing number of GPCR binders with an allosteric binding mode. Important advances in cryo-electron microscopy and receptor crystallization allowed already the identification of several allosteric binding sites that were difficult to be defined by other methods (Thal et al., 2018). Determination of GPCR structures bond to plant-derived molecules with suspected allosteric binding mode is likely to provide definitive proof for their binding mode in the future.
Plant-derived molecules as basis to design novel GPCR ligands

Several currently marketed drugs such as aspirin®, taxol®, quinine, morphine etc. are derived from natural sources (Muratspahić et al., 2019; Pirintsos et al., 2022). Given the richness and diversity of the molecules contained in plant extracts, it is not surprising that 424 over 1562 newly approved drugs by FDA a decade ago were derived from plants. This includes not only plant-derived molecules but also synthetic derivatives of them. Starting from an active natural ligand, the classical way to improve the effect of the molecule is to perform an SAR analysis. This technique has been employed to design and synthesize more potent and selective bile acid derivatives for the recently discovered TGR5 receptor (Sato et al., 2008) but also to identify a new class of coumarin-based GCRs activators and inhibitors (Fu et al., 2022). To further improve already well-documented plant-derived molecules with proven effects on human health, a large-scale library and fast screening technique are used like for the AT1 receptor and ETA receptor. More precisely, the technique of DNA-encoded library containing 32 000 phenolic acid-focused compounds for the AT1 receptor and 10 686 members derived from natural products for the ETA receptor were screened using the immobilized purified receptor method described above. The screening revealed a hit for each receptor which was evaluated in terms of binding, function and in vivo effect (Liang et al., 2021; Zhang et al., 2023).

Plants synthesize various types of molecules of which small molecules represent the majority of GPCR ligands. More recently, nature-derived peptides have gained significant attention for GPCR ligand development. In particular cyclotides have been discovered as interesting starting point for peptide-based GPCR drug discovery. They are disulfide-rich peptides with a unique cyclic cystine knot topology that confers them with remarkable structural stability.
and resistance to proteolytic degradation (Muratspahić et al., 2020). Indeed, Fahradpour et al. identified novel cyclotides present in C. ipecacuanha roots as antagonists of CRF1 receptor using a bioassay-guided fractionation approach combined with pharmacological and structural analysis (Fahradpour et al., 2017). In a review, Muratsphacic et al., have provided examples of how engineering cyclotides using molecular grafting may lead to the development of novel peptide ligands of GPCRs. This grafting technique consists in the insertion of a bioactive peptide epitope into a naturally occurring stable peptide scaffold, thereby generating a more stable peptide while retaining biological activity. This technique was applied to cyclotide as scaffolds to design cyclotide-based peptides targeting numerous GPCRs such as bradykinin receptors, melanocortin 4 receptor or CXC-motif-chemokine receptor 4 (Muratspahić et al., 2020). Once a new drug candidate has been identified and chemically optimized in in vitro assays and in vivo models, this candidate can go to the next step of the drug development the validation in clinical trials.

Conclusions

GPCRs are targeted by many synthetic molecules and are considered particularly interesting drug targets. A hand full of plant-derived molecules are also well-characterized GPCR ligands for which structural information exist. GPCR binding properties has been demonstrated or suggested for many other plant-derived molecules but their binding mode is generally poorly characterized. The rich chemical diversity of plant-derived molecules is likely to be an important source for the identification of new GPCR ligands and recent methodological advances are likely to accelerate the identification process and the determination of the
binding mode. Plant-derived molecules are also expected to continue to inspire the design of novel synthetic GPCR ligands as nicely exemplified by cyclotides.

AUTHOR CONTRIBUTIONS

Wrote or contributed to the writing of the manuscript: Labani N., Gbahou F., Lian S., Liu J. and Jockers R.

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Data Availability Statement
The authors declare that all the data supporting the findings of this study are contained within the paper and its Supplemental Data.

CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

FIGUR LEGEND

Figure 1. Superimposed GPCR structures obtained in the presence of endogenous or synthetic ligands and plant-derived molecules or their derivatives. (A-C) Side view of adenosine 2A receptor bound to (A) adenosine (blue) [PDB ID: 2YDO], or theophylline (green) [PDB ID: 5MZJ], (B) adenosine (blue) [PDB ID: 2YDO] or caffeine (green) [PDB ID: 3RFM], (C) adenosine (blue) [PDB ID: 2YDO] or XAC (green) [PDB ID: 3REY]. (D-E) Side view of cannabinoid CB1 receptor bound to (D) AMG315 (blue) [PDB ID: 8GHV] or AM841 (green) [PDB ID: 5XR8], (E) AMG315 (blue) [PDB ID: 8GHV] or AM11542 (green) [PDB ID: 5XRA]. (F) Top view of muscarinic acetylcholine M2 receptor bound to iperoxo (blue) [PDB ID: 4MQS] or NMS (green) [PDB ID: 5ZK8]. (G-H) Side view of GPR120 (G) bound to 9-hydroxystearic acid (9-HSA) (blue) [PDB ID: 8ID3] or linoleic acid (green) [PDB ID: 8ID4], (H) 9-HSA (blue) [PDB ID: 8ID3] or oleic acid (green) [PDB ID: 8ID6]. (I) Side view of 5-HT2B receptor bound to LSD (blue) [PDB ID: 7SRQ]. (J) Side view of κ-opioid receptor bound to dynorphin (blue) [PDB ID: 8F7W] or salvarine A (green) [PDB ID: 8DZP]. (K-L) Side view of the μ-opioid receptor (K) bound to endomorphin-1 (blue) [PDB ID: 8EF6] or morphine (green) [PDB ID: 8F7R], (L) endomorphin-1 (blue) [PDB ID: 8EF6] or mitragynine (green) [PDB ID: 7T2G]. H-bonds (blue lines). Adenosine, Ade; caffeine, Caff; Dynorphin, Dyn; endomorphin-1, EM-1; 9-hydroxystearic acid, 9-HAS; κOR, κ-opioid receptor; linoleic acid, LA; lysergic diethylamide.
acid, LSD; mitragynine, MG; μOR, μ-opioid receptor; morphine, MOR; N-methyl scopolamine, NMS; oleic acid, OA; theophylline, THE; xanthine amin congener, XAC.

Structure alignment and visualization was performed by PyMol.

References

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Integrating Traditional Medicine in Health Care.


Table 1: Selection of articles reporting *in vitro* evidence for direct binding of isolated plant-derived molecules to GPCRs.

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<th>Receptors</th>
<th>Primary G protein coupling</th>
<th>Class of molecule(s)</th>
<th>Molecule(s) tested</th>
<th>Plant species</th>
<th>Effect in <em>silico</em> / <em>in vitro</em> / <em>in vivo</em></th>
<th>Experimental model</th>
<th>Proposed action mode</th>
<th>Single conc./dose or K&lt;sub&gt;i&lt;/sub&gt;, IC&lt;sub&gt;50&lt;/sub&gt;, EC&lt;sub&gt;50&lt;/sub&gt;</th>
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<td>5-Hydroxytryptamine receptors</td>
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<tr>
<td>5-HT&lt;sub&gt;1A&lt;/sub&gt;</td>
<td>G&lt;sub&gt;o&lt;/sub&gt;</td>
<td>aporphine alkaloid</td>
<td>N-methyllaurotetanine</td>
<td><em>Eschscholzia californica</em></td>
<td><em>in vitro</em> CB with [&lt;sup&gt;3&lt;/sup&gt;H]8-OH-DPAT</td>
<td>full competition</td>
<td>K&lt;sub&gt;i&lt;/sub&gt; = 85 nM</td>
<td>Gafner et al., 2006</td>
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<tr>
<td>5-HT&lt;sub&gt;1A&lt;/sub&gt;</td>
<td>G&lt;sub&gt;o&lt;/sub&gt;</td>
<td>Alkaloid</td>
<td>geissoschizine methyl ether (GM)</td>
<td><em>Uncaria rhynchophylla</em></td>
<td><em>in vitro</em> + <em>in vivo</em> partial agonist (40%, GTPγS binding), improvement in behavioral test blocked by 5-HT&lt;sub&gt;1A&lt;/sub&gt; antagonist (WAY-100635)</td>
<td>partial agonist (40%, GTPγS binding), improvement in behavioral test blocked by WAY-100635/5-HT&lt;sub&gt;1A&lt;/sub&gt; antagonist</td>
<td>CB: K&lt;sub&gt;i&lt;/sub&gt; = 1 µM; GTPγS binding; EC&lt;sub&gt;50&lt;/sub&gt; = 1 µM; <em>in vivo</em> (300 µg/kg)</td>
<td>Nishi et al., 2012; Pengsuparp et al., 2001</td>
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<tr>
<td>5-HT&lt;sub&gt;1A&lt;/sub&gt;</td>
<td>G&lt;sub&gt;o&lt;/sub&gt;</td>
<td>Gramine</td>
<td>synthetic gramine derivatives</td>
<td></td>
<td><em>in vitro</em></td>
<td>Ca&lt;sup&gt;2+&lt;/sup&gt; agonistic effect</td>
<td>Ca&lt;sup&gt;2+&lt;/sup&gt;: EC&lt;sub&gt;50&lt;/sub&gt; = 0.47, 0.28, 0.46, 0.23 nM for gramine and derivatives 7, 19, 21</td>
<td>Yin et al., 2017</td>
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<td>5-HT&lt;sub&gt;1A&lt;/sub&gt;</td>
<td>G&lt;sub&gt;o&lt;/sub&gt;</td>
<td>Polyphenol</td>
<td>Proanthocyanidin flavan-3-ol, galloyl/hexahydroxyphenic acid esters of glucose</td>
<td><em>Croton lechleri etc.</em></td>
<td><em>in vitro</em> CB with [&lt;sup&gt;3&lt;/sup&gt;H]5-HT and Guinea pig brain membranes</td>
<td>competitive</td>
<td>CB: IC&lt;sub&gt;50&lt;/sub&gt; = 0.13 nM (procyanidin B-3)</td>
<td>Zhu et al., 1997; Phillipson, 1999</td>
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<tr>
<td>5-HT&lt;sub&gt;3C&lt;/sub&gt;</td>
<td>G&lt;sub&gt;o&lt;/sub&gt;/G&lt;sub&gt;i&lt;/sub&gt;</td>
<td>aporphine alkaloid</td>
<td>1857 (R)-asimilobine, 15781 (norunuciferine)</td>
<td><em>Stephania tetrandra</em></td>
<td><em>in vitro, in vivo</em> CB with [&lt;sup&gt;3&lt;/sup&gt;H]mesulergine, Ca&lt;sup&gt;2+&lt;/sup&gt;, β-arrestin, MD, mutagenesis, diet-induced obesity mouse model</td>
<td>competitive, partial agonists (1857, 15781) in Ca&lt;sup&gt;2+&lt;/sup&gt; on 5-HT&lt;sub&gt;3C&lt;/sub&gt;, (not 5-HT&lt;sub&gt;2A&lt;/sub&gt;, 5-HT&lt;sub&gt;2B&lt;/sub&gt;), 1857 does not recruit β-arrestin, 1857 reduces food intake</td>
<td>Ca&lt;sup&gt;2+&lt;/sup&gt;: EC&lt;sub&gt;50&lt;/sub&gt; = 308 nM (1857), EC&lt;sub&gt;50&lt;/sub&gt; = 653 nM (15781), <em>in vivo</em>: 10 mg/kg</td>
<td>Zhang et al., 2020</td>
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<tr>
<td>5-HT&lt;sub&gt;5A&lt;/sub&gt;</td>
<td>G&lt;sub&gt;o&lt;/sub&gt;</td>
<td>branched-chain saturated fatty acid</td>
<td>valerenic acid</td>
<td><em>Valeriana officinalis</em></td>
<td><em>in vitro</em> CB with [&lt;sup&gt;3&lt;/sup&gt;H]LSD, GTP shift assay</td>
<td>competitive</td>
<td>CB: K&lt;sub&gt;i&lt;/sub&gt; = 10.7 µM</td>
<td>Dietz et al., 2005</td>
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<td>5-HT&lt;sub&gt;5A&lt;/sub&gt;, 5-HT&lt;sub&gt;7&lt;/sub&gt;</td>
<td>G&lt;sub&gt;i&lt;/sub&gt;</td>
<td>hypericin, hyperforin</td>
<td>Hypericum perforatum</td>
<td></td>
<td><em>in vitro</em> CB with [&lt;sup&gt;3&lt;/sup&gt;H]LSD</td>
<td>competitive</td>
<td>CB 5-HT&lt;sub&gt;5A&lt;/sub&gt;: IC&lt;sub&gt;50&lt;/sub&gt;=97µM (hypericin), IC&lt;sub&gt;50&lt;/sub&gt;=22µM (hyperforin); CB 5-HT&lt;sub&gt;7&lt;/sub&gt;: IC&lt;sub&gt;50&lt;/sub&gt;=10µM (hypericin), IC&lt;sub&gt;50&lt;/sub&gt;=3µM (hyperforin)</td>
<td>Simmen et al., 2001; Simmen et al., 1999; Lundstrom et al., 2017</td>
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Acetylcholine receptors (muscarinic)
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<th>Receptor Type</th>
<th>Gα subtype</th>
<th>Subtype</th>
<th>Compound</th>
<th>Source</th>
<th>Assay Type</th>
<th>Method</th>
<th>IC50/EC50</th>
<th>CB: Kd (µM)</th>
<th>Dissociation Rate Constant (min⁻¹)</th>
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<td>isovaltrate</td>
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<td>in vitro</td>
<td>CB with [3H]-CCPA, GTPγS binding, electrophysiology on rat brain slices</td>
<td>CB: Kd = 2 mM, GTPγS: IC50 = 4.6 µM</td>
<td>Lacher et al., 2007</td>
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<td></td>
<td>A2α receptor</td>
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<td>curcumin</td>
<td>Curcuma longa</td>
<td>in vitro</td>
<td>platelet aggregation</td>
<td>CB: IC50 = 2 mM (procyanidin B-4); IC50 = 8 nM (rugosin-D)</td>
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<td>Adhesion class GPCRs</td>
<td>ADGRG3 (GPR97)</td>
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<td>curcumin</td>
<td>Curcuma longa</td>
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<td>Adrenergic receptors</td>
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<td>Gα and Gq/11</td>
<td>polyphenols</td>
<td>Croton lechleri etc.</td>
<td>in vitro</td>
<td>CB with [3H]-UK14304</td>
<td>CB: IC50 = 0.686 min⁻¹ (puerarin), 0.300 min⁻¹ (rosmarinic acid)</td>
<td>Liang et al., 2020</td>
<td></td>
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<tr>
<td></td>
<td>β1-AR</td>
<td>Gs</td>
<td>monoterpene</td>
<td>Zizia pungens</td>
<td>in vitro</td>
<td>identification from plant extract by affinity chromatography with purified β1AR, MD</td>
<td>MD into orthosteric binding site</td>
<td>n.d.</td>
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<td></td>
<td>β2-AR</td>
<td>Gi</td>
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<td>Pinellia ternate (epheidine), Aconitum carmichaeli (salsolinol, higeramine)</td>
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<td>Ultra-performance liquid chromatography with MS, reporter gene assay</td>
<td>CB: IC50 = 0.01 µM</td>
<td>Dong et al., 2013</td>
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<td>Angiotensin receptors</td>
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<td>Gq/11, Go</td>
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<td>CB: IC50 = 20 nM (Hit 1); inhibition of blood pressure (5, 15, 30 mg/kg)</td>
<td>Liang et al., 2021</td>
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<td>AT1</td>
<td>Gq/11, Go</td>
<td>Phenolic acids</td>
<td>library of 32,000 natural product derivatives</td>
<td>in vitro</td>
<td>library screening on immob. Halo-tagged AT1, CB with [125I]-Sar1-AngII, renovascular hypertensive rat model</td>
<td>CB: IC50 = 20 nM (Hit 1); inhibition of blood pressure (5, 15, 30 mg/kg)</td>
<td>Liang et al., 2021</td>
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<td>AT1</td>
<td>Gq/11, Go</td>
<td>steroid glycoside, polyphenol</td>
<td>ginsenoside Rg1, rosmarinic acid</td>
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<td>MD indicates that rosmarinic acid binds to the orthosteric binding site and ginsenoside Rg1 to an allosteric site at extracellular loop 2</td>
<td>n.d.</td>
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<td>Apelin receptor</td>
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<td>Liu et al., 2022</td>
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### Chemokine receptors

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<th>Modulator</th>
<th>Effect</th>
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<td>G&lt;sub&gt;i/o&lt;/sub&gt;</td>
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### Bile acid receptor

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<th>Receptor</th>
<th>G&lt;sub&gt;i/o&lt;/sub&gt;</th>
<th>Modulator</th>
<th>Effect</th>
<th>Methodology</th>
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<td>pentacyclic triterpene</td>
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<td>GPBA receptor (TGR5)</td>
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<td>(+)-resveratrol E, paconinol</td>
<td>Paeonia lactiflora</td>
<td>in vitro, in vivo</td>
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### Cannabinoid receptors

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<th>Modulator</th>
<th>Effect</th>
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<td>CB&lt;sub&gt;1&lt;/sub&gt;</td>
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<td>genistein</td>
<td>Glycine max</td>
<td>in vitro, in vivo</td>
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<td>CB&lt;sub&gt;1&lt;/sub&gt;</td>
<td>G&lt;sub&gt;i/o&lt;/sub&gt;</td>
<td>terpenes</td>
<td>α-humulene, geraniol, linalool, β-pinene</td>
<td>Cannabis sativa</td>
<td>in vitro, in vivo</td>
</tr>
<tr>
<td>CB&lt;sub&gt;1&lt;/sub&gt;/CB&lt;sub&gt;2&lt;/sub&gt;</td>
<td>G&lt;sub&gt;i/o&lt;/sub&gt;</td>
<td>cannabinoids</td>
<td>Δ9-THC, Δ9-THCa, CBD, CBDa, CBDD, CBG, CBC</td>
<td>Cannabis sativa</td>
<td>in vitro, in vivo</td>
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<tr>
<td>CB&lt;sub&gt;1&lt;/sub&gt;/CB&lt;sub&gt;2&lt;/sub&gt;</td>
<td>G&lt;sub&gt;i/o&lt;/sub&gt;</td>
<td>neolignans</td>
<td>4-O-methylhonokiol (1), magnolol (2), honokiol (3), tetrahydromagnolol (4)</td>
<td>Magnolia grandiflora</td>
<td>in vitro, in vivo</td>
</tr>
<tr>
<td>CB&lt;sub&gt;2&lt;/sub&gt;</td>
<td>G&lt;sub&gt;i/o&lt;/sub&gt;</td>
<td>366 natural products screened, pentacyclic triterpenoid</td>
<td>Celastrol purchased found in Piper nigrum</td>
<td>in vitro, in vivo</td>
<td>Agonist screen for CB&lt;sub&gt;2&lt;/sub&gt;/β-arrestin interaction, Ca&lt;sup&gt;2+&lt;/sup&gt;, cAMP, p-ERK, MD, mouse model of BLM-induced dermal fibrosis</td>
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<tr>
<td>Receptor Type</td>
<td>G&lt;sub&gt;i&lt;/sub&gt;</td>
<td>Ligand</td>
<td>Affinity column</td>
<td>in vitro</td>
<td>EC&lt;sub&gt;50&lt;/sub&gt;</td>
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<tr>
<td>Free fatty acid receptors</td>
<td>FFA1 (GPR40)</td>
<td>G&lt;sub&gt;i&lt;/sub&gt;</td>
<td>anthocyanins</td>
<td>in vitro</td>
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<td></td>
<td>FFA4 (GPR120)</td>
<td>G&lt;sub&gt;i&lt;/sub&gt;</td>
<td>long-chain free fatty acids</td>
<td>purchased</td>
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<td>FFA1 (GPR40)</td>
<td>G&lt;sub&gt;i&lt;/sub&gt;</td>
<td>fatty acid</td>
<td>purchased</td>
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<td></td>
<td>FFA4 (GPR120)</td>
<td>G&lt;sub&gt;i&lt;/sub&gt;</td>
<td>omega-3 fatty acids</td>
<td>purchased</td>
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<tr>
<td>Cholecystokinin receptor</td>
<td>CCK2R</td>
<td>G&lt;sub&gt;p&lt;/sub&gt;/11</td>
<td>cyclotides</td>
<td>in vitro</td>
<td></td>
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<tr>
<td>Corticotropin-releasing factor receptors</td>
<td>CRF1</td>
<td>G&lt;sub&gt;i&lt;/sub&gt;</td>
<td>hypericin, hyperforin</td>
<td>in vitro</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CRF1</td>
<td>G&lt;sub&gt;i&lt;/sub&gt;</td>
<td>cyclotides</td>
<td>in vitro</td>
<td></td>
</tr>
<tr>
<td>Dopamine receptors</td>
<td>D&lt;sub&gt;1&lt;/sub&gt;, D&lt;sub&gt;2&lt;/sub&gt;, D&lt;sub&gt;3&lt;/sub&gt;</td>
<td>G&lt;sub&gt;i&lt;/sub&gt;, (D&lt;sub&gt;1&lt;/sub&gt;); G&lt;sub&gt;i&lt;/sub&gt;, (D&lt;sub&gt;2&lt;/sub&gt;, D&lt;sub&gt;3&lt;/sub&gt;)</td>
<td>alkaloid</td>
<td>in vitro</td>
<td></td>
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<tr>
<td>Endothelin receptors</td>
<td>ETA</td>
<td>G&lt;sub&gt;p&lt;/sub&gt;/11</td>
<td>flavonoids</td>
<td>screening of extract</td>
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<td>Choerospondias axillaris</td>
<td>affinity column with purified ETA</td>
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</table>

| **CB with [125I]CXCL12, CXCL12-induced monocyte migration,** | **Competitive CXCR4 antagonist** | **CB: IC<sub>50</sub>=0.36 µg/ml, migration: IC<sub>50</sub>=7.5 µg/ml** |
| **CB with [125I]-artressin** | **Competitive, suspected antagonist (GTP/S binding assay, data not shown)** | **CB: IC<sub>50</sub>=300nM for hypericin, hyperforin is ineffective** |
| **Partial inhibition of CRF-induced response, no effect on forskolin- or AVP-stimulated response** | **CRE-luc reporter** | **Single conc. (26, 260 nM show partial inhibition in reporter gene assay** |
| **All molecules are agonists on Ca<sup>2+</sup> and ERK pathways** | **Insulin secretion IP production in INS-1E cells** | **IP: EC<sub>50</sub>=166 nM (D<sub>1</sub>), 1.4 µM (D<sub>2</sub>), 3.3 µM (D<sub>3</sub>)** |
| **G<sub>i</sub>/o** | **IP production** | **IP: EC<sub>50</sub>=8 μM** |

**Note:** The table contains information on the binding and functional effects of various ligands to different receptor types, including free fatty acid receptors, cholecystokinin receptors, endothelin receptors, and corticotropin-releasing factor receptors. The data includes EC<sub>50</sub> values for different ligands and their effects on various assays, such as insulin secretion, IP production, and chemotaxis. The table also references specific studies and research articles for further information.
### G protein-coupled estrogen receptor

<table>
<thead>
<tr>
<th>GPER (GPR30)</th>
<th>G&lt;sub&gt;α&lt;/sub&gt;</th>
<th>Isoflavone</th>
<th>Genistein</th>
<th>Purchased</th>
<th>In Vitro</th>
<th>AChE Activity, p-CREB, CRE-Luc Reporter</th>
<th>Genistein Induces cAMP Pathway and AChE Expression in PC12 Cells (the latter effect is blocked by G&lt;sub&gt;αS&lt;/sub&gt; (GPER Antagonist))</th>
<th>Reporter Gene: EC&lt;sub&gt;50&lt;/sub&gt; Low µM Range</th>
<th>Liu et al., 2018</th>
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### GPR55

<table>
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<tr>
<th>GPR55</th>
<th>G&lt;sub&gt;α&lt;/sub&gt;&lt;sub&gt;q/11-6&lt;/sub&gt;, G&lt;sub&gt;12/13&lt;/sub&gt;</th>
<th>Polyphenolic Pigment</th>
<th>Curcumin</th>
<th>Curcuma Longa</th>
<th>In Vitro</th>
<th>SRE-, SRF-RE-Luc Reporter in HEK Cells, MD</th>
<th>G&lt;sub&gt;αS&lt;/sub&gt; Stimulation, MD into Orthosteric Binding Site (Residue F190 Critical, Mutagenesis)</th>
<th>Reporter Gene: Single Conc. (5, 10 µM)</th>
<th>Harada et al., 2022</th>
</tr>
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### Lysophospholipid (LPA) receptors

<table>
<thead>
<tr>
<th>LPA1R, LPA3R</th>
<th>G&lt;sub&gt;α&lt;/sub&gt;</th>
<th>Fatty Acid</th>
<th>Lysophosphatidic Acid (LPA) (Isolated) and From Gintonin (Ginseng-Derived Glycolipoprotein Complex)</th>
<th>Panax Ginseng</th>
<th>In Vitro</th>
<th>Ca&lt;sup&gt;2+&lt;/sup&gt;, PC3 Cell Migration</th>
<th>Gintonin Stimulates Ca&lt;sup&gt;2+&lt;/sup&gt; Dependent PC-3 Cell Migration which is Blocked by LPA1/3R Agonist and Mimicked by LPA</th>
<th>Single Conc.: Gintonin (1 µg/ml), LPA (1 µM)</th>
<th>Choi et al., 2015</th>
</tr>
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### Melatonin receptors

<table>
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<tr>
<th>MT&lt;sub&gt;1&lt;/sub&gt;, MT&lt;sub&gt;2&lt;/sub&gt;</th>
<th>G&lt;sub&gt;α&lt;/sub&gt;</th>
<th>Polyprenyls</th>
<th>Gastrophyllin, Gastrophyllin (1–3)</th>
<th>Gastrodia Elata</th>
<th>In Vitro</th>
<th>Ca&lt;sup&gt;2+&lt;/sup&gt; Assay in Engineered Cells</th>
<th>Agonist (Comp 1)</th>
<th>EC&lt;sub&gt;50&lt;/sub&gt; = 76 µM (Comp 1 at MT&lt;sub&gt;1&lt;/sub&gt;)</th>
<th>Chen et al., 2019</th>
</tr>
</thead>
</table>

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<tr>
<th>MT&lt;sub&gt;1&lt;/sub&gt;, MT&lt;sub&gt;2&lt;/sub&gt;</th>
<th>G&lt;sub&gt;α&lt;/sub&gt;</th>
<th>Flavanols</th>
<th>Catechin and Epicatechin</th>
<th>Uncaria Rhynchophyla</th>
<th>In Vitro</th>
<th>Ca&lt;sup&gt;2+&lt;/sup&gt; Assay in Engineered Cells</th>
<th>Competitive Agonist Activity (Blocked by Luzindole Antagonist)</th>
<th>Catechin: EC&lt;sub&gt;50&lt;/sub&gt; = 26 µM (MT&lt;sub&gt;1&lt;/sub&gt;), 47 µM (MT&lt;sub&gt;2&lt;/sub&gt;), Epicatechin: EC&lt;sub&gt;50&lt;/sub&gt; = 156 µM (MT&lt;sub&gt;1&lt;/sub&gt;), 209 µM (MT&lt;sub&gt;2&lt;/sub&gt;)</th>
<th>Geng et al., 2019</th>
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<tr>
<th>MT&lt;sub&gt;1&lt;/sub&gt;</th>
<th>G&lt;sub&gt;α&lt;/sub&gt;</th>
<th>Oxyphenylated Ferulic Acid and Coumarin Derivatives</th>
<th>Oxyphenylated Ferulic Acid (1–4) and Umbelliferone (5–8) Derivatives</th>
<th>Cassia Cinnamon</th>
<th>In Vitro</th>
<th>CB with 2-[&lt;sup&gt;35&lt;/sup&gt;Cl]-Iodomelatonin, Cell Proliferation and Migration</th>
<th>Agonist, 8/11 Compounds are Competitive with 2-[&lt;sup&gt;35&lt;/sup&gt;Cl]-Iodomelatonin on MT&lt;sub&gt;1&lt;/sub&gt;</th>
<th>CB: K&lt;sub&gt;i&lt;/sub&gt; = 0.3–60 mM (Comp 3–7, K&lt;sub&gt;i&lt;/sub&gt; = 3 nM/1µM Biphasic (Comp 8), Partial (50%), Proliferation: IC&lt;sub&gt;50&lt;/sub&gt; µM Range</th>
<th>Hasan et al., 2017</th>
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<p>| MT&lt;sub&gt;1&lt;/sub&gt; | G&lt;sub&gt;α&lt;/sub&gt; | Gramine | Synthetic Gramine Derivatives | In Vitro | Ca&lt;sup&gt;2+&lt;/sup&gt; | Agonist | Ca&lt;sup&gt;2+&lt;/sup&gt;; EC&lt;sub&gt;50&lt;/sub&gt; 1.36, 0.51, 0.39, 0.50 mM for Gramine and Derivatives 7, 19, 21 | Yin et al., 2017 |
|----------------|----------------|----------------|---------------------------|---------|---------|--------------------------|------------------------------------------|------------------------------------------|------------------------------------------|</p>
<table>
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<tr>
<th><strong>Metabotropic glutamate receptors</strong></th>
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<tr>
<td>mGluR5</td>
<td>G&lt;sub&gt;k&lt;/sub&gt;11</td>
<td>10.7 kDa sweet protein</td>
<td>monellin</td>
<td>Dioscoreophyllum camunnsis</td>
<td>in vitro</td>
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<tr>
<td>mGluR5</td>
<td>G&lt;sub&gt;k&lt;/sub&gt;11</td>
<td>terpene</td>
<td>valerenic acid, isoborneol</td>
<td>Valeriana officinalis L.</td>
<td>in vitro</td>
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<td>MRGPRX2</td>
<td>G&lt;sub&gt;i/o&lt;/sub&gt;</td>
<td>isoflavone</td>
<td>genistein</td>
<td>purchased</td>
<td>in vitro, in silico, in vivo</td>
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<td>Mas-related GPRX2</td>
<td>G&lt;sub&gt;i/o&lt;/sub&gt;</td>
<td>coumarin</td>
<td>osthol</td>
<td>Ondium monnieri Cusson</td>
<td>in vitro, in silico, in vivo</td>
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<td>Neurotensin receptor</td>
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<td></td>
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<tr>
<td>NTS receptor</td>
<td>G&lt;sub&gt;k&lt;/sub&gt;11</td>
<td>cyclotide</td>
<td>cyclopychotide A: 31-residue cyclicpeptide</td>
<td>Psychotria longipes</td>
<td>in vitro</td>
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<td>Opioid receptors</td>
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<tr>
<td>δOR</td>
<td>G&lt;sub&gt;i/o&lt;/sub&gt;</td>
<td>peptides derived from large subunit of spinach D-ribulose-1,5-bisphosphate carboxylase/oxygenase</td>
<td>peptides: YPLDL and YPLDLF (Rubiscolin)</td>
<td>Spinacia oleracea</td>
<td>in vitro, in vivo</td>
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<tr>
<td>δOR, KOR, μOR, NOP</td>
<td>G&lt;sub&gt;i/o&lt;/sub&gt;</td>
<td>divers</td>
<td>82 natural compounds selected based on structural similarity to kown OR ligands</td>
<td>5 plant species (Carthamus tinctorius, Lepidium meyenii Walp etc.)</td>
<td>in vitro</td>
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<tr>
<td>δOR, kOR, μOR</td>
<td>G&lt;sub&gt;i/o&lt;/sub&gt;</td>
<td>BTIQ alkaloids</td>
<td>O-methylcoclaurine (5), N-methylcoclaurine (6), coclaurine (7), neferine (10)</td>
<td>Nelumbo nucifera</td>
<td>in vitro</td>
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<td>δOR, KOR, μOR</td>
<td>G&lt;sub&gt;i/o&lt;/sub&gt;</td>
<td>anthraquinone</td>
<td>hypericin, hyperforin</td>
<td>Hypericum perforatum</td>
<td>in vitro</td>
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<tr>
<td>kOR</td>
<td>G&lt;sub&gt;i/o&lt;/sub&gt;</td>
<td>neoclerodane diterpene</td>
<td>salvoniarin A</td>
<td>Salvia divinorum</td>
<td>in vitro</td>
</tr>
<tr>
<td>Class</td>
<td>G&lt;sub&gt;α&lt;/sub&gt;</td>
<td>Peptide/Compound</td>
<td>Source</td>
<td>Assay</td>
<td>Selective agonist</td>
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<tr>
<td>kOR</td>
<td>G&lt;sub&gt;α&lt;sub&gt;3;3,7,10&lt;/sub&gt;, cyclotides</td>
<td>Carpe 7, 8, 10-13, [T20K]&lt;sub&gt;3&lt;/sub&gt;kalata B1</td>
<td>in vitro</td>
<td>CB with [^3H]-DPN, CAMP, β-interrin</td>
<td>full agonists (inh. CAMP production) with allosteric action mode</td>
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<tr>
<td>µOR</td>
<td>G&lt;sub&gt;α&lt;sub&gt;3;3,7,10&lt;/sub&gt;, peptides with YPFV motif found in soy β-conglycine β-subunit (common to opioid peptide β-casomorphin-4)</td>
<td>soy β-conglycine β-subunit</td>
<td>in vitro</td>
<td>CB with [^3H]-DAMGO, GPI and MVD assays</td>
<td>competitive agonist</td>
</tr>
<tr>
<td>µOR</td>
<td>G&lt;sub&gt;α&lt;sub&gt;3;3,7,10&lt;/sub&gt;, aconitine alkaloids</td>
<td>ignavine</td>
<td>in vitro</td>
<td>CB with [^3H]-diprenorphine, cAMP, receptor internalization, tail flick test</td>
<td>competitive, MD indicates orthosteric binding site, PAM of DAMGO-induced internalisation, in cAMP essay and analgesic assay</td>
</tr>
</tbody>
</table>

**Odorant receptors**

| OR10J5 | G<sub>α</sub> | sesquiterpene | α-cedrene | Cupressus and Juniperus species | in vitro | cAMP, Ca<sup>2+</sup> binding mode not determined, most likely orthosteric, agonist on cAMP and Ca<sup>2+</sup> signaling | single conc. (10, 100µM) | Tong et al., 2017 |

**Orexin receptors**

| OX1, OX2 | G<sub>α</sub>| bisbenzyldioxquinoline alkaloid | neferine | Plumbula nemiiiius | in vitro, in silico | MD, virtual screening, SPR, gene expression profiling | SPR: K<sub>d</sub>=2 nM (OX1R), 10 nM (OX2R) | He et al., 2023 |

**P2Y receptors**

| P2Y1, P2Y12 | G<sub>α</sub>| polyphenols | salvianolic acid A, B and C | Salvia miltiorrhiza (Danshen) | in vitro, in silico | CB with [^3H]-MeSADP, cAMP, IP production, MD, virtual screening | competitive antagonists, docking on orthosteric binding site | CB (P2Y1): K<sub>i</sub>=15 (A), 23 (C) µM; CB (P2Y12): K<sub>i</sub>=20 (A), 36 (B), 15 (C) µM | Liu et al., 2018 |

**Rhodospin**

| Rho | G<sub>i</sub> | flavonoid | quercetin | purchased | in vitro | Modeling, protein stability, Rho activation kinetics, GTPγS binding | Allosteric modulator of rho bound to 9-cis-retinal; positive effect on stability and conformational properties of G90V mutant associated with retinitis pigmentosa | 1 µM | Herrera-Hernández et al., 2017 |

<p>| Rho | G&lt;sub&gt;i&lt;/sub&gt; | flavonoid | quercetin, myricetin, quercetin-3-rhamnoside and myricetin | purchased | in silico, in vitro | MD, thermal shift assay, spectroscopic assays, GTPγS binding, BRET oligomerization | Improved folding and stability causing faster entry of the retinal into ligand binding pocket of | 1-100 µM | Ortega et al., 2019 |</p>
<table>
<thead>
<tr>
<th>Ligand-Free Opsin (allosteric?)</th>
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<tbody>
<tr>
<td>Tachykinin receptor</td>
</tr>
<tr>
<td><strong>NK1</strong></td>
</tr>
<tr>
<td>Taste receptors</td>
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<td><strong>TAS2R</strong></td>
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<td>Vasopressin and Oxytocin receptors</td>
</tr>
<tr>
<td><strong>V1aR, OTR</strong></td>
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</tbody>
</table>

**Abbreviations**: AChE, acetylcholine esterase; AVP, arginine-vasopressin peptide; β-arrestin, β-arrestin recruitment; CB, competition binding; CRE, cAMP response element; CRF, Corticotropin-releasing factor; DIO, diet-induced obesity; GPI, guinea pig ileum; IP, inositol phosphate production; n.d., not determined; MD, molecular docking; MVD, mouse vas deferens; NFAT-RE, nuclear factor of activated T-cell response element; SPR, surface plasmon resonance; SRE, serum response element; SRFRE, serum response factor-response element.
Figure 1