Celtarys RESEARCH

LEGO like chemical conjugation

Innovative chemistry to illuminate biology

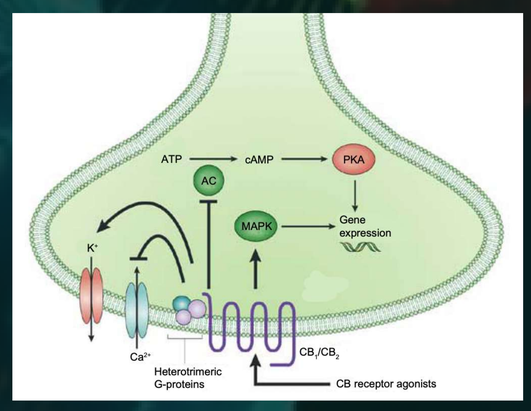
New fluorescent tools to evaluate the therapeutic potential of phytocannabinoids

Receptor binding modes characterization in drug discovery using "**customazible and innovative" receptor binding technologies**.

Novel properties of two new bioactive compounds of cannabis: **Δ9-THCA and Δ9-THCV**.

Speeding up the identification of potential new drugs acting on Cannabinoids receptors using **Celtarys fluorescent ligands**

- Cannabinoid receptors, **CB1 and CB2** are two extensively studied targets of the **GPCR family** for the treatment of numerous conditions, including inflammatory diseases, autoimmune disorders, pain, and cancer.
- **Cannabis sativa L.** has gained interest because, apart from Δ9-THC and CBD, as there are other compounds that are bioactive, by interacting with cannabinoid receptors and/or interacting with a variety of other GPCRs, e.g. GPR18, GPR55.
- Pharmacology of cannabinoids acting on **cannabinoid receptors is complex**. Recent data that have elucidated the structure of the cannabinoid receptors show that the **site of agonist binding** is **not readily available** to extracellular molecules, but the active compounds must enter through the lipid bilayer (1, 2). Also, it has been described the existence of exosites to which agonists of GPCRs may interact and regulate receptor functionality (3). Moreover **GPCRs may interact to form heteromers** whose functional properties are different from those of individually expressed receptors (4, 5).
- Despite the significance of these receptors, researchers **lack reliable analytical tools**, in particular to assess their tissue and **cellular distribution** and understand complex signalling mechanisms.
- Thanks to the use of **innovative receptor binding technologies designed by Celtarys, CELT-335 and CELT-331**, it was possible to characterize through **whole cell direct signaling pathway**s studies, new pharmacological properties derived from the action of Δ9-tetrahydrocannabinolic acid (Δ9-THCA) and Δ9- tetrahydrocannabivarin (Δ9-THCV) on CB1R, CB2R and CB1-CB2Hets.

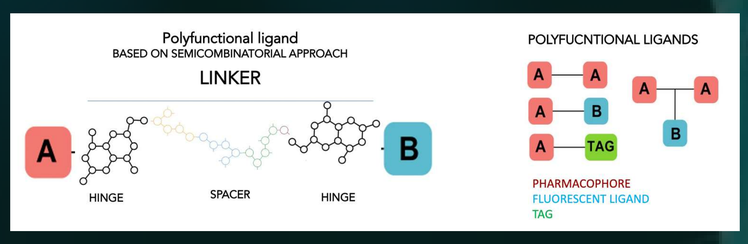


Agonist activation of a neuronal CB receptors and consequent downstream effects (6).

OUR TECHNOLOGY

Celtarys **accelerates pharmaceutical research** by providing unique fluorescent probes. Proprietary patented synthetic technology allows us to develop **tailor-made fluorescent ligands**.

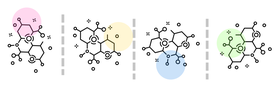
- Know how in MedChem for pharmacophore design.
- Proprietary libraries of more than **20 different hinges and spacers.**
- Design more than **400 different linkers at the same time.**
- Linkers with different **length and chemical properties**.
- Higher diversity in the chemical space.



OUR APPROACH

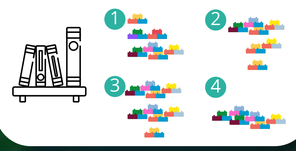
1 We start with the identification of the appropriate pharmacophore for each target, using an integrated approach that includes both **structure-ligand based** drug discovery for compound selection. Thanks to **in silico** experimental structure-activity study we identify the optimal position for pharmacophore **functionalization** and linker introduction. The optimal pharmacophore(s) are tipically identified among a set of **3-5 different chemical scaffolds**.

Pharmacophore identification



2 Once **identified the functionalized pharmacophore** with the best physicochemical and pharmacological profile, we prepare a **library of intermediates** (pharmacophore + linker) using our own LEGO-like Chemical conjugation technology. The biological evaluation of these compounds allows us to identify the optimal linker for the target of interest.

Linkers library generation



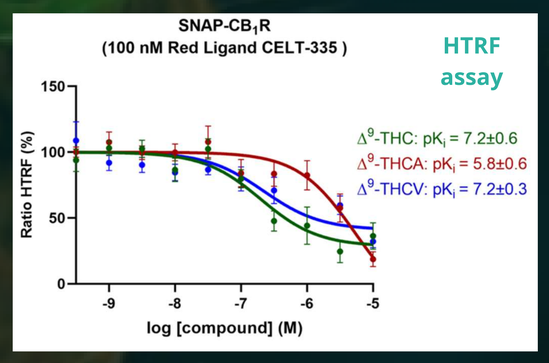
3 In the last phase we combine the previous identified precursors with fluorophores suitable for the kind of assay of interest. The activity of the final molecules, measured in a binding or functional assay, allows us to select the best one(s).

Fluorescent ligand synthesis

OUR WORK

Study effects of new cannabis bioactive compounds **Δ9-THCA and Δ9-THCV** on CB1 and CB2 receptors, Δ9-THC and Δ9-THCV showed, in **living HEK-293T** cells, a higher affinity for CB1R than for CB2R, and Δ9-THCA consistently showed less affinity than the other two compounds (7).

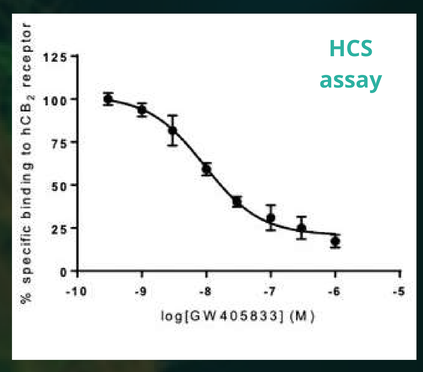
HTRF assay



CELT-335: is a dual ligand for CB1 and CB2 receptors with high affinity [CB1 **Ki: 44.8nM**, CB2 **Ki: 7.4 nM**]. It is labelled with a red emitting fluorescent tag (SulfoCy5) and was designed and optimised for **HTRF-competition binding assays (8).**

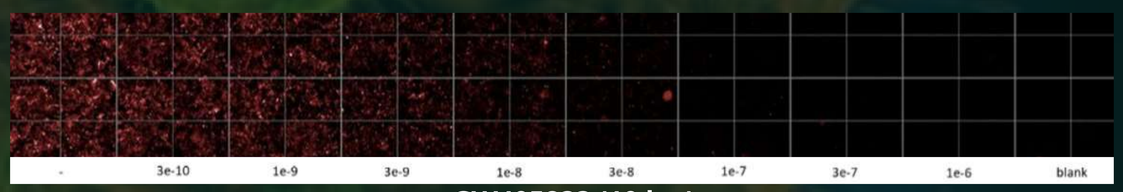
In this other experiment, we evaluated the **concentration-response** curves of GW405833, agonist of the human CB2 receptor, generated from fluorescence intensity of **CELT-331** in a HCS assay in HEK-293 cells expressing human CB2 receptor.

HCS assay



Competition binding between CELT-331 (**at 30nM**) and the CB2 receptor agonist GW405833 (in the range of 1mM to 0.3nm) in HEK-293 cells expressing human CB2 receptor.2

CELT-331: is a selective CB2 ligand exhibiting high affinity [CB2 **Ki: 75.9 nM**]. CELT-331 is labelled with a red emitting fluorescent tag (SulfoCy5) and was specifically optimised for **High Content Screening assay (8)**.



GW405833 (10 log)

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