

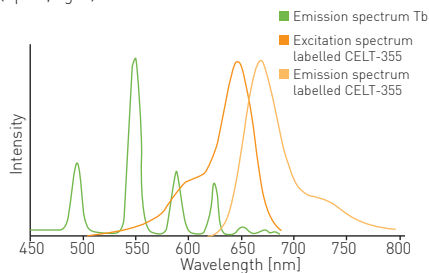
# Differential binding of $\Delta^9$ -tetrahydrocannabinol derivatives to type 1 cannabinoid receptors (CB1)

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- CELT-335 is a dual (CB1/CB2) fluorescent ligand that serves as a TR-FRET acceptor compatible with terbium
- Natural cannabinoids differentially bind to CB1 and CB2 receptors resulting in qualitatively different effects
- CB1-ligand interactions were detected with the PHERAstar<sup>®</sup> FSX at high sensitivity

## Introduction

Cannabinoid receptors are G protein-coupled receptors (GPCRs) that lead to diverse cellular events mediated through different cell signaling pathways. Two main types of cannabinoid receptors exist: CB1 and CB2. CB1 receptors are mainly found in the brain and central nervous system, where they play a role in regulating mood, memory, pain, appetite and motor function. CB2 receptors reside in the immune system and peripheral tissues and are mainly linked to modulating inflammation, immune responses and other functions of the body's defence system. Cannabinoids are potential tools to combat a variety of diseases including different neurological or metabolic conditions. This interest is driving efforts to understand the pharmacology of naturally occurring and synthetic cannabinoids at the receptor level. The results to date show that when different cannabinoids bind to their receptors, they produce distinct effects depending on which signaling pathway is activated. Moreover, the signaling pathway activation depends on the agonist structure and the monomeric or heteromeric cannabinoid receptor states. Here, the PHERAstar FSX microplate reader was used to detect differential binding of natural cannabinoids to CB1 based on TR-FRET assays using terbium and the CELT-335 fluorescent ligand developed by Celtarys Research SI (Spain; fig. 1).



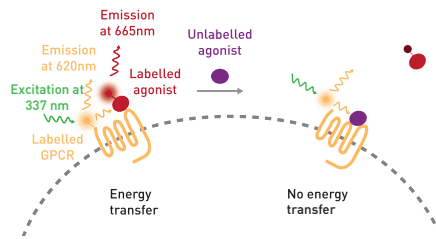
**Fig. 1:** Combinational optical spectra of terbium (Tb) and CELT-335: The terbium (donor) emission spectrum overlaps with the CELT-335 (acceptor) excitation spectrum (spectral overlap).

This ligand was used in assays to assess the pharmacological properties of  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC),  $\Delta^9$ -tetrahydrocannabinolic acid ( $\Delta^9$ -THCA) and  $\Delta^9$ -tetrahydrocannabivarin ( $\Delta^9$ -THCV) on CB1.

## Assay Principle

The fluorescent ligand CELT-335 is a full agonist that binds to the orthosteric site of human cannabinoid receptors and bears a highly hydrophilic fluorophore compatible with TR-FRET. The tagged agonist binds tightly to the CB1 GPCR labelled with terbium. When agonist and receptor come

into proximity, energy is transferred based on a spectral overlap of terbium emission and CELT-335 excitation and light is emitted at 665 nm with terbium as the TR-FRET donor (fig. 2).



**Fig. 2:** Assay Principle: TR-FRET assay using CELT-335, a dual (CB1/CB2) fluorescent ligand that serves as a TR-FRET acceptor.

## Materials & Methods

- HEK-293 T cells (lot 612 968, ATCC)
- SNAP-tag<sup>®</sup> (New England Biolabs)
- Tag-lite (Cisbio-Perkin Elmer)
- CELT-335 (Celtarys Research SI)
- PHERAstar FSX microplate reader (BMG LABTECH)

### Experimental procedure

HEK-293 T cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) and maintained at 37°C in a humidified atmosphere of 5% CO<sub>2</sub> as previously reported.<sup>1</sup>

Competition binding experiments were performed in HEK-293 T cells transfected with 1  $\mu$ g cDNA for SNAP-CB1Ra and receptors were labelled with terbium as a TR-FRET donor. Competition binding curves were obtained by TR-FRET using 100 nM CELT-335 and 0–10  $\mu$ M  $\Delta^9$ -THC,  $\Delta^9$ -THCA and  $\Delta^9$ -THCV.

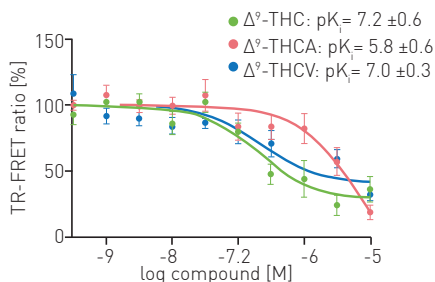
The TR-FRET ratio was calculated based on the ratio of signal at 665 nm (acceptor) X 10,000 divided by signal at 620 nm (donor). Data were normalized to the TR-FRET ratio at the highest value (set to 100 %).

### Instrument Settings

Optic settings	Time-resolved fluorescence, plate mode endpoint, SDE	
	Optic Module	Ex: 337 Em1: 665 Em2: 620
Integration time	Delay: 60 $\mu$ sec, Time: 400 $\mu$ sec	
General settings	Number of flashes	40
	Settling time	0.1 s

## Results & Discussion

TR-FRET assays provided sensitive and robust measurements for competition experiments looking at the binding of CELT-335 and cannabinoid substrates to living HEK-293 T cells expressing CB1. Competition binding curves were obtained using 100 nM CELT-335 and increasing concentrations of  $\Delta^9$ -THC,  $\Delta^9$ -THCA and  $\Delta^9$ -THCV (0 – 10  $\mu$ M). Figure 3 shows that competition was similar for  $\Delta^9$ -THC and  $\Delta^9$ -THCV. The  $pK_i$  values obtained for  $\Delta^9$ -THC and  $\Delta^9$ -THCV were  $7.2 \pm 0.6$  and  $7.2 \pm 0.3$ , respectively, whereas the affinity for  $\Delta^9$ -THCA was considerably lower ( $pK_i$   $5.8 \pm 0.6$ ).



**Fig. 3:** TR-FRET competition assay between CELT-335 [TR-FRET acceptor] and  $\Delta^9$ -THC,  $\Delta^9$ -THCA and  $\Delta^9$ -THCV. Data represent the mean  $\pm$  SEM (n = 5 in triplicates).

The data obtained using CELT-335 as TR-FRET acceptor are comparable to those reported in radioligand binding assays (Table 1).

**Table 1 Comparison of binding affinities using radioligand binding or TR-FRET assays.** Radioligand binding data are from [2,3].

Compound	Affinity ( $pK_i$ )	
	Radioligand binding assay	TR-FRET
$\Delta^9$ -THC	7.3-7.4	7.2
$\Delta^9$ -THCV	7.12 [ $K_i=75.4$ nM]	7.2 [ $K_i=63$ nM]
$\Delta^9$ -THCA	5.5 [ $K_i=3.1$ $\mu$ M]	5.8 [ $K_i=1.6$ $\mu$ M]

## Conclusion

CELT-335 is a robust TR-FRET acceptor readily coupled with terbium as donor. CELT-335 showed high affinity for the CB1 cannabinoid receptor subtype and binding affinity values obtained for  $\Delta^9$ -THC,  $\Delta^9$ -THCA and  $\Delta^9$ -THCV were comparable to values reported in the literature.<sup>2,3</sup> TR-FRET assays provide a reliable way to measure binding affinities for cannabinoid and other receptors and the PHERAstar FSX is the ideal microplate reader to detect even the smallest changes in GPCR-ligand interactions.

## References

1. Raich I *et al.*, Similarities and differences upon binding of naturally occurring  $\Delta^9$ -tetrahydrocannabinol-derivatives to cannabinoid CB1 and CB2 receptors, *Pharmacological Research* [2021] 174: 105970, doi: 10.1016/j.phrs.2021.105970
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