

Investigating Minor Phytocannabinoids using Kinetic Signalling Assays

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INTRODUCTION

- Medicinal cannabis has been used by humans for millennia and is indicated for many health disorders¹.
- The major pCBs, delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD) are well characterised. However, there are nearly 150 pCBs which remain understudied¹.
- Phytocannabinoids exert their effect, through many targets, one of which is, a G-Protein coupled receptor, Cannabinoid 1 receptor (CB1)².

This project aimed to develop a biosensor to determine the pharmacological effect of understudied minor phytocannabinoids at the CB1 receptor.

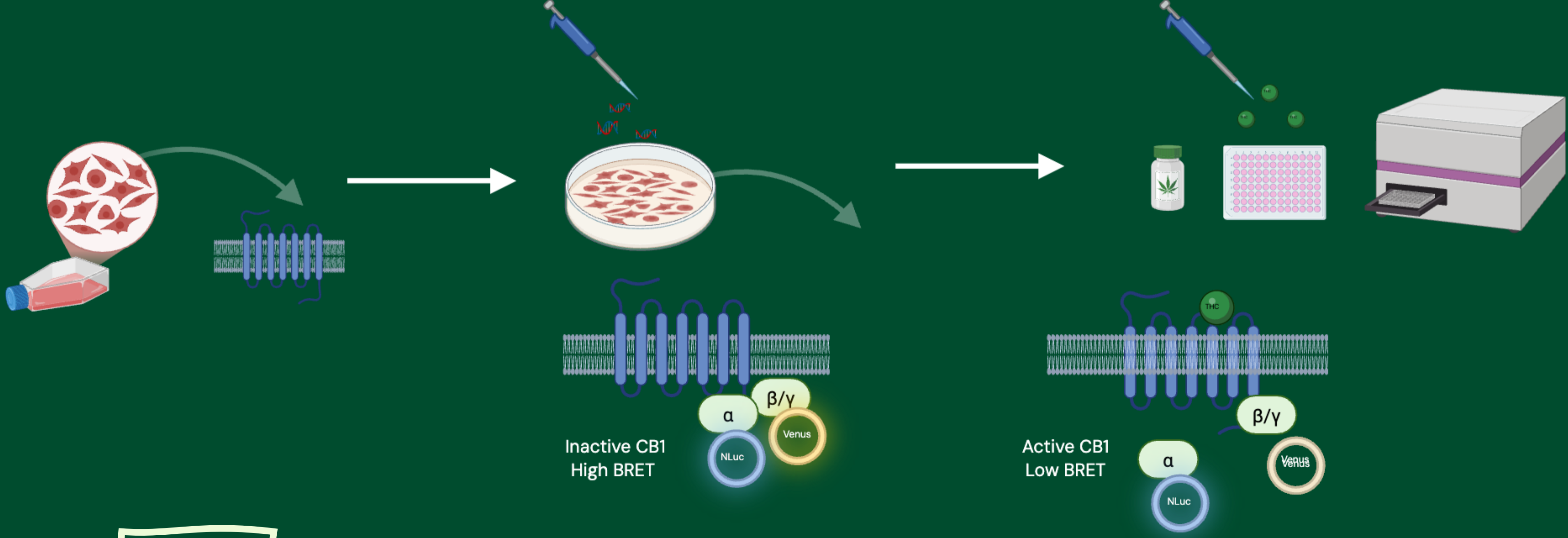
METHODOLOGY

To investigate the activity of minor pCBs, a biosensor measuring CB1 activation was utilised.

Stable Human CB1 in HEK293T cells

G-CASE BRET GPCR sensor

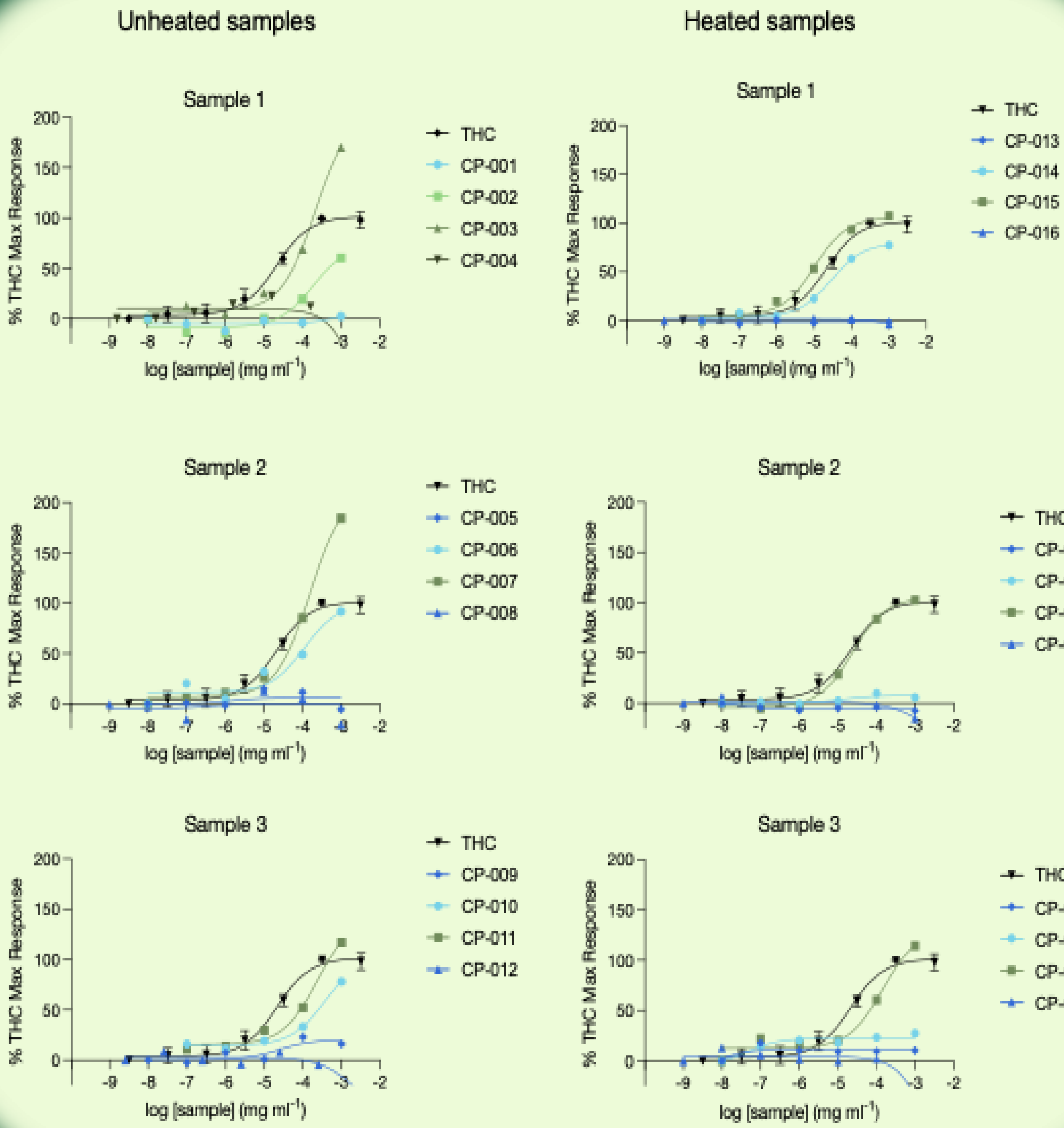
Test crude Cannabis Sativa L samples
THC positive control



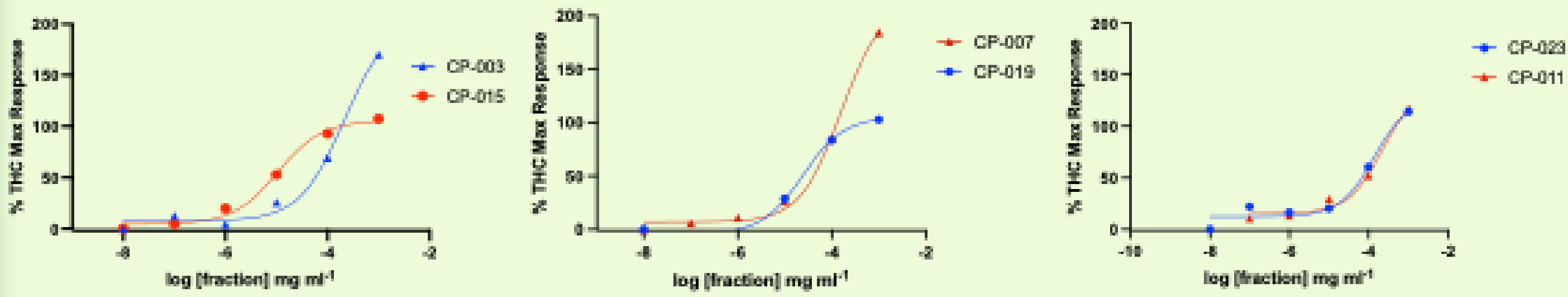
RESULTS 1.2
Efficacy and potency values

Unheated Fraction	CP-002	CP-003	CP-006	CP-007	CP-010	CP-011	THC
logEC ₅₀ *	0.2681	-5.946	-6.451	-4.739	-2.688	-3.897	-7.18
E _{max} **	107.8	169.3	60.41	170.8	135.6	110.2	
Heated Fraction	CP-014	CP-015	CP-018	CP-019	CP-022	CP-023	
logEC ₅₀ *	-4.715	-5.084	NR	-4.735	NR	-4.288	
E _{max} **	69.46	98.95	NR	95.92	NR	90.84	

RESULTS 1.1
Initial sample screening



RESULTS 2
Investigate the effect of heat on samples



DISCUSSION

- The biosensor was validated, as THC potency was comparable to previous literature³.
- We identified *Cannabis Sativa L.* fractions that displayed activity at CB1.
- Three fractions displayed efficacy that was comparable, if not greater, than THC. This suggests that minor phytocannabinoids have pharmacological activity at CB1.

FUTURE DIRECTIONS

- Phytocannabinoids may signal via an alternative pathways, that are not detected by this biosensor^{4,5,6}. Further characterisation of fractions using bioassays that measure alternative signalling pathways.
- Further investigation should be conducted with other established CB1 drugs, to validate the biosensor.
- Bioassays with higher throughput should be developed, to facilitate screening of more *Cannabis Sativa L.* samples.

CONCLUSION

- A medium throughput screening assay to identify CB1 activation by *Cannabis Sativa* fractions was established and validated.
- Further biosensor development, allowing for alternative read outs and higher throughput should be conducted.

SIGNIFICANCE

This assay enables screening of previously uncharacterised samples containing understudied phytocannabinoids, generating novel knowledge to support medicinal cannabis research.

