# *In vitro* and *in silico* biological target screening of cannabinoids

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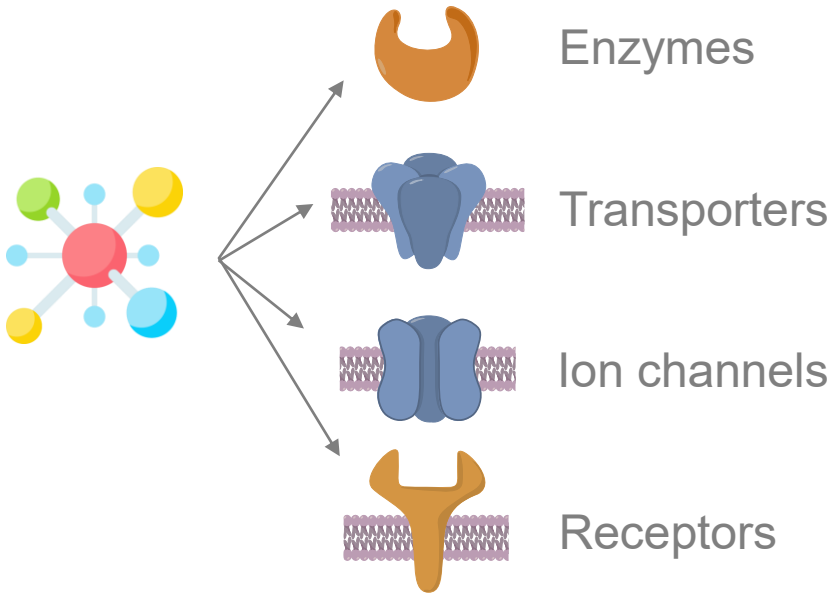
U.S. FOOD & DRUG ADMINISTRATION

Human Foods Program
Office of Laboratory Operations & Applied Science
Office of Chemistry & Toxicology

# Biological targets and adverse outcome pathways

**Molecular Initiating Event** → **Key Events** ... → **Adverse Outcome**

*Interactions between chemicals and biological targets*

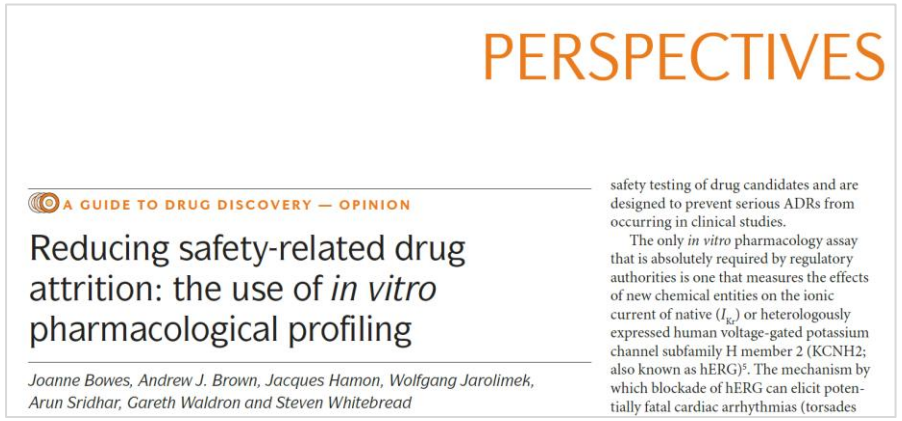



*Changes in cellular, tissue, or organ function*

*Observed toxicity*

Predict binding by *in silico* methods
Measure binding by *in vitro* methods

# Safety-related biological target screening panel



PERSPECTIVES

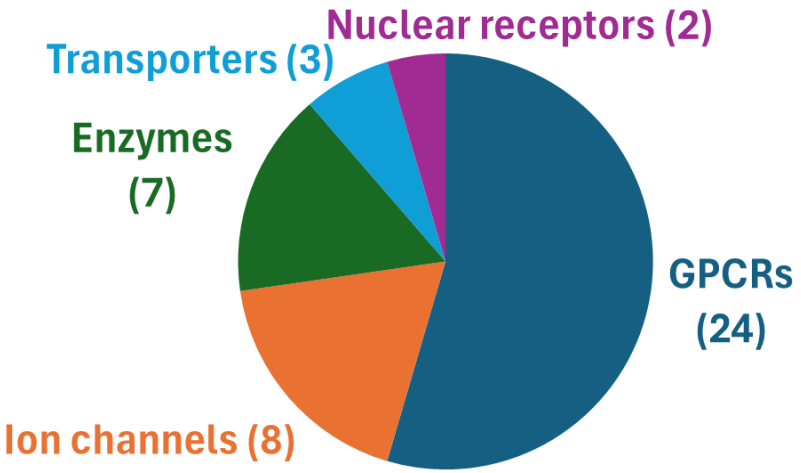
A GUIDE TO DRUG DISCOVERY — OPINION

Reducing safety-related drug attrition: the use of *in vitro* pharmacological profiling

*Joanne Bowes, Andrew J. Brown, Jacques Hamon, Wolfgang Jarolimek, Arun Sridhar, Gareth Waldron and Steven Whitebread*

safety testing of drug candidates and are designed to prevent serious ADRs from occurring in clinical studies.

The only *in vitro* pharmacology assay that is absolutely required by regulatory authorities is one that measures the effects of new chemical entities on the ionic current of native ($I_{Kr}$) or heterologously expressed human voltage-gated potassium channel subfamily H member 2 (KCNH2; also known as hERG)[5]. The mechanism by which blockade of hERG can elicit potentially fatal cardiac arrhythmias (torsades




- Acetylcholine
- Adenosine
- Andrenergic
- Cannabinoid
- Dopamine
- GABA
- Glutamate
- Histamine
- Opioid
- Serotonin

- Calcium
- Chloride
- Potassium (hERG)
- Sodium

- Acetylcholinesterase
- Cyclooxygenases
- Monoamine oxidase
- Phosphodiesterases

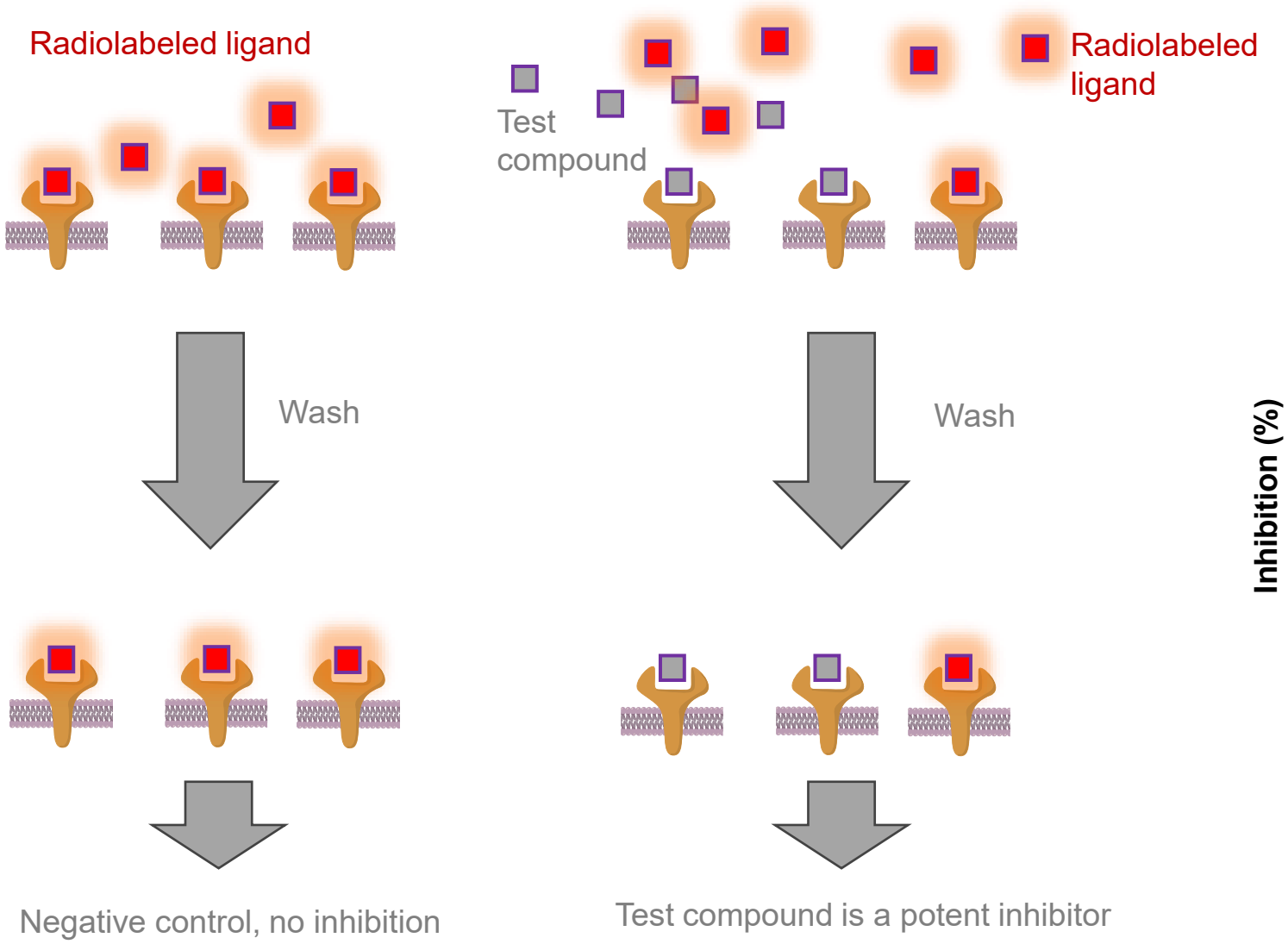
- Dopamine
- Norepinephrine
- Serotonin

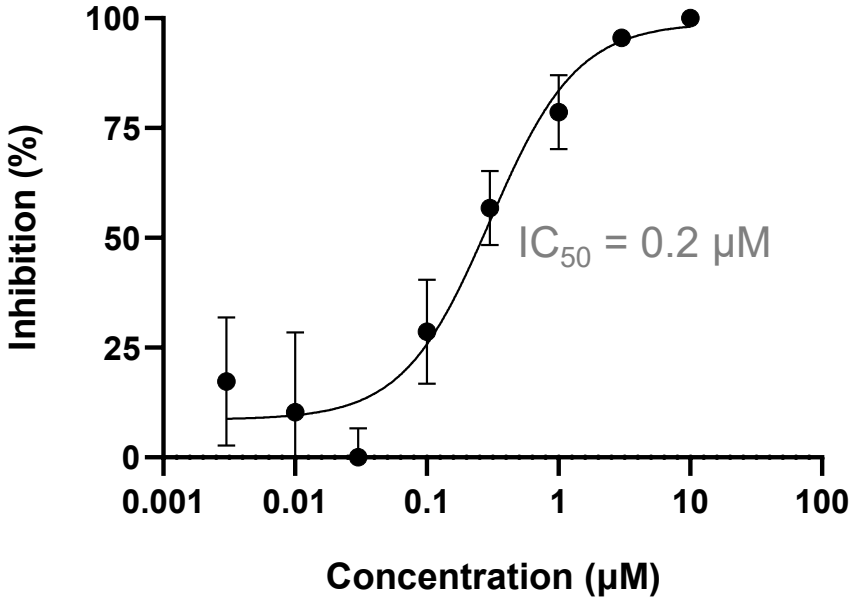
- Androgen
- Aryl hydrocarbon

Bowes et al, *Nat Rev Drug Discov* 2012, 11, 909-922

Brennan et al, *Nat Rev Drug Discov* 2024, 23, 525-545

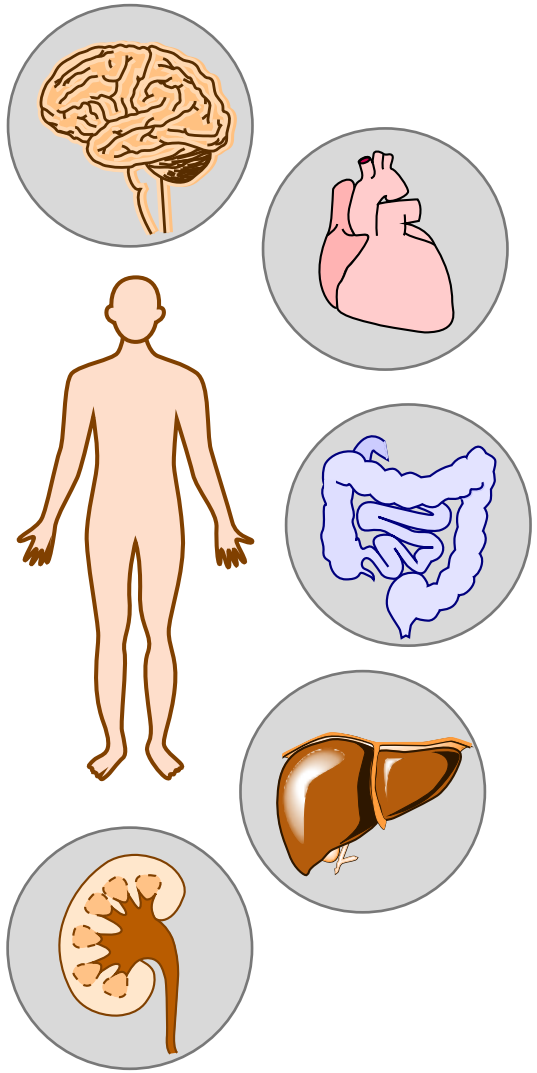
# In vitro assays for measuring target binding



# CB1 cannabinoid receptor

# CB1 receptor and adverse health effects



- Arrhythmia
- Bradycardia
- Cardiotoxicity
- Cardiovascular disorder
- Tachycardia

- Diarrhoea
- Nausea
- Vomiting

- Bronchodilation
- Dyspnoea
- Respiratory depression
- Hypertension
- Hypotension

- Aggression
- Agitation
- Anxiety
- Delirium
- Depression
- Drug abuse
- Drug dependence
- Dysphoria
- Eating disorder
- Euphoric mood
- Hallucination
- Intentional self-injury
- Nervousness
- Panic attack
- Paranoia
- Psychotic disorder
- Sleep disorder

- Cognitive disorder
- Disturbance in attention
- Dizziness
- Headache
- Hypokinesia
- Memory impairment
- Motor dysfunction
- Nervous system disorder
- Neurotoxicity
- Sedation
- Seizure
- Somnolence
- Hypothermia

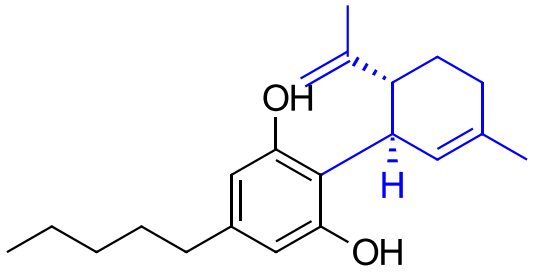
Level of evidence (Off-X database)

Medium High Very high

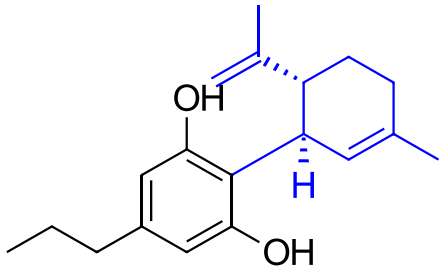
Clarivate Off-X database, www.targetsafety.info

# CB1 receptor: Non-THC type cannabinoids

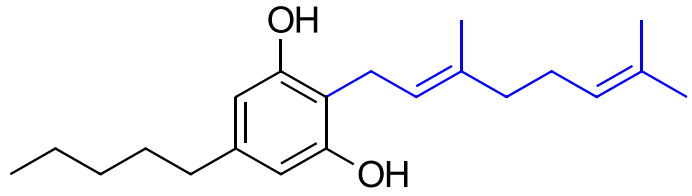
Weak agonists at CB1 receptors (micromolar range) and do not cause intoxicating effects




CBD
Ki ~ 4 μM




CBDV
Ki > 10 μM

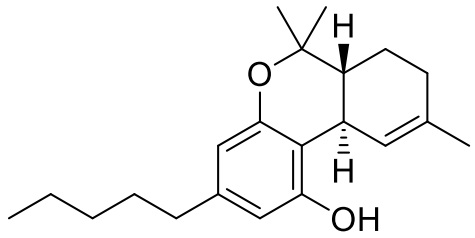



CBG
Ki ~ 1 μM

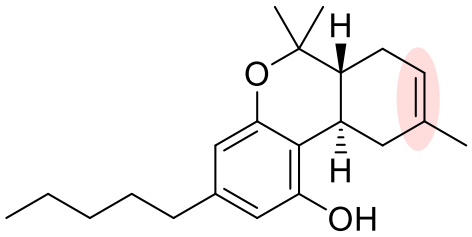
Nelson et al, *J Med Chem* 2020, 63, 12137-12155.

PB Samson, *J Nat Prod* 2021, 84, 142-160

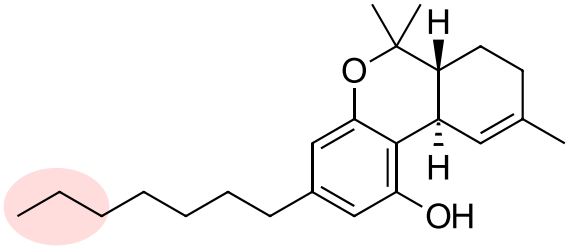
# CB1 receptor: THC-type cannabinoids



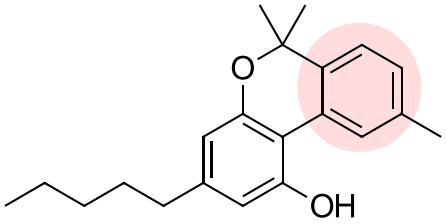
Δ9-THC
Ki ~ 30 nM



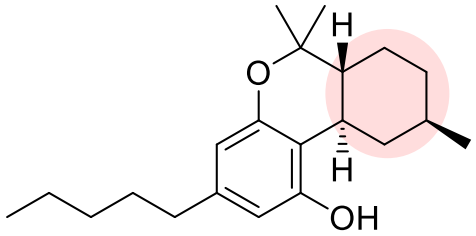
Δ8-THC
Ki ~ 30 nM



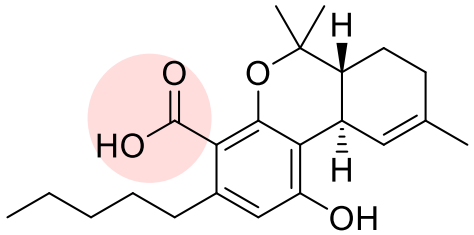
Δ9-THCP
Ki ~ 1 nM



CBN
Ki ~ 220 nM



HHC
Ki ~ 150 nM



Δ9-THCA-A
Ki ~ 800 nM


C Prandi et al, *Molecules* 2018, 23,1526.

EW Bow et al, *Persp Med Chem* 2016, 8

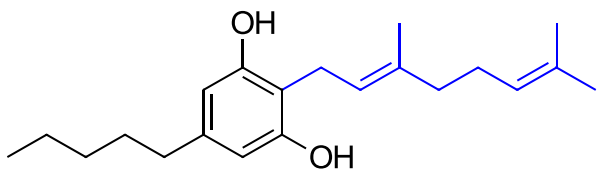
PB Samson, *J Nat Prod* 2021, 84, 142-160

RK Razdan, *Pharmacol Rev* 1986, 38(2), 75-149

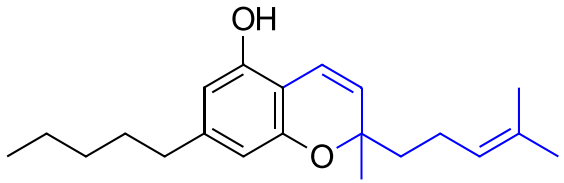
MH Ree et al, *J Med Chem* 1997, 40, 3228–3233.

# Additional safety-related biological targets

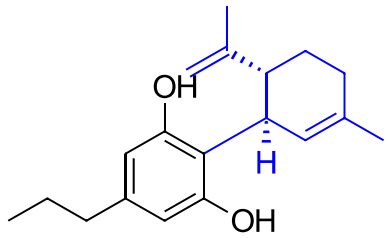
# Cannabinoids selected for in vitro screening



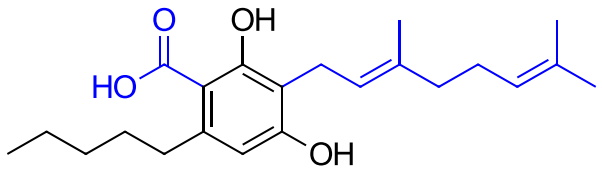

CBG



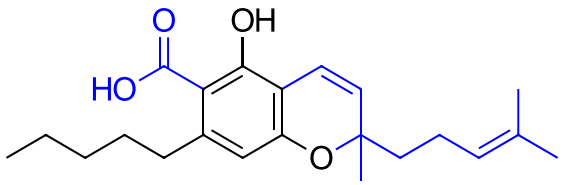

CBC



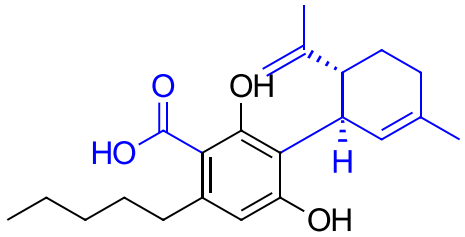

CBDV



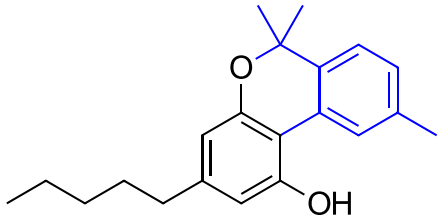

CBGA




CBCA

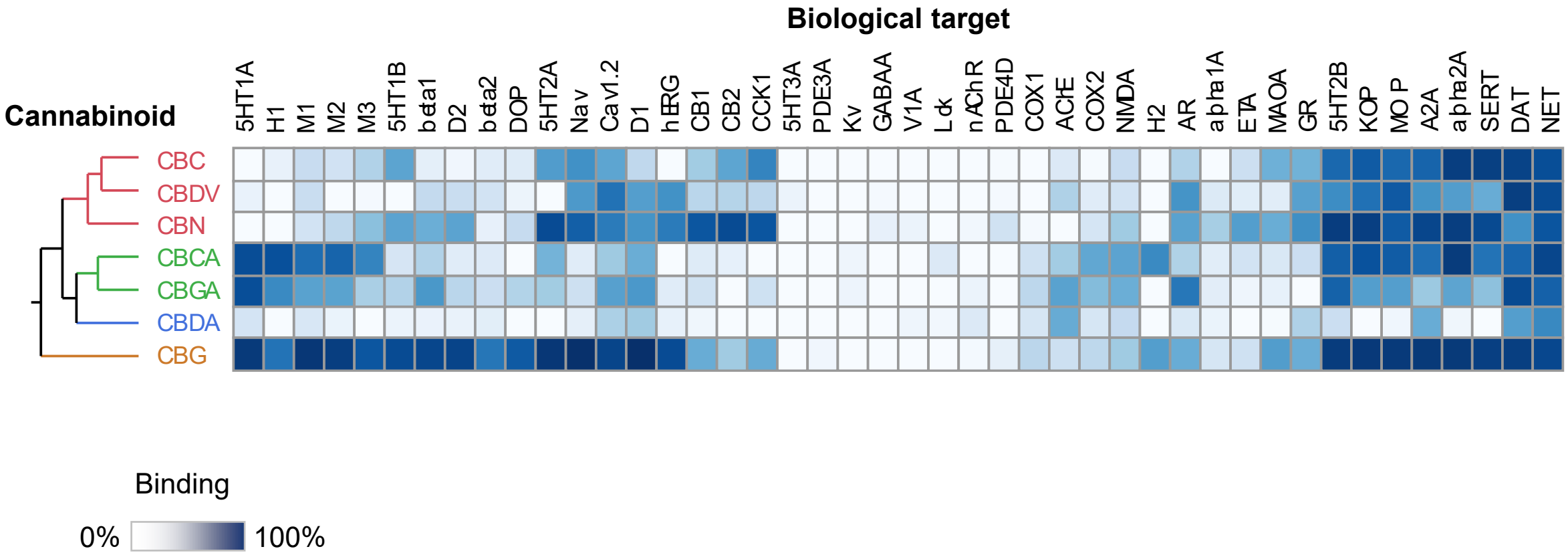



CBDA



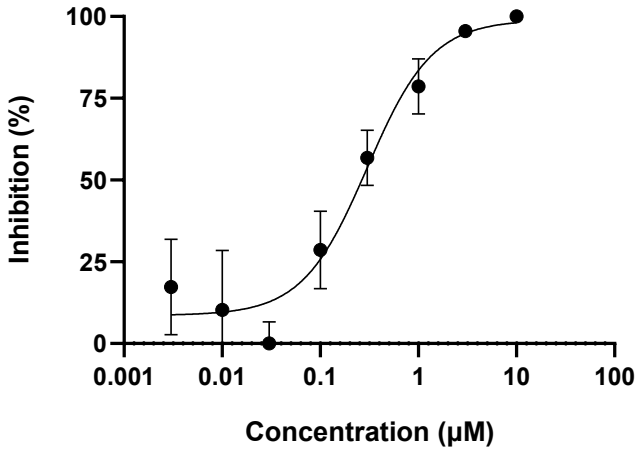

CBN

# Target binding to cannabinoids (10 µM)



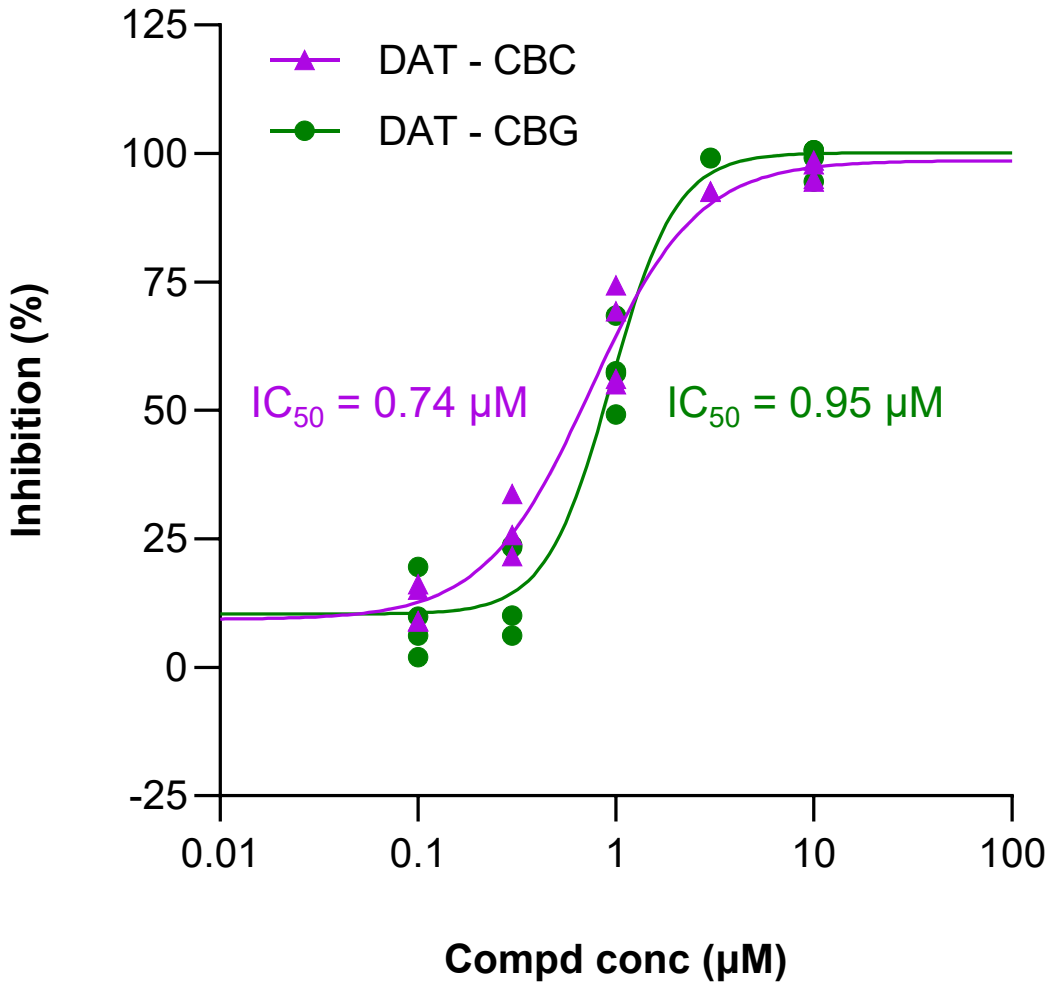

M Santillo et al, *J Nat Prod* 2024, 87, 2881-2886.

# Binding potency ($IC_{50}$, μM) from conc-resp curves




| | CBG | CBDA | CBGA | CBCA | CBN | CBDV | CBC |
|---|---|---|---|---|---|---|---|
| **5-HT2A** | 1.88 | | | | 4.89 | | |
| **DAT** | 0.95 | | 3.88 | | | 3.00 | 0.74 |
| **H1** | | | | 5.70 | | | |
| **hERG** | 3.06 | | | | | | |
| **MOP** | 1.38 | | | 10.80 | 3.21 | 6.42 | |
| **NET** | 2.45 | | | 3.22 | 1.15 | 2.66 | 1.95 |
| **SERT** | 1.66 | | | | 3.35 | | 6.26 |

# Most potent binding (dopamine transporter, DAT)



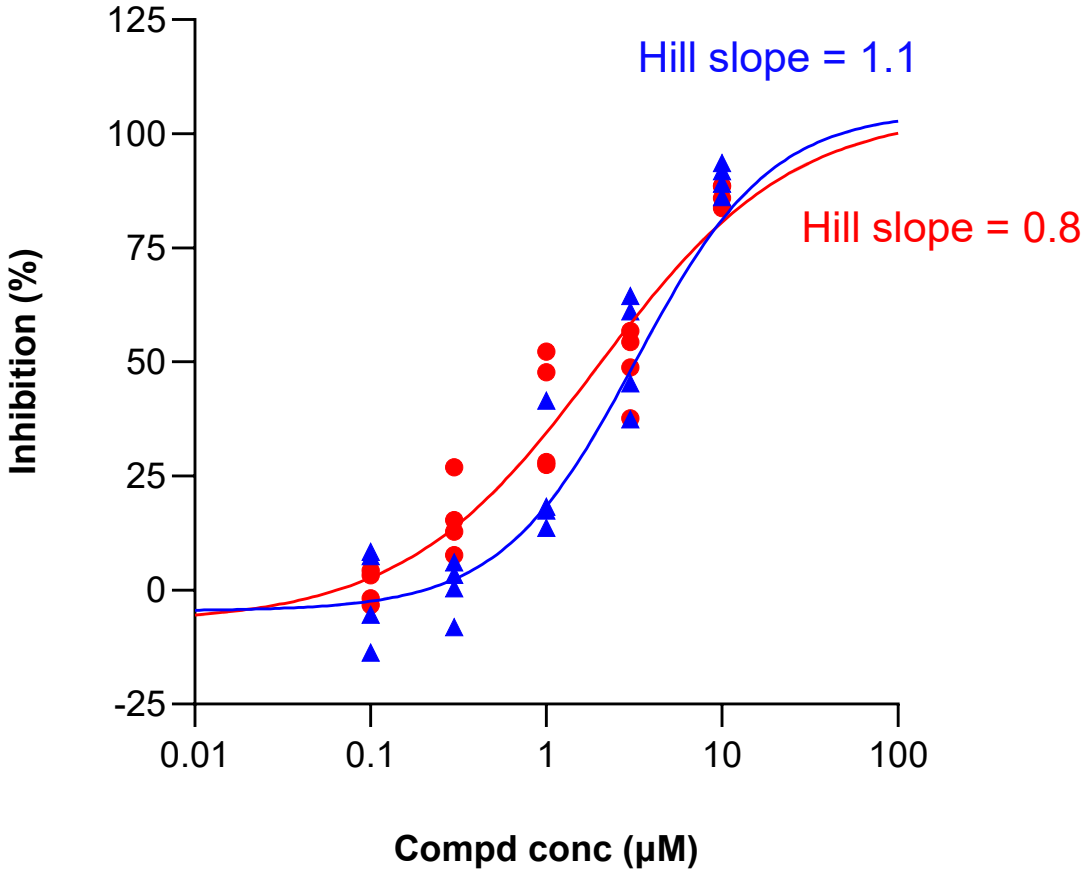

**Adverse effects for DAT inhibition**

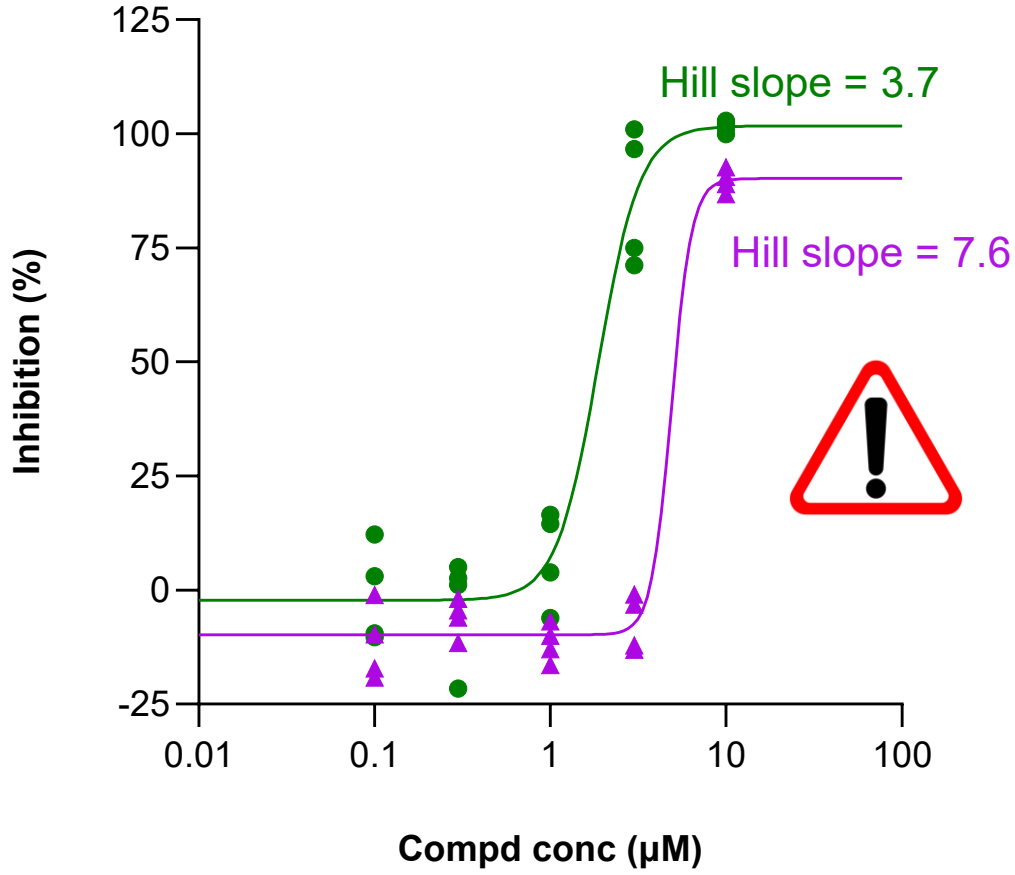
| | |
|---|---|
| Anxiety | Tachycardia |
| Depression | Dry mouth |
| Drug abuse | Nausea |
| Drug dependence | Dyskinesia |
| Insomnia | Dystonia |
| Psychostimulation | Headache |
| Stereotypy | Parkinsonism |
| Acne | Psychomotor hyperactivity |
| Hyperhidrosis | Seizure |
| | Tremor |

Level of evidence (Off-X database) High Very high

Clarivate Off-X database, www.targetsafety.info

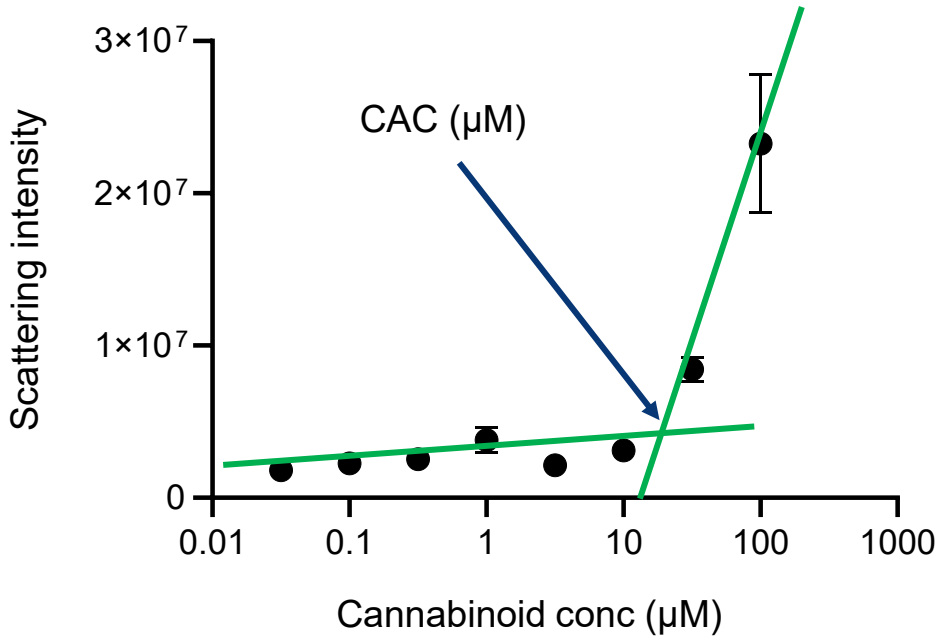
# Differences for Hill slopes



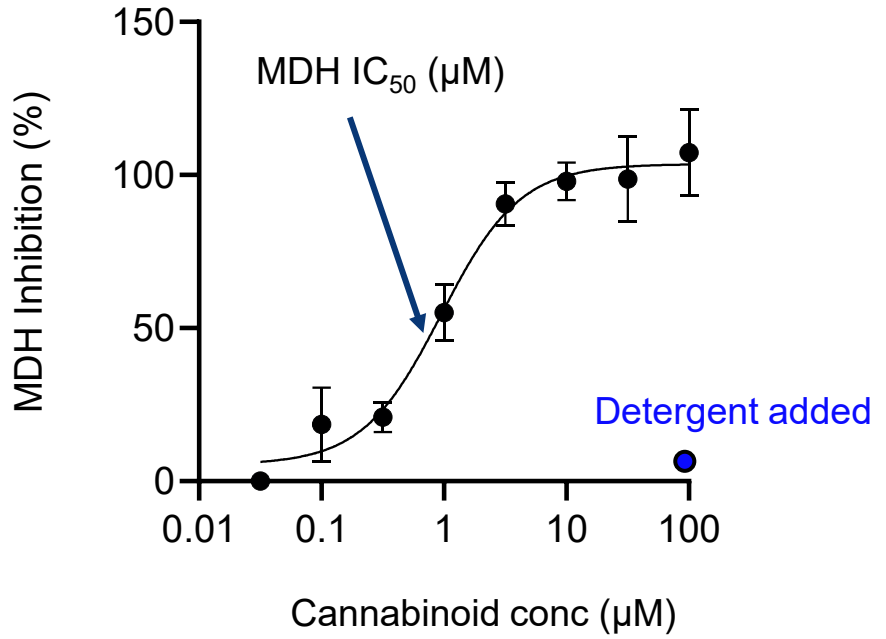
# Methods to determine colloidal aggregation

## Dynamic light scattering



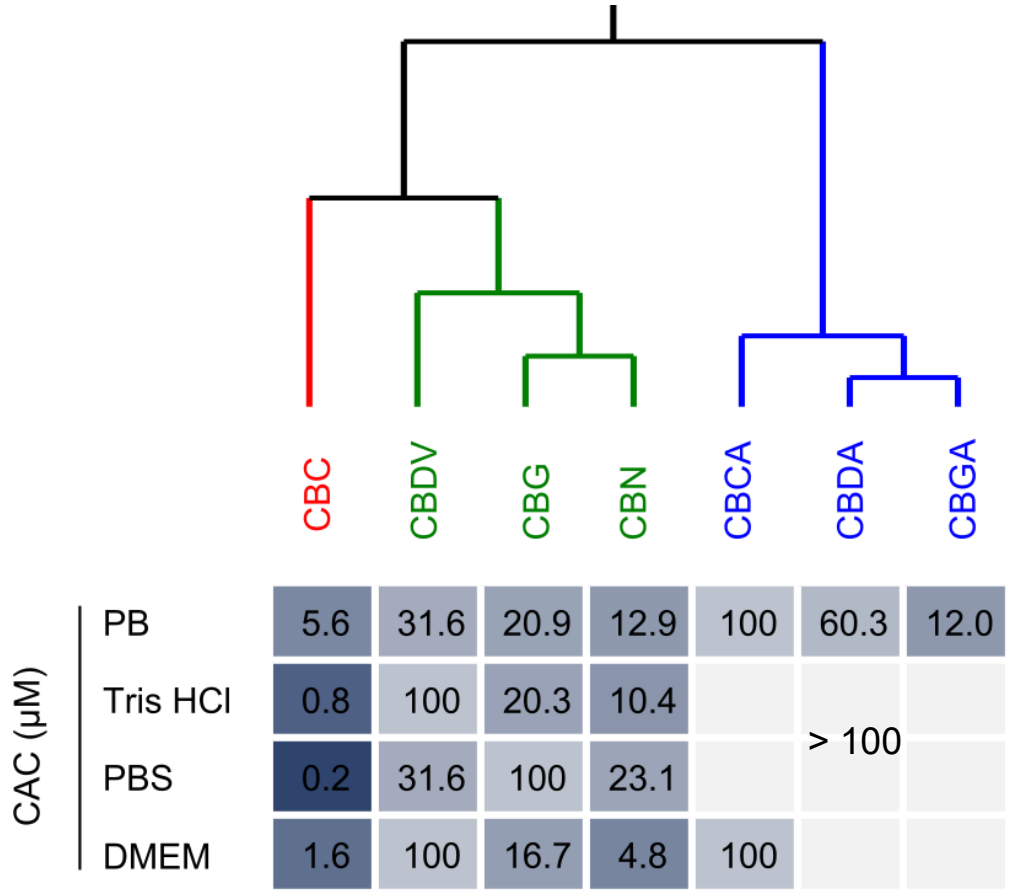

Determined **critical aggregation conc (CAC)** of cannabinoids in PB, Tris HCl, PBS, DMEM

## Detergent-sensitive malate dehydrogenase (MDH) inhibition assay




**MDH $IC_{50}$** reflects aggregation conc

# Summary of colloidal aggregation of cannabinoids

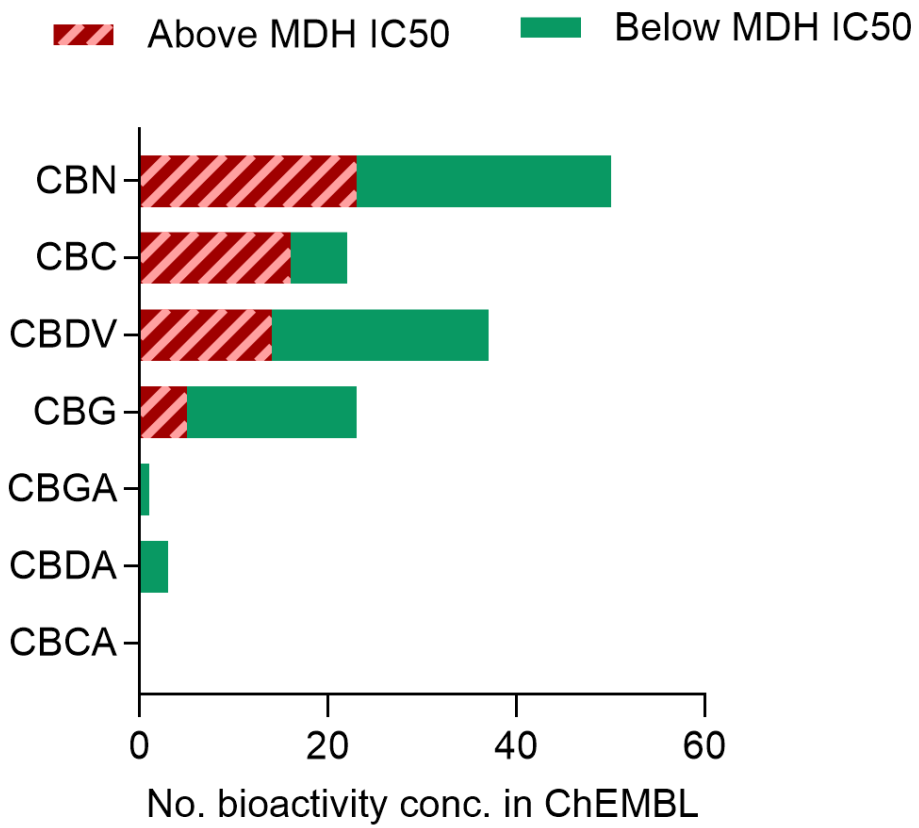



Aggregation in pure buffers and media do not reflect exact conditions in biological target assays, so aggregation must be confirmed for each assay

# Implications of cannabinoid aggregation beyond this study

ChEMBL 2.8 M compounds 24.2 M bioactivities

| Compound | Assay | $IC_{50}$, $EC_{50}$, or $K_i$ (μM) |
|---|---|---|
| CBDV | SH-SY5Y cell viability | 55.00 |
| CBC | TRPA1 channel agonism | 0.06 |
| CBDV | CYP1A1 inhibition | 1.60 |
| CBG | CB2 receptor binding | 2.70 |
| … | … | … |



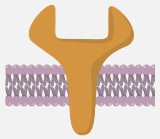
# In silico target predictions

Query compound
(unknown binding)

Molecular features

QSAR model

Chemotargets
**CLARITY®**



Predicted compound-target binding
(Ki or $IC_{50}$, μM)

- Peroxisome proliferator-activated receptors (PPAR)
- Transient receptor potential channels (TRP)
- Retinoic acid receptors
- Steroidogenic factor-1
- Arachidonate lipoxygenases

M Santillo et al, *J Appl Toxicol* 2023, 43, 1476-1487.

# Conclusions and future directions

- There are over 100 cannabinoids, many of which lack safety data
- Screening interactions between cannabinoids and safety-related biological targets is a rapid, inexpensive, high-throughput means to identify potential adverse health effects
- Despite similarity among cannabinoids, subtle differences among their structures lead to changes in biological target binding profiles
- Depending on the targets and concentrations tested, cannabinoids may nonspecifically inhibit targets due to colloidal aggregation, leading to false positives
- Aggregation needs to be evaluated in specific assay conditions for various target assays as confirmation
- Biological target binding data can prioritize subsequent testing, support read across predictions of toxicity, and identify cannabinoids that may contribute to additive (cumulative) adverse health effects

FDA

# Acknowledgements


- Jeffrey Yourick
- Robert Sprando
- HFP Scientific Computing Team


- Brian Shoichet
- Isabella Glenn
- Lu Paris