UNIVERSITÄTS KLINIKUM FREIBURG

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Metabolism of new synthetic cannabinoids and detection of metabolites in urine samples

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Agenda

Scope of research - Why is metabolism a field of interest?

Some basics - What is metabolism?

Methods - How to discover metabolites?

Metabolism of new synthetic cannabinoids - Important facts

Further goals and challenges - What are the next steps?

Scope

Why is metabolism a field of interest?

Prevalence of
new synthetic cannabinoids

?

?

?

Abstinence control
- Drivers license
- Conditions of probation
- Withdrawal therapy

?

Need for drug screening methods

Identification of consumed substance

?

Urine is the most common material for drug screening

No parent compound detectable in urine!

Better **understanding of drug effects**

Active metabolites?

Studies on metabolism

Identification of main metabolites

Metabolites are mostly **detectable** for a **longer period** then their parent compounds

Optimised drug screening methods

Prove of consumption

Metabolism

General basics

What is drug metabolism? (from Greek: μεταβολή *metabolē*, "change")

Organism detoxification by a set of xenobiotic-metabolising enzymes. In human there are two phases of metabolism.

Phase I: Functionalisation

(mainly oxidation by CYP 450 isoenzymes)

Phase II: Conjugation

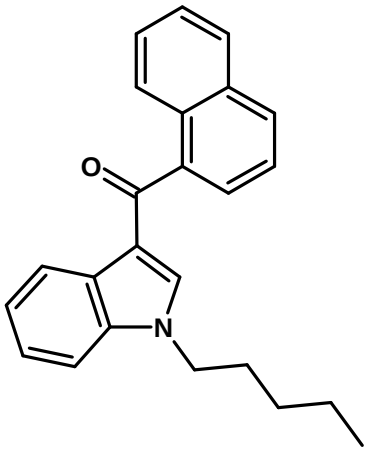
(mainly with glucuronic acid by glucuronidyltransferases)

Metabolism

General basics

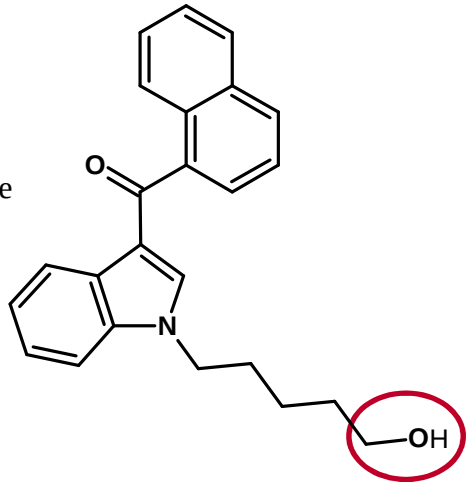
Phase I

Phase II



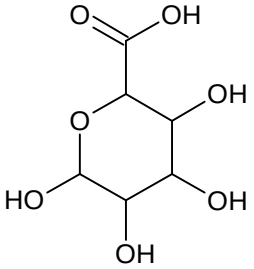
JWH-018

CYP 450 enzyme



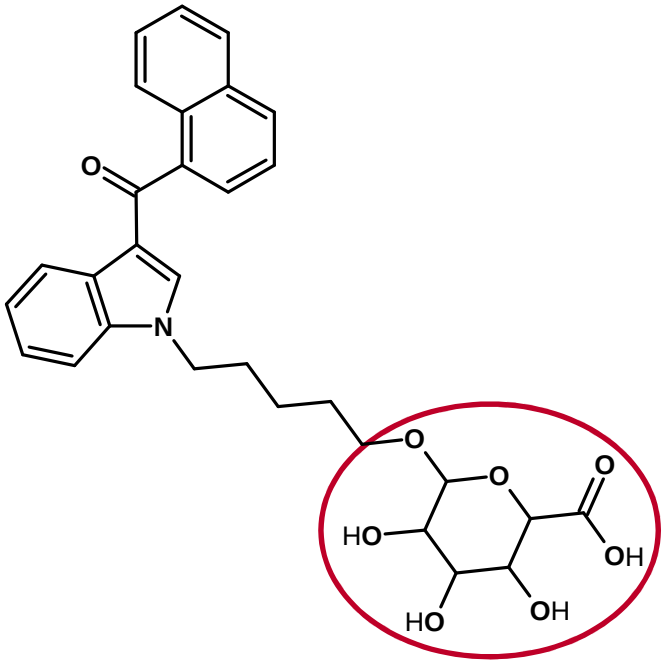
JWH-018 5-Hydroxy-pentyl metabolite

Glucuronic acid



Glucuronyltransferase

Glucuronidase



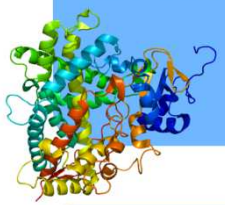
JWH-018 Glucuronide metabolite

Solubility in water

Methods

How can we discover metabolites?

in vitro

Isolated CYP 450 isoenzymes	**Human liver microsomes (HLM)**	**Human hepatocytes**
		
Phase I metabolites	**Phase I** metabolites	**Phase I+II** metabolites

Process of metabolites identification

Our approach of choice

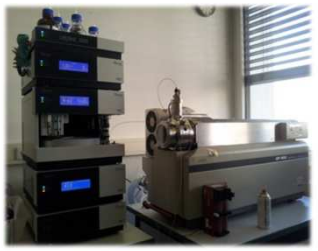
New synthetic cannabinoid

Human liver microsomes (*in vitro*)

LC-MS/MS identification method

Hypothetical main metabolites

Upgrade LC-MS/MS screening method



Detected in human urine sample (*in vivo*)

LC-MS/MS identification method

Actual human main metabolites

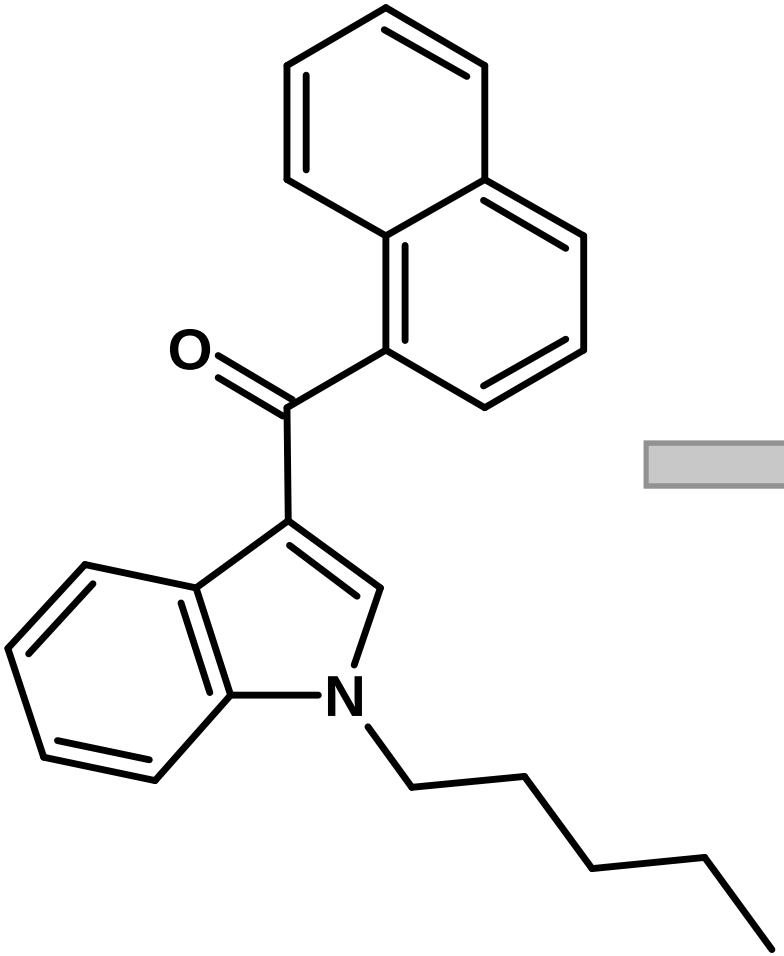
New knowledge of metabolic pathways

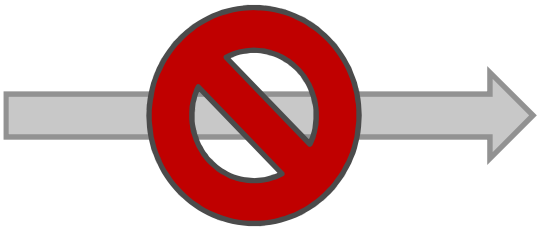
Optimised LC-MS/MS screening method

Metabolism of synthetic cannabinoids

Some important facts

1.





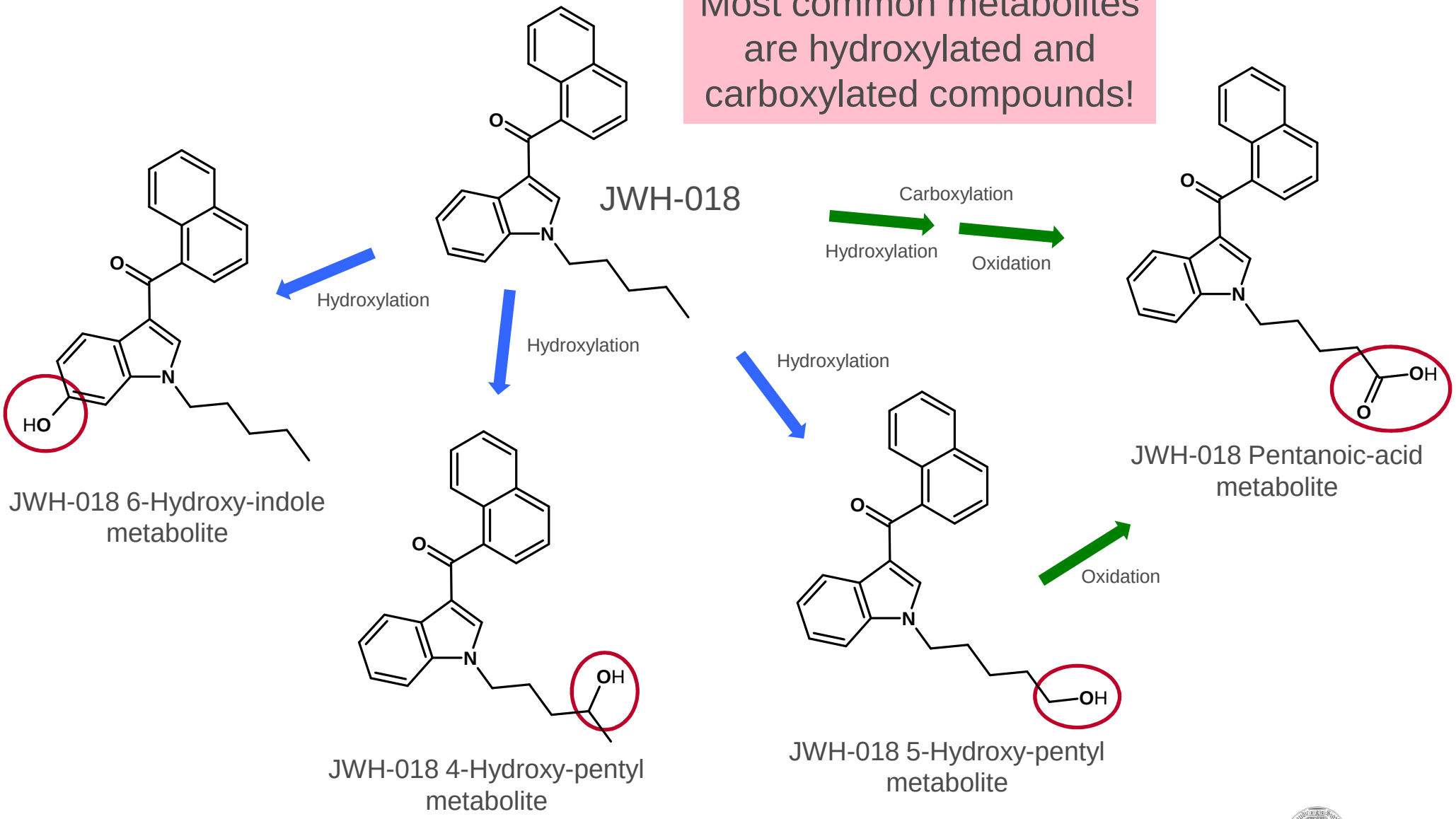
Parent compounds are mostly not detectable in urine samples! (e.g. JWH-substances)

Metabolism of synthetic cannabinoids

Some important facts

2.

Most common metabolites are hydroxylated and carboxylated compounds!



JWH-018 6-Hydroxy-indole metabolite

JWH-018 4-Hydroxy-pentyl metabolite

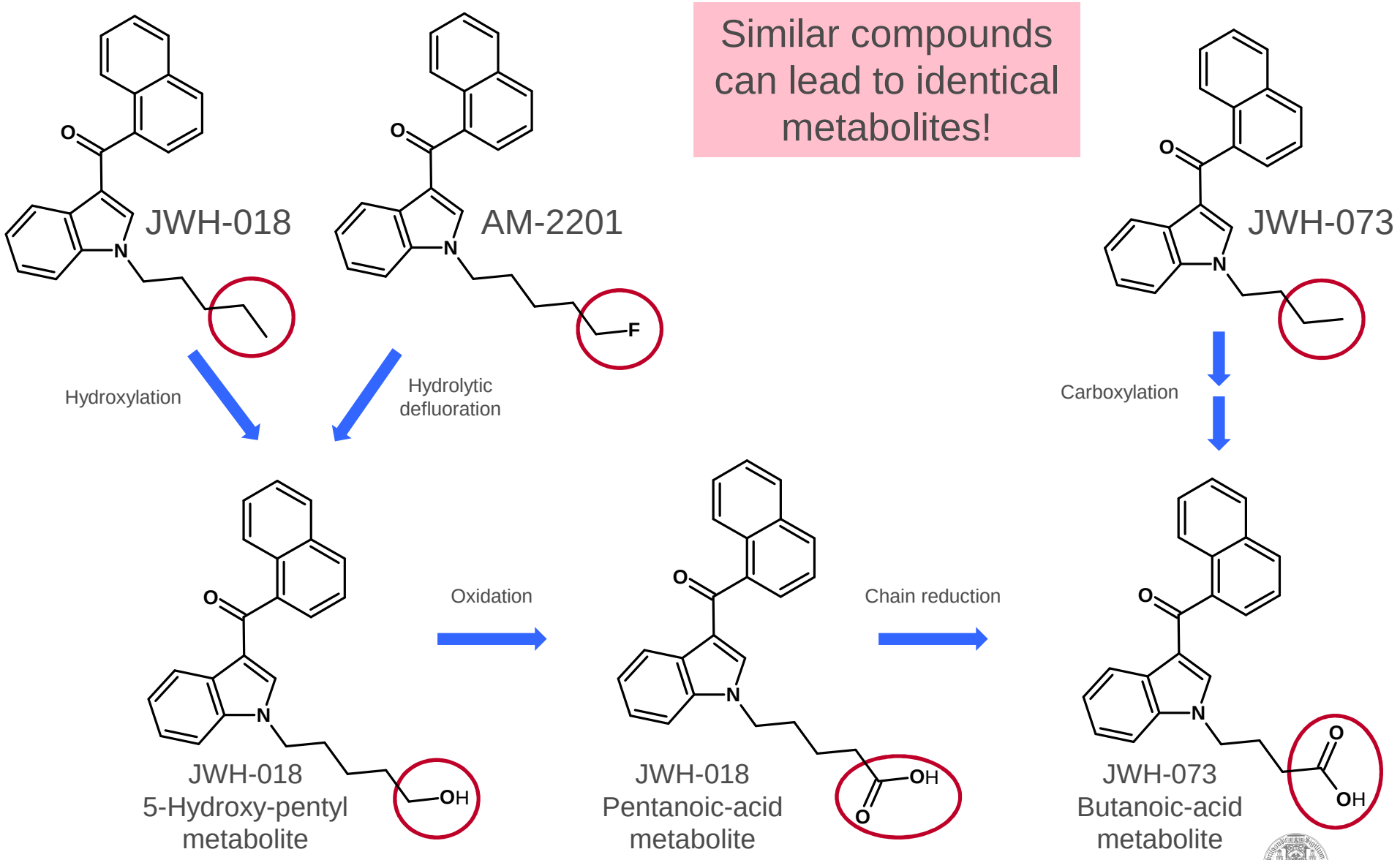
JWH-018 5-Hydroxy-pentyl metabolite

JWH-018 Pentanoic-acid metabolite

Metabolism of synthetic cannabinoids

Some important facts

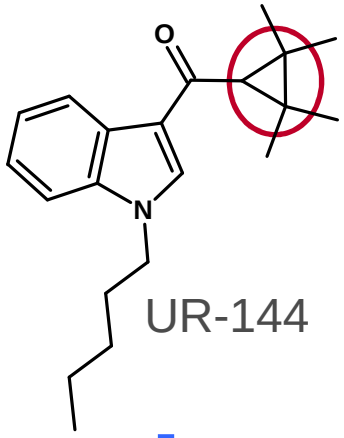
3.



Metabolism of synthetic cannabinoids

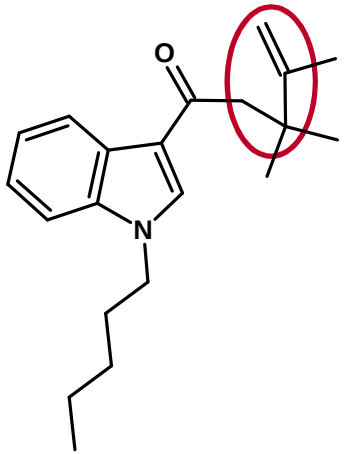
Some important facts

4.

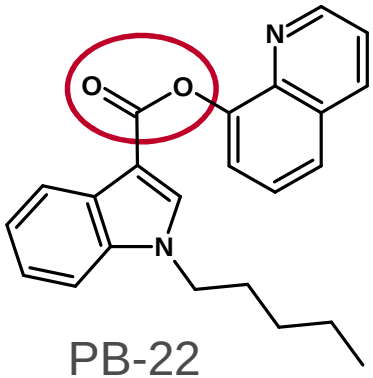


UR-144

Isomerisation

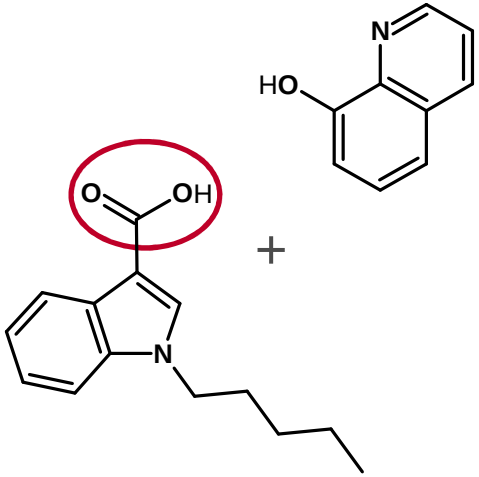


Unstable bonds in a compound can be cleaved
- by pyrolytic reactions (smoking)
- by enzymes in the organism
- during sample preparation or analysis



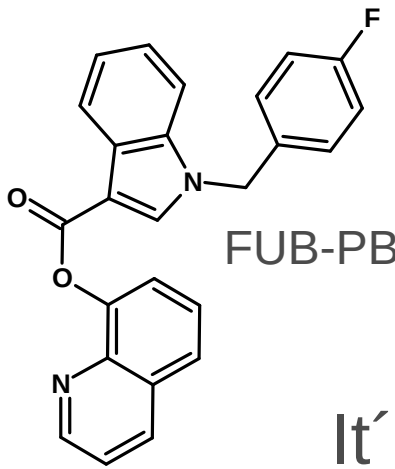
PB-22

Ester hydrolysis

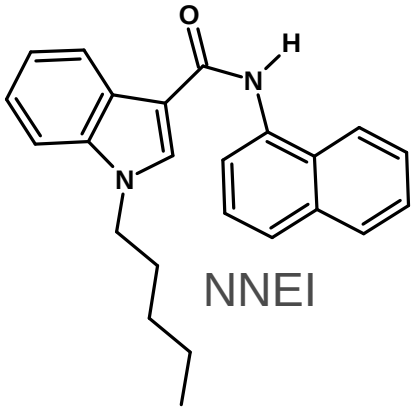


Further goals and challenges

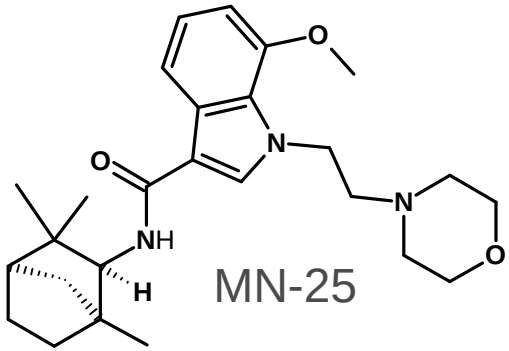
Monthly new compounds to investigate!



FUB-PB-22

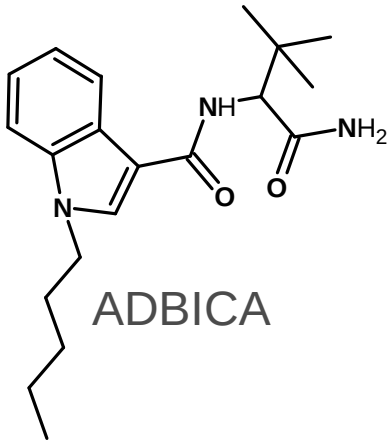


NNEI

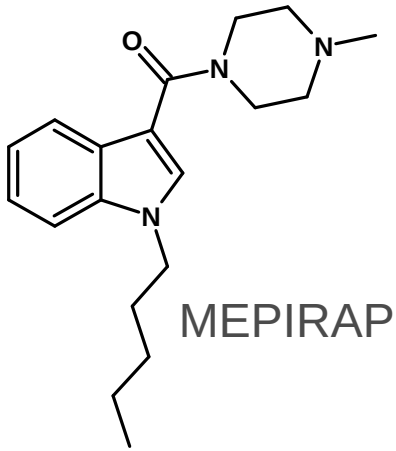


MN-25

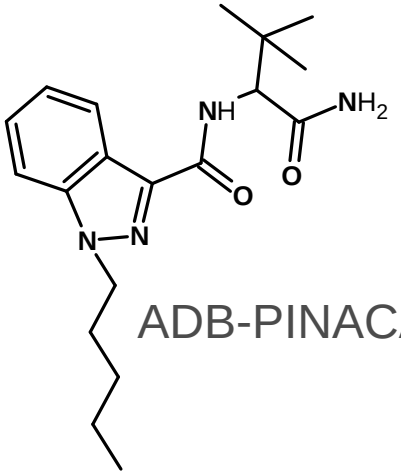
It´s like a bottomless pit…



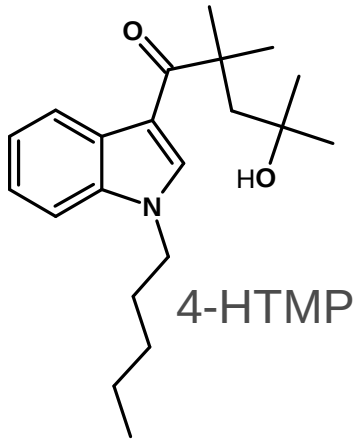
ADBICA



MEPIRAPIM



ADB-PINACA



4-HTMPIPO

Further goals and challenges

Still a lot to do!

- Keeping the screening method up to date
- Faster update of the method
- Optimisation of the metabolite identification process
- Development of new screening methods (based on LC-MS-ToF) to deal with the rapidly increasing number of analytes

Thank you for your attention!

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