

ACTIVITY-BASED TESTING: AN INNOVATIVE TECHNIQUE FOR DRUG TESTING



 @christophestove

New Psychoactive Substances

Activity-based assays: what?

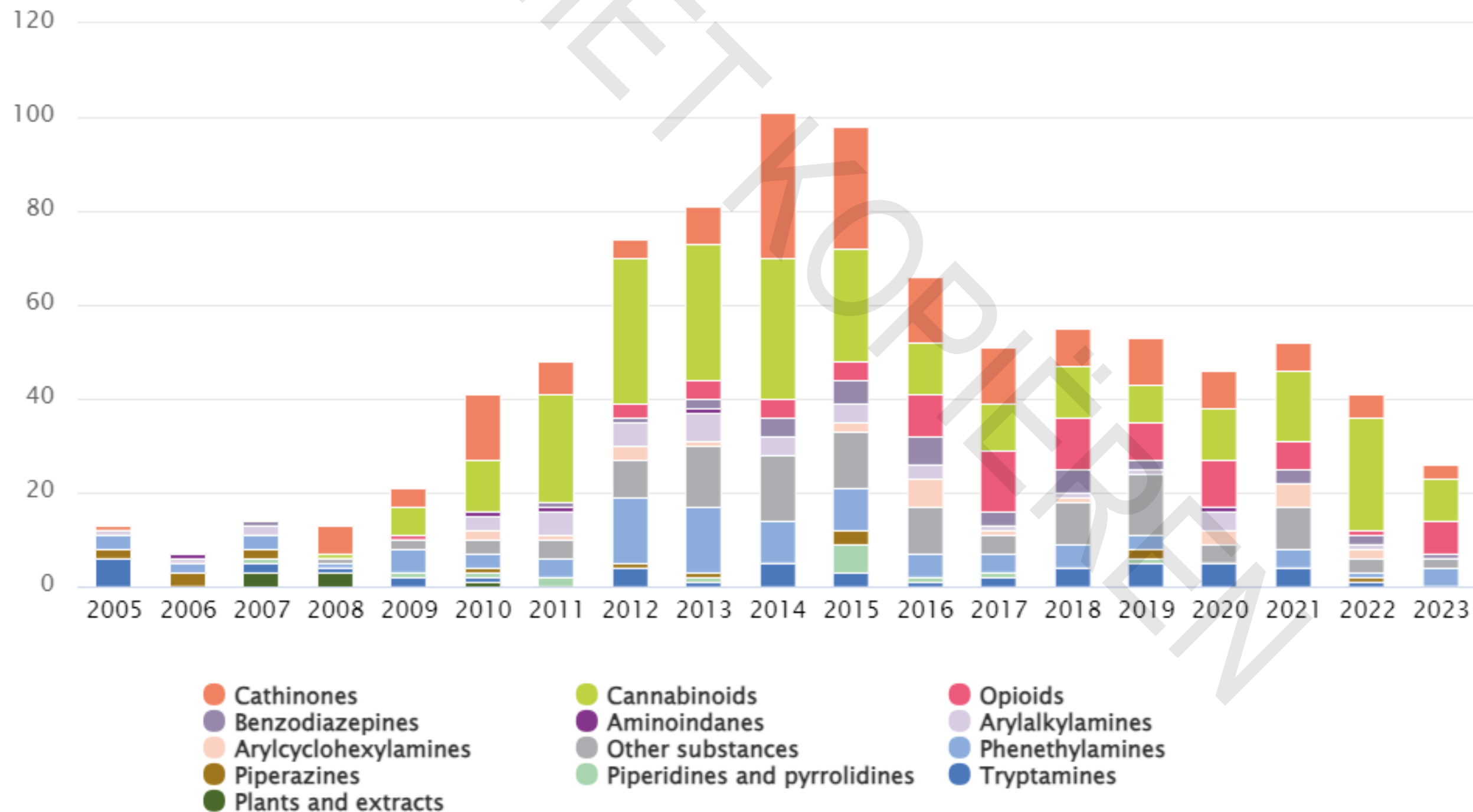
Applications of activity-based assays: Screening & Characterization

Conclusions

Activity-based assays: the NPS issue



Number of new psychoactive substances (NPS) notified for the first time:

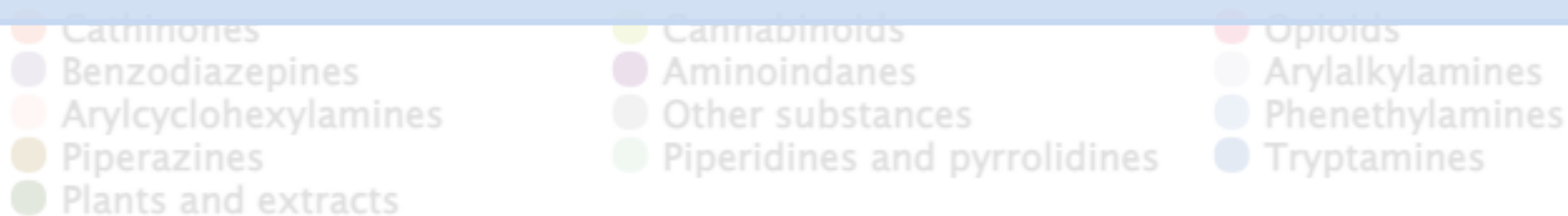


“Substances of abuse, either in pure form or a preparation, that are not controlled by the 1961 Convention on Narcotic Drugs or the 1971 Convention on Psychotropic Substances, but which may pose a public health threat” (UNODC)

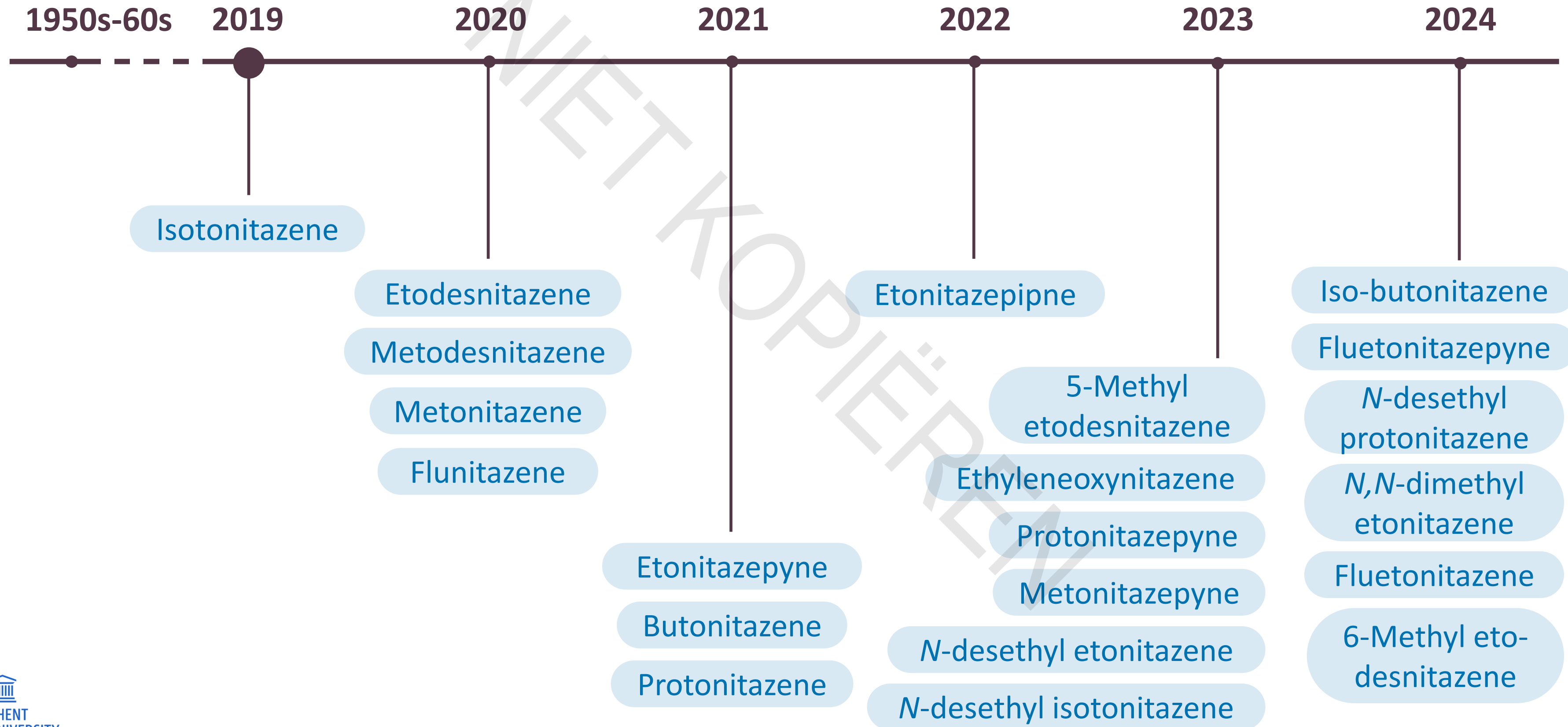


New psychoactive substances (NPS) notified for the first time:

- **Continuously changing** panel
- **Uncharacterized** at the time of emergence
- Often highly potent drugs ~ **low concentrations** in biofluids
- Traditional approaches to drug screening may fail to detect NPS:
 - Possibly little to **no cross-reactivity** in immunoassays
 - Difficult to keep **mass spectral libraries** up to date
- **Limitations to non-targeted (GC-MS, LC-(HR)MS) screening**



NPS: an ever changing landscape – the example of nitazene synthetic opioids



NPS: an ever changing landscape – synthetic cannabinoid receptor agonists

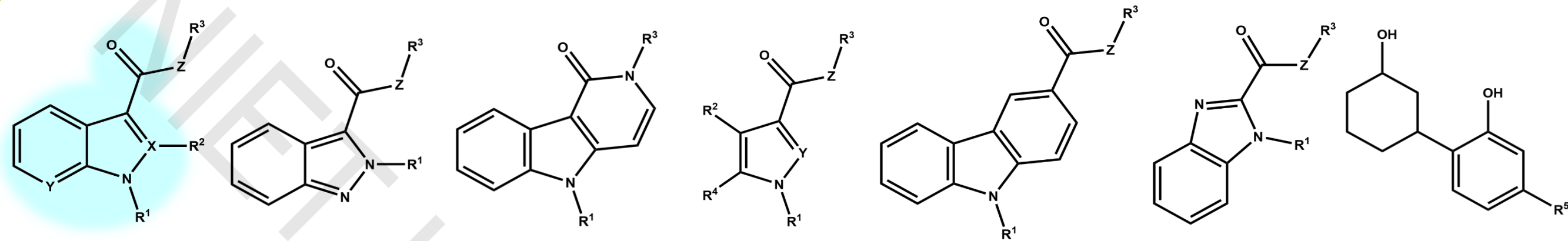


JWH-018

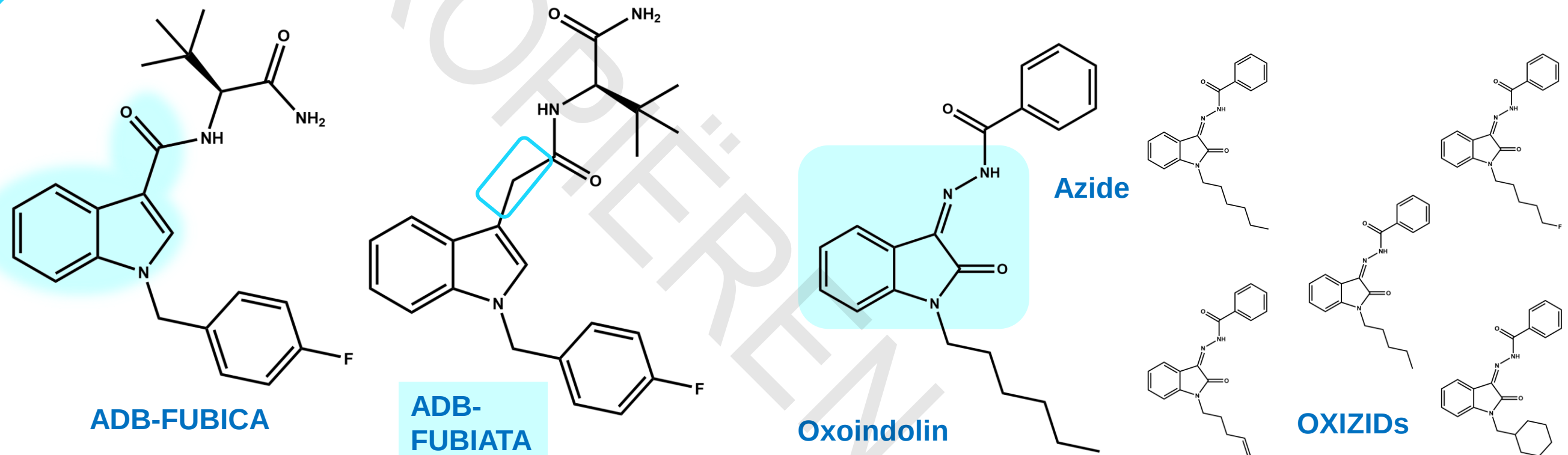
Head

Core

Tail

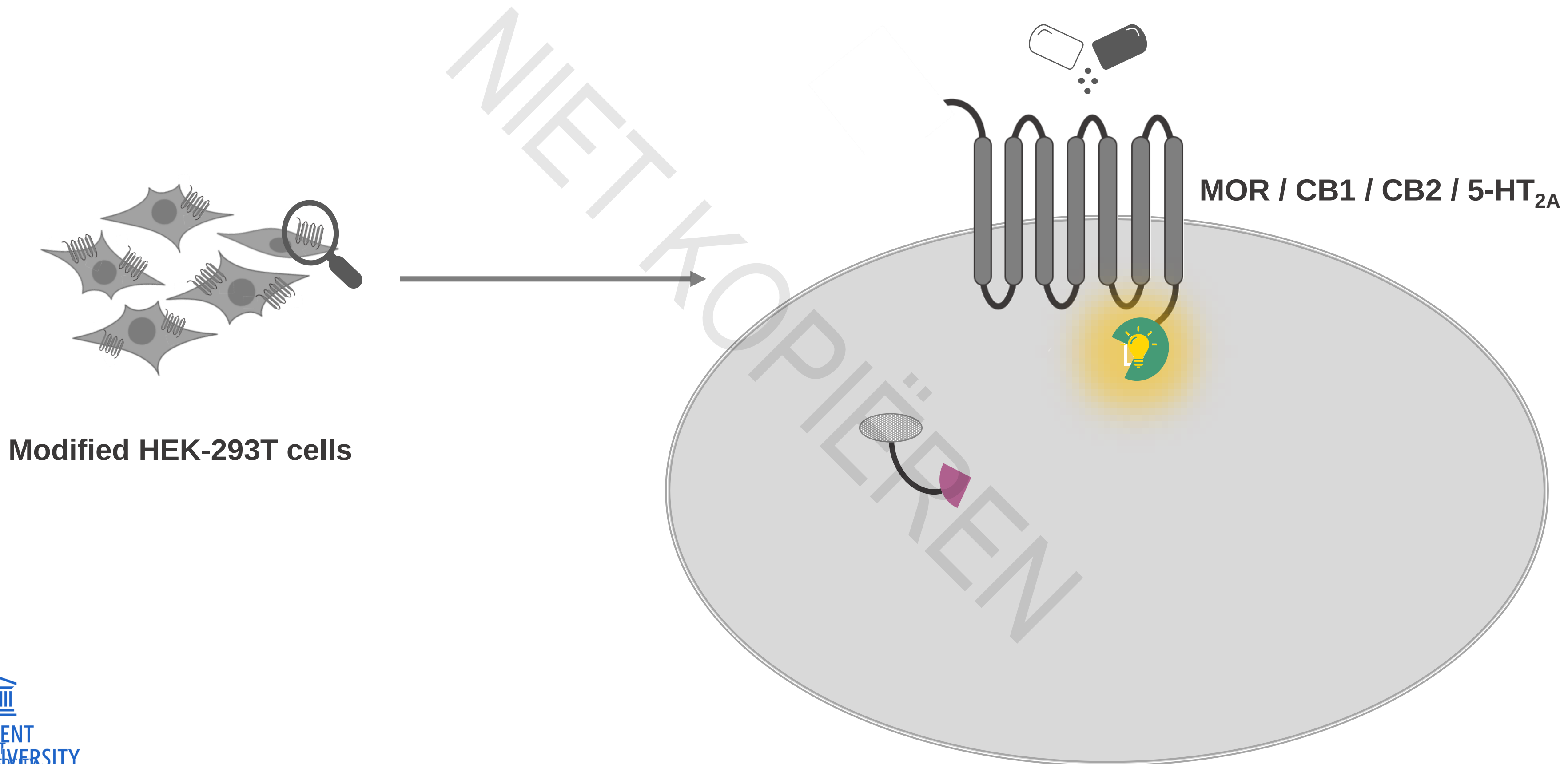


'China ban' on SCRA

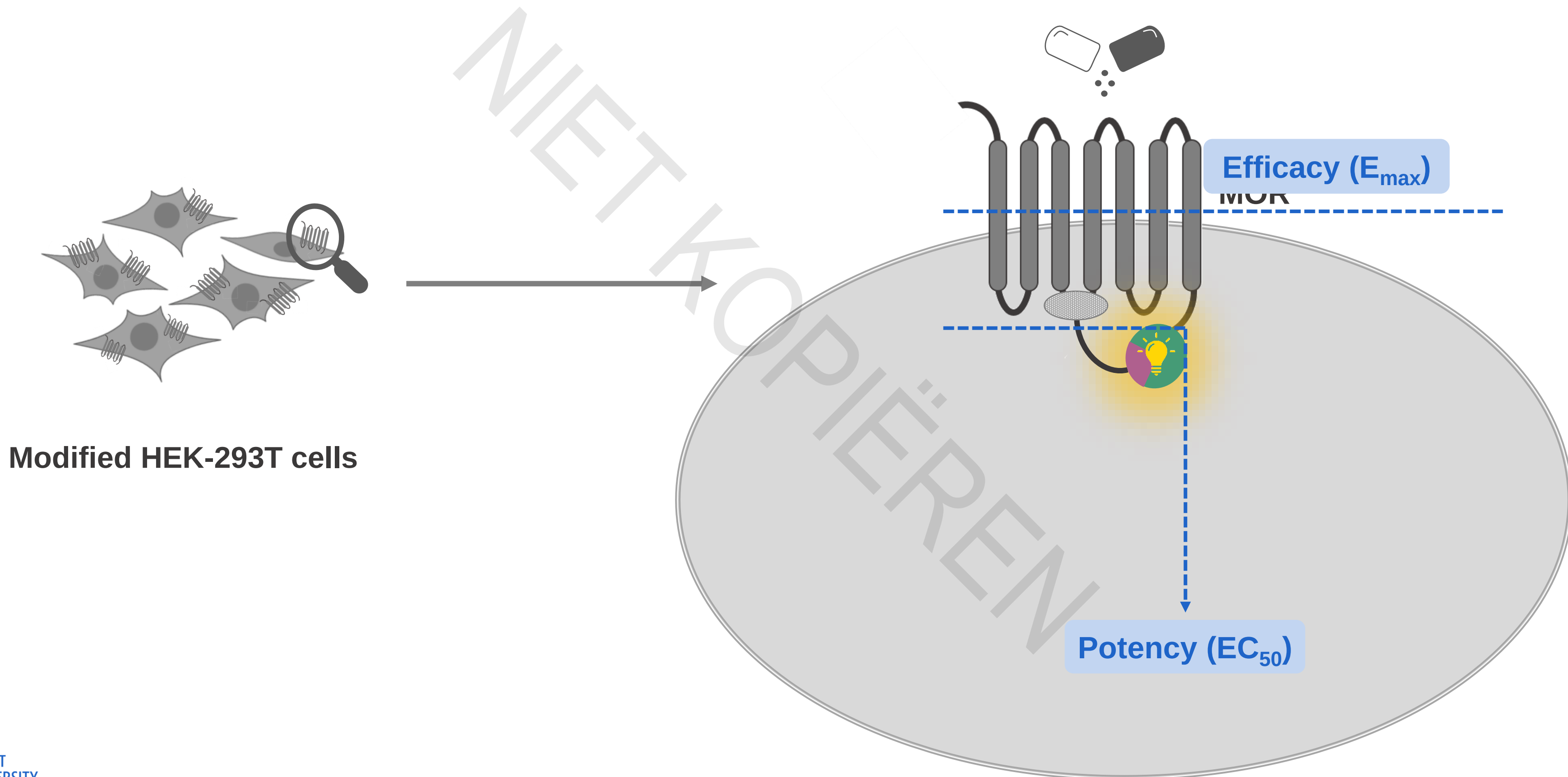


-  New Psychoactive Substances
-  **Activity-based assays: what?**
-  Applications of activity-based assays: Screening & Characterization
-  Conclusions

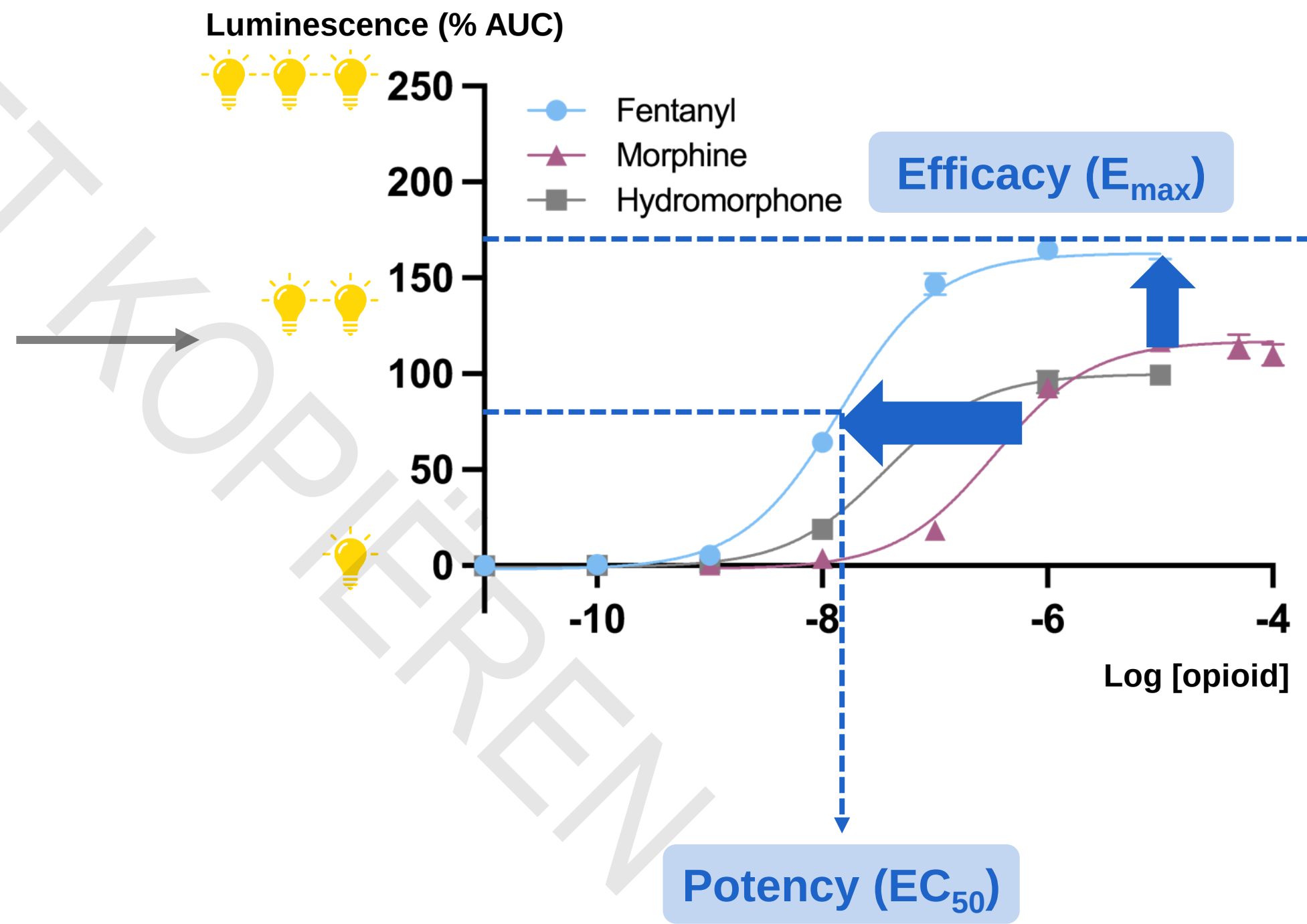
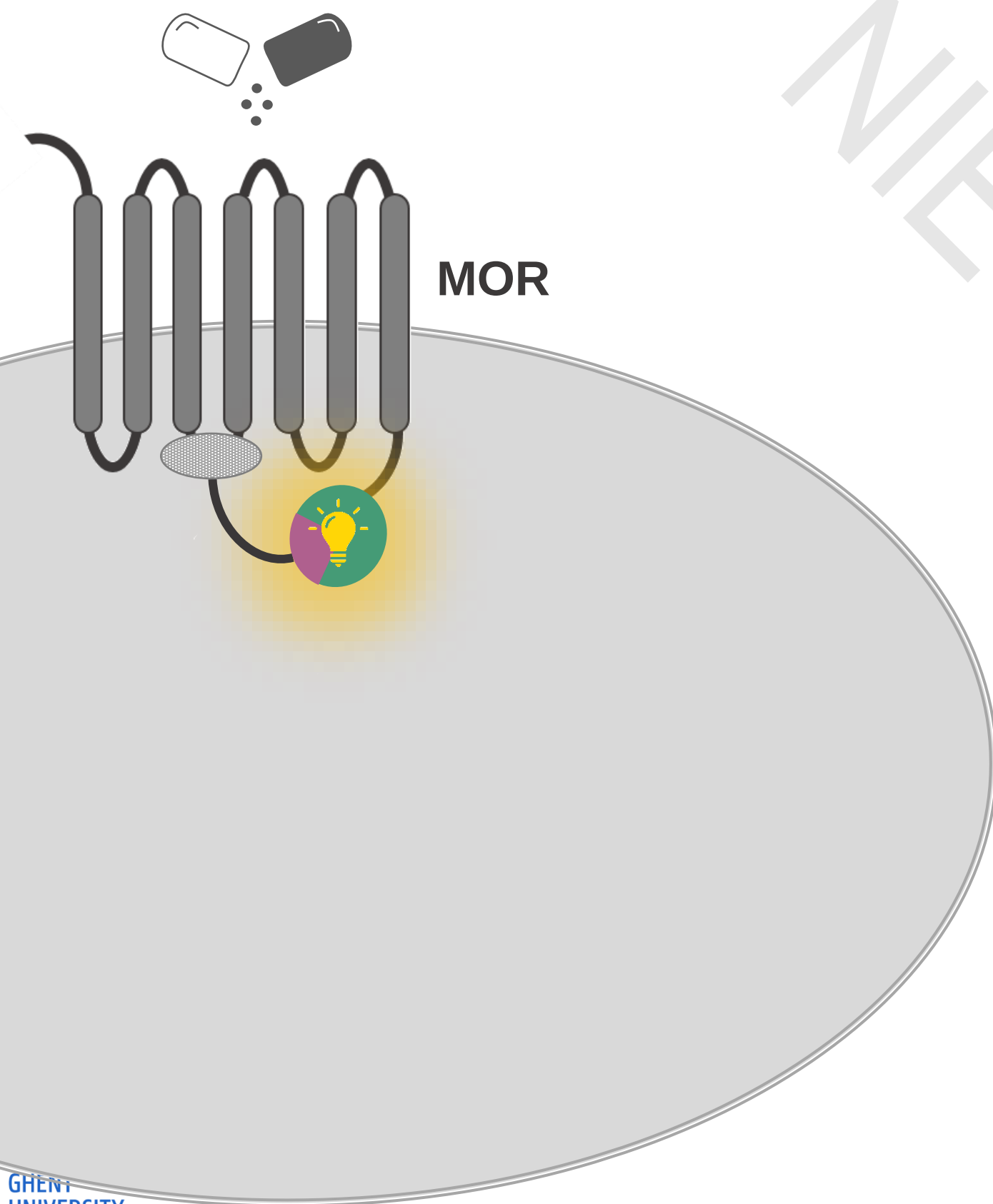
In vitro functional characterization of new psychoactive substances



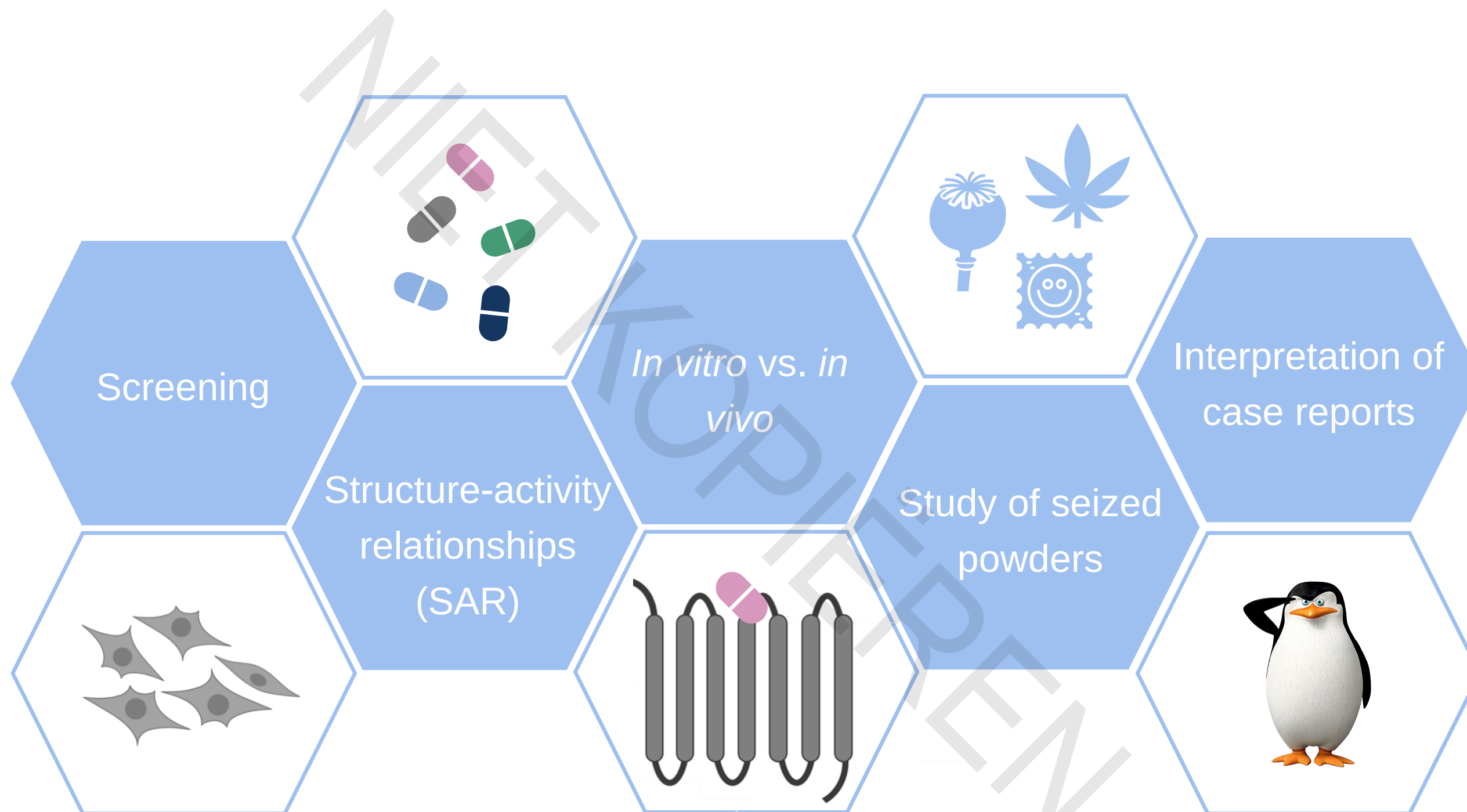
In vitro functional characterization of new psychoactive substances

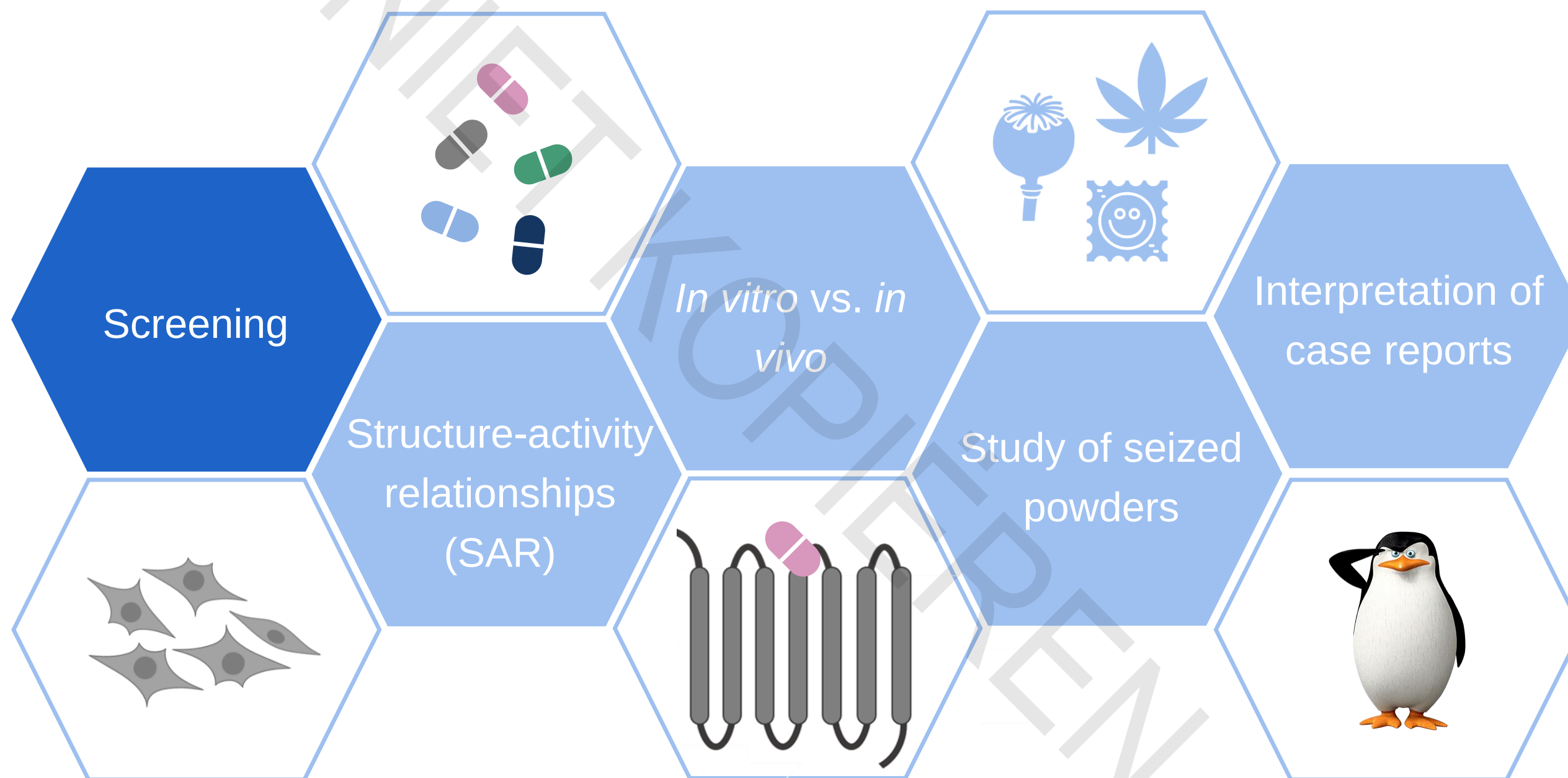


In vitro functional characterization of new psychoactive substances

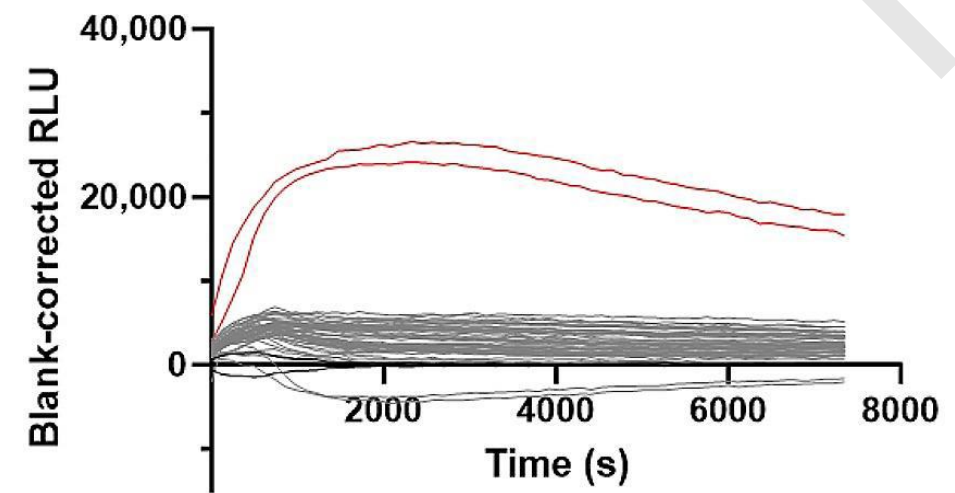
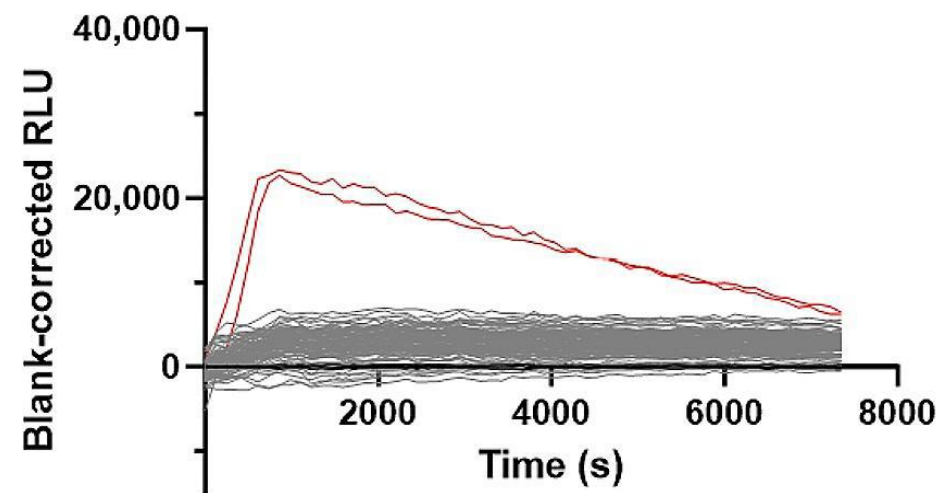
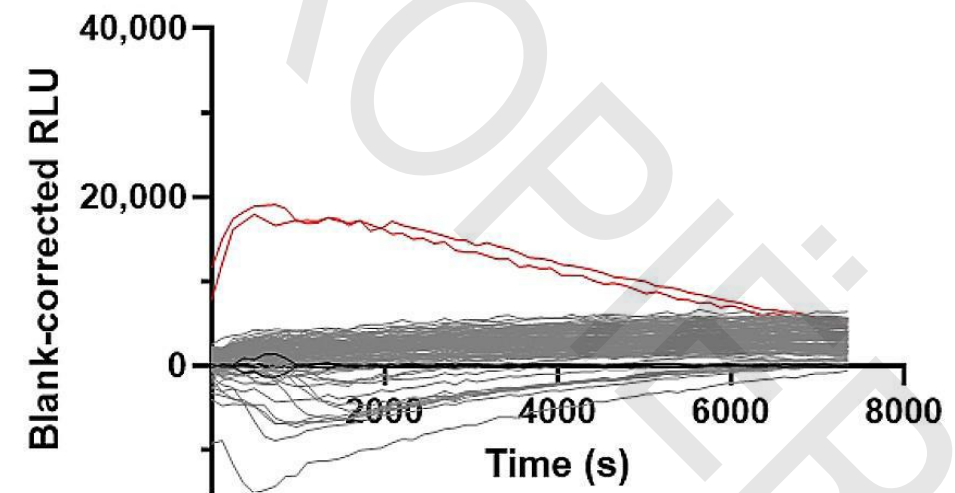
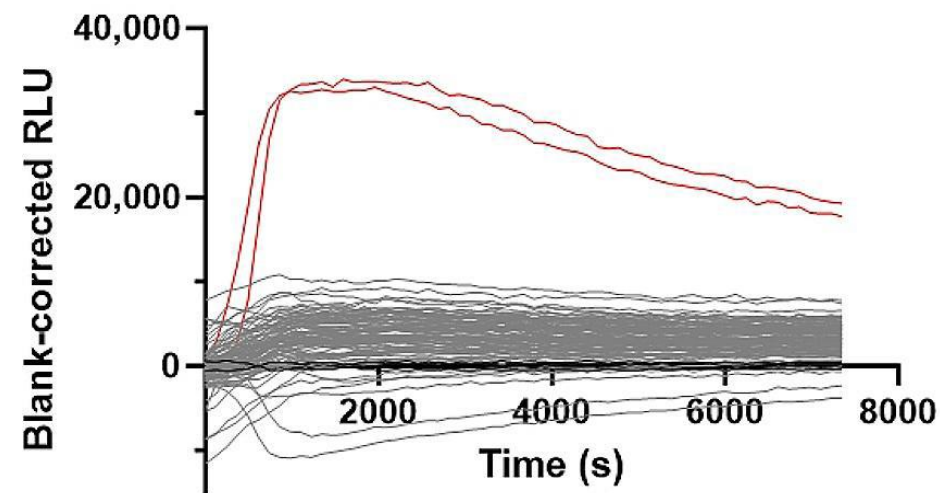
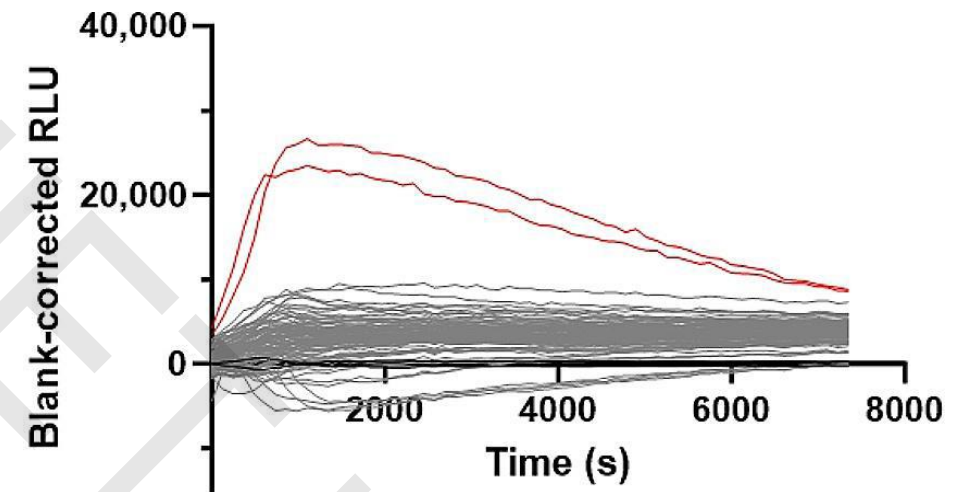
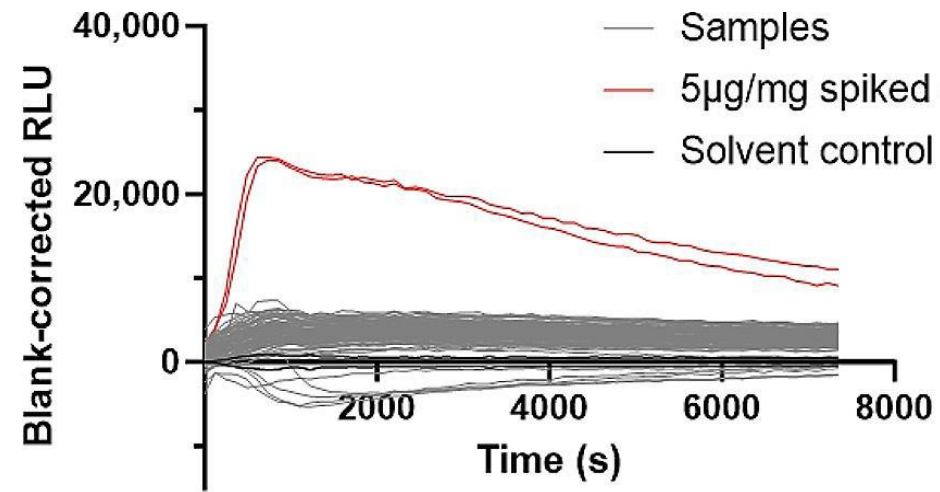


-  New Psychoactive Substances
-  Activity-based assays: what?
-  **Applications of activity-based assays: Screening & Characterization**
-  Conclusions



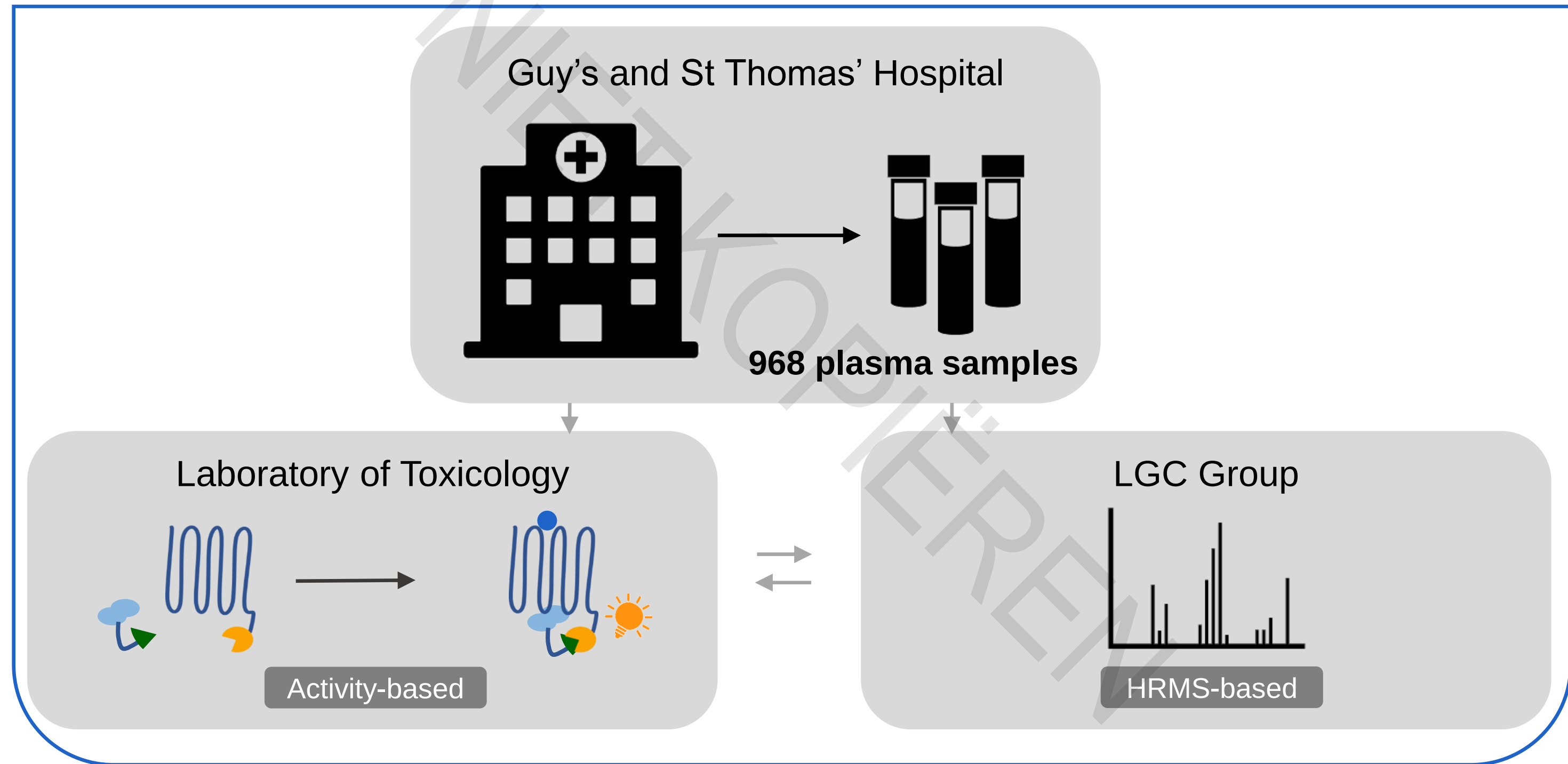


Screening of seized products for cannabinoid activity

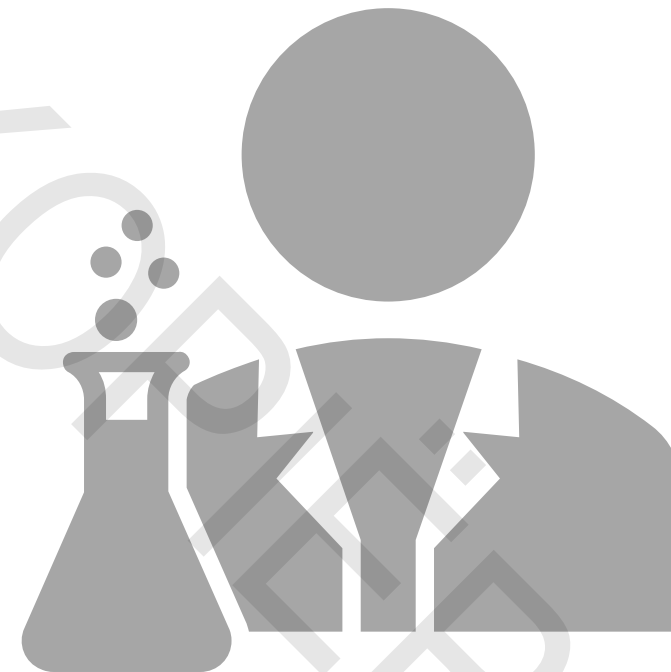
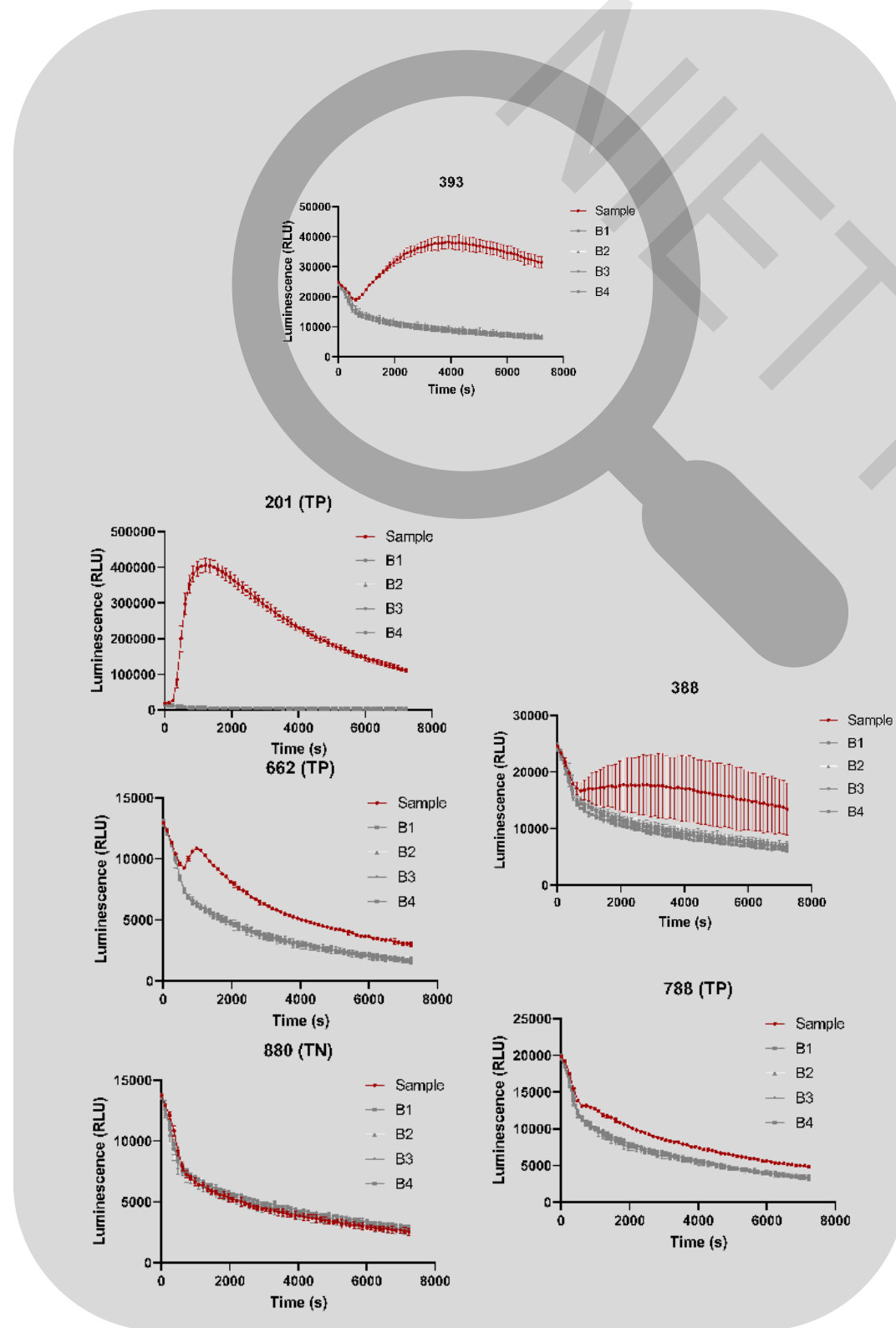


Activity-based testing of
herbal samples seized at a
big Belgian music festival





Screening: synthetic cannabinoid receptor agonists



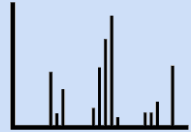
Screening: synthetic cannabinoid receptor agonists



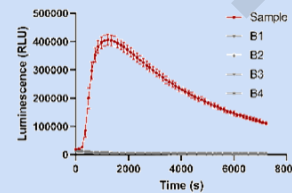
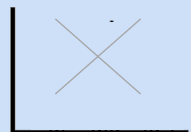
Bioassay

Analytical

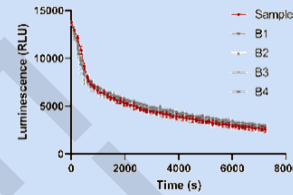
SCRA positive



SCRA negative



Positive scoring



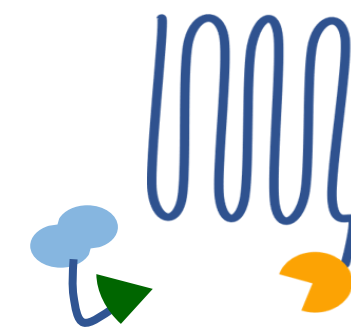
Negative scoring

True Positive:
141

False Negative:
8

False Positive:
12

True Negative:
807

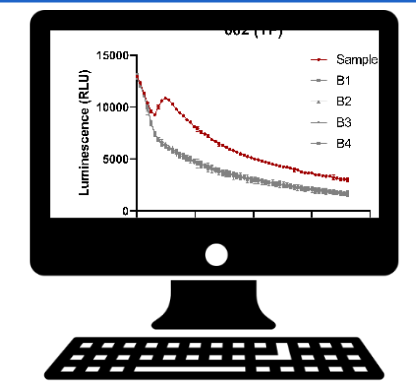


Sensitivity

 **94.6%**

Specificity

 **98.5%**



Sensitivity

 **94%**

Specificity

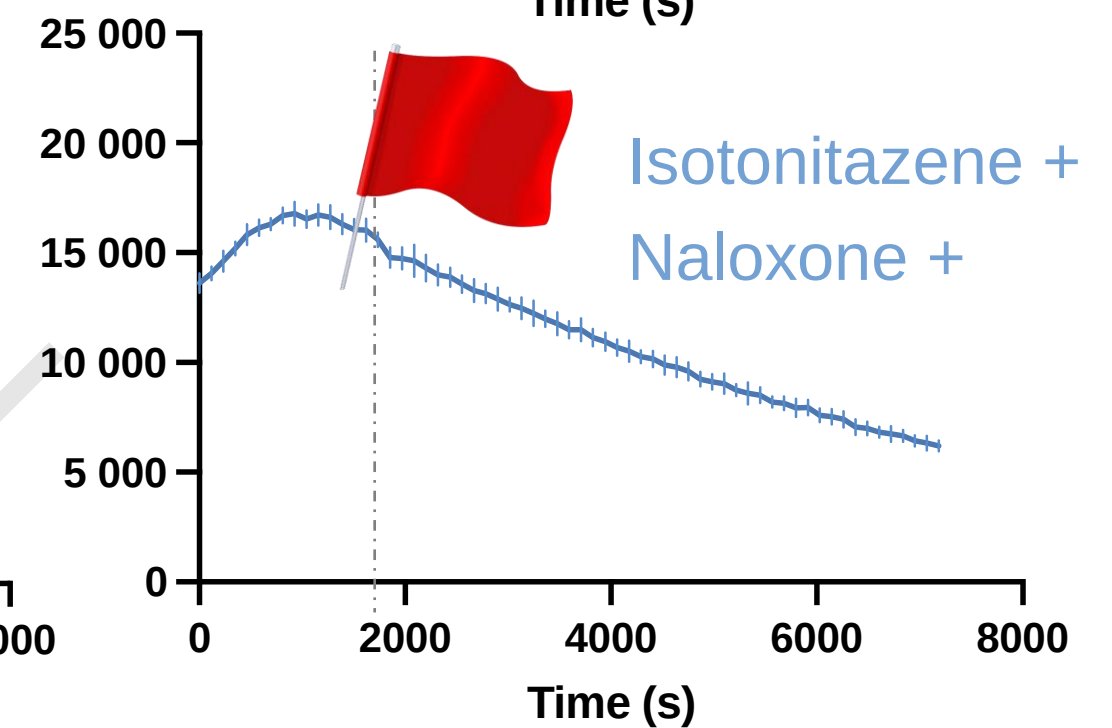
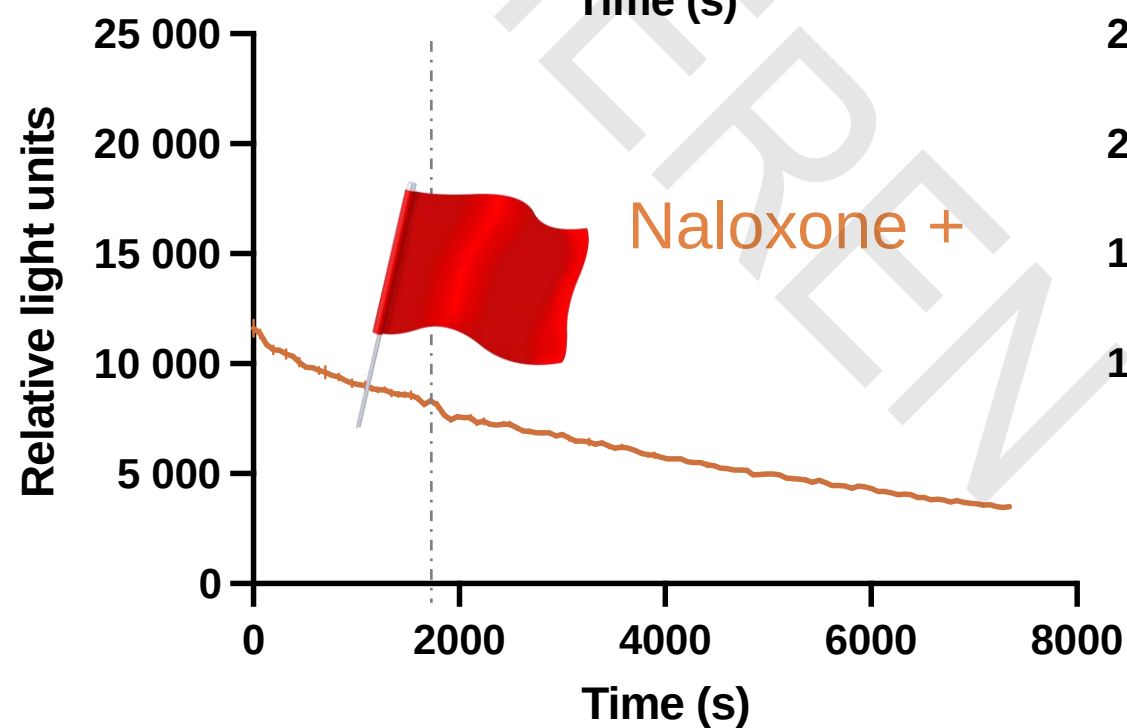
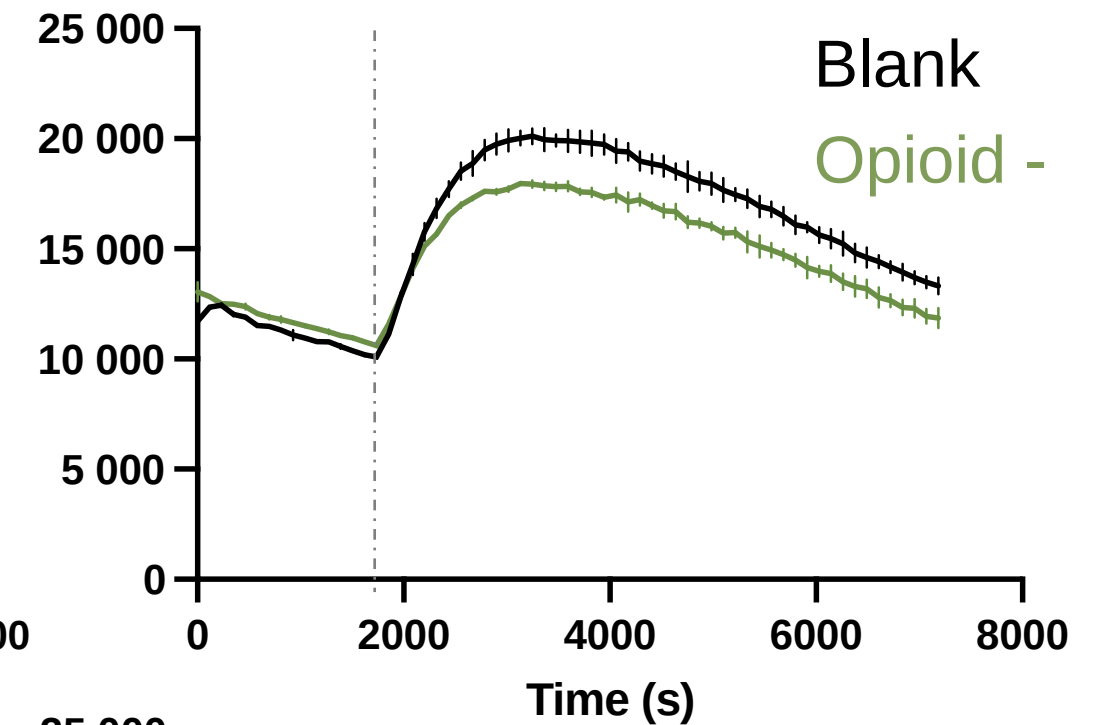
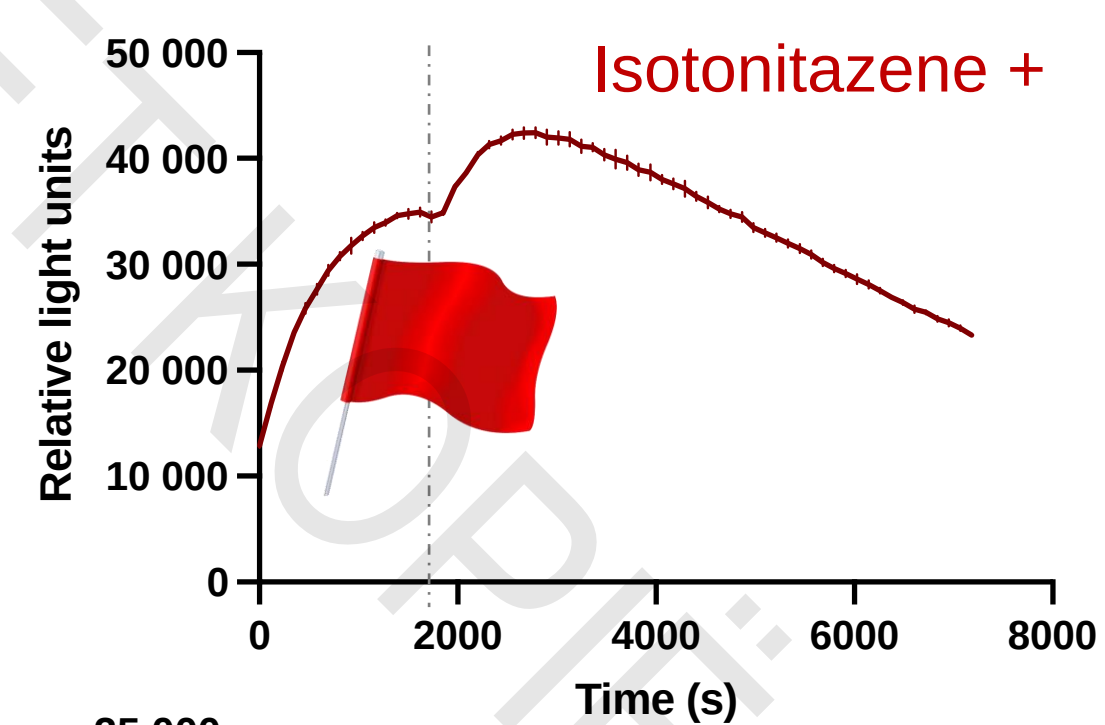
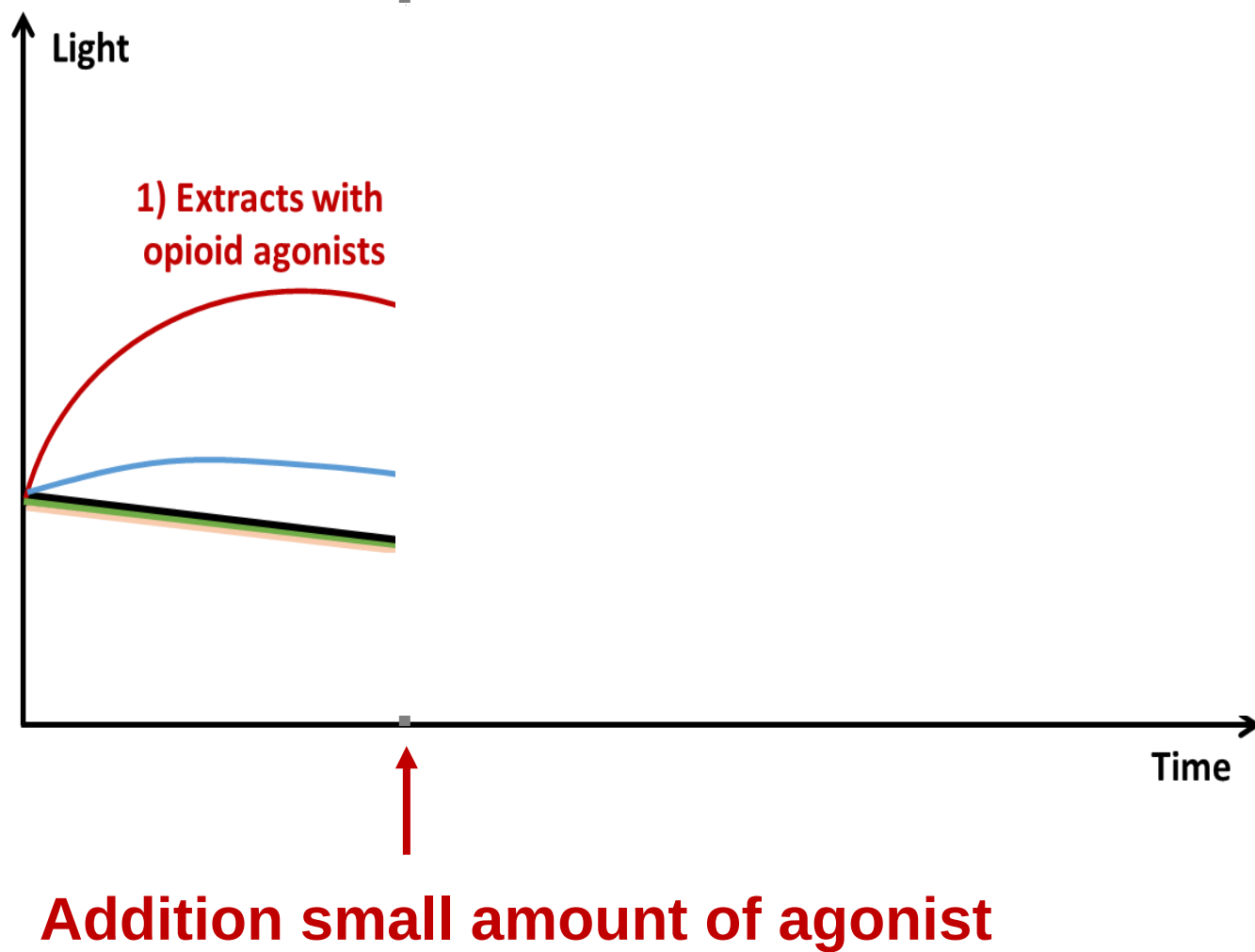
 **94%**

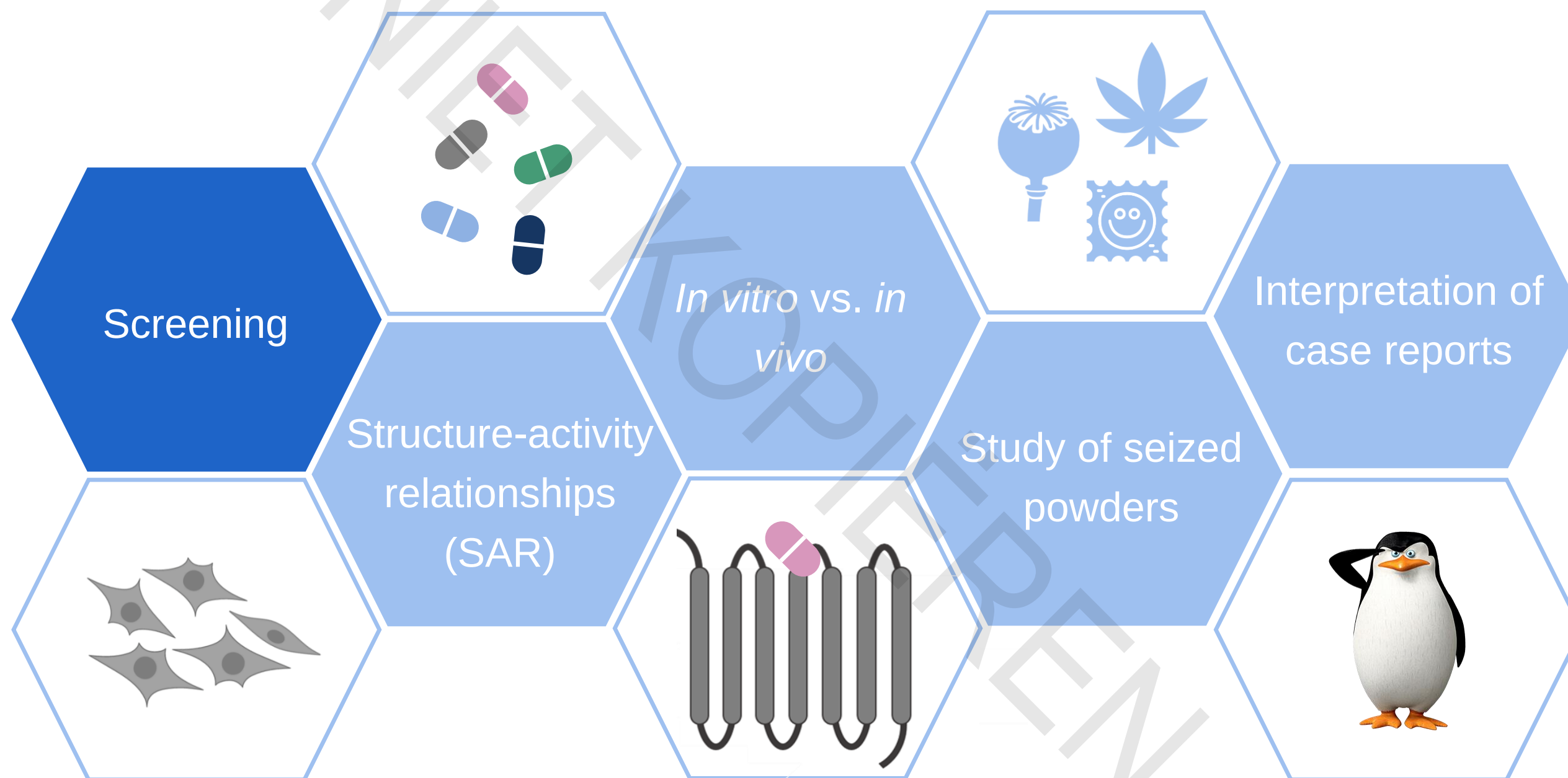
Screening of biological samples for activity: opioid activity

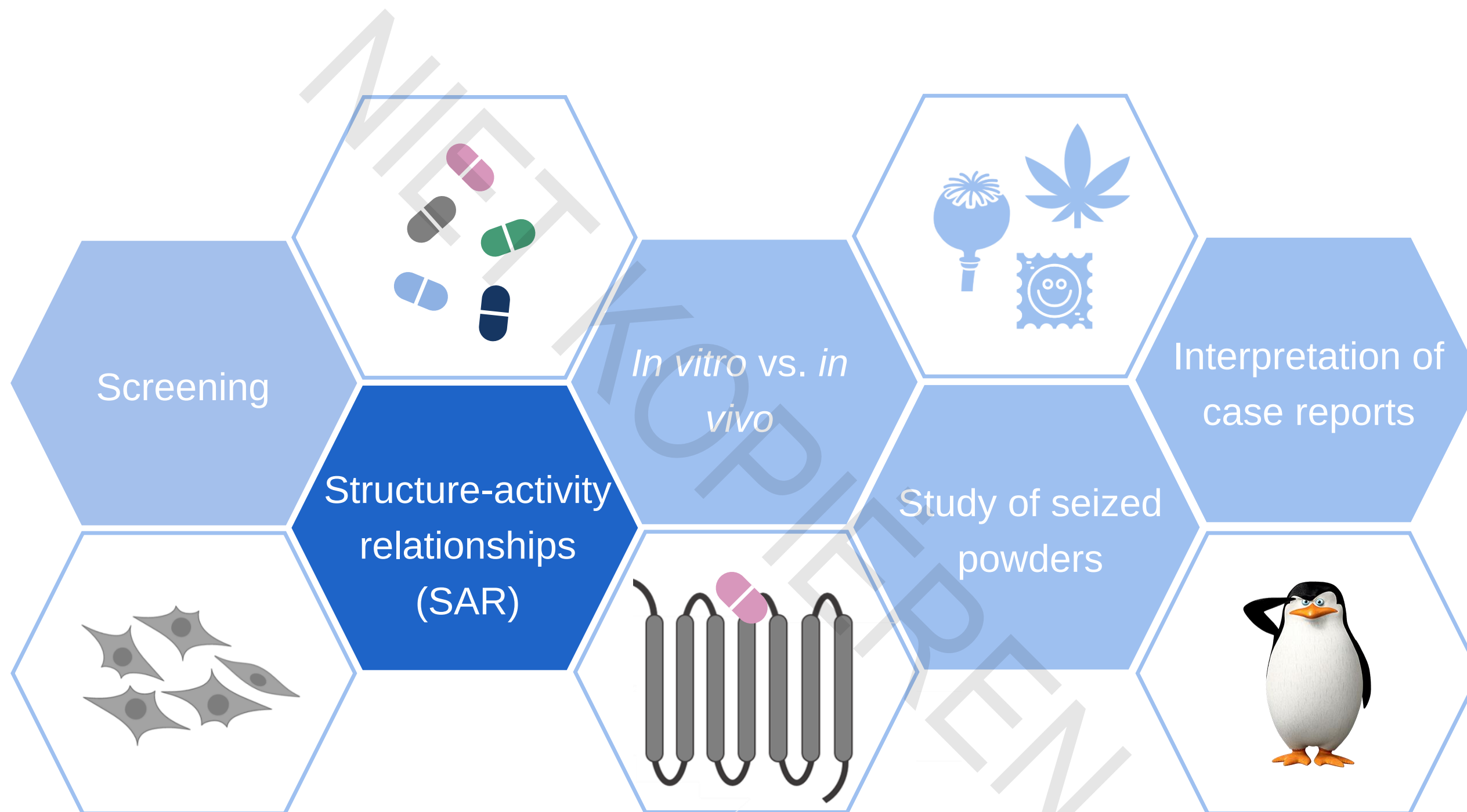


Theory

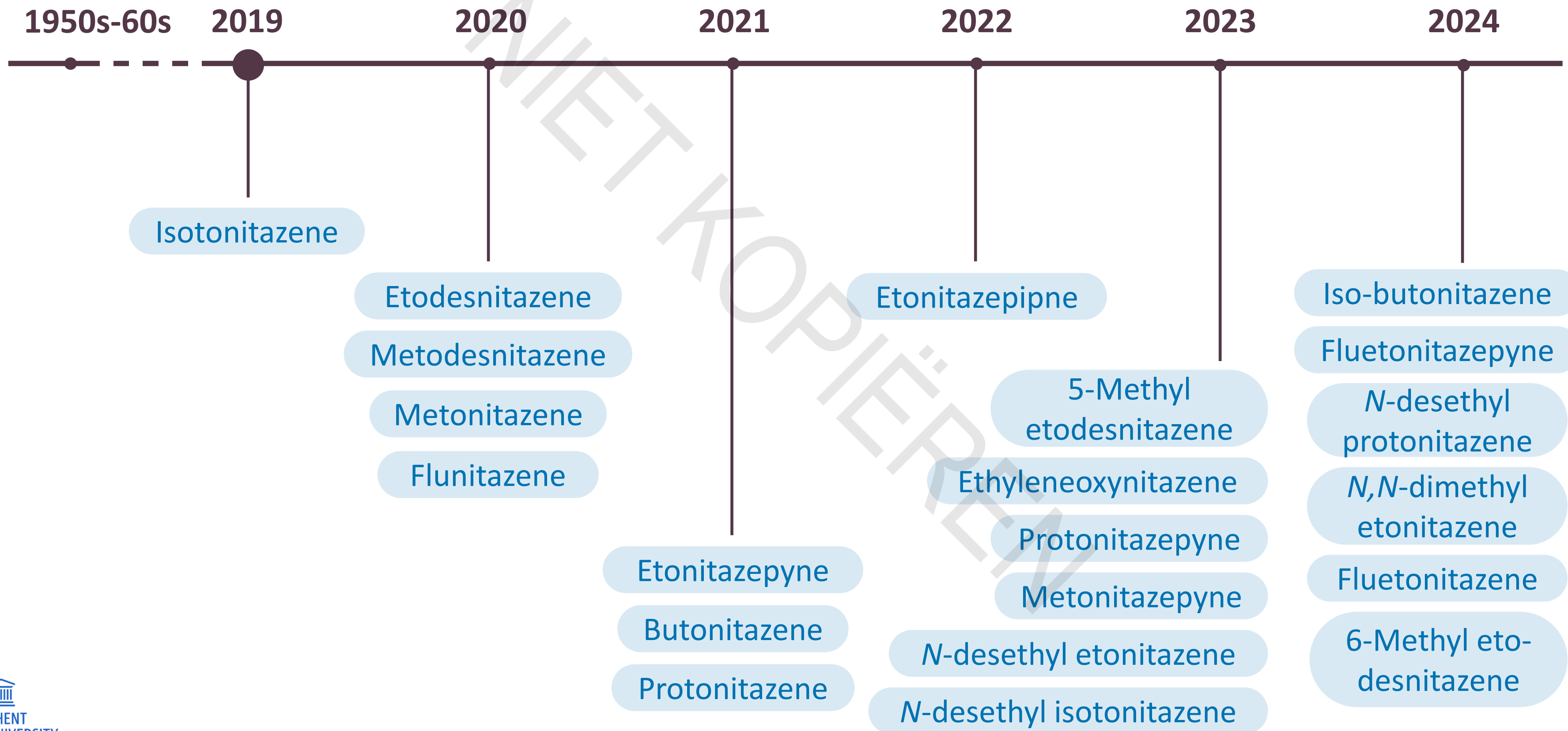
Practice



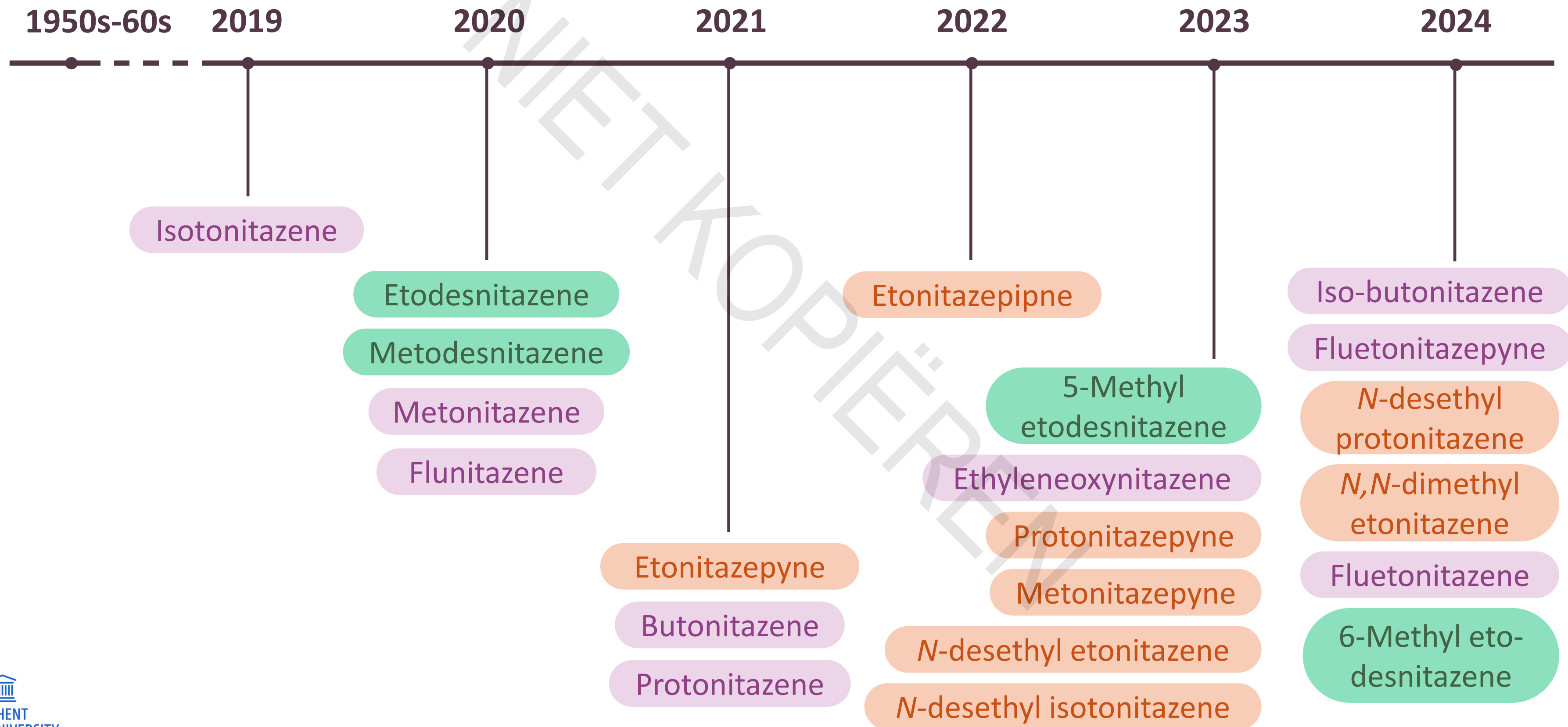




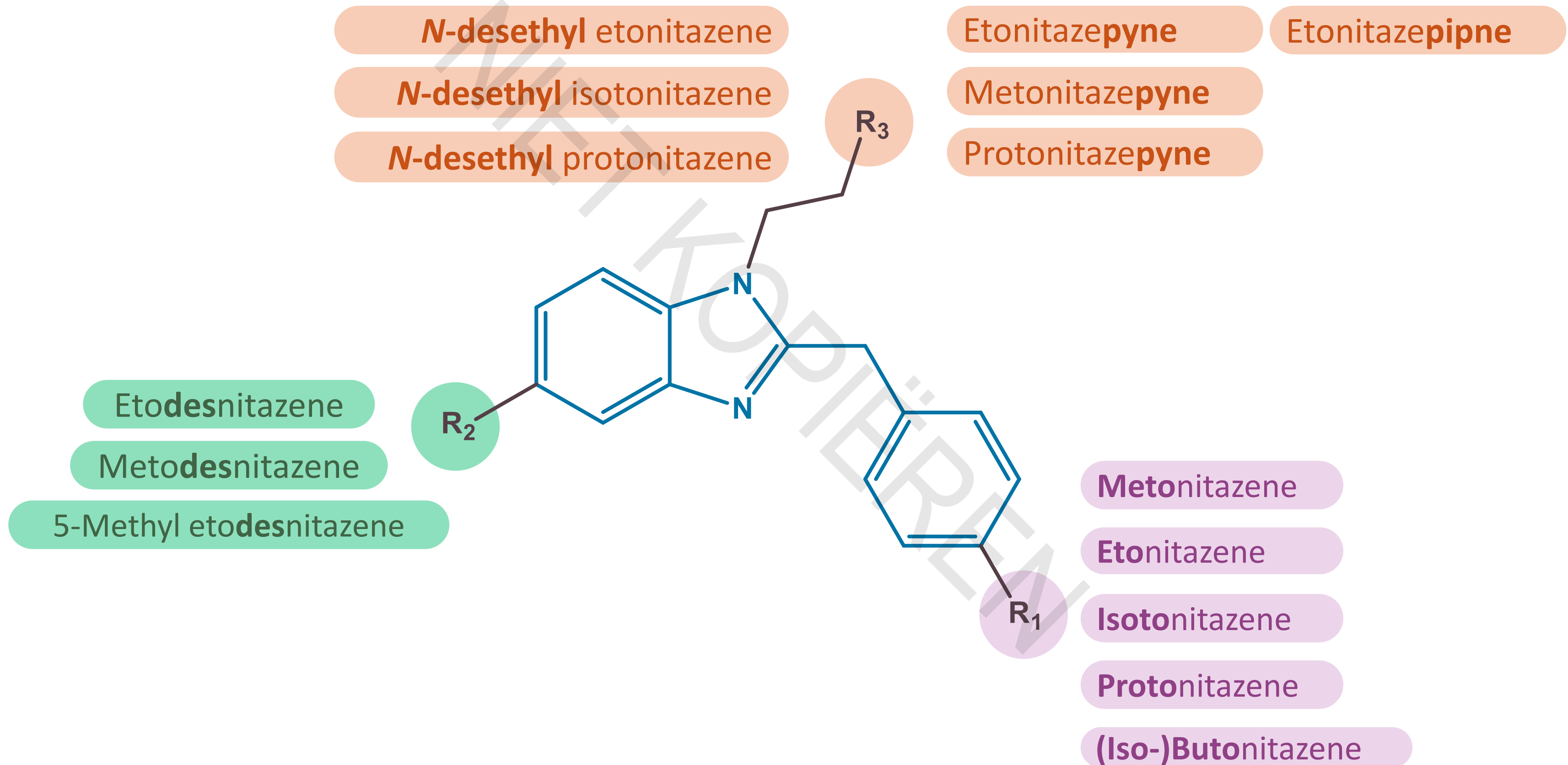
Nitazene opioids: structure-activity relationship



Nitazene opioids: structure-activity relationship

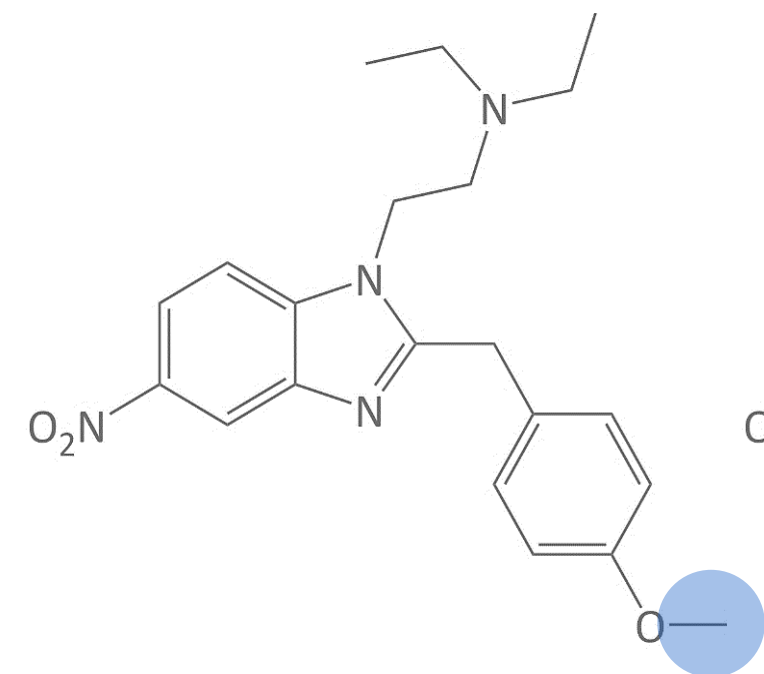


Nitazene opioids: structure-activity relationship

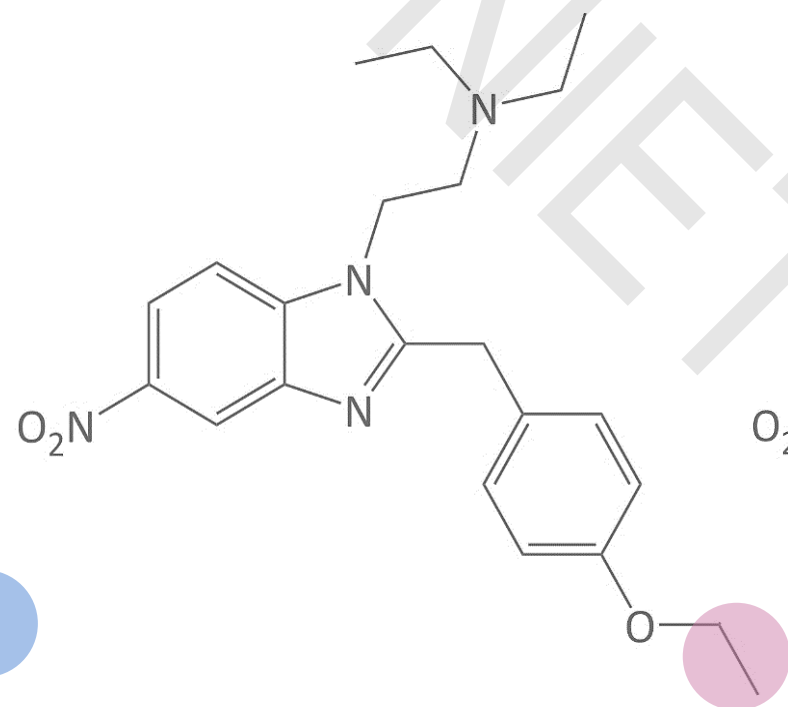


Nitazene opioids: structure-activity relationship: R1 group

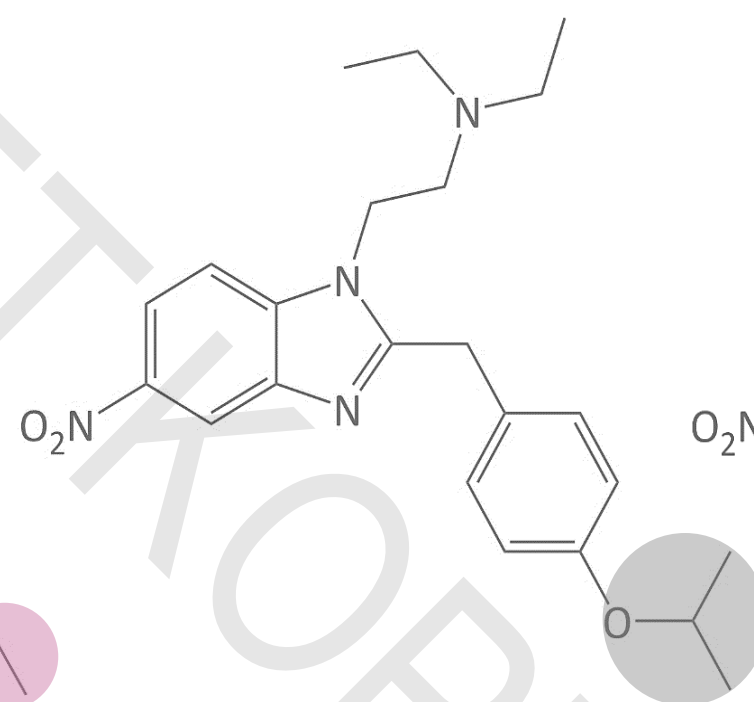
Metonitazene



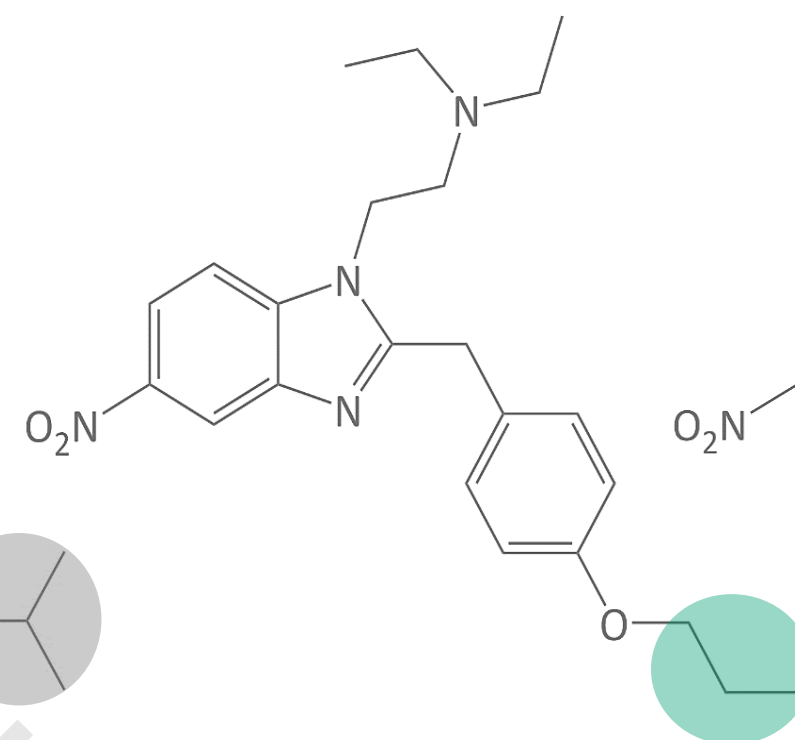
Etonitazene



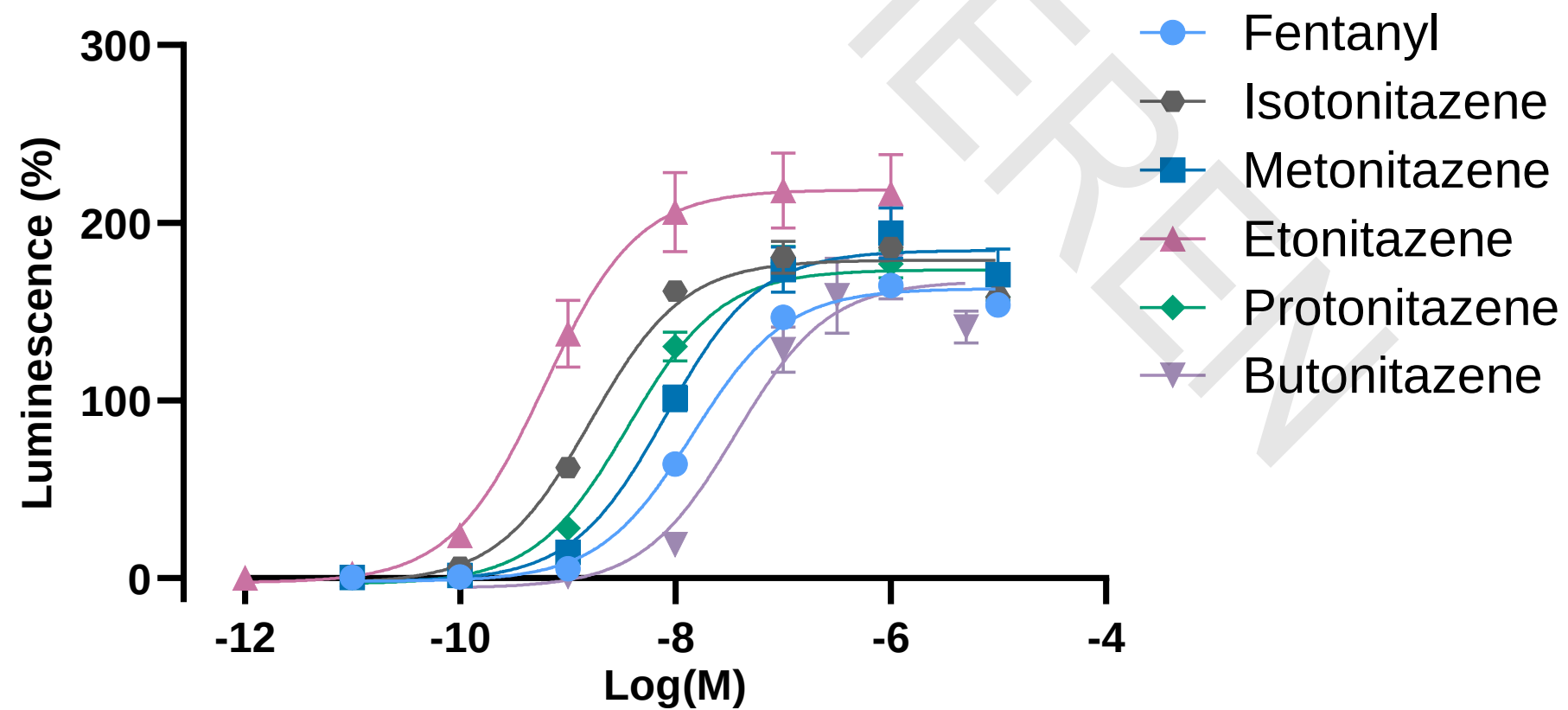
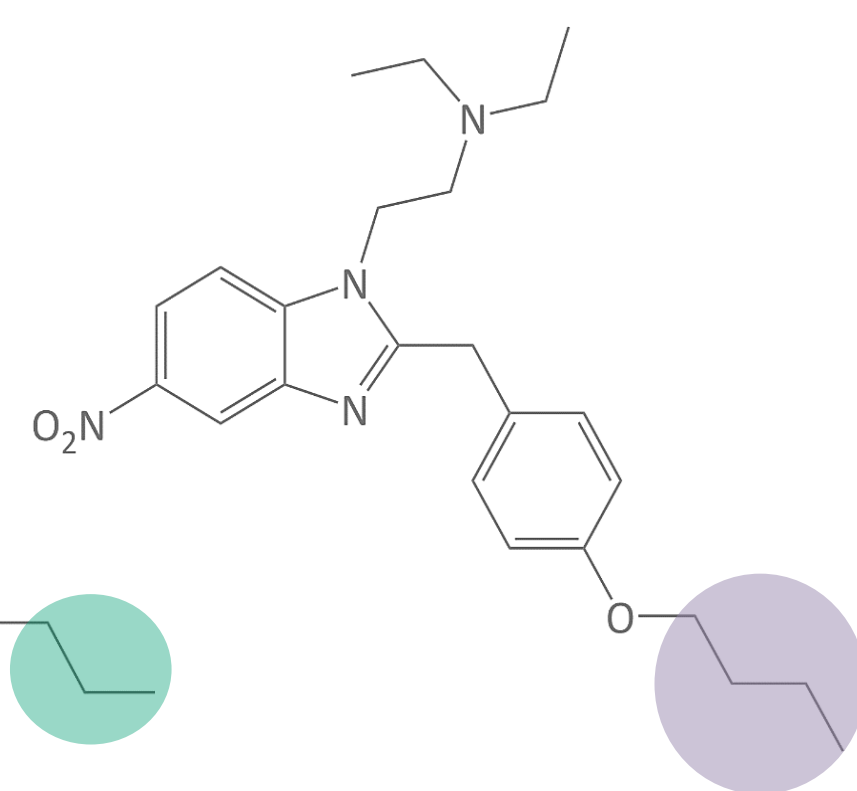
Isotonitazene



Protonitazene



Butonitazene



Nitazene opioids: structure-activity relationship

N-desethyl etonitazene

N-desethyl isotonitazene

N-desethyl protonitazene

Etonitazepyne

Etonitazepipne

Metonitazene

Protonitazene

**Various
modifications
well-tolerated**

Etodesnitazene

Nitro group

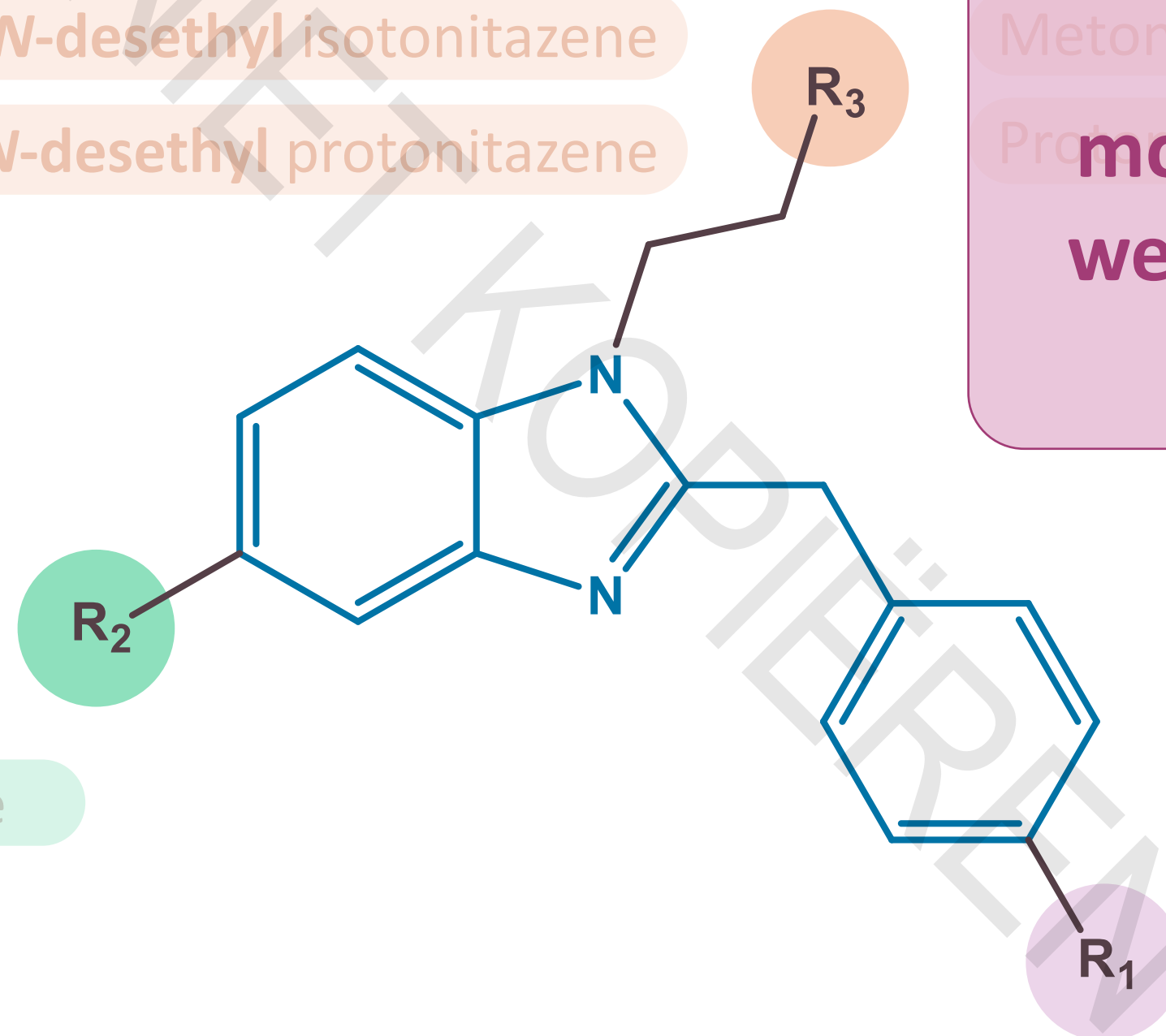
5-Methyl etodesnitazene

Metonitazene

**Relatively short
or more compact
alkoxy side chain**

Protonitazene

(Iso-)Butonitazene



Nitazene opioids: from structure-activity relationship to 'prophetic' nitazenes

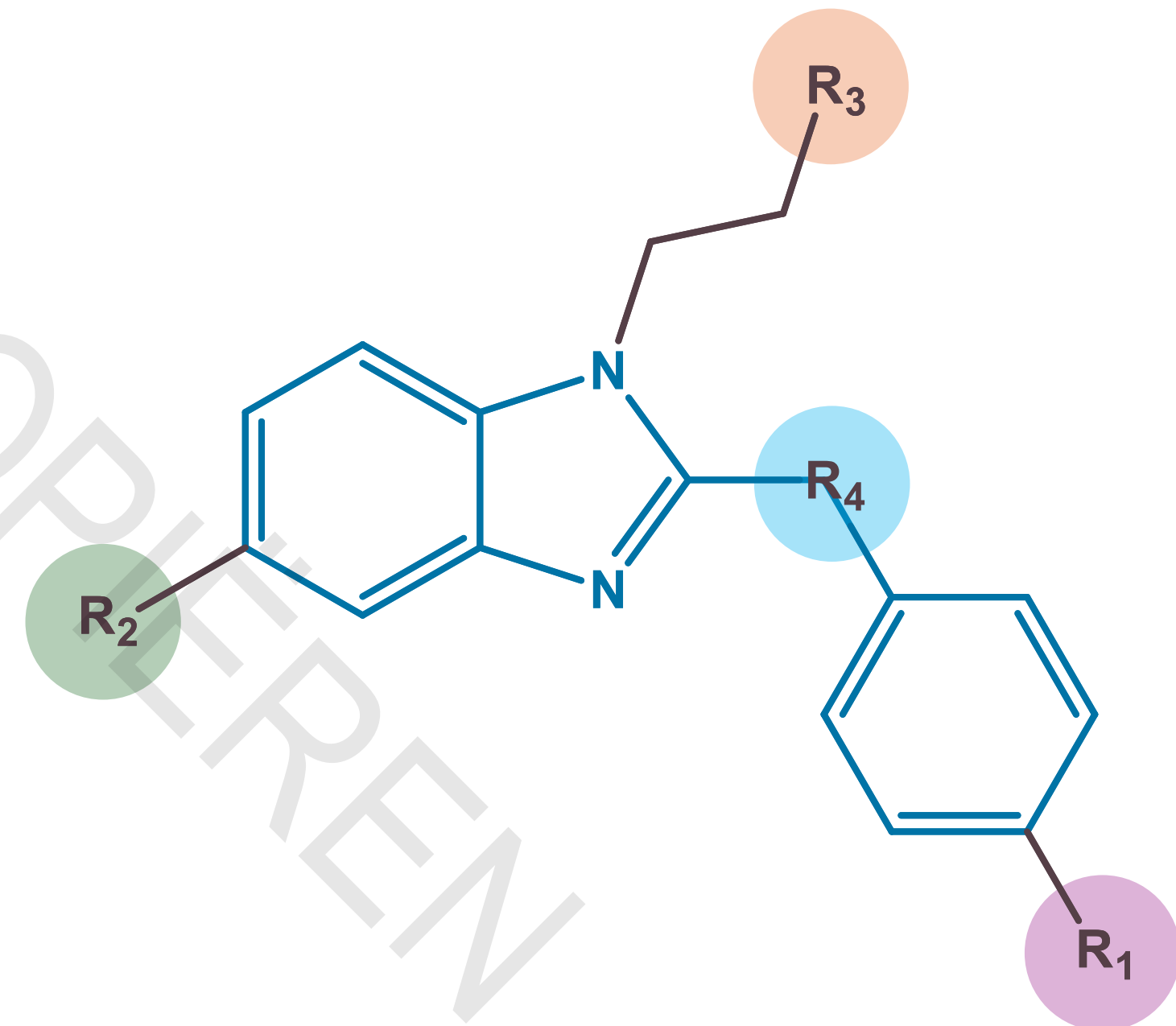
Existing literature + knowledge of structure-activity relationships

Ease of synthesis

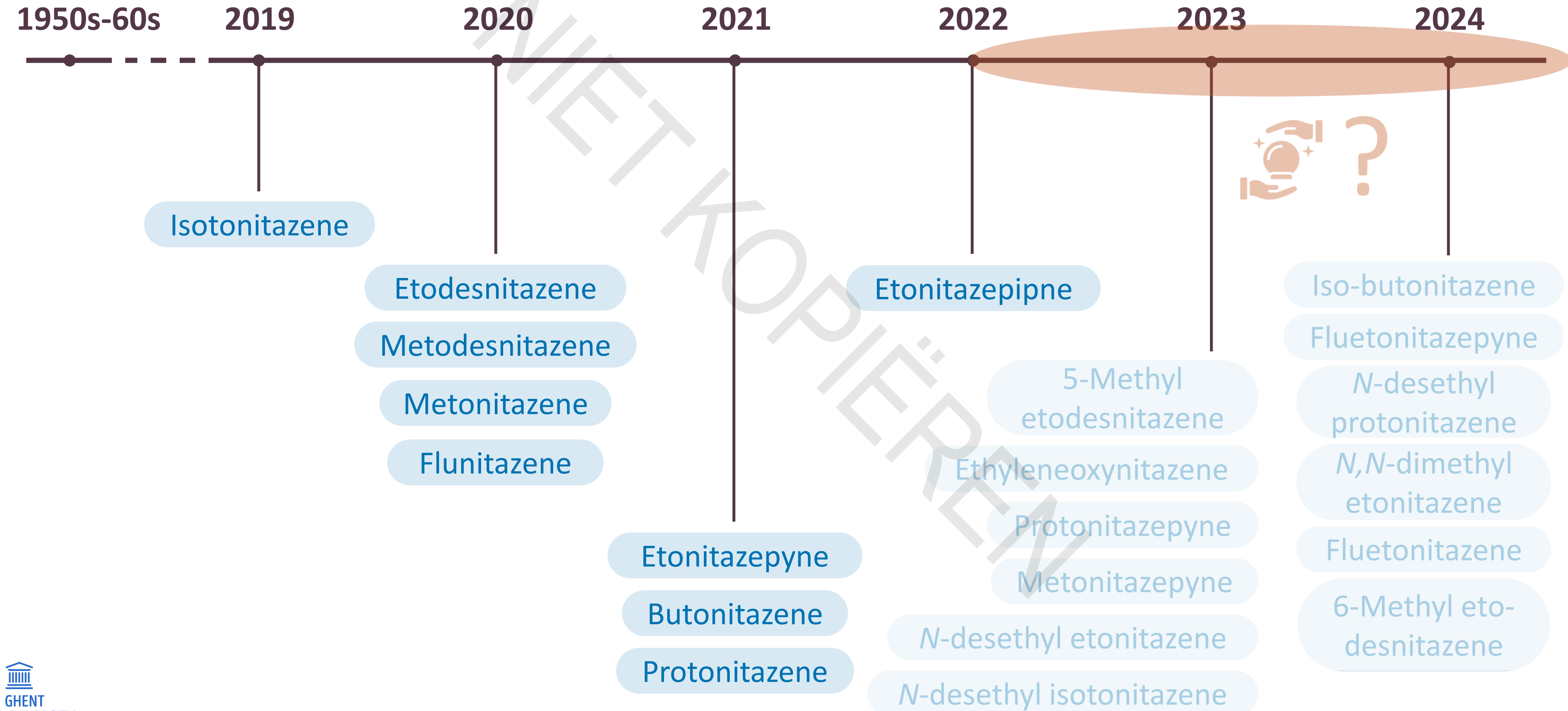
15 'prophetic' nitazenes

In vitro

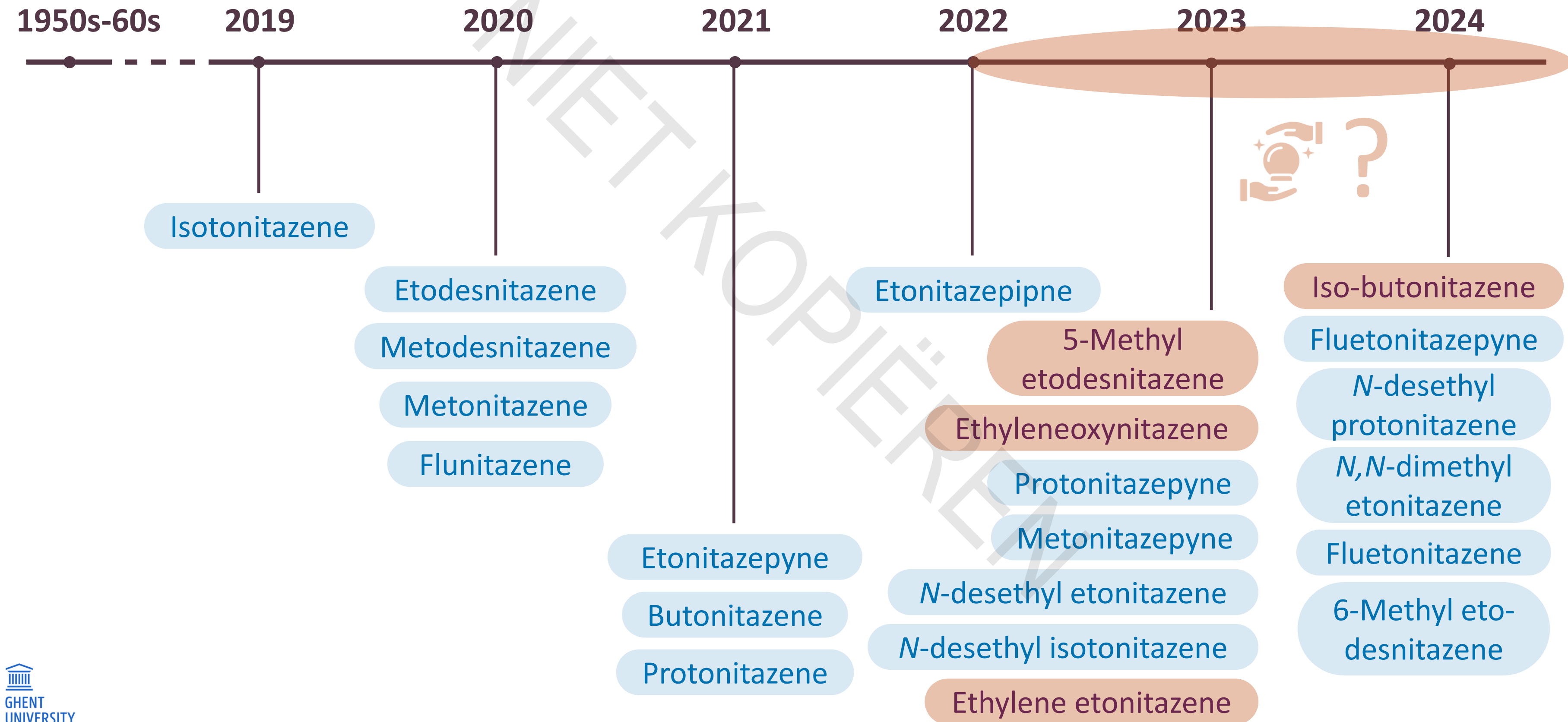
In vivo



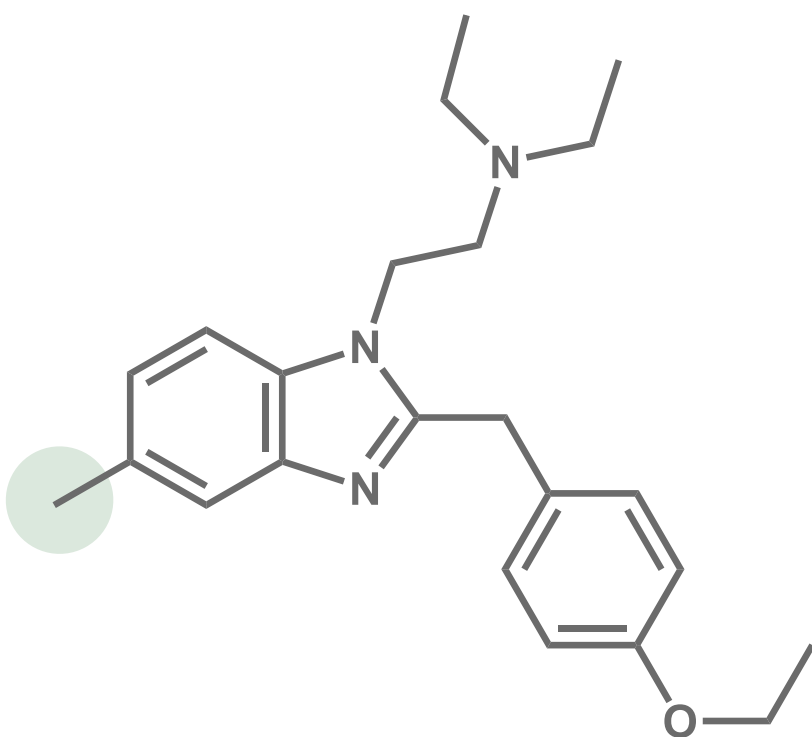
Nitazene opioids: from structure-activity relationship to 'prophetic' nitazenes



To date, 4 prophecies came true...

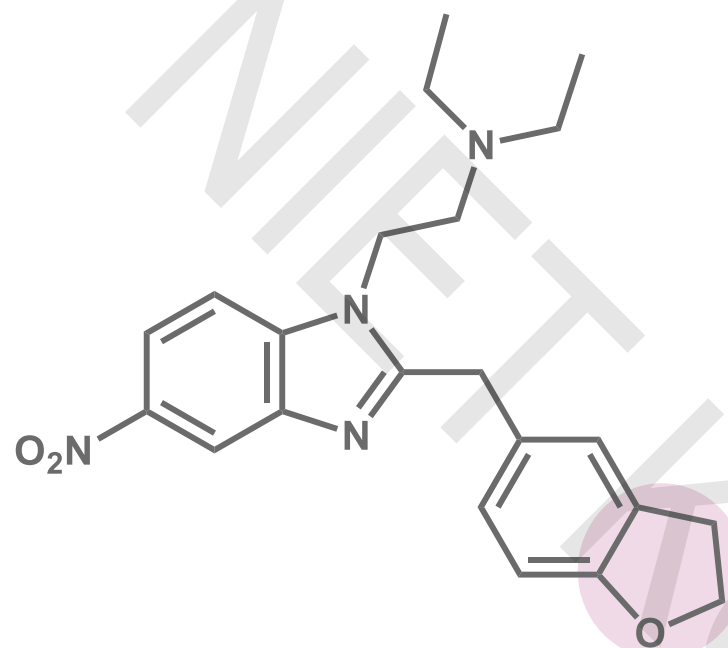


To date, 4 prophecies came true...



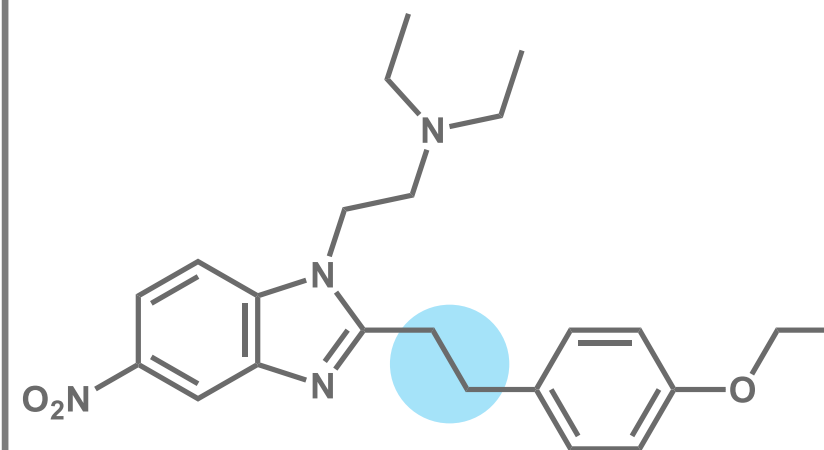
5-Methyl etodesnitazene

2022
(Finland + USA)



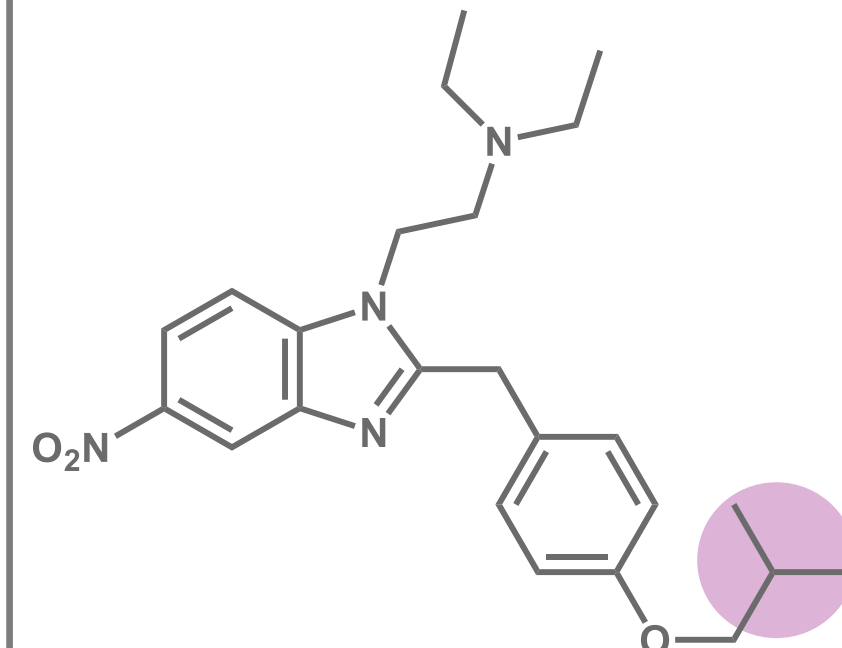
Ethyleneoxynitazene

2023
(Canada, Estonia, Latvia)



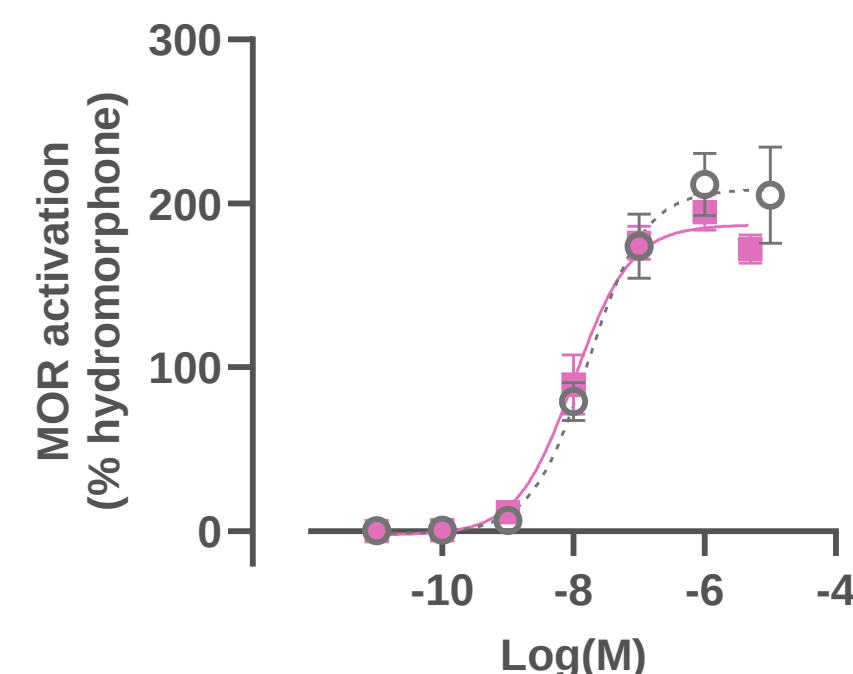
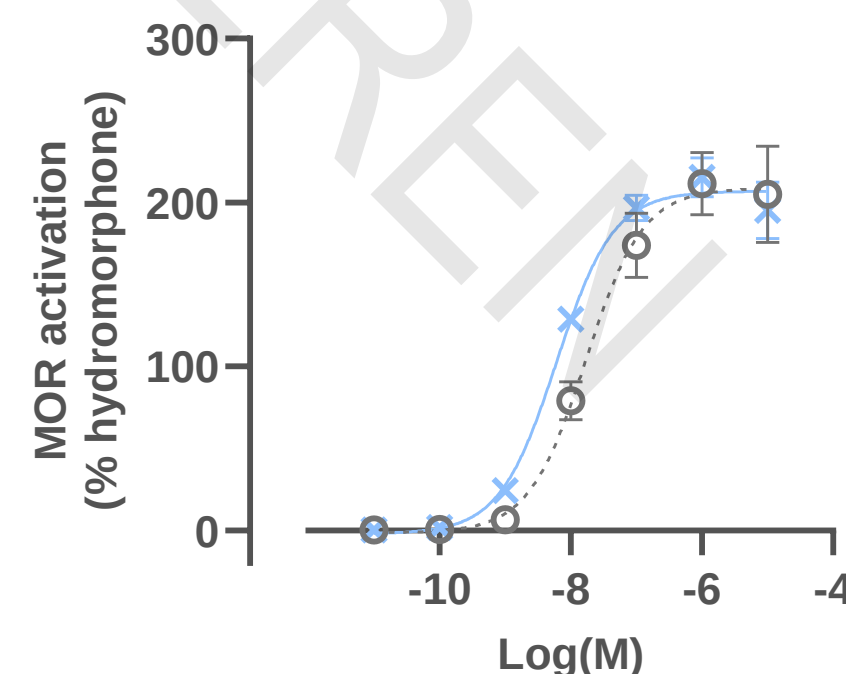
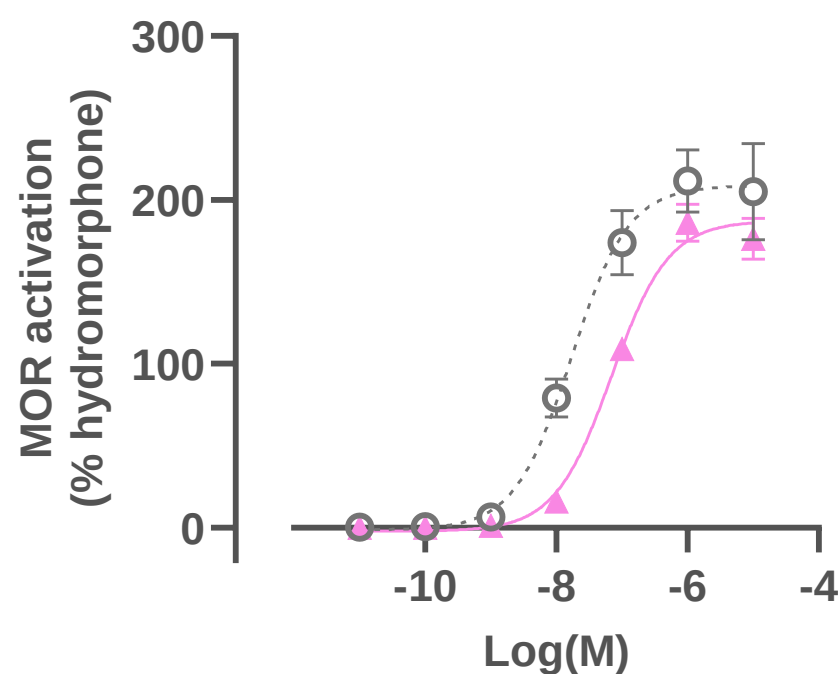
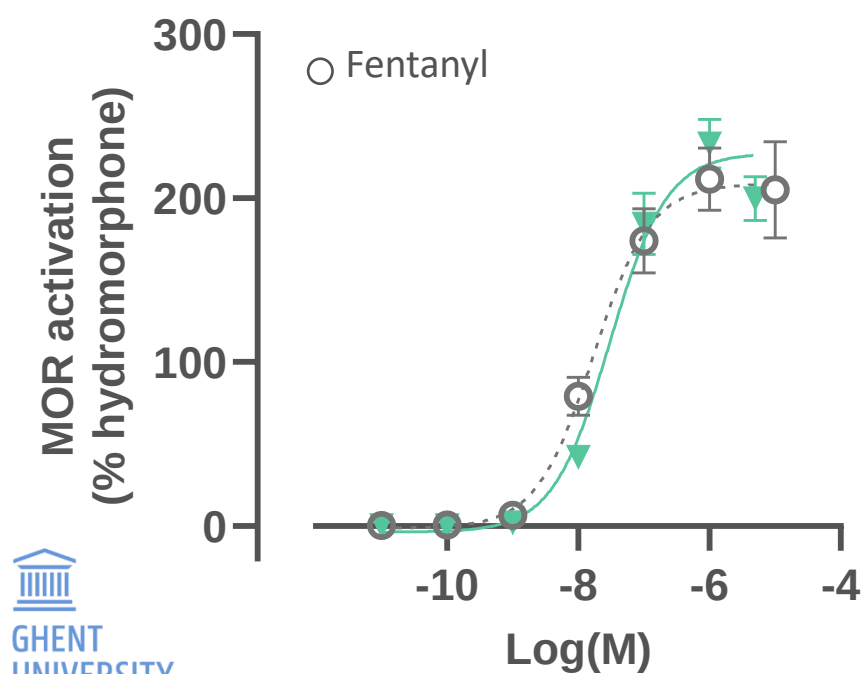
Ethylene etonitazene

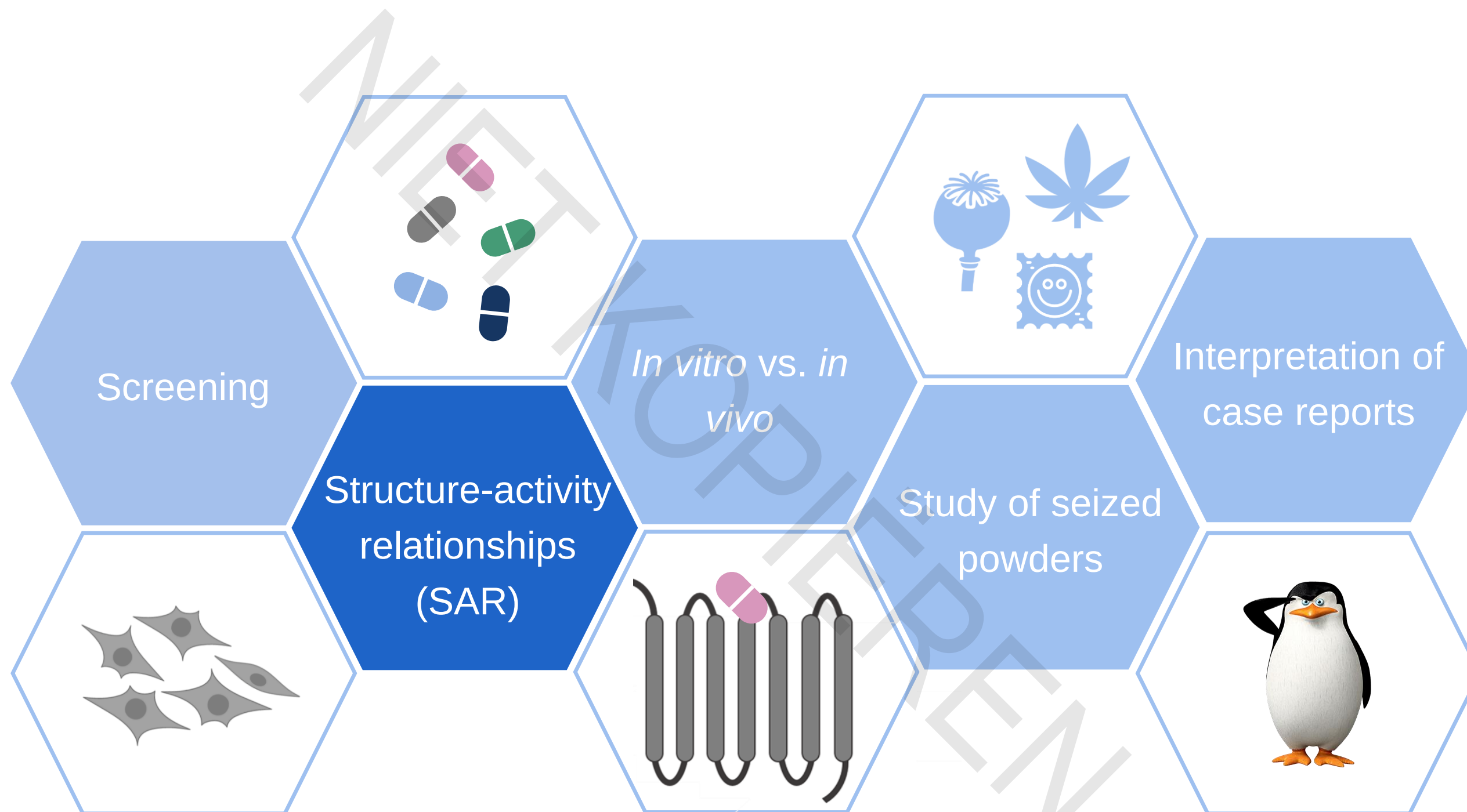
2024
(USA)

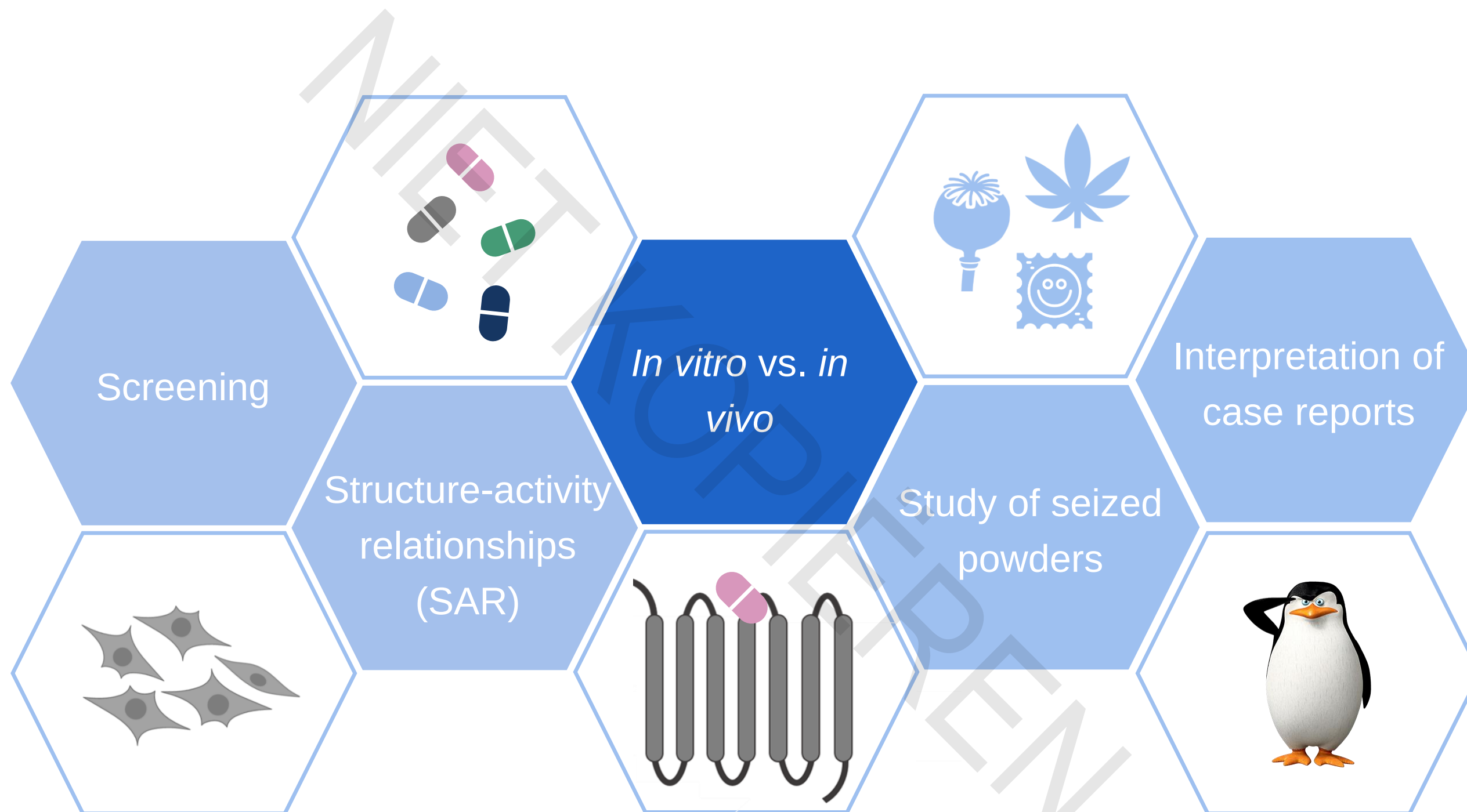


Iso-butonitazene

2024
(Europe)





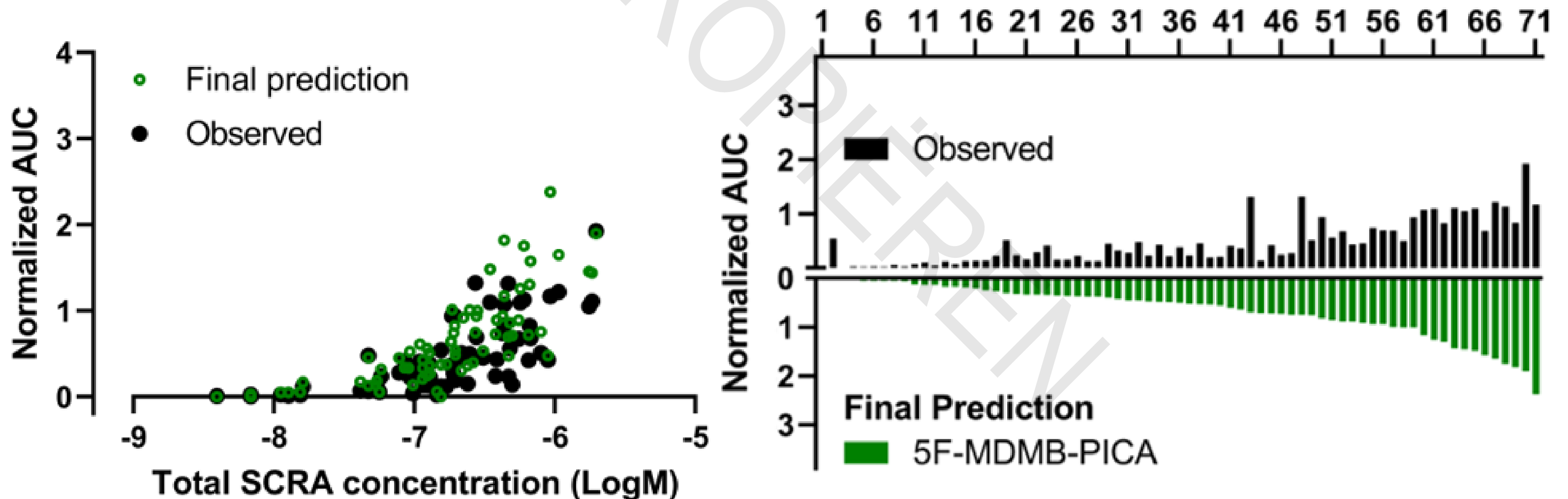


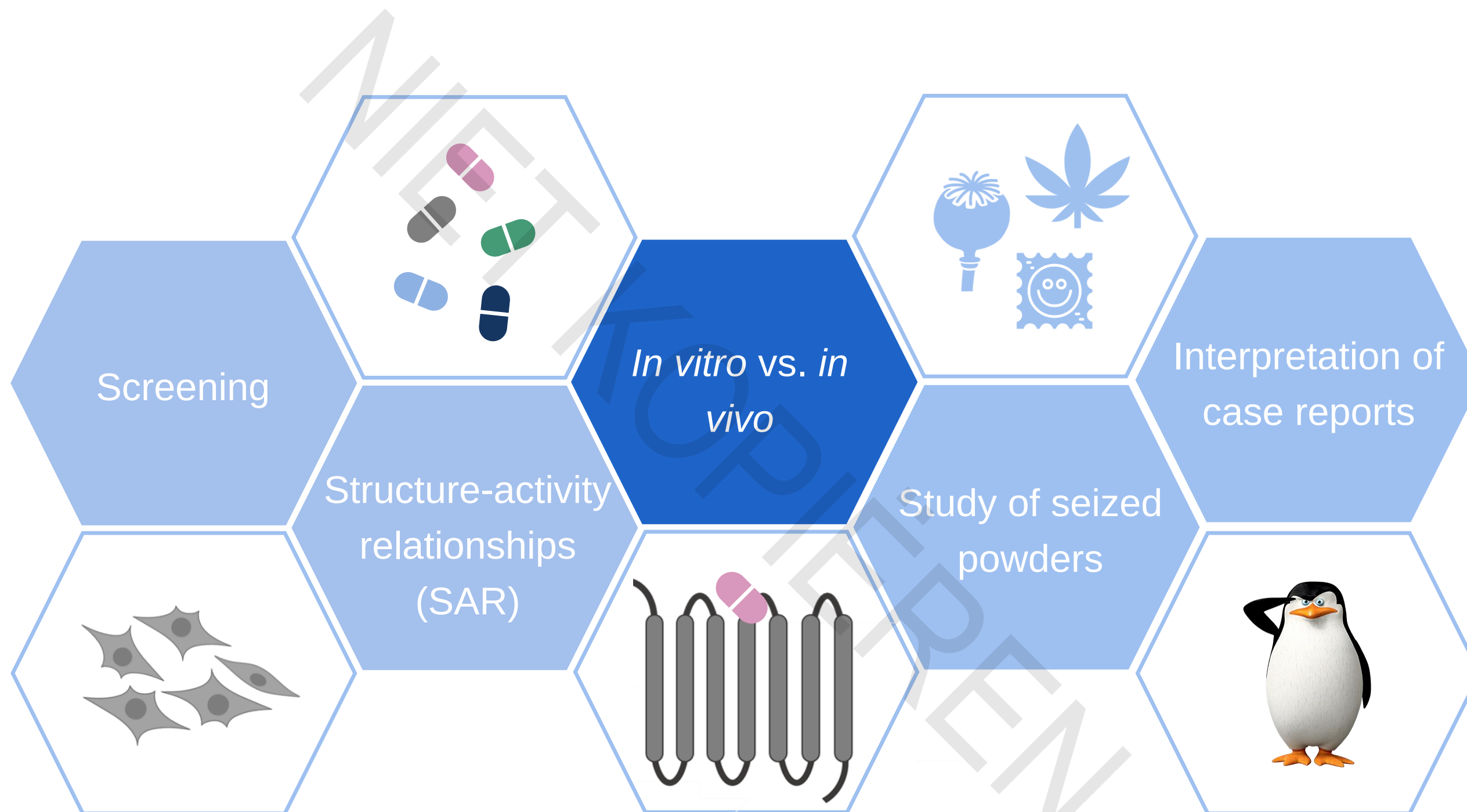
In vitro vs. *ex vivo*: synthetic cannabinoid receptor agonists

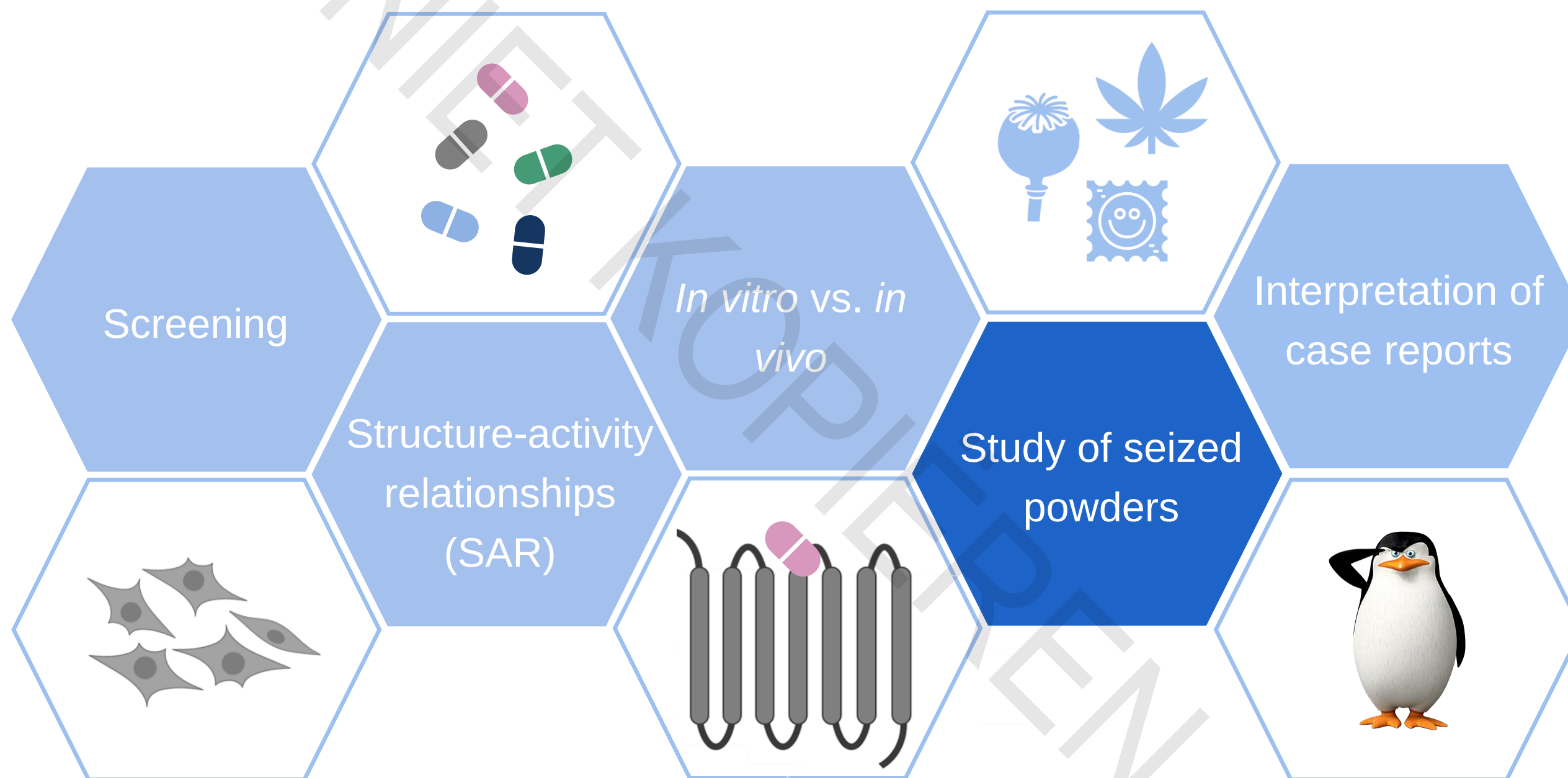
Cohort of 71 patient samples with as only SCRA 5F-MDMB-PICA or its metabolites

Determination of:

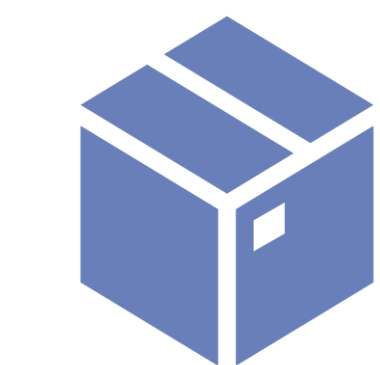
- Concentrations 5F-MDMB-PICA & metabolites
- *In vitro* activity of reference compounds
- *Ex vivo* activity of serum extracts



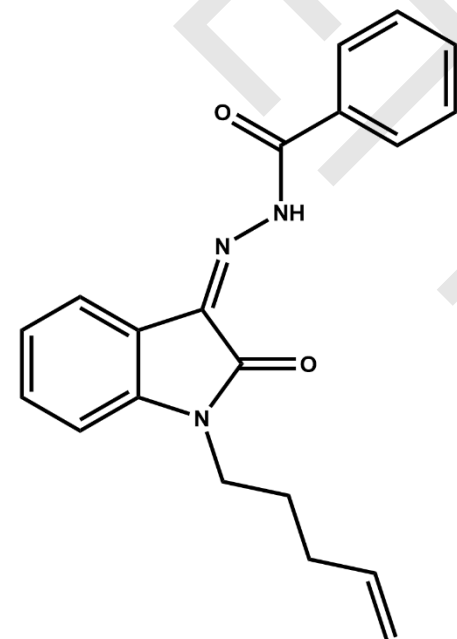




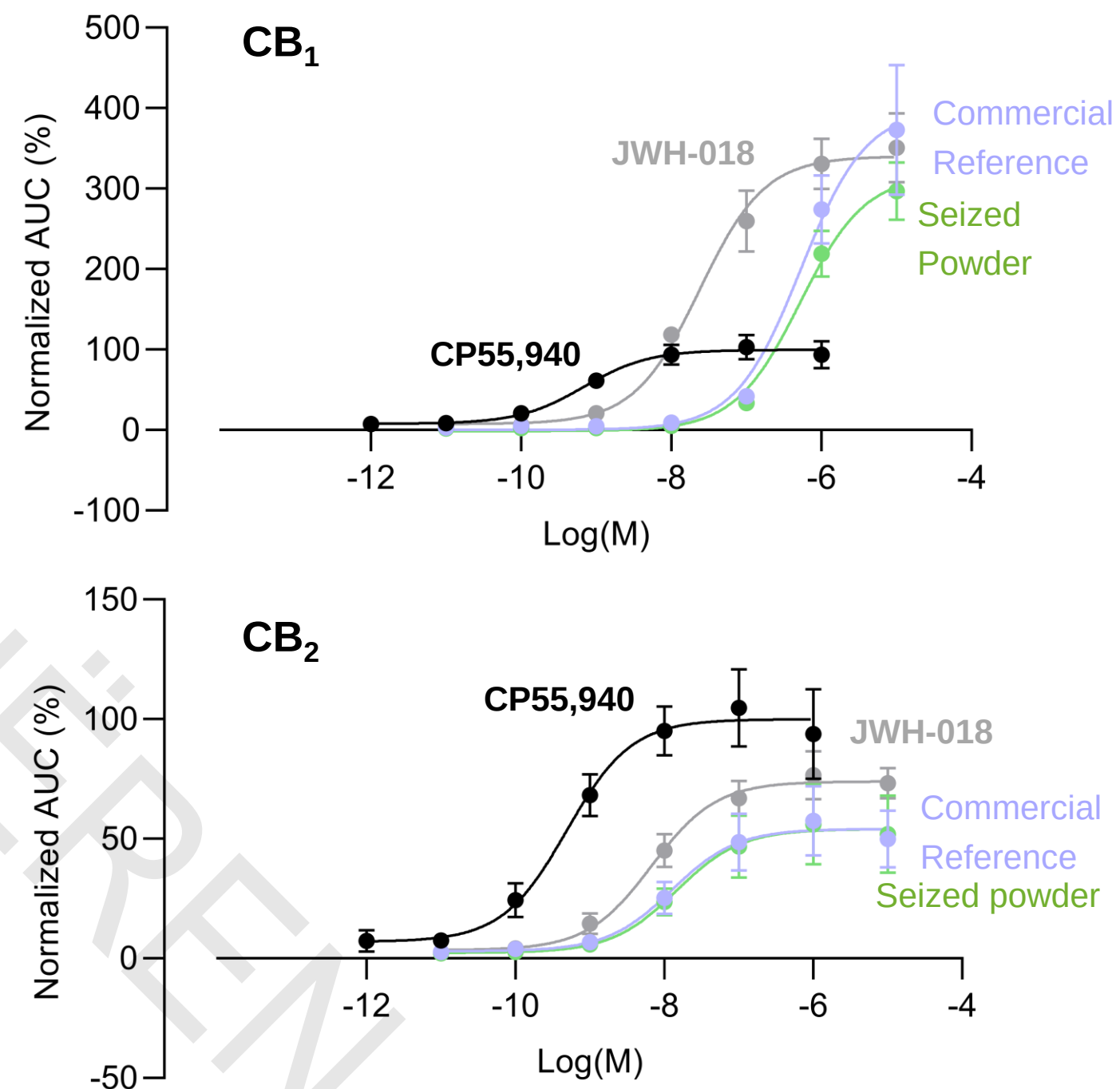
Study of seized powders: assessment of activity / purity



Federal
Public Service
FINANCE
CUSTOMS AND EXCISE



BZO-4en-POXIZID
(seized powder)

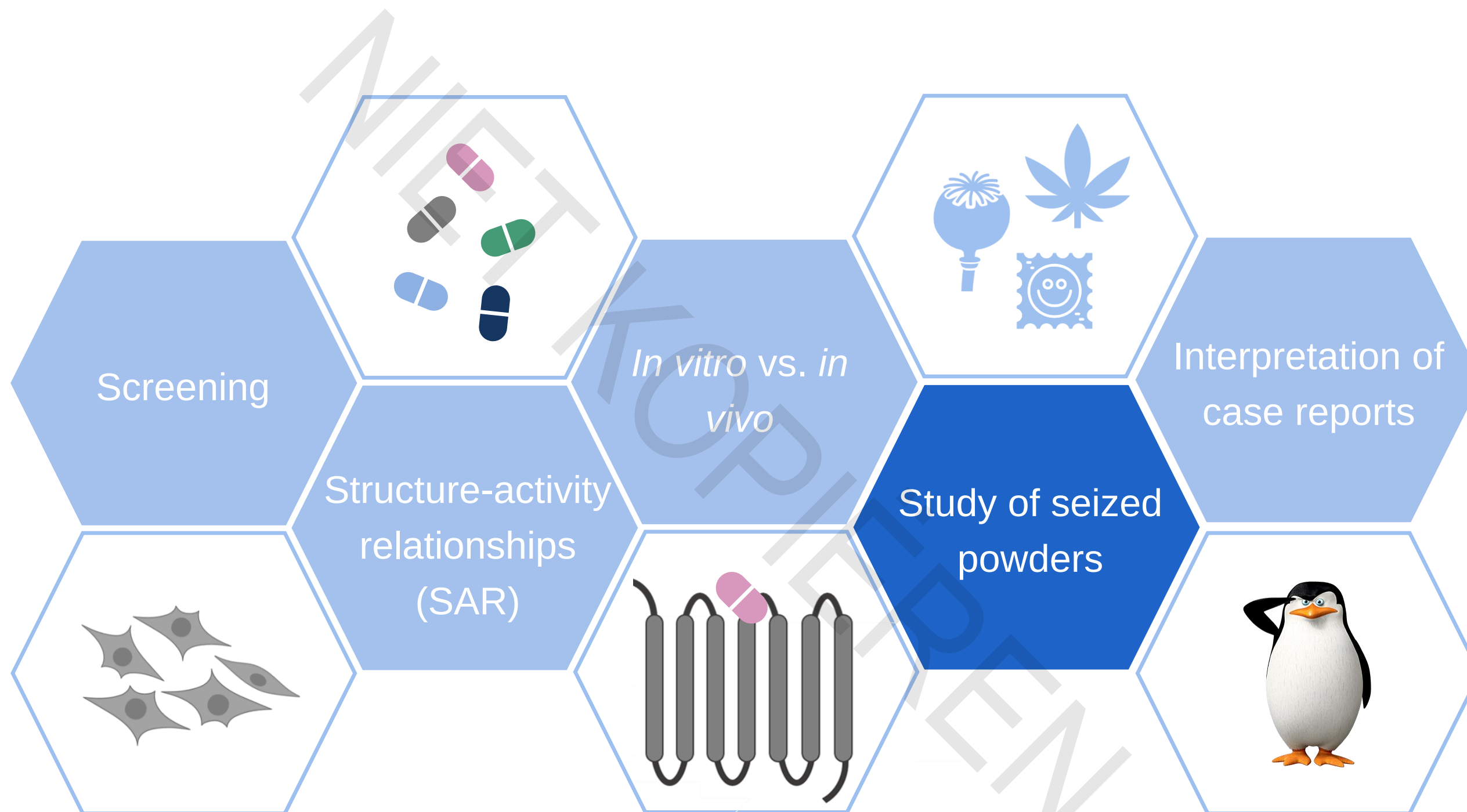


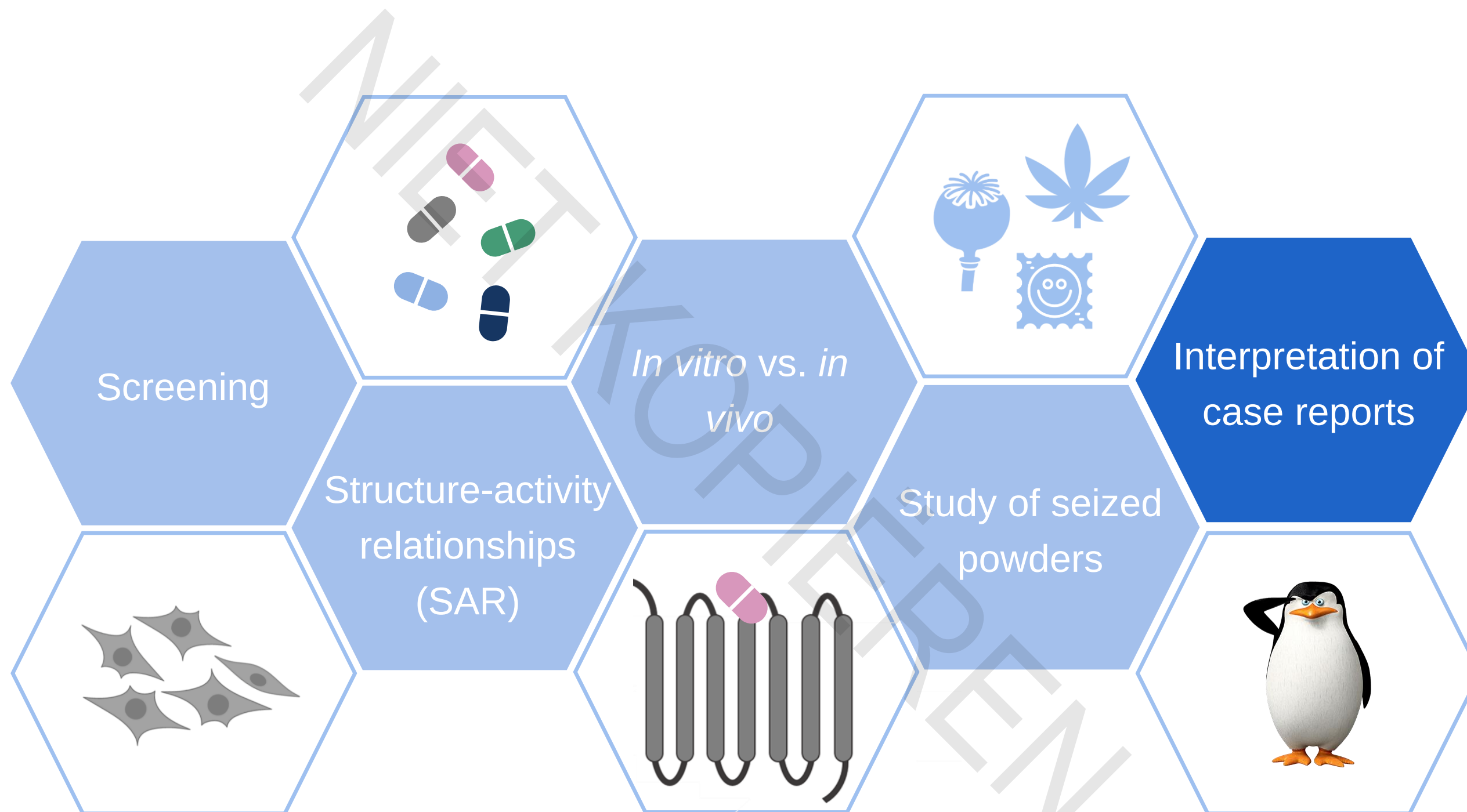
Purity supported by analytical data (GC-MS, LC-QTOF-MS, FTIR and HPLC-DAD)

Expressing the 'strength' of a tablet/powder relative to a reference

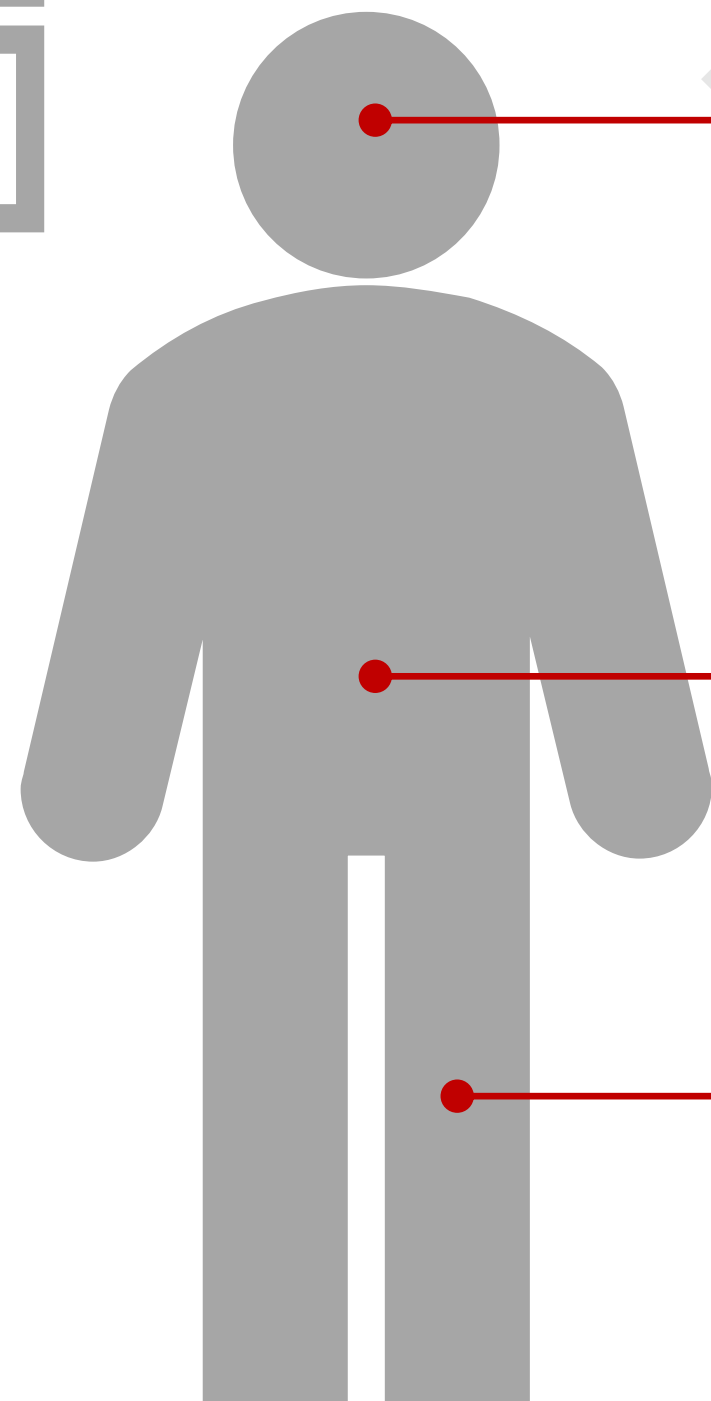


‘fentanyl equivalents’





Interpretation of an etonitazepipne case



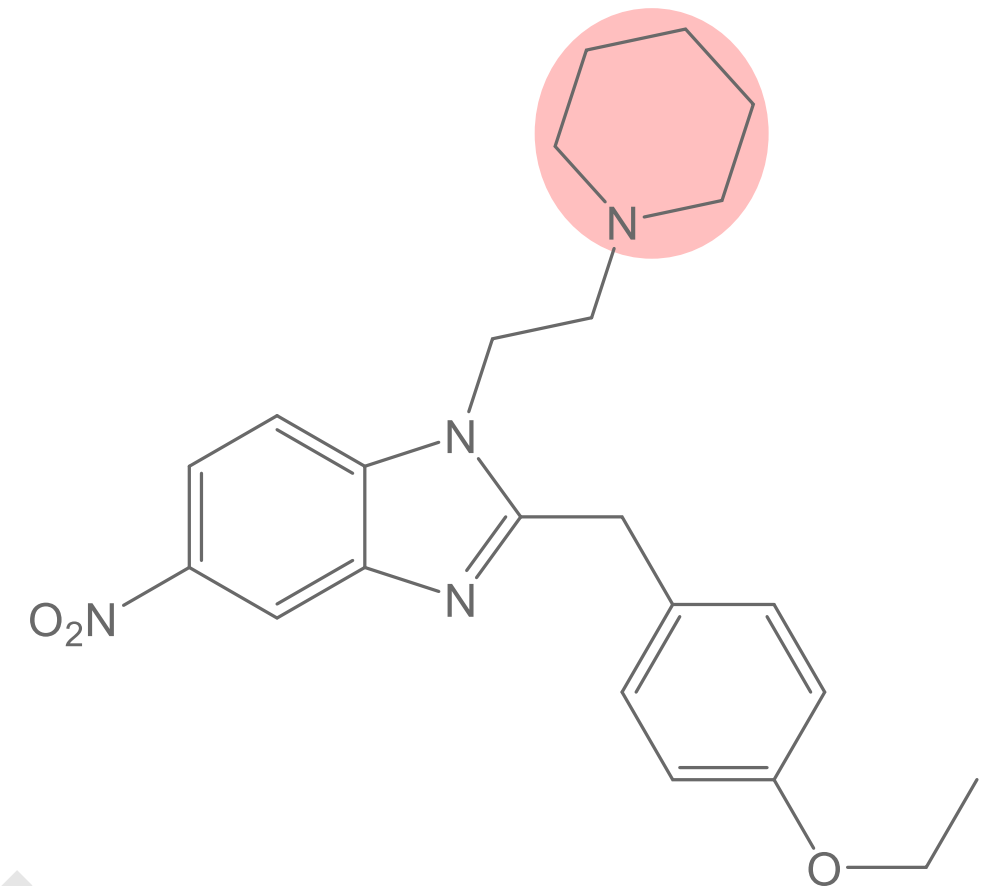
History of anxiety

Severe abdominal pain,
nausea, cramping

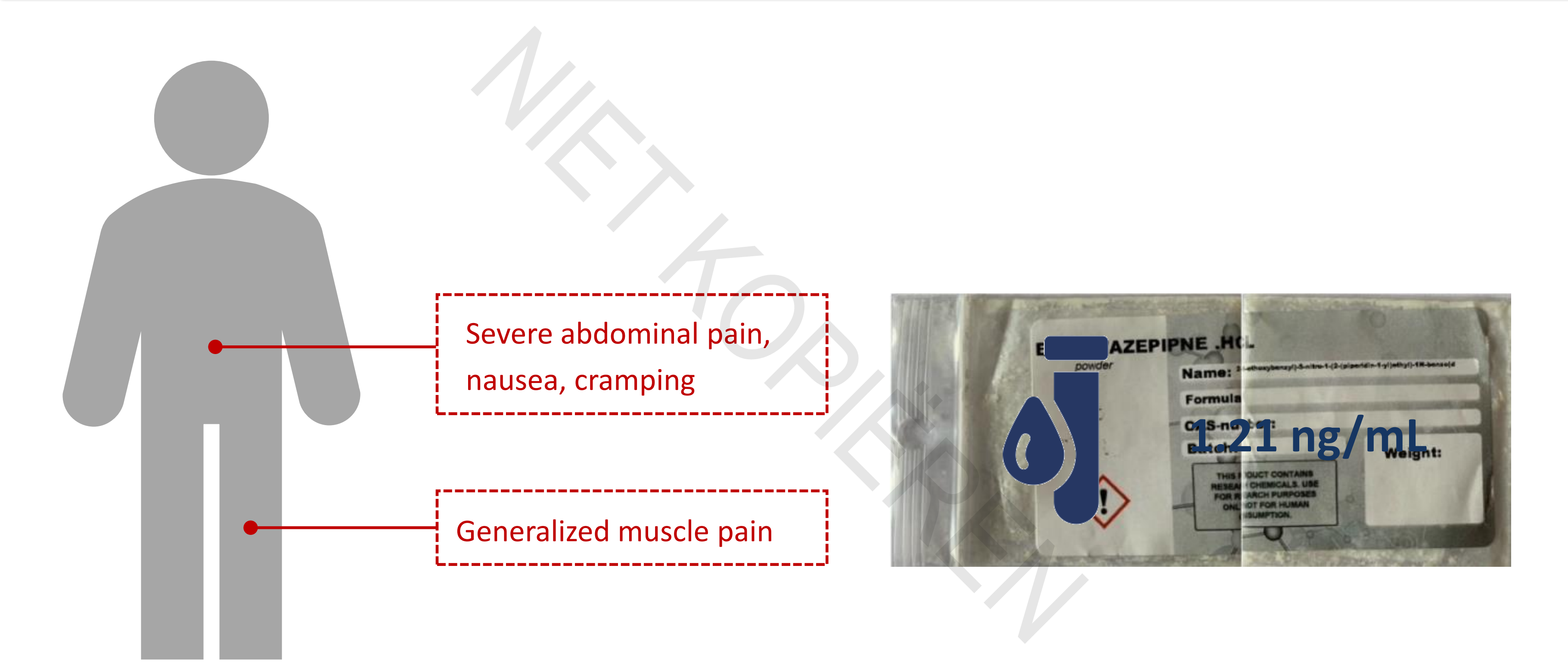
Generalized muscle pain

25 y/o male

Etonitazepipne
(*N*-Piperidinyl
etonitazene)

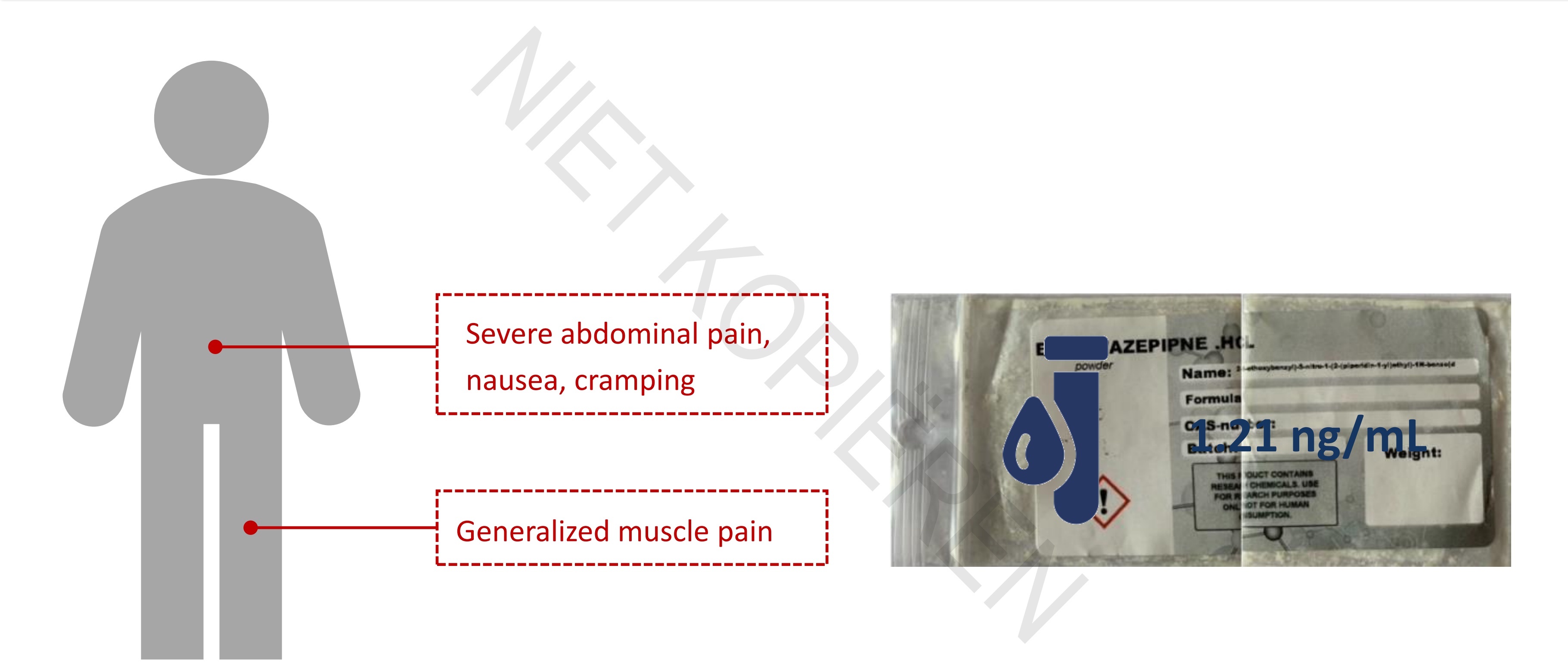


Interpretation of an etonitazepipne case

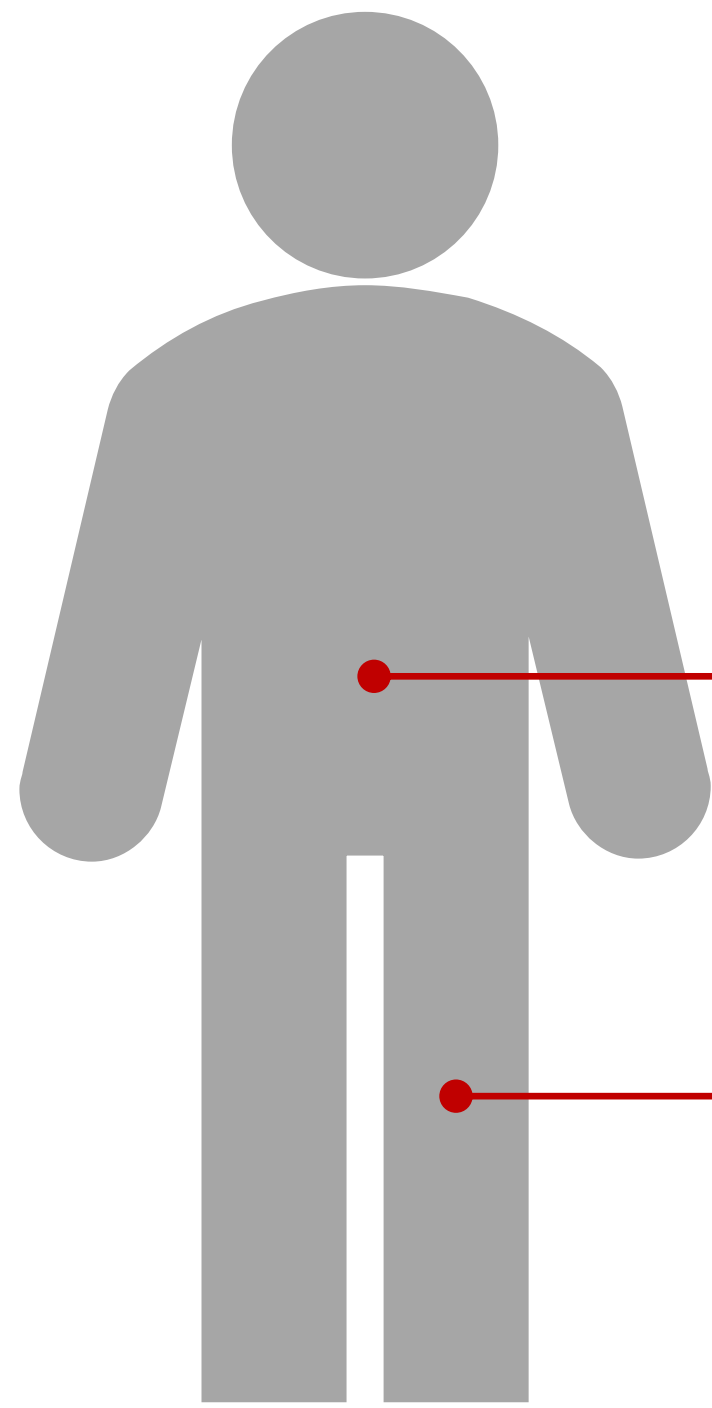


Severe abdominal pain,
nausea, cramping

Generalized muscle pain



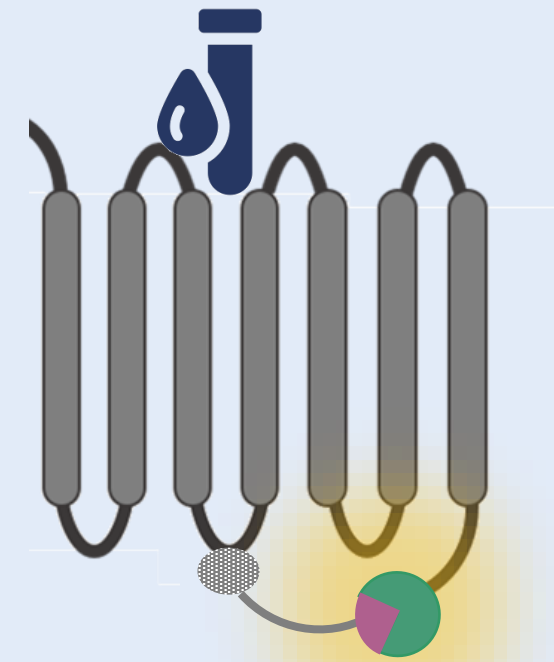
Interpretation of an etonitazepipne case



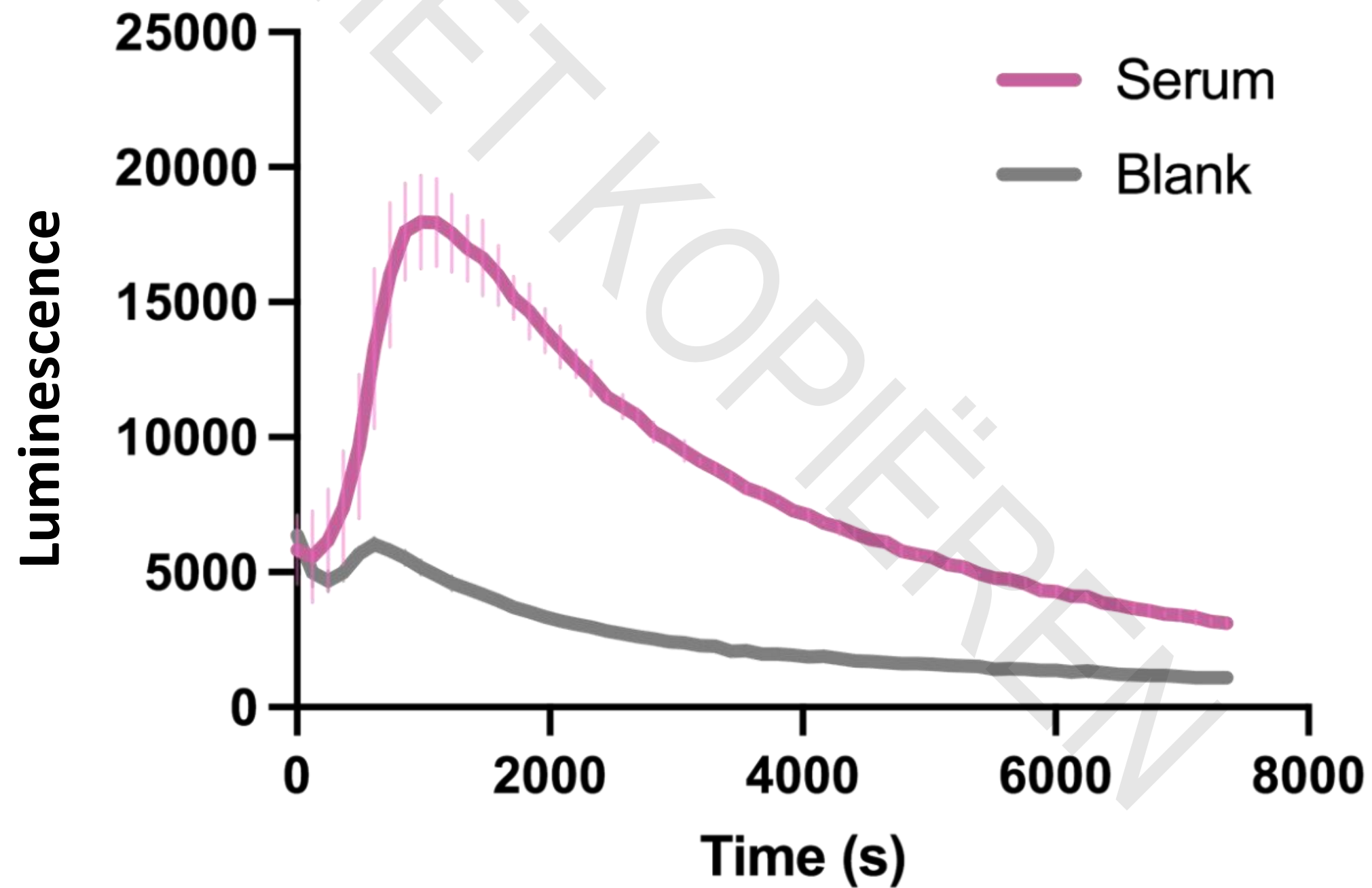
Severe abdominal pain,
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Generalized muscle pain

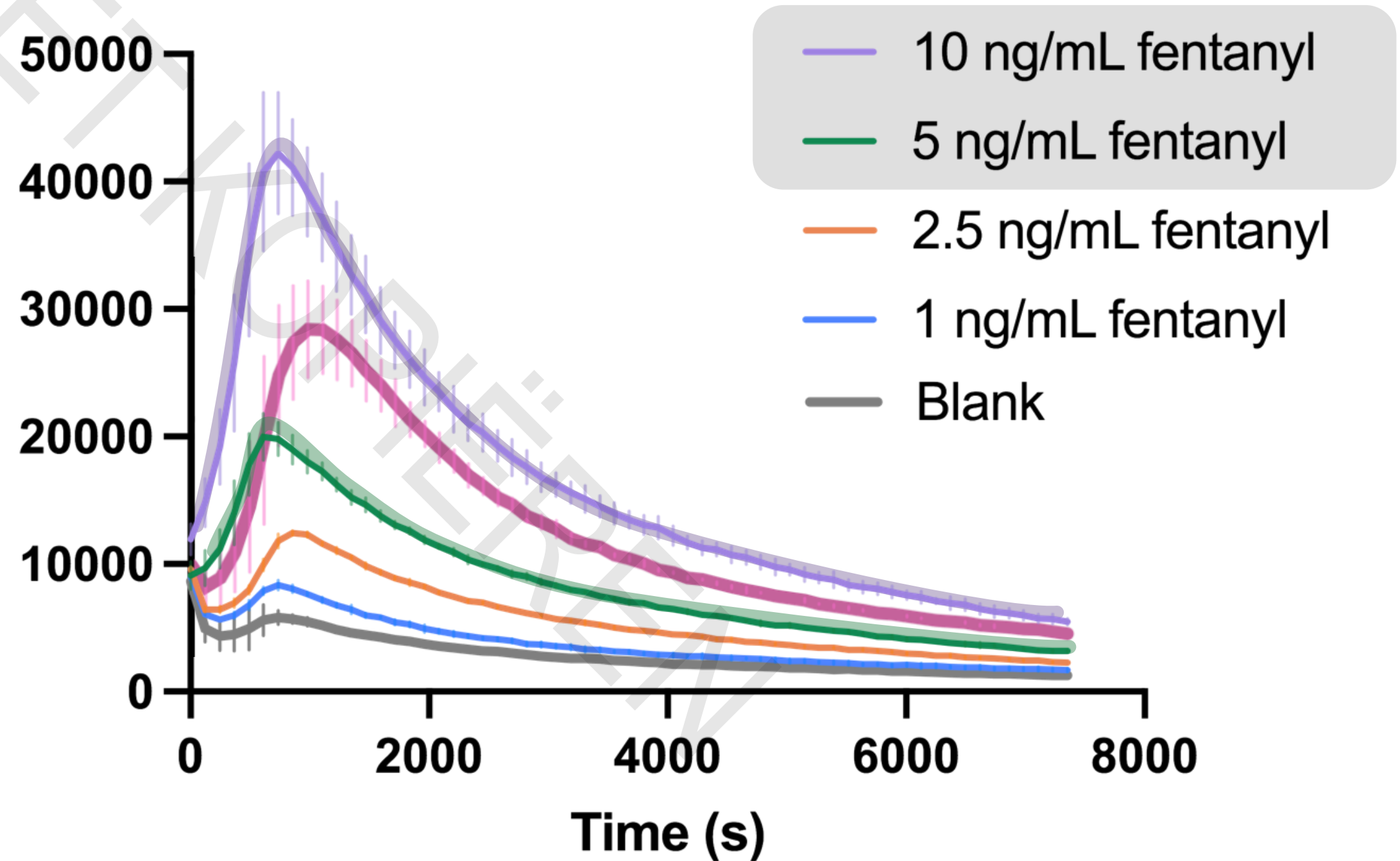
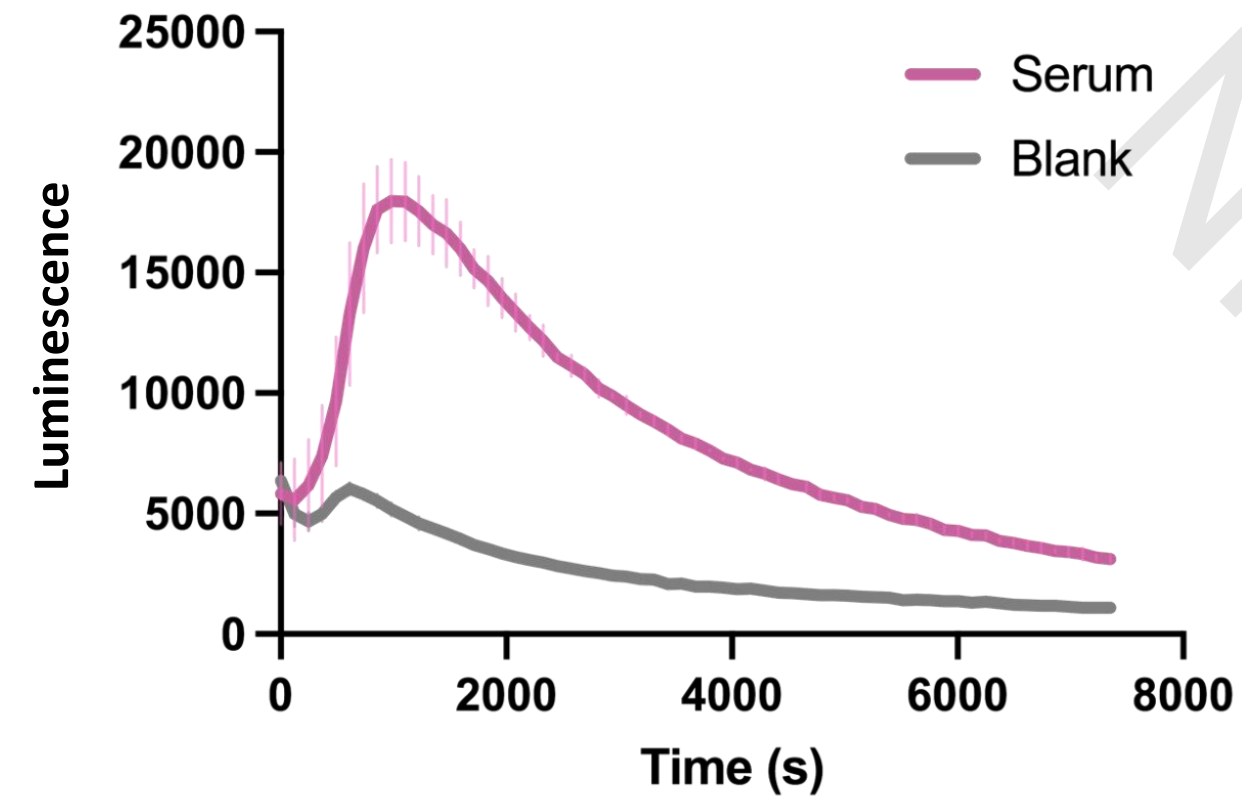
Opioid “activity
equivalents”



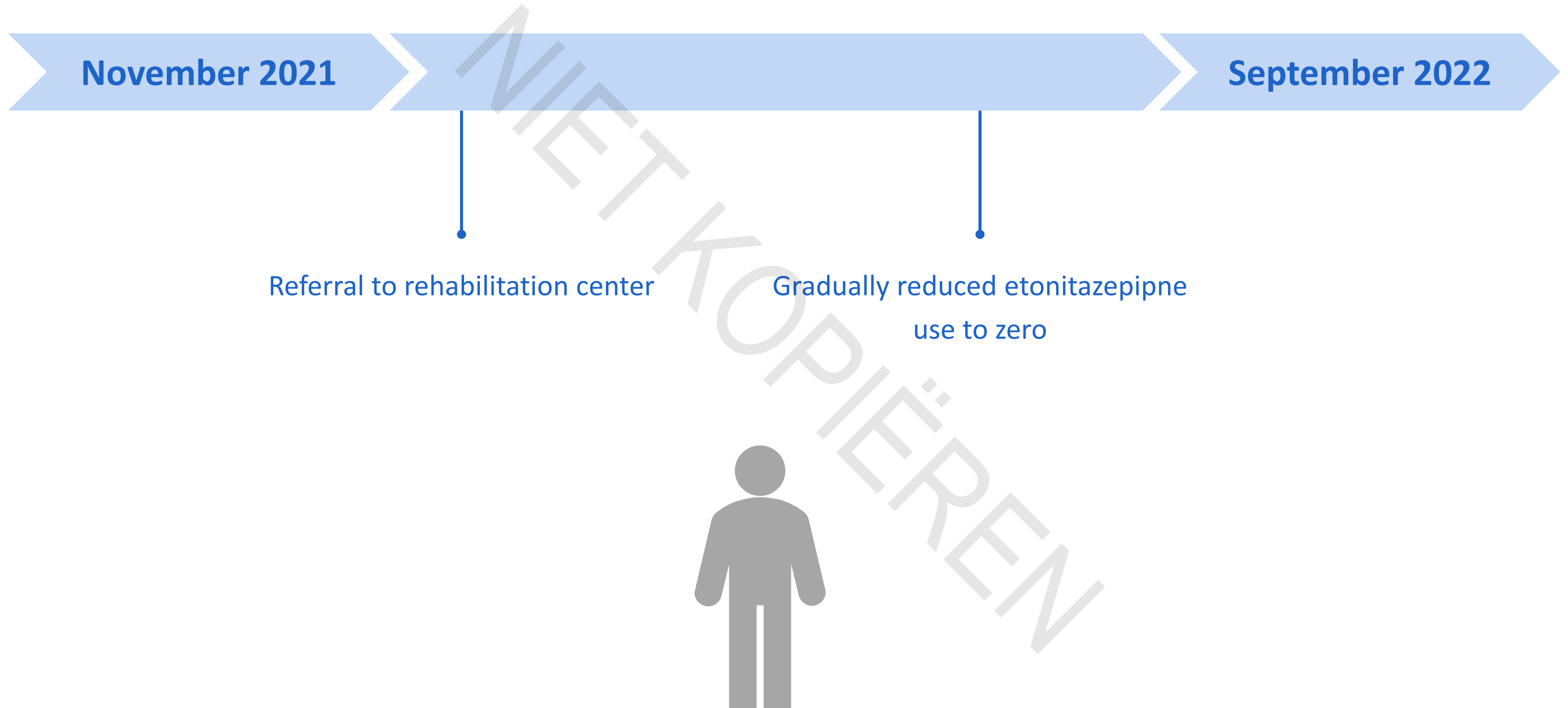
Interpretation of an etonitazepipne case



Interpretation of an etonitazepipne case



Interpretation of an etonitazepipne case



Interpretation of an etonitazepipne case

November 2021

September 2022



Successful-Ad6094 · 5d ago

Hey man. Just saw your post on accident.

I was extremely addicted to etonitazepipne, in dosages that were pretty much insane. At some point, I was just snorting the pure powder, and I reached a point where I just couldn't take enough to not get any withdrawals. I finally gained enough courage to get to my doctor, and I told him it was pretty much equipotent to fentanyl. He told me to just keep taking what I was taking and pointed me to an addiction specialist, who put me on suboxone. I suffered for a day before I could take the suboxone, but after I was on it for two days, I kinda felt free again. I started at 16mgs and for some weird reason, today was actually the last day of me taking it (was taking 0.5mg for two weeks now)

If you're serious about withdrawing, its best to swallow your pride and just get help. I've tried 10 times and I eventually just had to get back on it harder and harder. Its a very dangerous substance. I started to actually inject pure fent at some point to just stave of withdrawals.

I was taking about 10-15mgs a day by the way. I gave a sample of my eto to my doctor who got it analyzed, and a professor made a paper on PubMed about it. (Google "First identification etonitazepipne" and you'll get the article, they actually did a great job identifying it and analyzing the MOR activity, equivalency to morphine, etc.

You got this, if you need more help, send me a message. Kinda went fast over it. TLDR I was on 15mg+ a day, got put on suboxone 16mg, tapered over 6 months!! Until today and tommorows my first day without an opiate since a year and half ago.

Vandeputte & Verougstraete *et al.* (2022),
Arch. Toxicol.

-  New Psychoactive Substances
-  Activity-based assays: what?
-  Applications of activity-based assays: Screening & Characterization
-  **Conclusions**

Increasing **complexity** of the NPS market

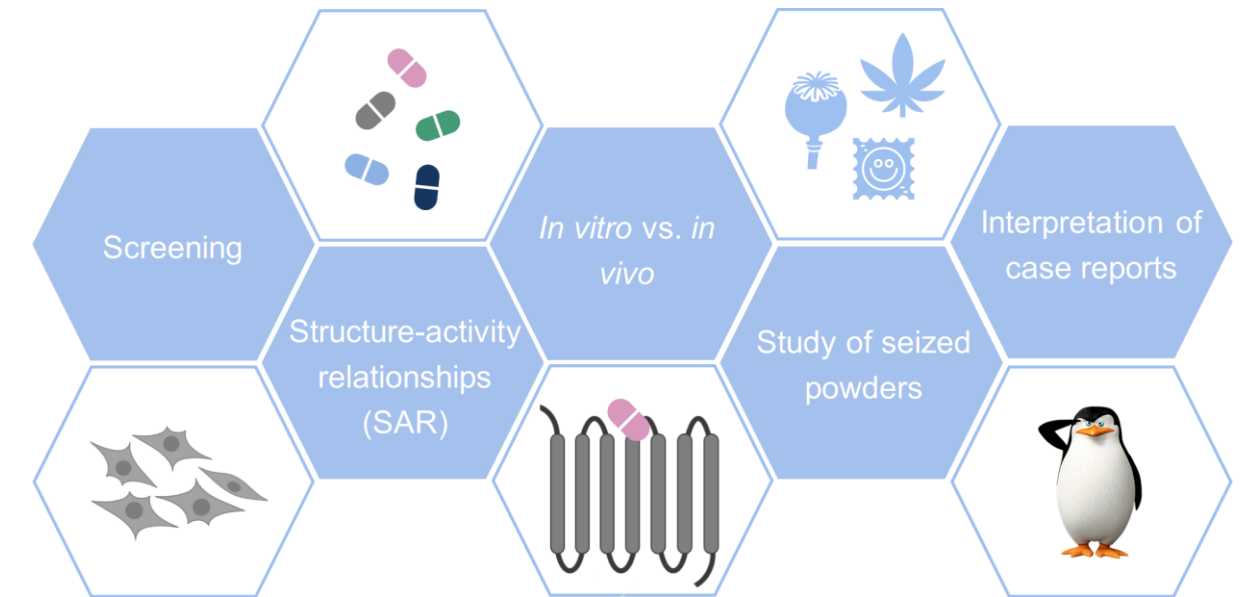
Bioassays to monitor receptor activation

Activity-based **detection** allows universal screening for synthetic opioids & cannabinoid receptor agonists

- allows to rule out *relevant* presence in forensic cases
- confirmation of positive cases still required

Activity-based **characterization: prioritization & early risk assessment**

Global & multidisciplinary partnerships to combat the NPS problem





Penguins of Madagascar, 2014

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... and the MANY collaborators from all over the globe