

NPSsay

Development and implementation of a new psychoactive substances receptor activation assay

DURATION
15/12/2016 - 15/03/2019

BUDGET
150 000 €

PROJECT DESCRIPTION

CONTEXT

About 450 new psychoactive drugs (NPS), with the largest group being the synthetic cannabinoids, have appeared on the worldwide drug market the last decade. The major challenge is the continuous chemical development of NPS, making it difficult to obtain up-to-date techniques for monitoring of these substances in bulk form or in biological samples. At the moment, the customs and toxicological laboratories work with high-end equipment such as nuclear magnetic resonance spectrometry and (time-of-flight) mass spectrometry to structurally elucidate NPS or detect these compounds. However, these techniques are time-consuming, tedious and expensive. Quick and inexpensive, high-throughput-compatible tests, capable of demonstrating the presence of an NPS would increase the ability of the public organizations to respond fast to this global problem. Although at the moment some known NPS can be detected via rapid immunological tests, these tests are quickly outdated as they target a chemical structure and cannot cope with the continuous evolution in NPS structure. In addition, they often lack sensitivity. As a result, the public organisations are always a step behind. Development of high-throughput screening techniques focusing on their activity, rather than their chemical structure is of major importance as Belgium will publish a generic legislation shortly, making a broad range of NPS illegal.

OBJECTIVES

Within this project, a stable cell system for a novel *in vitro* bio-assay, will be set up and deployed, allowing the detection of 'unknown' NPS in bulk materials or biological matrices (e.g. urine, oral fluid), based on their ability to activate G-protein coupled receptors (GPCRs). The developed technique, a bio-assay, will help to ensure an up-to-date detection for several public organizations, not only resulting in a better knowledge-database but also allowing a better assessment of potential harm of NPS.

METHODOLOGIE

First, a bio-assay detecting synthetic cannabinoids will be developed. In a second stage, the activity-based screening will be extended to other recently emerging synthetic drugs, such as synthetic opioids. Ultimately, the NICC will evaluate the test's applicability and added value in the context of a routine forensic laboratory. The bio-assay that will be developed utilizes the concept of functional complementation, based on the NanoLuc[®] Binary Technology (Promega), which makes use of inactive subunits of the NanoLuc[®] Luciferase, Large BiT (LgBiT) and Small BiT (SmBiT). These subunits will be coupled to the two proteins of interest, being the GPCR on the one hand and the β -arrestin 2 protein (β arr2) on the other (Figure 1). Both fusion proteins will be expressed in the cell. Upon activation of the GPCR, the β arr2-fusion protein is recruited to the receptor, which results in functional complementation (i.e. re-association of SmBiT with LgBiT) of the NanoLuc[®] Luciferase, which can be easily monitored via luminescence.

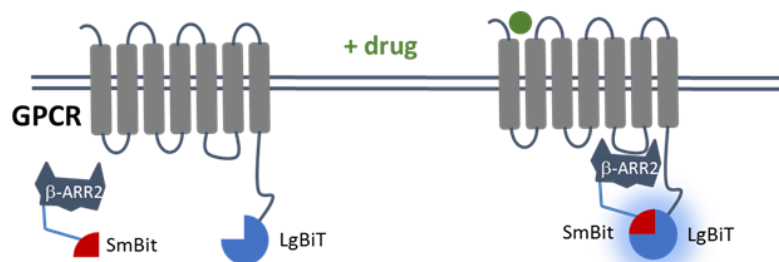


Figure 1 : Example of a set-up on the developed bio-assay.

NPSsay

IMPACT

With the known limitations of broad implementation of current structure-based methods, the public organizations are always a step behind. It is difficult and costly to broadly implement up-to-date structure-based (via liquid chromatography coupled to tandem or high resolution mass spectrometry) screening. Therefore, it is known that positive cases are missed. Within this project, a stable cell system will be set up and deployed, allowing screening in 96-well format (hence high-throughput-compatible) of synthetic cannabinoids or other NPS in bulk materials or biological matrices, based on their ability to activate their respective receptors. As the screening is based on activity rather than upon chemical structure, this would allow the detection of all NPS, regardless if their structure is known. As far as we are aware, the application of 'activity-based' screenings assays is a novel approach that has not been used in a toxicological/forensic setting so far, anywhere in the world.

The impact of this project clearly lies in the increase of the detection capabilities of NPS for customs, public health and forensic laboratories. The up-to-date detection will result in the possibility to direct action or information gathering for several public organizations such as the Departments of Justice, Public Health, Finances and Interior Affairs. Because detection of NPS is a worldwide problem, and given the novelty of the approach to be used, the impact of the project will likely not remain limited to Belgium, but may be extended on a global scale.

DESCRIPTION OF FINISHED PRODUCTS OF RESEARCH AT SHORT AND MEDIUM TERM.

The obtained results will be shared with the national stakeholders such as the Departments of Justice, Public Health, Finance (customs) and Interior Affairs as well as the international forensic community.

- A. Publications in scientific journals and presenting the results at conferences.
- B. Participation in workshops for public organizations to present the results of the project:
 - Participation in the workshop 'Strategies for the Detection of Synthetic Cannabinoids in Biological Specimens' hosted at the 55th Annual meeting of International conference of The International Association of Forensic Toxicology (TIAFT), joint meeting with the Society of Forensic Toxicologists (SOFT), Boca Raton, Florida, US, 06/01/2018. Bioassay-based screening of synthetic cannabinoids: adding a new spice to the toxicologist's palette. (Cannaert A). (oral presentation for international group of toxicologists).
- C. Implementation in the NICC workflow for seizures and biological samples: foreseen in 2018-2019.

CONTACT INFORMATION

Coordinator

Sarah Wille
NICC / Afdeling Toxicologie
INCC / Département Toxicologie
Sarah.Wille@just.fgov.be

Partners

Christophe Stove
UGent/ Afdeling Toxicologie
Stove@UGent.be

Annelies Cannaert
NICC / Afdeling Toxicologie
INCC / Département Toxicologie
Annelies.Cannaert@just.fgov.be
Annelies.Cannaert@UGent.be

LINKS

<https://nicc.fgov.be/>