Results overview:

1. Selecting potential synthetic cannabinoids compounds after scientific literature analysis.

Firstly, a thorough scientific database interrogation, using the following three criteria, was necessary for choosing the suitable synthetic cannabinoids for achieving our project objectives.

1. High CB1 and CB2 receptor affinity, highly selective for CB1
2. Available/ unavailable scientific data regarding their effects on chronic neuropathic pain, but also the scarce existing information about the toxicology of these compounds.
3. The Limit of detection (LoD) by liquid chromatography coupled to mass spectrometry (LC/MS), allowing an accurate measurement of their levels in biological samples.

As a result, 4 synthetic cannabinoids were selected and then included in our research.

Secondly, further literary research was conducted regarding major phytocannabinoids and their mechanism of action through CB1 and CB2 receptors, considering both clinical and preclinical studies.

While initially all attention was directed towards the endocannabinoid system, more recent studies suggest that many of the clinically proven effects of phytocannabinoids are based on intrinsic mechanisms that do not necessary involve CB receptors. Recent studies show that major phytocannabinoids and their derivatives also interact with non-cannabinoid receptors, being responsible for their pharmacological action in diseases such as Alzheimer's, Epilepsy, depression, neuropathic pain, certain types of cancer and diabetes.

However, further studies are needed before fully understanding these compounds' mechanisms. In the quest of enhancing their pharmacological effects, not only should we explore CB receptors or phytocannabinoids' structure modulation, but also more recently discovered courses of action.

Considering all these findings, it seems entirely possible to achieve our project's objective through naturally occurring cannabinoids, studying their potential effects on CB, CB2 receptors as well as on other receptor types. Therefore, a natural cannabinoid, with two different routes of administration, was also included in our efficacy study on chronic neuropathic pain.

In addition, the mechanism of action and the effects on chemotherapy-induced chronic neuropathic pain of the 4 selected synthetic cannabinoids will be studied

2. Compound formulation for acute toxicity assessment. Selection and optimization of experimental lots.

In order to evaluate the toxicity of the selected compounds, two routes of administration will be used (oral and intraperitoneal).

Regarding the tested doses, compound evaluation and specialized literary research were carried out. Given the complete lack of information on the toxicity of our chosen compounds and the limited existing toxicity studies on other synthetic cannabinoids, existing efficacy studies on structurally-related compounds were taken into account.

In the matter of compound formulation, we considered the possibility of their solubilization and/or incorporation in suitable forms for each of the two administration routes. An appropriate formulation method was established after a thorough scan through literature. For oral administration, a suspension by

using methylcellulose, carboxymethylcellulose or gum tragacanth allows drug delivery while maintaining their biodisponibility and avoiding stomach irritation. The administered suspension volume is calculated according to animal's weight (following internal working protocols).

For the intraperitoneal route of administration, the compounds will be solubilized in the most suitable solvent available, in the minimum effective dose so as not to produce unwanted pharmacological effects when mixed with saline solution.

3. **Creating the working protocol for the acute in vivo toxicology and safety screening stage for C1-C4.**

The protocol for the acute toxicity studies was finalized.

Acute toxicity assessment will be carried out according to OECD guidelines no. 423. This method estimates the range of values for the lethal dose 50 (LD50 - the dose that would lead to the death of 50% of the animals), while also evaluating the lowest dose at which quantifiable adverse reactions occur (organ toxicity or observable adverse effects during animal monitoring).

Rats' general behaviour will be individually noted at specific time intervals after treatment, according to the internal protocol. The number of deaths, removals from study and euthanasia for welfare reasons will be recorded during this period, and the LD50 value will be determined according to OECD guidelines.

Clinical biochemistry and haematology profiles will be determined using blood samples collected prior to animals' euthanasia.

All study animals will undergo a complete and detailed gross necropsy, which includes a careful examination of the external body surface, all orifices, cranial, thoracic, and abdominal cavities and their contents. The most important tissues will be preserved in an appropriate fixation medium according to tissue type for subsequent histopathological examination.

4. **Preliminary research for deciding on the working procedures for the following stages of our project.**

The following research has been carried out prior to the practical stages in order to:

1. Develop protocols for evaluating compound efficacy in chronic neuropathic pain.

2. Establish a working protocol for chronic toxicity evaluation for the compound that has the best results in the first two stages.

3. Maximize the reproductibility of the combined, transdermal nano-delivery system model by ß-cyclodextrin esterification.

4. Optimize PET-MRI acquisition parameters as to visualize the interest areas in the brain/CNS with the aim of tracking the studied compounds in the body through imaging techniques.

5. **Single dose toxicity assessment and LD50 calculation for the 4 compounds.**

All work protocols for this phase have been developed, and the phase will begin as soon as the manufacturers deliver the selected compounds for the study.