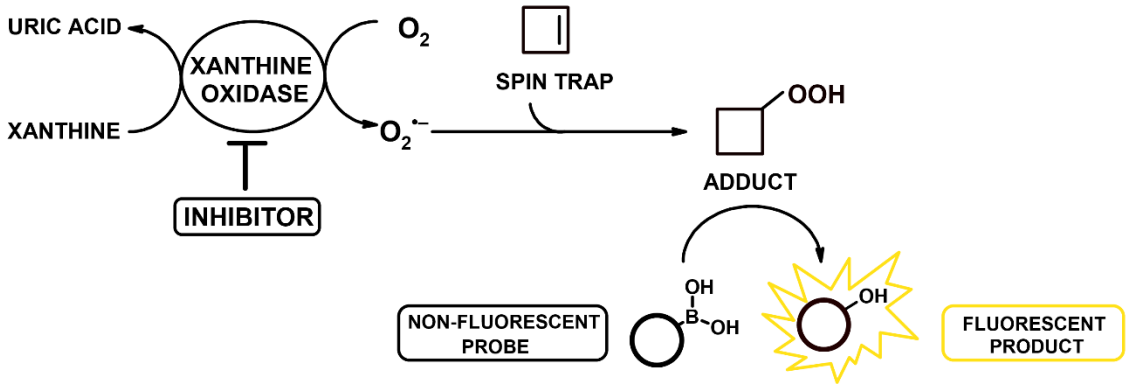
Development of novel screening method for identifying xanthine oxidase inhibitors – towards new drug candidates for gout

Xanthine oxidase (**XO**) enzyme plays a crucial role in the breakdown of purine nucleotides, which are the building blocks of DNA and RNA. As a result of xanthine oxidase physiological activity, uric acid and reactive oxygen species (**ROS**), such as superoxide radical anion ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2), are formed. Reactive oxygen species is a group of biological oxidants being the products of molecular oxygen metabolism. **XO** is also an important pharmacological target of modern therapy, especially due to its central role in the development of hyperuricemia in the body, i.e. too high concentration of uric acid in the blood. This, in turn, leads to the development of gout. According to the study published in 2024[1], the global prevalence of gout more than doubled from 22.1 mln in 1990 to 53.9 mln in 2019. The results of many studies show that increased activity of xanthine oxidase also plays an important role in many other pathological conditions, including chronic heart failure[2], and tissue and organ damage associated with post-ischemic reperfusion[3].

Xanthine oxidase inhibitors currently available on the European and American markets are characterized by a wide range of side effects[4–7]. There is a need to find effective inhibitors that could be used as drugs, inter alia, for the treatment of gout associated with hyperuricemia. The systematic screenings of large libraries of compounds may be a promising and powerful approach to identify potent candidate **XO** inhibitors, which could subsequently serve as novel chemical scaffolds for medicinal chemistry-based structure optimization to maximize both inhibitory efficiency and *in vivo* safety of the compounds.

The aim of the proposed project is to develop a novel efficient method to identify the xanthine oxidase inhibitors that would be suitable for high-throughput screening (**HTS**) methods. Taking into account the limitations of currently used assays in a **HTS** approach, this project is dedicated to the development of a novel and reliable method for the specific and sensitive detection of the superoxide radical anion. Integrating this method with others aimed at finding xanthine oxidase inhibitors can lead to the development of a protocol for a high-throughput screening assay set with a low false-positive rate.

The BOronate Oxidation Spin Trapping (**BOOST**)[8] is based on combining the use of spin traps (that react with the free radicals to form a more stable molecule called a spin adduct) and boronate fluorogenic probes (compounds that could be oxidized by a spin adduct to fluorescent product). The idea of the mechanism is shown in Scheme 1.



Scheme 1 Idea of BOOST technique

The addition of a compound that is an xanthine oxidase inhibitor will reduce the intensity of the fluorescent signal compared to the system without this compound. For compounds without such activity, the signal will remain the same. This effect will allow many compounds to be quickly tested for xanthine oxidase inhibitory activity.

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