Steroid compounds are commonly associated with doping in sport. However, the group of steroid compounds is much larger. In the human body, several hundred different steroids have been detected so far, which are responsible for regulating almost all physiological processes. This group also includes widely used in medicine anti-inflammatory drugs, but also sex hormones, contraceptives and aromatase inhibitors. In view of the fact that their action is usually multi-directional, it is aimed at obtaining such compounds that show the highest desired biological activity while at the same time the weakest side effects. It is known that the introduction of a hydroxyl group, major at positions 7α, 11α, 11β, and 16α, significantly changes their biological activity. The steroids with the lactone moiety in the D ring were the first inhibitors of aromatase, enzyme transforming male (androgens) into the female sex hormones (estrogens). These inhibitors are used to treat hormone-dependent cancers (breast and prostate), but they are also commonly used by people who practice strength and bodybuilding sports.

Because steroid compounds are composed of at least 17 carbon atoms connected in four rings, it is difficult to introduce targeted modifications by chemical synthesis. The solution is biotransformations - transformations of compounds carried out by the enzymatic apparatus of microorganisms, e.g. filamentous fungi. Microorganisms have the advantage over chemical synthesis that they do not require multistep processes using toxic reagents that are difficult to utilise. Biotransformations occur under mild conditions, in an aqueous environment and give products with a specific spatial configuration, which in medicine is of considerable importance. However, it is very important to look for new microorganisms capable of specific transformation of substrates. In the proposed project, species of filamentous fungus infecting insects (*Isaria fumosorosea*, *Isaria farinosa*, *Beauveria bassiana* and *Beauveria caledonica*) will be used for the first time to transform modified steroid compounds. Entomopathogenic fungi are used as biopesticides to regulate crop pests population, and their safety in relation to humans has been thoroughly tested and confirmed.

Steroid compounds naturally occurring in the human body will be modified by chemical synthesis by replacing the oxygen atom in the carbonyl and hydroxyl groups with a sulfur atom. In this way, also biologically active thiosteroids will be formed, which will then be subjected to biotransformations with the help of the above-mentioned entomopathogenic fungi. In this way, new steroid compounds not described in the literature will be obtained. All starting compounds as well as chemical and microbiological obtained compounds will be tested to determine their ability to inhibit aromatase. In addition, all steroids will be used in cytotoxicity studies against normal and cancer cell lines.

The aim of the project is to study the metabolism of steroid compounds containing thiol or thioketone group in the entomopathogenic cultures of filamentous fungi and compare it with the metabolism of chemically unmodified compounds. In addition, the relationship between biological activity and the structure of the compounds obtained will be examined.

The use of biotransformation for modification of compounds containing the thioketone group may lead to new derivatives difficult to obtain by chemical synthesis and have higher or distinctly altered biological activity relative to the substrates. The comparison of the transformation of unmodified compounds (naturally occurring in the human body) with the chemical synthesis products will allow for a detailed understanding of the metabolism of entomopathogenic fungi. Because biotransformations are considered to be models of mammalian metabolism, it will be possible to predict with a high probability the metabolism of potential steroid drugs in the human body.

Planned studies will also provide valuable information on the relationship between inhibitory activity relative to human aromatase and the structure of modified steroid compounds containing the thioketone moiety. The results will expand the current state of knowledge on the structure of aromatase inhibitors and may give a new direction for the development of this group of drugs. The SRB test will determine the cytotoxicity of these compounds in relation to normal and cancer cell lines, which may be the next step towards a better understanding of this group of compounds.