introduces the cis steroid confirmation is 3-oxo-5β-steroid-Δ4-dehydrogenase (AKR1D1) [3]. The mutation within the AKR1D1 gene may lead to liver cholestasis which when untreated can progress to steatosis [4]. Interestingly, AKR1D1 is not only involved in bile acid pathways but also in androgens and progestogens clearance. Thereby synthetic testosterone derivatives like anabolic–androgenic steroids (AAS) may pose a real threat of altering the physiological function of the AKR1D1. AAS are often taken off-label in high dosage regiments and can therefore pose a target and pathway-specific homeostasis thread. In our research, we investigated commonly used AAS and their impact on endogenous testosterone metabolism performed by the AKR1D1. We have found that nandrolone, clostebol, methasterone, drostanolone, and methenolone alter the AKR1D1 activity of endogenous testosterone when assessed by the *in vitro* assay at 5uM concentration in a statistically significant manner. Whereas structures of nandrolone suggest that it might be metabolized by AKR1D1, molecular scaffolds of clostebol, methasterone, drostanolone, and methenolone indicate that competitive inhibition may occur. This disturbance of the AKR1D1 activity may lead to a shift in bile acid profiles which can then relate to bile acid deficiency syndrome. In this contribution, we shed light on the structure-activity relationship of the AAS in the scope of the AKR1D1 enzyme.

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P17-27
Green synthesis of nanostructured bioactive glass for dental applications

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Calcium sodium phosphosilicate (known as bioactive glass) is a biomaterial commonly used in dental care products and bone tissue engineering applications due to biocompatibility, bone-forming and dentin sensitivity reduction capability. Bioactive 45S5 glass, so-called NovaMin, comprises of 45% SiO_2, 24.5% Na_2O, 24.5% CaO, and 6% P_2O_5 (wt%). It is traditionally synthesized by wet chemical methods such as melt-quenching and sol-gel which requires high temperature heating and the use of a strong acid catalyst, which brings into the question of the possibility of introducing toxic acid residues into the final product. Therefore, there is a clear need to develop environmental-friendly bioactive glass synthesis methods or to modify existing ones in a way to uplift their environmental friendliness. To satisfy this need, we *greenized* traditional sol-gel method by replacing an acid catalyst with an environment-friendly alternative and successfully used it for the synthesis of nano-structured 45S5 bioactive glass. The synthesized bioactive glass was characterized in terms of composition, size, surface charge, morphology and mesoporous structure using Scanning Electron Microscope (SEM), Transmission Electron Microscope (TEM), Dynamic Light Scattering (DLS), Brunauer-Emmett-Teller (BET), Ultraviolet–Visible–Near Infrared (UV–Vis–NIR) microplate spectrophotometer and X-ray spectroscopic methods (XRD and EDX). Apatite formation capability of the bioactive glass was investigated in simulated body fluid (SBF). *In vitro* toxicity tests were performed to assess the cytotoxic potential of the synthesized bioactive glass (of three different particle sizes) on Saos-2 human osteosarcoma cell line, while their functionality was tested by a set of mineralization experiments. Results indicate that bioactive glass synthesized through green methods do not induce any significant toxicity on Saos-2 cell line and can be safely used in dental applications. We also observed slight size-dependent differences in both cytotoxic and mineralization effects of nanostructured bioactive glass.

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Assessment of the properties of the strigolactone analog (GR24) against human red blood cells

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Strigolactones are phytohormones responsible for the stimulation of seed germination, moreover, they play a regulatory role in the plant development process by affecting the root structure, leaf aging and secondary growth. The latest literature reports show that the newly discovered antioxidant properties of strigolactones against animal cells make it possible to use these phytohormones as potential compounds with protective properties against normal blood cells exposed to toxic compounds.

The cytotoxicity of GR24 against erythrocytes, the main human blood cells, which damage can be lethal to the organism, has not been determined yet.

The aim of this study is to evaluate the cytotoxicity of synthetic strigolactone analogue (GR24) against human red blood cells – erythrocytes. Erythrocytes were isolated from erythrocyte-leukocyte platelet buffy coats purchased from the Regional Centre of Blood Donation and Blood Treatment, Lodz, Poland. Next, erythrocytes suspensions in PBS with 5% hematocrit were treated with GR24 and stimulated for 24 hours in 37°C. The concentration range for GR24 was 10; 50; 100; 250, 500 µM.

Hemolysis and methemoglobin formation were assessed by spectrophotometric measurements at visible range. Acetylcholinesterase (AChE) activity was measured spectrophotometrically (at 412 nm) as the rate of acetylthiocholine iodide hydrolysis. Phosphatydylserine (PS) externalization was assessed with Annexin V-FITC fluorometric test by flow cytometry.

Human erythrocytes acetylcholinesterase (AChE) is located on the external surface of the cell membrane. AChE is linked to the erythrocyte membrane through a glycosylphosphatidylinositol (GPI). The AChE activity may serve as a sensitive biomarker for substance toxicity. On the other hand, preservation of membrane phospholipid asymmetry plays a key role in the proper functioning of erythrocytes. Maintaining phosphatidylserine (PS) in the inner leaflet of the membrane lipid bilayer seems particularly important. Therefore, both parameters are necessary to determine the safety of GR24 for use in human cells.

The obtained results indicate that GR24 is non-toxic to human erythrocytes up to the concentration of 100 µM. Higher concentration of GR24 (500 µM) slightly increases hemolysis, up to 14%.

To sum up GR24 is non-toxic to essential human blood cells, such as erythrocytes. This lays the foundation for further studies in which the potential protective properties of GR24 against erythrocytes treated with brominated flame retardants will be assessed.

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