Summary

Tissue hypoxia is a pathological state which accompanies different types of disorders and is a direct cause of damage, which can lead to tissues, organs or the whole body's death. We can differentiate hypoxia according to its principal cause (respiratory hypoxia, blood-related hypoxia, circulatory hypoxia or histotoxic hypoxia). Regardless of the cause, there exist universal mechanisms induced by oxygen decrease in cells. In order to compensate the lack of oxygen supply, the organism can take advantage of a set defensive measures, such as oxygen reserves in the blood, employment of the hypoxia induced factor, and furthermore modification of cellular track and an expansion of certain genes to decrease oxygen consumption on this level. If the cells deplete all the oxygen reserves, they will die unless they receive new supplies. In the case of mild hypoxia, the organism has time to start the process of programed cell death, which mitigates negative consequences. However, if the factor acted abruptly, a cascade of events induced during hypoxia will include processes which damage the cell (disturbances in energy metabolism, electrolyte balance) and, in consequence, will lead to necrotic death, with the appearance of inflammation. Due to the high likelihood of such disorders (coronary artery disease, cancer) connected to various pathological processes and potentially fatal character, continuous work needs to be done in order to protect the patients from their negative consequences.

1,3,4-thiadiazole group compounds, investigated in Cell Biology Department, belong to synthetic derivatives of heterocyclic thiadiazol system. The literature provides information on their broad spectrum of applications in the industry and science as dyes, complexing factors and also effective antibacterial, antifungal, antiparasite, anticancer substances, etc. Some of these are used in clinical therapy.

Within the scope of this dissertation two substances from this group, namely 4-(5-methyl-1,3,4-thiadiazol-2-yl)benzene-1,3-diol (MTDBD, C1) and 4-(5-heptyl-1,3,4-thiadiazol-2-yl)benzene-1,3-diol (HTDBD, C7), were studied according to the difference in length of the acyl-chain substituent. A series of techniques were used for the experiments, such as ultraviolet-visible spectroscopy (UV-Vis), fluorescence spectroscopy and Fourier transformed infrared spectroscopy (FTIR). Other employed techniques were resonance light scattering (RLS), circular dichroism (CD) or differential scanning calorimetry (DSC). Spectroscopic methods are based on certain interaction of electromagnetic waves of different

wavelength with the investigated sample. The absorption of a part of radiation is related to the increase of electron energy or functional group vibration, which provides information about the state of the investigated substance and its properties. Spectral methods allow for non-invasive and fast measurements which are also very reliable, repeatable and have a high level of precision.

At the beginning the compounds were analysed with respect to the variation of polarity and hydrogen ion concentration of the environment, and also with respect to their own concentration and temperature. As a result, and with the help of theoretical analysis, their spectral characteristic were determined, and the description and interpretation of molecular organization of monomers and higher aggregated structures was performed in different environments. This information served for the second part of investigation results interpretation, namely model system. Both 1,3,4-thiadiazol derivatives underwent experiments with the use of liposomal systems created using dipalmitoylphosphatidylcholine (DPPC). Since it can often be found in biological membrane structures, this lipid became an initial source of information on the molecular basis for the interaction of the analysed molecules with the DPPC membrane. The data on the interaction of thiadiazole and lipid molecules in the area of all functional groups, membrane penetration or the molecular organization of the compounds in liposomal environment were gathered using all the above mentioned methods. Basing on the results from the two experimental parts, the C7 compound was rated as the one offering better selectivity to enter an interaction with lipid membrane and allowing for a more precise rating of its state with the use of employed methods.

As a result it has been decided to use it in biological experiments involving animals. During the experiment carried out by scientists from the above mentioned department (with the consent of the I Local Ethics Committee in Lublin, no. 31/2012, 15th June 2012, project title: "Anticonvulsant and neuroprotective potencial of selected compounds from the 1,3,4-thiadiazole group in the model of brain hypoxia/ischemia and the model of excitotoxicity in rats") done on rats in the normobaric hypoxia/ischemia model (with the use of three combinations of C7 doses, samples of biological material (bool plasma, heart and kidney) was taken which was then analysed within this dissertation using spectroscopy measurements. Due to fluorescent properties, the C7 compound could be confirmed in all the three samples. It verified that fluorescent spectroscopy method can be used in for rapid detection of the studied compound even in complex biological system and that it has the ability to distribute through biological barriers (despite the fact that the mechanism of migration was not the scope of this work). Further spectroscopy analysis using FTIR method showed that there are differences in

spectra of hypoxic and control animals, which could be seen on the level of second derivative of spectra. However, the method did not allow to determine the specific marker of oxygen deprivation state. What is important, it was possible to show that applying compound C7 in investigated animals decreased or completely mitigated the effect of oxygen deprivation, which could be seen on spectral level. The results described in the section dedicated to those studies cell protective properties of the studied substance, especially the lowest dose applied.