Dear Reviewer,

We would like to express our sincere appreciation for your careful attention to our manuscript and for the suggested improvements and valuable comments. We have revised the manuscript according to your remarks. Please find below the detailed description of the revision with our point-by-point answers.

Reviewer: The manuscript describes the synthesis and PDT ability of new derivatives of porphyrazine. The study was planned well but still, some important data is missing as well as some inconsistencies within the presented results.

Point 1: Check English, for ex. Line 83- In the study [39] showed how, and following lines. Connective words are missing. A similar error has been found in several other places.

Response 1: Corrected. In addition, we rely on editing by MDPI's translators.

Quotation: *We have previously shown that cyanoporphyrazines can be efficiently loaded in nanoscale and submicron containers of different nature. Encapsulation of hydrophobic cyanoporphyrazine dye into liposomes of different lipid composition promoted their high uptake by tumor cells while preserving the photoinduced toxicity of the PS [50]. The dyes of cyanoporphyrazine group were also successfully loaded into amphiphilic polymer brushes and, due to the presence of such a container, were selectively accumulated and retained in tumors, providing subsequent successful photodynamic therapy [51],[49]. The potential of cyanoporphyrazines was fully shown on the example of vaterite particles [52]. The unique property of these dyes, namely, the dependence of the fluorescence lifetime on the microenvironment, allowed visualization of porphyrazines release from vaterite in real time in vivo. The obtained results arousing interest in further modification and study of cyanoporphyrazines not only as potential agents for PDT, but also as sensors to assess the dynamics of drug release from the transport carrier.*

Point 2: Figure 2: The dye concentration used for obtaining these spectra is missing.

Response 2: Corrected. (Line 281 in the revised version of the manuscript).

Quotation: Figure 2. *Absorption (a) and fluorescence (b) spectra of* ***PzNPh*** *and* ***FePzNPh*** *(both at 5 μM) in glycerol. The fluorescence was excited at* λ_{ex} *570 nm.*

Point 3: It was reported that viscosity influences the photo-activity of dyes. Thus, how are the results in glycerol relevant to clinical testing? Further, in uptake studies, the author claims that no fluorescence was detected in culture media, however, the result in the water tells a different story.

Response 3: The studied compounds (**PzNPh** and **FePzNPh**) are characterized by the dependence of the lifetime of the excited state and the quantum yield of fluorescence on the viscosity of the medium. According to a number of literature sources, it is known

that the viscosity values of the cytoplasm of living cells ranges from 2 cP to 100 cP [1, 2]. These values are corresponding with the viscosity range of glycerin-ethanol mixtures (~10-70% of glycerin concentration at room temperature) [3].

The fluorescence spectra of **PzNPh** and **FePzNPh** were studied using a high sensitivity (2 pM fluorescein typical) Synergy MX plate reader. This made it possible to get a very low fluorescence intensity emission spectra in water.

The accumulation of **PzNPh** and **FePzNPh** by cells was studied using a confocal microscope Axio Observer Z1 LSM 710 NLO/Duo (Carl Zeiss, Germany). Its detector sensitivity is much lower than the Synergy MX plate reader in use. So, in cells experiment, the fluorescence signal of both **PzNPh** and **FePzNPh** solutions could not be distinguished from electronic noise in the growth medium.

References:

1. Fushimi K, Verkman AS. Low viscosity in the aqueous domain of cell cytoplasm measured by picosecond polarization microfluorimetry. J Cell Biol. 1991 Feb;112(4):719-25. doi: 10.1083/jcb.112.4.719

2. Luby-Phelps K, Mujumdar S, Mujumdar RB, Ernst LA, Galbraith W, Waggoner AS. A novel fluorescence ratiometric method confirms the low solvent viscosity of the cytoplasm. Biophys J. 1993 Jul;65(1):236-42. doi: 10.1016/S0006-3495(93)81075-0

3. J. B. Segur and Helen E. Oberstar Viscosity of Glycerol and Its Aqueous Solutions Ind. Eng. Chem. 1951, 43, 9, 2117–2120

Point 4: The accumulation rate is slower for Fe complex, based on fluorescence intensity detected within cells. However, the fluorescence spectra already suggested that the Fe complex generates low fluorescence compared to non-modified compound. How has this been considered to determine the uptake?

Response 4: In this study, we assessed the rate of reaching the maximum possible brightness value, but not the absolute concentrations of photosensitizers in the cell. This is due to the dependence of the fluorescence quantum yield on the viscosity of the medium. To avoid misinterpretation of the results by readers, we have added the statement into the article text. (Lines 321-324 in the revised version of the manuscript).

Quotation: *However, since the fluorescence brightnesses of **PzNPh** and **FePzNPh** differ even at the same viscosity values (Figure 2), we cannot make absolute estimates regarding the concentration of the studied dyes in the cell.*

Point 5: The compound purity and structural characterization data are missing.

Response 5: We have added data on the purity of the compounds and their structural characterization. (Lines 133-139; 141-150 in the revised version of the manuscript).

Point 6: Physiochemical characteristics such as solubility in different mediums and stability are also relevant data if the aim is to utilize them for PDT.

Response 6: PzNPh and **FePzNPh** are characterized by low solubility in water and glycerol. **PzNPh** shows good solubility in acetonitrile and tetrahydrofuran, but little solubility in toluene. As for **FePzNPh**, it is rather slightly soluble in all the solvents listed above. Both compounds show high solubility in dimethylsulfoxid (DMSO). In this regard, the stock aqueous solution for research contained about 20 wt.% DMSO and was further diluted by deionized water for the experiments. After preparing the compounds and before adding them to cell cultures, we controlled the solubility and retention of the compounds by the shape of the absorption and fluorescence spectra. No sighs of aggregation was detected in these conditions.
Since no quantitative measurement of **PzNPh** and **FePzNPh** was performed, we did not include a qualitative assessment into the manuscript.

For the study, we used solutions of photosensitizers that were stored for no more than three months, the stability of the compounds was confirmed by the absence of sediment and the preservation of their spectral properties and photodynamic activity.

Point 7: Study missing to prove that these compounds release ROS, which is essential for PDT.

Response 7: In the present work we aim to analyze the photophysical and photobiological properties of **PzNPh** and **FePzNPh**. The study of reactive oxygen species (ROS) generation was not included in the objectives of our study, which is primarily associated with the peculiarities of the photophysical properties of porphyrazines. As mentioned in the article, porphyrazines belong to the class of fluorescent molecular rotors. The probability of formation of particular states of the pz molecule (excited singlet planar and excited singlet twisted, with one to four twisted groups, triplet planar and twisted) are strongly dependent on viscosity, as well as their lifetimes. The common methods based on measuring in low viscous organic or water solutions lead to very significant underestimation of the singlet oxygen quantum yield, up to inability to detect them. Thus, to provide relevant information, a large-scale investigation in the physical chemistry of the processes and their dependence on viscosity must be performed.