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FULL ARTICLE

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Effect of novel porphyrazine photosensitizers on normal and tumor brain cells

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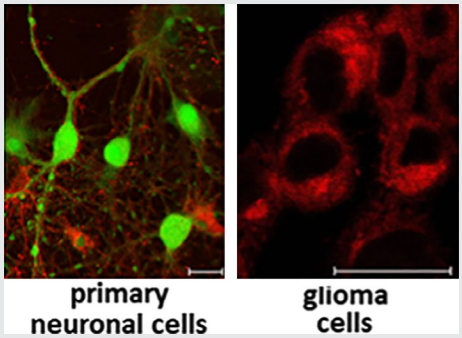
Abstract



Photodynamic therapy (PDT) is a clinically approved procedure for targeting tumor cells. Though several different photosensitizers have been developed, there is still much demand for novel photosensitizers with improved properties. In this study we aim to characterize the accumulation, localization and dark cytotoxicity of the novel photosensitizers developed in-house derivatives of porphyrazines (**pz I-IV**) in primary murine neuronal cells, as well as to identify the concentrations at which **pz** still effectively induces death in glioma cells yet is nontoxic to nontransformed cells. The study shows that incubation of primary neuronal and glioma cells with **pz I-IV** leads to their accumulation in both types of cells, but their rates of internalization, subcellular localization and dark toxicity differ significantly. **Pz II** was the most promising photosensitizer. It efficiently killed glioma cells while remaining nontoxic to primary neuronal cells. This opens up the possibility of evaluating **pz II** for experimental PDT for glioma.

KEYWORDS
cell death, cytotoxicity, GL261 glioma cells, neurooncology, photodynamic therapy, photosensitizer, primary neuronal cultures

1 | INTRODUCTION

Malignant gliomas are the most common type of primary brain tumors. They represent one of the worst types of human cancers, with one of the poorest 5-year overall survival rates among all human cancers [1, 2]. Importantly, current multimodal treatment for gliomas (eg, chemotherapy, targeted therapy, radiotherapy) fails to improve prognosis, which prompted scientists to look for novel means of treating this disease. The difficulty in developing new treatment modalities for gliomas is that the brain tissue adjacent to the tumor must be preserved and damage to the nontransformed brain tissues must be minimized, even when micro metastases are present, because of the generally high sensitivity of normal brain tissue to most physical and chemical treatment agents [3].

Photodynamic therapy (PDT) is a clinically approved, minimally invasive, multistep procedure that uses the toxicity

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of singlet oxygen ($^1O_2$) and other reactive oxygen species generated by a reaction between a highly safe photosensitizer accumulated in tumor cells and a light with a specific wavelength corresponding to an absorbance band of the photosensitizer it excites [4]. Although several different photosensitizers or their precursors, including the hematoporphyrin derivatives porfimer sodium, temoporfin, verteporfin and 5-ALA, have been used clinically to treat different brain tumors, including high grade gliomas [5–7], there is still a need for new photosensitizers with improved properties but low cytotoxicity for nontransformed brain tissues. Porphyrazines (**pz**) or tetracyanoporphyrazines, which are tetrapyrrole macrocycles similar to porphyrins and phthalocyanines, have recently emerged as a useful class of photosensitizers suitable for PDT and with excellent uptake and retention properties [8, 9]. **Pz** differ from porphyrins in that they contain meso nitrogen atoms rather than carbon atoms and differ from phthalocyanines because their β-pyrrole positions are open for substitution. These differences confer physical properties that are distinct from those of porphyrins and phthalocyanines [10].

Recently, several novel **pz** photosensitizers with cyano and aryl substituents in the periphery of the tetrapyrrole macrocycle have been described [8, 11, 12]. The unique feature of the cyanoarylporphyrazines (hereafter referred to as **pz**) is that their photophysical properties are highly sensitive to the viscosity of the medium. It has been shown that **pz** with *para*-fluorophenyl groups possess properties that enable their dual use as sensitizers as well as viscosity markers in PDT, thus providing a new type of diagnostic and dosimetry tool in PDT treatment [8]. Moreover, the photodynamic properties of **pz** can be significantly improved by chemical design of the peripheral aryl groups [13, 14]. In his study, we analyzed four cyanoarylporphyrazines (**pz I-IV**; Figure 1A) that are highly potent in PDT.

Administration of photosensitizers and subsequent PDT can damage normal neurons and glial cells, leading to unwanted neurological consequences [15]. This dictates the need to compare the effects of photosensitizers on these cells and on cancer glioma cells. Therefore this study has two aims. First, it aims to characterize the accumulation, localization and dark cytotoxicity of the novel photosensitizers we developed in-house from groups **I-IV** of **pz** (Figure 1A) in primary murine neuronal cells. Second, it aims to analyze the concentrations at which **pz I-IV** are effective in inducing cell death in glioma cells but are nontoxic to nontransformed neuronal cells. This knowledge will allow us to select the safest **pz** for future experimental PDT of brain cancer.

2 | MATERIALS AND METHODS

2.1 | Ethics statement

All experiments were approved by the Bioethics Committee of Lobachevsky University and carried out in accordance with Act708n (23 082010) of the Russian Federation National Ministry of Public Health, which states the rules of laboratory practice for the care and use of laboratory animals, and Council Directive 2010/63 EU of the European Parliament (September 22, 2010) on the protection of animals used for scientific purposes. C57BL/6J mice were killed by cervical vertebra dislocation, their embryos were surgically removed, and then they were decapitated.

2.2 | Isolation of the primary neuronal cultures

Primary cells were obtained from the cerebral cortex of embryos obtained from C57BL/6J mice (day 18 of gestation) and cultured on coverslips (18 × 18 mm) pretreated with polyethyleneimine solution (1 mg/mL; Sigma-Aldrich, P3143) according to a previously developed protocol [16]. In brief, after surgical isolation, embryonic cerebral cortices were mechanically dissected in PBS followed by incubation for 20 minutes in 0.25% trypsin-ethylenediaminetetraacetic acid (EDTA, Invitrogen, 25200-056). The suspension of dissociated cells was centrifuged at 1000 rpm for 3 minutes,

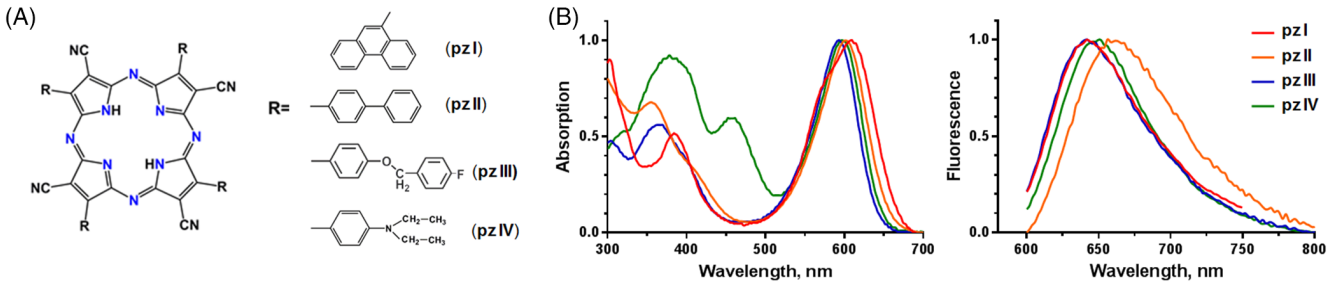

FIGURE 1 A chemical structure and absorption spectra of pz I-IV. A, Chemical structure of tetra(4-fluorophenyl)tetracyanoporphyrazines. B, Absorbance and fluorescence spectra of photosensitizers obtained by spectrofluorometry. As **pz I-IV** showed very weak fluorescence in water, the **pz I-IV** fluorescence was analyzed in an ethanol/glycerol mixture with a viscosity of about 45 cP. All four compounds have a red emission (640-660 nm). **pz II** showed the strongest long-wave fluorescence and **pz IV** the strongest shortwave fluorescence

and the pellet was resuspended in Neurobasal medium (Invitrogen, 21103-049) supplemented with 2% B27 (Invitrogen, 17504-044), 0.5 mM L-glutamine (Invitrogen, 25030-024), and 5% fetal bovine serum (FBS; PanEco, K055, Russia). The suspension was placed on coverslips at an initial density of 4500 cells/mm^2. Half of the medium containing 0.4% of FBS was replaced every third day.

2.3 | Glioma cell line

Murine glioma GL261 cells were cultured at 37°C under 5% CO_2 in DMEM containing 4.5 g/L glucose and supplemented with 2 mM glutamine, 100 µM sodium pyruvate, 100 U/mL penicillin, 100 µg/L streptomycin and 10% FBS (Fisher Scientific, 10082147). At the end of the exponential growth period, the cells were removed using a solution of trypsin-versine (1:3) and reseeded. The multiplicity of seeding was 1:10, and the cell density was 1.0×10^5 cells/mL. Experiments were carried out after the third passage. The viability of primary neuronal cultures and GL261 glioma cells was maintained at 35.5°C under 5% CO_2 in a humidified atmosphere in a cell culture incubator (Sheldon Manufacturing).

2.4 | Spectra acquisition

This study used cyanoarylporphyrazines with 9-phenanthrenyl (**pz I**), 4-biphenyl (**pz II**), 4-(4-fluorobenzyoxy)phenyl (**pz III**) and 4-diethylaminophenyl (**pz IV**) groups in the aryl frame of the macrocycle (Figure 1A). Absorption and fluorescence spectra of **pz I-IV** were registered using Synergy MX Microplate Reader (BioTek) in black 96-well microplates with a clear glass bottom (Falcon Imaging; Corning). Fluorescence was excited at 590 nm. Since the fluorescence quantum yield of **pz I-IV** in nonviscous media is very low, the fluorescence was measured in ethanol-glycerol solution (2:3).

2.5 | Analysis of dark cytotoxicity in primary neuronal cultures

To estimate the dark toxicity effects, the photosensitizers were added to the culture medium on day 14 of the primary neuronal culture development at concentrations of 0.1, 1, 10, 50 and 100 µM. Then, the cell cultures were placed in a CO_2 incubator in the dark.

Cell culture viability was evaluated on days 3 and 7 after **pz I-IV** application. Viability was calculated as the number of dead cells stained with propidium iodide (PI) (Sigma-Aldrich, P4170) relative to the total number of cells stained with bisbenzimide (Invitrogen, H3570) [17]. PI binds to double-stranded DNA by intercalating between base pairs, but it is excluded from cells with intact plasma membranes (ie, viable cells). PI and bisbenzimide were added to the culture medium to final concentrations of 5 and 1 mg/mL, respectively, 30 minutes before assessing viability. The cultures stained with fluorescent dyes were observed using a Leica DMIL HC inverted fluorescence microscope (Leica, Germany) with a 10x/0.2Ph1 objective.

2.6 | Analysis of cell death by MTT assay

Cell death was induced by PDT after treatment of the cells with **pz I**, **pz II**, **pz III**, or **pz IV**. GL261 glioma cells were seeded in 96-well glass-bottom plates (Corning) at 6×10^3 cells per well and grown overnight. The glioma cells were then incubated with the different **pz** photosensitizers in serum-free medium at concentrations ranging from 0.001 to 70 µM for 4 hours, followed by irradiation with a light dose of 20 J/cm^2 in photosensitizer-free medium using a LED light source at a power density of 20 mW/cm^2 (λ_{ex} 615–635 nm) [18]. Cells loaded with the different photosensitizers were handled either in the dark or in subdued light. After PDT, the cells were cultured in complete medium for 24 hours and cell death was analyzed by MTT assay (Alfa Aesar, L11939.06) according to the manufacturer's instructions. Optical density was measured at 570 nm. Control cells were cultured in the same conditions but without photosensitizers.

2.7 | Accumulation dynamics and subcellular distribution of pz

To analyze accumulation dynamics, GL261 cells were seeded in 96-well glass-bottom plates (Corning) at 10^4 cells per well and grown overnight. Then, the cells were incubated with 10 µM of the different photosensitizers in serum-free culture medium for 2 to 4 hours, followed by washing with PBS and confocal image acquisition. The same was done for primary neuronal cultures.

Due to the complex morphology of primary neuronal cultures, we used a fluorescent calcium-sensitive dye (Oregon Green 488 BAPTA-1AM "OGB1"; Invitrogen, O-6807). This dye reveals the cytoplasm of neurons and glial cells and makes it possible to track the accumulation of pz I-IV even in the thinnest outgrowths of nerve cells [19]. OGB1 (0.4 mM) was added to the culture medium 40 minutes before confocal image acquisition. OGB1 was excited at 488 nm and emission was recorded in the range of 500 to 530 nm. The fluorescence of **pz I-IV** was excited at 594 nm and recorded in the range of 600 to 670 nm.

For colocalization analysis of **pz I-IV**, the following dyes (all from Thermo Fisher Scientific) were added for 30 minutes: 0.5 µM LysoTracker Green DND-26 (L7526), 0.5 µM ER-Tracker (E34251) and 0.5 µM MitoTracker Green FM (M7514). The fluorescent dyes were added to the viable cells

after their incubation with the different photosensitizers (**pz I-IV**). The cells were stained according to the manufacturer's instructions. Fluorescence of the stained organelles was excited by an argon laser at 488 nm and registered in the range of 500 to 560 nm.

Intracellular distribution of **pz I-IV** was studied by using the LSM 710 Axio Obzerver Z1 DUO NLO laser scanning microscope (Carl Zeiss, Germany). The images were obtained using an LD C-Apochromat water immersion objective lens 40×/1.1.

2.8 | Statistical analysis

Statistical analysis was performed in GraphPad Prism (v.6.0). Cell death was analyzed by ANOVA followed by t-criteria with Bonferroni correction.

3 | RESULTS AND DISCUSSION

3.1 | Spectra characterization

First, we analyzed the absorption and fluorescence spectra of **pz I-IV**, which belong to the family of tetra(aryl)tetra(cyano) porphyrazines (Figure 1A). The absorption peaks of **pz I-IV** in aqueous solution were all in the shortwave region (Soret band) and the long-wave region (Q-band) of the spectrum (Figure 1B). Some bathochromic shift of Q-band that we observed for **pz II** and even more for **pz III** is due to the contribution of the additional peripheral aryl substituent to the total π-conjugated aromatic system of the macrocycle. The value of this contribution depends on the aromatic system length, its geometry and the angle which the aryl substituent forms with the macrocyclic ring plane.

It should be noted that **pz I-IV** belong to the class of "molecular rotors," and their fluorescence depends on the rate of intramolecular rotation [8]. Intramolecular rotation is found to be the main nonradiative way of the excited state relaxation for "molecular rotors" in the environment of low viscosity. Thus, **pz I-IV** showed very weak fluorescence in water (data not shown). But the fluorescence intensity of those dyes increased very strongly in the environment of high viscosity because viscous media prevent segmental intermolecular mobility. Therefore, we analyzed the **pz I-IV** fluorescence in an ethanol/glycerol mixture with a viscosity of about 45 cP (Figure 1B). All four compounds have a red emission (640–660 nm). Porphyrazine with peripheral biphenyl groups (**pz II**) showed the strongest long-wave fluorescence and **pz IV** with diethylamino group at *para*-position of the phenyl substituent demonstrated stronger shortwave fluorescence.

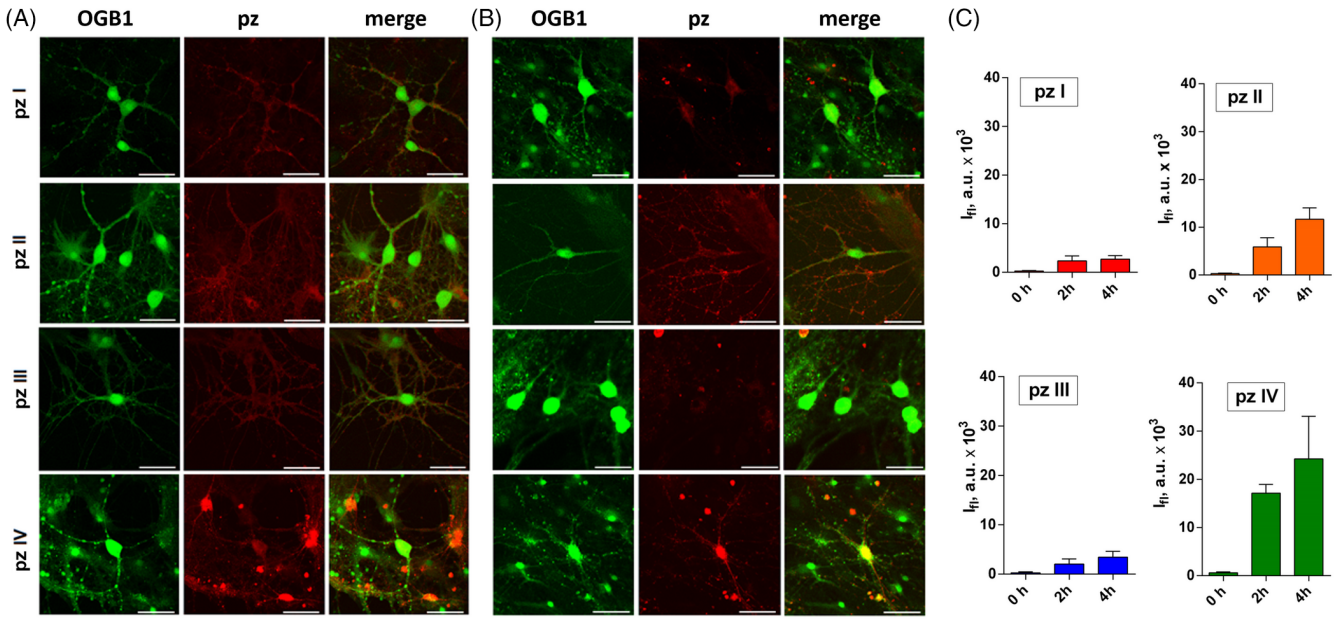

FIGURE 2 Uptake of pz I-IV by primary neuronal cells. Cellular uptake was assessed by confocal microscopy after 2 hours, A and 4 hours, B of incubation with the different **pz I-IV** (5 μM) on day 14 of culture development in vitro. Green channel: primary cultures were prestained with Oregon Green 488 BAPTA-1 AM (OGB1) to visualize the cytoplasm of neurons and glial cells in the culture. Red channel: fluorescence of photosensitizer. Merged: overlay of the fluorescence channels. Scale bars, 50 μm. C, The fluorescence intensity (I_{fl}) of the cells after incubation with the **pz I-IV**. Of note, all four photosensitizers were substantially present in neurons and glial cells in primary cultures 2 hours after **pz I-IV** addition. It is assumed that the size and hydrophobicity of the molecule are important determinants of the rate of dye penetration through the plasma membrane. **Pz IV**, being the most compact molecule, had the highest internalization rate

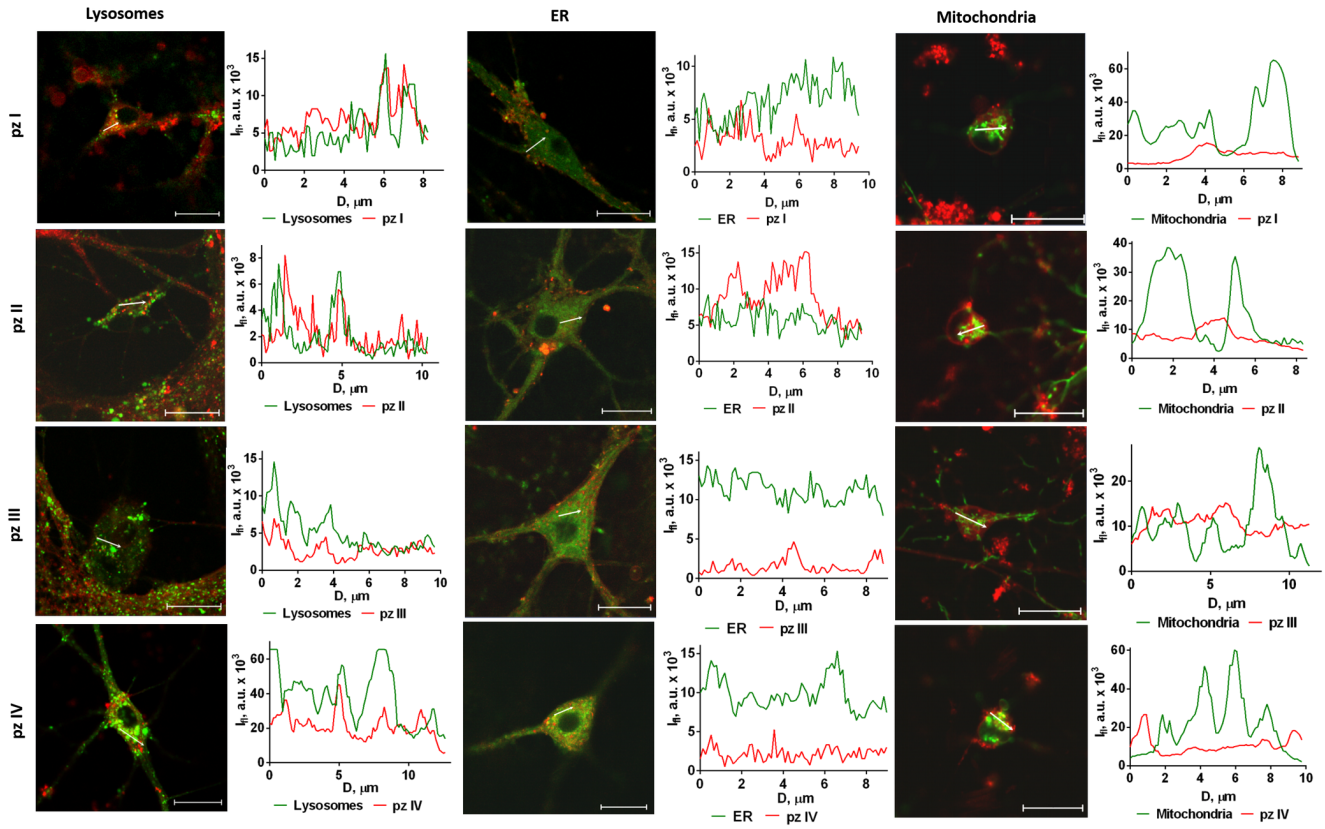

FIGURE 3 Subcellular distribution of pz I-IV in primary neuronal cells. Analysis was done 2 hours after the start of incubation with the different photosensitizers. The following dyes were used: LysoTracker Green (DND-26) for lysosomes; MitoTracker Green for mitochondria; ER-Tracker for ER. *In graphs*: red line—fluorescence intensity of photosensitizer; green line—fluorescence intensity of dye in the indicated cellular compartment ($I_{fl,}$ a.u. $\times 10^3$). All four **pz** accumulated in vesicles, mostly in lysosomes, and the most hydrophobic (**pz I** and **pz II**) were detected in the endoplasmic reticulum as well. Scale bars, 20 μm

3.2 | Cellular uptake and subcellular localization of pz I-IV in GL261 glioma cells and primary neuronal cells

We used confocal microscopy to analyze the rate of uptake of **pz I-IV** into primary neuronal cells and GL261 glioma cells. First, we analyzed the rate of **pz I-IV** accumulation into primary neuronal cells. We found that all four photosensitizers were substantially present in neurons and glial cells in primary cultures 2 hours after **pz I-IV** addition (Figure 2). The size and hydrophobicity of the molecule seem to be the important determinants of the rate of dye penetration through the plasma membrane. **Pz IV,** as the most compact molecule, had the highest internalization rate. Analysis of the subcellular localization of **pz I-IV** revealed that all four **pz** accumulated in vesicles, mostly in lysosomes (Figure 3), whereas the most hydrophobic (**pz I** and **pz II**) were detected in the endoplasmic reticulum as well. Importantly, it has been shown that subcellular localization of photosensitizers in the endoplasmic reticulum could indicate their ability to induce an immunogenic form of cancer cell death [20–23]. Next, we found that incubation of GL261 glioma cells for 2 hours with any of the four photosensitizers was sufficient for accumulation of a substantial amount of **pz** in the cells (Figure 4A), suggesting that use of these **pz** in PDT will effectively kill GL261 glioma cells. Notably, **pz I** and **pz IV** had the highest internalization rate. The difference between neurons and glioma cells may be due to a significant difference in the relative contributions of the internalization pathways in these types of cells.

3.3 | Analysis of the dark toxicity of pz I-IV in primary neuronal cultures

A basic requirement for development of PDT for gliomas is determination of the photosensitizer concentrations that can effectively kill cancer glioma cells without being toxic to native (nontumor or nontransformed) cells. The reason is that a photosensitizer delivered to a tumor during PDT will also reach intact cells in its vicinity. Moreover, photosensitizers may possess dark toxicity (ie, light-independent toxicity), which can limit their use in vivo [24]. Therefore, we analyzed the dark cytotoxicity of the newly synthesized **pz I-IV** photosensitizers for primary neuronal cultures to allow

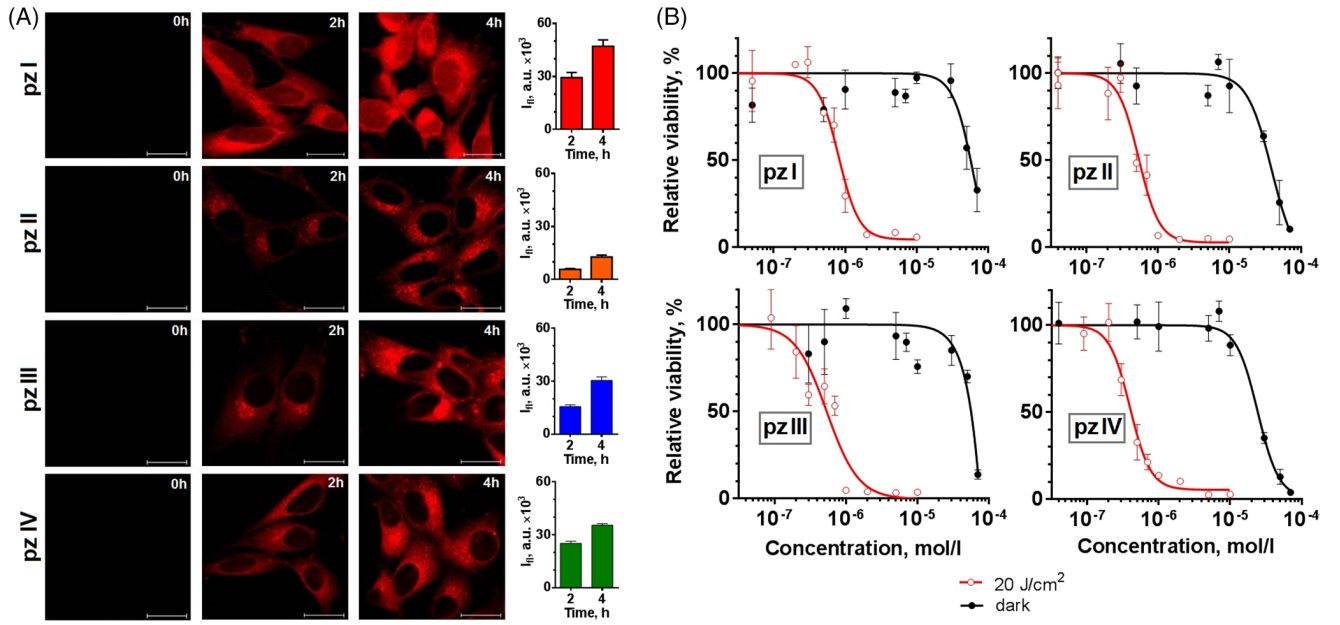

FIGURE 4 Analysis of the interaction of pz I-IV with GL261 glioma cells. A, Representative confocal images and fluorescence intensities (I_{fl}) of glioma GL261 cells 2 and 4 hours after incubation with **pz I-IV** (10 μM). Scale bars, 20 μm. Fluorescence intensity before addition of the photosensitizers did not exceed 300 a.u. Two hours of incubation with any of the four photosensitizers was sufficient for accumulation of a substantial amount of **pz** in the cells. **Pz I** and **pz IV** had the highest internalization rate. B, MTT analysis of GL261 glioma cell death after PDT. Dark toxicity (black lines) was analyzed after incubation of GL261 glioma cells with the photosensitizers in serum-free medium for 24 hours. For photoinduced toxicity (red lines), GL261 glioma cells were first incubated with the respective **pz** (**I-IV**) in serum-free medium for 4 hours and then exposed to light: λ_{ex} 615-635 nm, 20 J/cm^2. MTT assays were performed 24 hours after irradiation. The data represent the mean values ± SD (n = 3). **Pz I-III** showed no dark toxicity up to 30 μM and **pz IV** up to 10 μM. Analysis of phototoxicity allowed determination of the IC_{50} values, which are presented for the four **pz** in Table 1

us to estimate possible toxic effects of **pz I-IV** and to select the most suitable photosensitizer for PDT of glioma. The cytotoxic effects of photosensitizers in the short time frame are of low interest, because the cells of the central nervous system are characterized by certain patterns of neural network degradation [25]. Therefore, to analyze the cytotoxic effects, we selected two principal time points for assessment of delayed cytotoxicity, namely, days 3 and 7. Day 3 after addition of the photosensitizer reflects the period of a primary wave of cell death resulting from the direct cytotoxic effect of the photosensitizer on neuronal cells. In contrast, day 7 after incubation with the photosensitizer is characterized by the second wave of cell death due to the loss of a considerable quantity of neuronal intercommunications [3]. In our previous work, we have shown that the number of the dead cells reaches maximum on day 7 [25]. Analysis of the dark cytotoxicity of **pz I-IV** showed that high concentrations of the photosensitizers have a strong cytotoxic effect on primary neuronal cells (Figure 5). On the seventh day after addition of 100 μM of the **pz** to the primary neuronal cells, the following percentages of dead cells were found (Figure 5): **pz I**, 98% ± 1.21%; **pz II**, 95.9% ± 3.54%, **pz III**, 97.65% ± 3.65%; **pz IV**, 87.39% ± 2.06%. Even 10-fold lower concentration of the photosensitizers (10 μM) had a pronounced cytotoxic effect on the primary neuronal cells. The fraction of dead primary neuronal cells on the seventh day exceeded 30% (Figure 5): **pz I**, 30.09% ± 7.68%; **pz II**, 35.63% ± 3.00%; **pz III**, 60.45% ± 5.04%; **pz IV**, 55.78% ± 3.74%. However, 0.1 μM of **pz I** or **pz IV** did not have a pronounced cytotoxic effect on primary neuronal cells. The viability of the cells in the experimental groups was comparable to that of untreated cells. Notably, addition of the same concentration of **pz II** or **pz III** had a somewhat lower cytotoxic effect, but it was significant relative to the absence of treatment. The percentages of dead cells were 10.63% ± 1.55 for **pz II**, 11.41% ± 1.66 for **pz III**, and 4.51% ± 0.49 in the nontreated cultures. Analysis of dark cytotoxicity revealed that though the four photosensitizers have similar chemical structures, they have relatively different cytotoxicities for primary neuronal cells. Of note, the cytotoxic effect does not strictly correlate with the rate of **pz** uptake. While **pz I** and **pz III** had the lowest internalization rates, through this analysis we identified **pz I** and **pz II** as having the lowest dark cytotoxicity for nontransformed primary neuronal cells. Other factors, including localization, potential interference with metabolic pathways, and steric interaction with biological macromolecules, are assumed to be important. A low dark toxicity of photosensitizers is

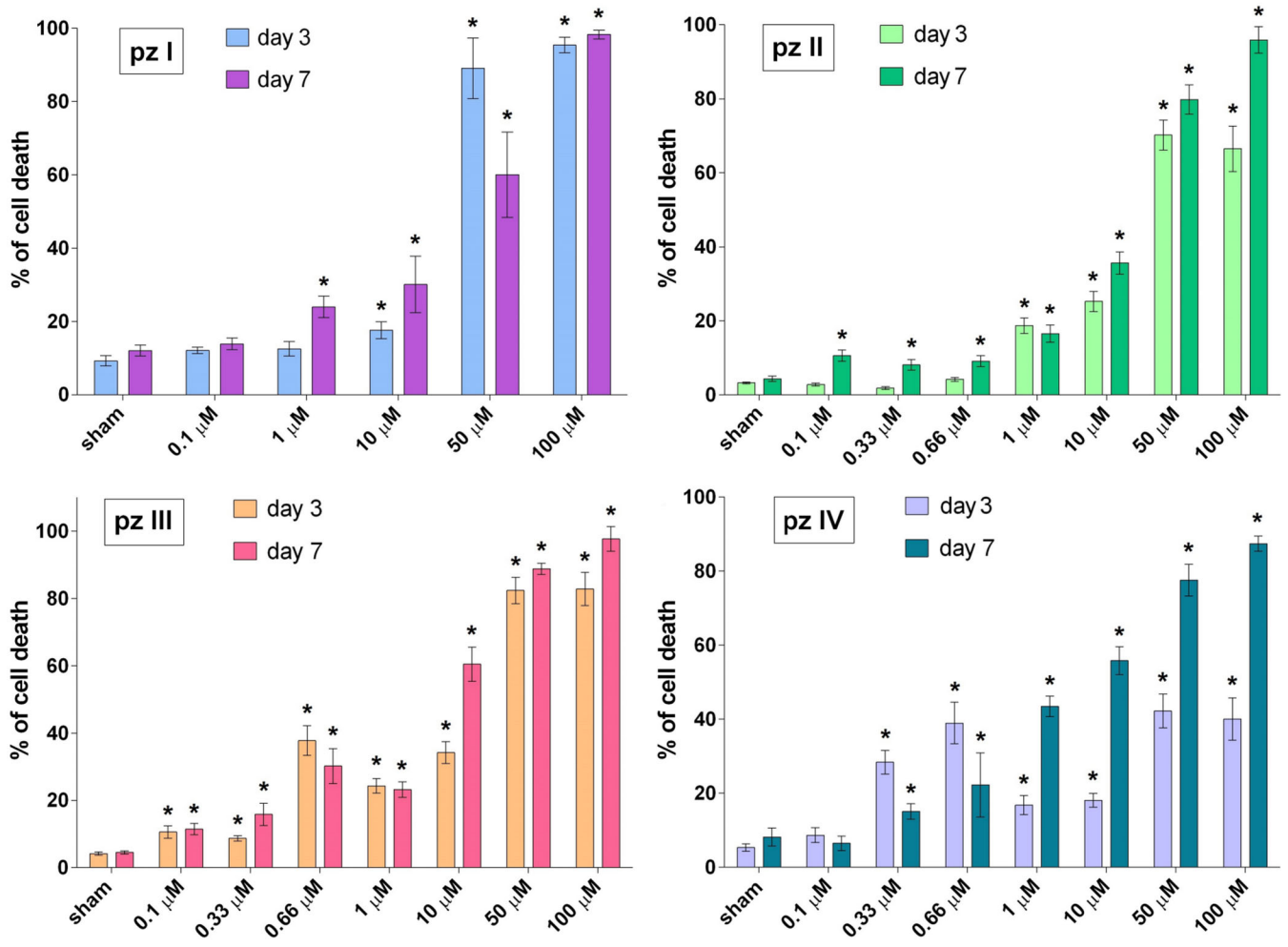

FIGURE 5 Dark cytotoxicity of pz I-IV to primary neuronal cells. Analysis was performed after incubating the cells with the different photosensitizer for 3 and 7 days. High concentrations (more than 10 μM) of **pz I-IV** had a strong cytotoxic effect on primary neuronal cells. The fraction of dead cells on day 7 after starting incubation with different **pz** exceeded 30%. **pz I** and **pz II** had the lowest dark cytotoxicity. A low dark toxicity of photosensitizers for nontumor cells is especially important when they are used in vivo. The data represent the mean values ± SEM from three independent experiments. Statistical significance was calculated by using ANOVA test, $*P < .05$

TABLE 1 Comparison of dark cytotoxicity in primary neuronal cultures with the IC_{50} phototoxicity effect of GL261 glioma cells

Photosensitizer	IC_{50} for GL261, μM [95% confidence interval]	Dark cytotoxicity for the primary neuronal cultures in the area of IC_{50} of GL261
pz I	1.18 [0.92; 1.51]	2—Average
pz II	0.56 [0.44; 0.70]	1—Light
pz III	0.54 [0.31; 0.92]	3—Significant
pz IV	0.39 [0.29; 0.50]	2—Average

TABLE 2 Cytotoxicity score according to the ISO 10993-5:2009

Scale of cytotoxicity	Number of dead cells, %	Interpretation of cytotoxicity
0	0% to 10%	Not-cytotoxic
1	10% to 20%	Light
2	20% to 30%	Average
3	More than 30%	Significant

especially important when they are used in vivo. In this context, it has been shown that intratumoral injection of large amounts of a hematoporphyrin derivative into rat brain induced strong dark toxicity in normal brain while tumors seemed unaffected [26].

3.4 | Comparative analysis of dark toxicity and phototoxicity effects of pz I-IV

For PDT to have minor side effects, the photosensitizer should have minimal dark cytotoxicity for normal neuronal cells but a pronounced therapeutic effect after PDT at the same concentration. We examined the possibility of inducing cell death in the murine glioma cell line, GL261. The

cells were pretreated for 4 hours with the different **pz** photosensitizers and then irradiated with a light dose of 20 J/cm^2 using a LED light source (power density of 20 mW/cm^2, λ_{ex} 615–635 nm). The control GL261 cells were incubated in the dark with the same doses of photosensitizers for 28 hours (4 + 24). **Pz I-III** showed no dark toxicity up to 30 μM and **pz IV** up to 10 μM. (Figure 4B). Irradiation with 20 J/cm^2 resulted in cell death at photosensitizer concentrations not exceeding ~1 μM (Figure 4B). The IC_{50} values for the four **pz** are shown in Table 1.

Next, the IC_{50} concentrations of **pz I-IV** in GL261 cells (when irradiated at a dose of 20 J/cm^2) were correlated to their IC_{50} concentrations in dark toxicity (ie, without PDT) on the primary neuronal cultures (Table 1). For this analysis, we used a cytotoxicity score according to ISO 10993-5:2009 (https://www.iso.org/standard/36406.html) (Table 2). Of the four **pz**, **pz II** had the lowest cytotoxicity for primary neuronal cells (IC_{50} = 0.56 μM; Table 1). **Pz I** and **pz IV** had IC_{50} of 1.18 and 0.39 μM, respectively, and at these concentrations they had an average cytotoxicity on primary neuronal cells (Table 1). In contrast, **pz III** had the highest cytotoxicity for primary neuronal cultures (IC_{50} = 0.54 μM; Table 1). This analysis demonstrated that **pz II** is the most promising photosensitizer, as it can efficiently kill GL261 glioma cells without being toxic for primary neuronal cells.

4 | CONCLUSIONS

Ideally, a photosensitizer should easily penetrate through the hemato-encephalic barrier and not be absorbed in significant amounts in healthy tissues, and it should have maximal cytotoxic effect on malignant cells but no systemic toxic effects [4]. The risks of adverse reactions and death of healthy neuronal cells associated with the currently known photosensitizers dictate the need for new photosensitizers that better match the requirements described above. In that context, we comparatively studied the effects of cyanoarylporphyrazines with 9-phenanthrenyl (**pz I**), 4-biphenyl (**pz II**), 4-(4-fluorobenzyoxy)phenyl (**pz III**) and 4-diethylaminophenyl (**pz IV**) groups in the aryl frame of the macrocycle on primary neuronal cultures and tumor glioma cells. We have shown that during 4 hours of incubation with **pz I-IV**, all four **pz** accumulated in primary neuronal cells and in glioma cells, but to different extents. These four photosensitizers differ significantly in the rate of their internalization, subcellular localization and dark toxicity. Our results demonstrate that **pz II** is the most promising photosensitizer, efficiently killing GL261 cells but showing no toxicity to primary neuronal cells. This finding opens up prospects for testing **pz II** in PDT of glioma in experimental therapy in the future.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

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