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Synthesis and Sonodynamic Activity of Ferrocenyltriazoleporphyrin

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The ferrocene-containing triazoleporphyrin was synthesized by the [3+2] dipolar cycloaddition of tetraphenylporphyrinazide with ferrocenylethyl propargyl ether. The cytotoxicity of the compound to Escherichia coli was studied. It was shown that the obtained compound exhibits a pronounced sonodynamic effect under the action of ultrasound.

Keywords: Porphyrin, ferrocene, triazole, ultrasound, sonodynamic therapy.

Синтез и сонодинамическая активность ферроценилтриазолпорфирина

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Ферроценсодержащий триазолопорфирин синтезирован по реакции [3+2]-диполярного циклоприсоединения тетрафенилпорфириназида с ферроценилэтилпропаргиловым эфиром. Изучена цитотоксичность соединения по отношению к Escherichia coli. Показано, что полученное соединение проявляет выраженный сонодинамический эффект под действием ультразвука.

Ключевые слова: Порфирины, ферроцен, 1,2,3-триазол, ультразвук, сонодинамическая терапия.

Introduction

The problem of cancer treatment is one of the leading places in scientific research. Sonodynamic therapy (SDT) is a promising selective method that allows targeting of tumor cells with special drugs (sonosensitizers) activated by the ultrasound.[1–3] Unlike photodynamic therapy (PDT) SDT has the advantage of affecting tumors located deep in organs and tissues. The method of sonodynamic therapy also allows to influence the foci of inflammation, and can be very effective in the treatment of such socially significant diseases as "diabetic foot".[4] The search for suitable sonosensitizers is a problem to be solved. Porphyrin compounds are now used as sonosensitizers.[5,6] Moreover, the structures of porphy-

rins are subjected to various chemical modifications in order to identify and select the best. Modification of porphyrins with ferrocene compounds makes it possible to obtain systems with unique properties.[7–10] Otherwise heterocyclic derivatives of ferrocene exhibit a variety of biological activity, including antitumor, while being low toxic compounds.[11–17] Moreover, the membranotropy of the ferrocene group and the redox properties of ferrocene, manifested in cells, can positively affect the porphyrin system.

Experimental

Chemicals used were reagent grade and were used as received without further purification. 5-(*p*-Azidophenyl)-10,15,20-triphenylporphyrin was synthesized according to described method.[18] The solvents were purified according to standard procedures and were distilled just before use. The mass spectra were obtained by the electron impact method on a FINNIGAN POLARIS Q instrument (USA), the temperature of the ionization chamber was 250 °C, the energy of ionizing electrons was 70 eV, and the electrospray method was used on a Thermo Finnigan instrument under standard conditions (electrospray ionization, acetonitrile, capillary voltage 4.5 kV). The NMR spectra were recorded on an AVANCE spectrometer with operating frequencies of 400 MHz for protons, and 100 MHz for ^{13}C nuclei, in $CDCl_3$ at 30 °C. For calibration, ^{13}C signals and residual protons of deuterium solvents were taken. The purity of the isolated compounds was checked by TLC on Silufol UV 254, Sorbfil, 25 DC-Alufolien, and Kieselgel 60 F_{254} plates. Preparative chromatography was performed on neutral alumina (Brockmann activity grade II; from Reanal), Kieselgel 60 F254 (Merck), or Kieselgel (0.035–0.070 μm, 90 Å; Acros).

Ferrocenylethylpropargyl ether, ***1****.* To a mixture of *α*-ferrocenyl ethanol (0.46 g, 2.0 mmol) and propargyl alcohol (0.144 mL, 2.5 mmol) CAN (10 mg, 0.9 % mol) was added under stirring at room temperature. The mixture was stirred for 15 min until the reaction was complete (TLC control). Then the reaction mixture was poured into water (20 mL), extracted with ethyl acetate (25 mL), washed with water. The organic phase was dried over $MgSO_4$, filtered, and evaporated.[19] Yield: 80 %, yellow oil. Anal. Calc. for $C_{15}H_{16}FeO$: C 67.19, H 6.01. Found: C 67.25, H 6.15 %. ^1H NMR δ_H ppm: 1.58 (d, J = 6.5 Hz, 3H, CH_3), 2.40 (t, J = 2.4 Hz, 1H, CH), 4.10 (dd, J_1=15.8 Hz, J_2 = 2.4 Hz, 1H, CHH), 4.14 (dd, J_1 = 15.8, J_2 = 2.4 Hz, 1H, CHH), 4.19 (s, 5H, C_5H_5), 4.21 (m, 2H, C_5H_4), 4.28 (m, 2H, C_5H_4), 4.60 (q, J = 6.6 Hz, 1H, CH). ^{13}C NMR δ_C ppm: 22.0 (CH_3), 55.1 (CH_2), 68.3 (C_5H_4), 69.8 (C_5H_4), 70.4 (C_5H_4), 70.9 (C_5H_4), 71.3 (C_5H_5), 71.8 (CH), 73.9 (≡CH), 80.4 (─C≡), 91.9 (*ipso*-C_5H_4).

Zinc 4-((ferrocenylethoxy)ethyl)-1-(5-(p-aminophenyl)-10,15,20-triphenylporphyrin)-1H-1,2,3-triazole), ***3****.* To a mixture of the ferrocenylethylpropargyl ether (1 mmol) and copper acetate hydrate (3 mg, 1.4 mol %) in toluene (5 mL) the zinc tetraphenylporphyrinazide **2** (1 mmol) was added at room temperature. The reaction mixture was stirred at ambient temperature; the progress of the reaction was monitored by TLC. The reaction mixture was cooled to room temperature and then poured into water (20 mL) and ethyl acetate (10 mL). The organic layer was separated and the aqueous phase was extracted with ethyl acetate (10 mL). The combined organic fractions were dried ($MgSO_4$) and the solvent was removed. The residue was chromatographed (silica gel, methylene chloride). Yield 75 %, purple powder. M.p. > 250 °C. ^1H NMR δ_H ppm: 1.30 (t, J = 6 Hz, 3H, CH_3), 4.18 (s, 5H, C_5H_5), 4.24 (s, 2H, C_5H_4), 4.31 (s, 2H, C_5H_4), 4.10 (m, 1H, CH), 4.65 (s, 2H, CH_2), 7.71–7.80 (m, 9H, Ph), 8.03–8.05 (d, J = 8 Hz, 2H, Ph), 8.17–8.20 (d, J = 8 Hz, 6H, Ph), 8.39–8.40 (d, J = 8 Hz, 2H, Ph), 8.64–8.79 (m, 8Hαβ, Py). ^{13}C NMR δ_C ppm: 15.60 (CH_3), 57.30 (-CH_2-), 63.19 (C_5H_4), 68.48 (C_5H_4), 68.70 (C_5H_5), 84.16 (CH), 84.57 (*ipso*-C_5H_4), 119.40 (C_{tr}), 118.77 (TPP), 119.20 (TPP), 126.71 (*o*-Ph), 127.85 (TPP), 128.38 (TPP), 134.67 (*m*-Ph), 135.82 (TPP), 138.83 (TPP), 139.72 (TPP), 141.20 (TPP), 149.28 (TPP), 150.12 (TPP), 152.32 (TPP), 159.32 (C*tr*).

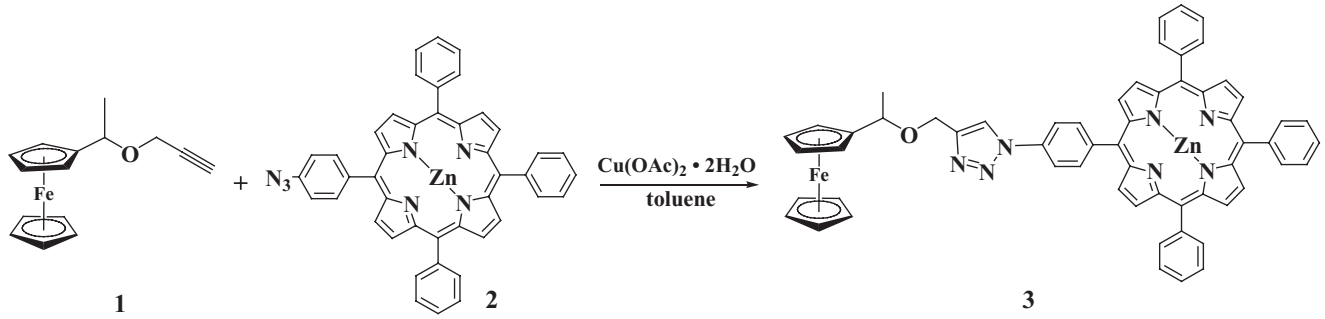
Sonodynamic experiment. The test culture (*Escherichia coli*) was grown at 37 °C on PCA Standard Methods agar. A suspension of bacterial cells in a sterile isotonic solution was prepared by washing them off from the gel substrate. A 5 mL portion of a bacterial cell suspension containing a compound to be tested was placed into a vessel with a sound-transmitting bottom. The vessel was placed into a water bath maintained at 37 °C over an ultrasonic source (0.88 MHz), and the suspension was subjected to ultrasonic irradiation over a period of 5 min at an intensity of 1.5 W/cm^2. A 1 mL sample was then withdrawn and transferred onto a Petri dish charged with a nutrient medium. The Petri dish was placed into an air thermostat (37 °C), and the growth of bacteria was estimated after 24 h by counting colonyforming units in the test and control samples. Each experiment was performed in duplicate. The results were presented as control/test percentage.

Results and Discussion

Ferrocene-containing porphyrins are poorly studied as bioactive, especially sonodynamic, agents. Recently we have obtained original ferrocene-containing porphyrins, which were firstly (previously such compounds were not studied under the conditions of sonodynamic therapy) studied for sonodynamic activity on *Staphylococcus aureus*.[20] Both its own cytotoxic effect and pronounced amplification under the ultrasound were revealed. Obviously, further research of such complexes will lead to the creation of new original drugs. Existing methods for the synthesis of ferrocene-modified porphyrins are multistage, with low yields and sometimes difficult to reproduce; others require expensive reagents. In our previous research, we investigated the reaction of the [3+2] dipolar addition of various aromatic azides to ferrocenylalkyl propargyl ethers. We have shown that the reaction proceeds with the best yield in the presence of an electron-withdrawing substituent in the aromatic ring. In our case, we used rather stringent conditions for the reaction, since the porphyrin ring is an electron donor relative to the azido group of tetraphenylporphyrin. At the same time in our studies we showed that both ferrocene derivatives and aminotetraphenylporphyrin have sonodynamic activity.[21] But despite the fact that their cytotoxicity is quite high, the triad of ferrocene-heterocycle-porphyrin has a more pronounced cytotoxic effect under the influence of ultrasound. That is, in our case, we can talk about the synergistic action of all components in ferrocenylheterocyclic porphyrins.

So, in this work we have carried out [3+2]-cycloaddition reaction between ferrocene derivative **1** and porphyrinazide **2** (Scheme 1). The product was obtained in 75 % yield. It is important to mention that in order to avoid the incorporation of copper into porphyrin azidoporphyrin was metallated with zinc acetate. Because when using catalytic amounts of copper acetate the terminal triple bond was not activated, and when an equivalent amount of copper acetate was added, the target product with copper was formed in the porphyrin ring.

The target compound **3** was studied in sonodynamic experiments and cytotoxic effect was revealed under ultra-



Scheme 1. The synthesis of ferrocenyl-containing 1,2,3-triazoleporphyrin.

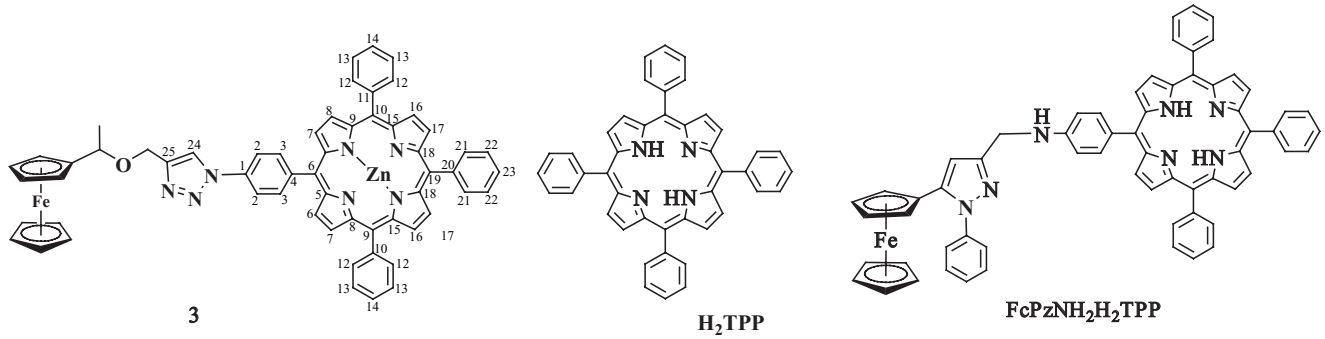


Figure 1. Porphyrins for ultrasound experiment.

sound (Table 1). In this case, the choice of *Escherichia coli*, rather than cancer cells, demonstrates the principal opportunity of using ferrocene-modified porphyrins in the method of sonodynamic therapy because the morphology of these cells coincides. Due to poor solubility in water, the substances were dissolved in a mixture of DMSO – water, choosing the optimal working concentration (Table 1). Table 1 shows the number of dead cells when irradiated with ultrasound without a substance. It is known that ultrasound itself has some cytotoxic effect. The fourth column shows the number of dead cells when processing drugs under the influence of ultrasound. So, we compared the activity of **3** with starting **H_2TPP** (5,10,15,20-tetraphenylporphyrin) and **FcPzNH$_2$H$_2$TPP** synthesized earlier. [20] The latter has another heterocyclic spacer, pyrazole between ferrocene and porphyrin. Of the three compounds presented, drug **3** has the greatest effect, showing the death of a larger number of cells (Figure 1, Table 1). These primary studies intended to identify, in principle, the cytotoxic effect of ferrocenemodified porphyrins under ultrasound. We have not yet studied how exactly one or another fragment of a molecule (ferrocene, porphyrin or heterocyclic spacer) affects sonodynamic activity. However, based of literature, suppose that the porphyrin part of the molecule is responsible for sonodynamics. Modification by ferrocene is manifested in various aspects: improving transport properties due to the lipophilicity of the ferrocene core; a very significant decrease in the toxicity of molecule with the introduction of a ferrocene fragment.

Conclusions

[3+2]-Cycloaddition reactions of ferrocenylethyl propargyl ether with porphyrinazide was carried out in toluene using copper acetate. Sonodynamic effect of ferrocene-modified porphyrin **3** was studied on *Escherichia coli*. The insolubility of this compound in water complicates the experiments with them. Alternative use of oils or DMSO sometimes negatively affects the state of experimental cell cultures, which are already extremely sensitive to external factors. The need for water-soluble preparations led us

Table 1. The results of ultrasound experiment on *Escherichia coli* (DMSO μL/10mL).

Substance	DMSO mL/10mL	US control, % death	US+sample, % death	Effect	Standard deviation
3	300	20	41	21	0.84
H_2TPP	300	20	38	18	0.72
FcPzNHH$_2$TPP[20]	300	20	29	9	0.47

to a new stage in the development of appropriate synthetic methods. Nonetheless, ferrocene-containing porphyrins are promising compounds for consciousness based on them drugs for sonodynamic therapy of cancer diseases and inflammatory processes.

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