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The ‘click’ synthesis of new cytotoxic conjugate based on *meso*-arylporphyrin and Erlotinib

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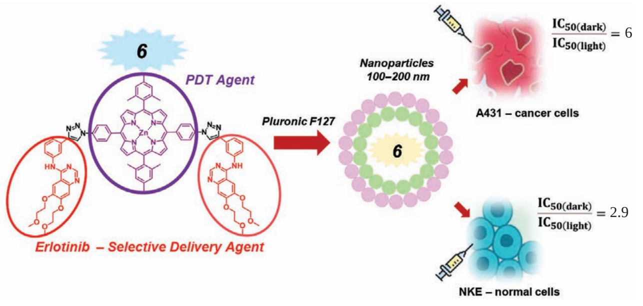
New phototherapeutic agents based on synthetic porphyrin-type tetrapyrroles and small-molecule-targeted tyrosine kinase inhibitor Erlotinib have been synthesized based on the scheme involving ‘click’ reaction between bis(4-azidophenyl)-containing zinc porphyrinate derivative and *N*-(3-ethynylphenylamino)-6,7-bis(2-methoxyethoxy)-quinazoline. The final compound and its precursors were tested as potential photosensitizers for targeted photodynamic therapy (PDT), the conjugate having shown photoinduced cytotoxicity IC_{50} for NKE cells 0.86 ± 0.017 μM and for A431 cells 0.54 ± 0.011 μM.



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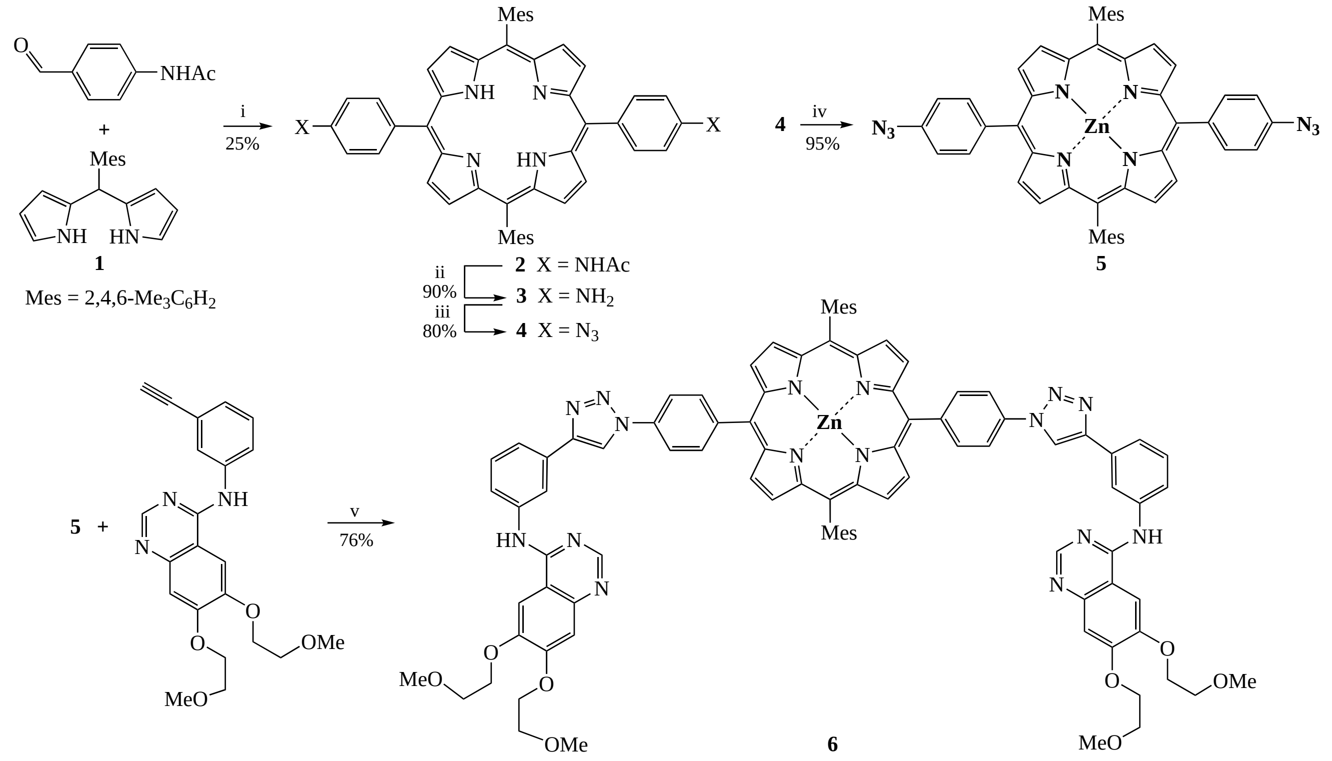
Photodynamic therapy (PDT) utilizes application of three non-toxic agents: photosensitizers (PSs), laser irradiation, and oxygen dissolved in tissues. Upon irradiation PS generates reactive oxygen species (ROS) causing the destruction of vital structures of tumor cells.[1,2] Photodynamic therapy was successful in the treatment of a wide range of neoplasia of various etiologies as well as non-oncological diseases.[3] Despite the promising and innovative nature of PDT in cancer therapy, its widespread use is limited due to the low selectivity of first- and second-generation photosensitizers towards tumor cells and, consequently, the need to increase therapeutic doses of drugs to achieve positive dynamics. Such highly toxic doses tend to affect healthy tissues, causing serious undesirable side effects and reduction of the therapeutic effect of PDT. The development of photodynamic agents capable of selective accumulating in cancer cells is a relevant direction for improving antitumor photodynamic therapy.[4] Synthetic porphyrins are widely used as photoactive agents due to their photophysical characteristics and are universal starting platforms for creating compounds with specified characteristics.[5–7]

One of the ways to regulate the biodistribution of photosensitizers and, consequently, to increase their overall efficacy during systemic administration is to modify PSs with ligands that specifically bind to target molecules and are recognized by receptors overexpressed in tumor cells.[8–11] Monoclonal antibodies, aptamers, peptides, glycoconjugates were reported among such targeting agents.[9,10,12,13]

Epidermal growth factor receptors (EGFRs) have received attention as the most promising ligand for active targeting due to their overexpression in a wide range of tumors and involvement in the processes of proliferation, angiogenesis, and metastatic spread of cancer cells.[14–16] Most of known small-molecule-targeted tyrosine kinase inhibitors (TKIs) of EGFRs are derivatives of 4-anilinoquinazoline. Such TKIs block the action of epidermal growth factor receptors overexpressed in tumors and can be used as high-affinity ligands for targeted PDT.[17–19]

Combining high selectivity of tyrosine kinase inhibitors to tumor cells with therapeutic activity of photosensitizers by obtaining their conjugates is an urgent task that allows one to achieve significant progress in the treatment of oncology by PDT.[20,21] Antitumor activity of photosensitizers based on phthalocyanines,[22] chlorins[23,24] containing TKI fragments has been documented. However, the search for the optimal structure of PS-TKI inhibitors remains an important direction of improvement of antitumor PDT requiring interdisciplinary scientific research.

In this work, we have prepared a new hybrid phototherapeutic agent combining porphyrin and small-molecule tyrosine kinase inhibitor Erlotinib. Copper(I)-catalyzed azide–alkyne cycloaddition (CuAAC) reaction was chosen for the conjugation, namely, for the cycloadditon of commercially available Erlotinib bearing ethynyl groups with azido-substituted *meso*-arylporphyrin of the *trans*-ABAB type.[25,26] We have previously reported on the efficacy of this bioorthogonal approach for the synthesis of porphyrin-type targeted photosensitizers.[27] Currently, most *meso*-substituted porphyrins are prepared by either the Lindsey monopyrrole condensation[28,29] or the Adler reaction.[30] However, the application of these approaches to the synthesis of *trans*-ABAB porphyrins is limited due to the formation of by-products.[31]



Scheme 1 *Reagents and conditions*: i, $CHCl_3$, $BF_3 \cdot OEt_2$, Ar, 1 h, then DDQ, room temperature, 2 h; ii, EtOH, HCl (3 : 2, v/v), 78 °C, 48 h; iii, CF_3CO_2H, $NaNO_2/H_2O$, 0 °C, 15 min, then NaN_3/H_2O, 25 °C, 1 h; iv, $Zn(OAc)_2 \cdot 2H_2O$, CH_2Cl_2, MeOH, 25 °C, 5 h; v, $CuSO_4 \cdot 5H_2O$, sodium ascorbate, THF–H_2O (8 : 5, v/v), 64 °C, 6 h.

Considering the described difficulties, a two-step method for the cyclization of α-substituted dipyrrolylmethane **1** with 4-acetamidobenzaldehyde [2 + 2], similar to the classical Lindsey method, was chosen for the preparation of the porphyrin precursor (Scheme 1). It was previously described that the use of sterically hindered mesityl(dipyrrolyl)methane **1** allows one to minimize scrambling in the course of the MacDonald [2 + 2] condensation.[32] The subsequent DDG oxidation of the resulting porphyrinogen provided porphyrin **2** in 25% yield. Further acidic *N*-deacetylation, diazotization of thus obtained diamine **3** and treatment of the intermediate bis-diazonium salt with NaN_3 afforded diazido-substituted *trans*-ABAB porphyrin **4** (see Scheme 1). Next, it seemed necessary to introduce Zn^{II} cation into the cavity of the porphyrin macrocycle **4** to enable preventing copper chelation during the ‘click’-reaction as well as anticipating of increasing the quantum yields of singlet oxygen compared to free-base photosensitizers.[33] The formation of the complex with Zn^{II} was confirmed on the basis of electronic absorption spectra, where four Q-bands were degenerated into two due to the increased symmetry of the macrocycle. The key Cu^{I}-catalyzed azide–alkyne cycloaddition was carried out by conjugation of Erlotinib modified with terminal triple bond to diazido Zn^{II} porphyrinate **5** at 64 °C in a THF/H_2O mixture. An aqueous mixture of copper sulfate pentahydrate (0.6 equiv.) and sodium ascorbate (1.2 equiv.) was selected as the reaction promoter, which afforded conjugate **6** in 76% yield.

The main photophysical properties of thus obtained porphyrins are given in Table 1. In the electronic absorption spectrum of target conjugate **6**, a bathochromic shift of 4–6 nm can be observed compared with the porphyrin precursors **2**–**5**. The Soret band of **6** is located at λ_B = 430 nm, and the Q-bands are observed at λ_Q = 562 and 606 nm (see Table 1 and Figure S15 of the Online Supplementary Materials). Our previous work[27] showed decrease in the molar extinction coefficient (lgε) when Erlotinib molecule was introduced into the A3B cationic porphyrin due to the aggregation processes. In this work, molar extinction coefficient of the final Zn^{II} complex **6** is almost identical to that of the precursor **5** (see Table 1). The luminescence spectrum of conjugate **6** is also characterized by a 5 nm bathochromic shift of bands relative to the precursor **5** (see Figure S15).

The poor solubility of Erlotinib (0.1 mg ml^{-1} in 1 : 9 solution of DMF/PBS, PBS is phosphate-buffered saline) and mostly hydrophobic porphyrin core complicates its biological tests under the physiological conditions. To improve its solubility, the Pluronic F127 micelles were used. Thin solid film of compound **6** and Pluronic F127 was prepared using known method.[34] The nanoparticles were purified using gel-exclusion chromatography

Table 1 Available UV-VIS data of synthesized porphyrins in DMSO.

Sample	λ_{abs}/nm (lgε)[a]					λ_{em}/nm	
	Soret	QI	QII	QIII	QIV	Q(0,0)	Q(0,1)
2	424 (5.82)	519 (4.53)	558 (3.44)	594 (3.26)	651 (3.23)	607	659
3	425 (5.84)	520 (4.42)	561 (3.43)	597 (3.22)	650 (3.19)	608	660
4	426 (5.81)	522 (4.40)	560 (3.41)	599 (3.30)	653 (3.18)	607	660
5	426 (5.72)	–	562 (4.16)	603 (3.76)	–	611	663
6	430 (5.74)	–	562 (4.22)	606 (3.89)	–	616	664

[a] ε – molar extinction coefficient.

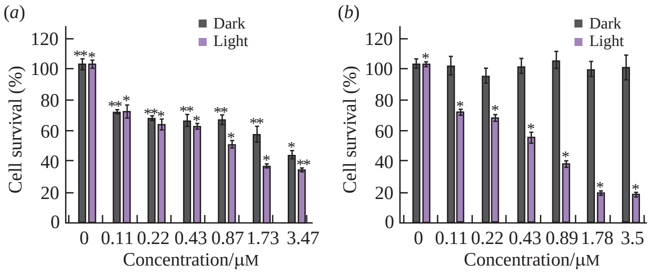


Figure 1 Dark and photoinduced toxicity of conjugate **6** for (*a*) NKE and (*b*) A431. Cells were irradiated for 90 min using the Medical Therapy Philips TL 20W/52 lamp (irradiation dose of 8.073 J cm^{-2}). Incubation of cells with the compound for 24 h in serum-free medium. Symbols */** indicate statistically significant differences in cell survival relative to the values with zero concentration of the compound ($p < 0.05$)

and have 100–200 nm size according to the dynamic light scattering and scanning electron microscopy (Figures S16, S17). The target conjugate **6** (as micelles) was incubated with both cancer and non-cancer cell lines, *e.g*., human epidermoid carcinoma A431 cells and normal human kidney embryos NKE. Dark and photoinduced toxicity of the conjugate was evaluated by standard MTT assay using 8.073 J cm^{-2} irradiation dose. Dark cytotoxicity IC$_{50}$ for NKE cells is 2.5 ± 0.073 μM, and 0.86 ± 0.017 μM when irradiated. For human epidermoid carcinoma A431 cells, no dark cytotoxicity was shown within the solubility, and photoinduced cytotoxicity for compound **6** was found at 0.54 ± 0.011 μM (Figure 1 and Table S1). In fact, conjugate **6** exhibited photoinduced cytotoxicity increased 2.9-fold compared to dark values and more than 6-fold for the cancer cell line. The obtained IC$_{50}$ values are a characteristic of porphyrins,[35] which is important considering the presence of cytotoxic Erlotinib. Photoinduced toxicity of the compounds can be compared to the previously published one.[36] In the cited work, the ratio between light and dark toxicity for compound Hu–CuTPP was 1.3 for the EGFR-expressing 4T1 cell line and normal LO2 cells. Thus, the herein obtained data on the toxicity of conjugate **6** are in accordance with the literature, and the target conjugate can be interesting for the further investigation in the biological system.

In conclusion, we have synthesized porphyrin-based conjugate containing two Erlotinib molecules *via* the ‘click’ reaction of the relevant azido and ethynyl counterparts. This synthetic approach characterizes by high yields and convenient processing. The *in vitro* studies have shown a greater toxicity for cancer A431 cells to the action of the resulting conjugate compared to that for the normal NKE cells.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2024.09.019.

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