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Novel amphiphilic cationic porphyrin and its Ag(II) complex as potential anticancer agents

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ABSTRACT

In the present study we have synthesized a novel amphiphilic porphyrin and its Ag(II) complex through modification of water-soluble porphyrinic structure in order to increase its lipophilicity and in turn pharmacological potency. New cationic non-symmetrical *meso*-substituted porphyrins were characterized by UV–visible, electrospray ionization mass spectrometry (ESI-MS), ^{1}H NMR techniques, lipophilicity (thin-layer chromatographic retention factor, R_f), and elemental analysis. The key toxicological profile (*i.e.* cytotoxicity and cell line-(cancer type-) specificity; genotoxicity; cell cycle effects) of amphiphilic Ag porphyrin was studied in human normal and cancer cell lines of various tissue origins and compared with its water-soluble analog. Structural modification of the molecule from water-soluble to amphiphilic resulted in a certain increase in the cytotoxicity and a decrease in cell line-specificity. Importantly, Ag(II) porphyrin showed less toxicity to normal cells and greater toxicity to their cancerous counterparts as compared to cisplatin. The amphiphilic complex was also not genotoxic and demonstrated a slight cytostatic effect via the cell cycle delay due to the prolongation of S-phase. As expected, the performed structural modification affected also the photocytotoxic activity of metal-free amphiphilic porphyrin. The ligand tested on cancer cell line revealed a dramatic (more than 70-fold) amplification of its phototoxic activity as compared to its water-soluble tetracationic metal-free analog. The compound combines low dark cytotoxicity with 5 fold stronger phototoxicity relative to Chlorin e_6 and could be considered as a potential photosensitizer for further development in photodynamic therapy.

1. Introduction

Porphyrins are known to preferentially accumulate in tumor tissue, a highly desirable feature for anticancer therapy [1–5]. Many photosensitizers (PSs) have been created based on numerous structural modifications plausible on porphyrinic macrocycle and successfully applied in photodynamic therapy of cancer [4–8]. Due to their high phototoxicity and tumor targeting ability, porphyrins were linked to various anticancer moieties to selectively transport and destroy the tumor tissue both with and without light applications [9–14]. At present, the search for potent chemotherapeutics (agents used without light application) among this class of compounds became also an area of vigorous exploration. In fact, for the last decade, anticancer activity of a series of metalloporphyrins has been a subject of several research groups' studies. A series of Au(III) tetraarylporphyrins were synthesized and tested as potential chemotherapeutical agents for cancer treatment [15–17]. They exerted higher potency than cisplatin in killing human cancer cells [18], which led the authors to proceed successfully with *in vivo* studies [15]. Anticancer activity of water-soluble cationic Mn(III) complexes of *meso*-tetrakis(2-*N*-substituted pyridyl)porphyrins alone or as a part of combinatorial treatment was demonstrated in a series of *in vivo* cancer models such as the skin, brain, breast and prostate [19–23]. Two types of mechanisms, *i.e.* pro- and/or antioxidative, have been suggested to be possibly involved in the anticancer action of Mn(III) porphyrins, which were initially developed as superoxide dismutase mimics [23–27]. Kawakami's group have reported that Fe(III) complexes of *meso*-substituted cationic porphyrins are also promising

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anticancer agents able to selectively destroy cancer cells [28]. They have demonstrated that iron porphyrin uses intracellular high level of superoxide anion as a target molecule to induce selective cancer cell death [29–31].

Lately we have shown that Ag(II) complexes of water-soluble cationic *meso*-tetrakis(*N*-substitutedpyridyl)porphyrins could also be considered as a potential new class of chemotherapeutic agents [32–35]. A number of water-soluble, *meso*-substituted cationic pyridylporphyrins and their metallocomplexes bearing various central metal atoms (Ag, Zn, Co, and Fe) in porphine ring and functional groups (allyl, oxyethyl, butyl, and methallyl) at the nitrogen atom in pyridine ring were synthesized and screened *in vitro* as potential anticancer agents [32–35]. The AgTAll4PyP, which includes Ag as a central metal atom and allyl functional group at the periphery was identified as the most cytotoxic metalloporphyrin (Fig. 1). Synthesized porphyrins and metallocomplexes were tested also on their photodynamic activities. The most phototoxic porphyrin was revealed to be the allyl group containing free-base porphyrin, H_2TAll4PyP (Fig. 1) [35].

In the present work, aiming to improve the bioavailability, and in turn anticancer (dark and photo-induced) activity of (metallo)porphyrins, we have designed and synthesized a new amphiphilic type of porphyrin, H_2TriAll4PyMVanC_{16}P and its Ag(II) complex, AgTriAll4PyMVanC_{16}P (Fig. 1) via incorporation of long hydrophobic chain into the *meso*-position of the porphine ring. The combination of hydrophobic and hydrophilic substituents in the porphyrinic structure was documented earlier to improve compounds' pharmacological and pharmacokinetic properties [5,8,36–39]. The insertion of polar substituents into the macrocycle carrying also hydrophobic moieties makes the molecule an amphiphilic species with sufficient water-solubility to allow its systemic administration *in vivo*, while retaining high tendency to penetrate through a lipid barrier of cytoplasmic membrane of tumor cells and localize at intracellular compartments [5,36]. In addition, the presence of electrically charged functional groups generates electrostatic repulsion, thereby preventing the formation of aggregates which otherwise would drastically modify the porphyrin bioactivity [40]. Hence, due to the introduction of long alkyl chain and the loss of single positive charge (Fig. 1), synthesized porphyrin H_2TriAll4PyMVanC_{16}P and its Ag(II) complex, AgTriAll4PyMVanC_{16}P, gain in the lipophilicity as compared to their tetracationic analogs. In turn, purposed structural variations intensify their phototoxicity (for free-base porphyrin) and chemotherapeutic (for Ag(II) complex) activity. The activities of new porphyrins (H_2TriAll4PyMVanC_{16}P and AgTriAll4PyMVanC_{16}P) as potential photosensitizing and chemotherapeutic agents were studied and compared with those of tetracationic porphyrins (H_2TAll4PyP and AgTAll4PyP) (Fig. 1).

2. Experimental

2.1. Chemicals and equipments

Pyrrole, 4-hydroxy-3-methoxybenzaldehyde (vanillin), 4-pyridinecarboxaldehyde (97%), $AgNO_3$, 3-bromopropene (allylbromide), acetonitrile, heptafluorobutyric acid (HFBA), and *N*,*N*-dimethylformamide anhydrous of 99.8% purity were purchased from Sigma-Aldrich Chemie GMBH, Germany. The chemotherapeutic drug cisplatin was obtained from EBEWE Pharma Ges.m.b.H.Nfg.KGA-4866 Unterach, Austria, and Chlorin e_6 was kindly provided by Dr. Gyulkhandanyan (Institute of Biochemistry, NAS RA). All chemicals were used as received without further purification. H_2TAll4PyP and its Ag complex were synthesized according to the methods earlier published in [35].

The structure and purity of compounds synthesized were determined by NMR, electronic absorption spectroscopy, elemental analysis and thin-layer chromatography. Analytical thin-layer chromatography was performed on silica-coated plastic plates (1:1:8 = KNO_3-saturated H_2O:H_2O:CH_3CN (v/v) mobile phase is used for the water soluble and amphiphilic (metallo)porphyrins and chloroform:methanol system for organosoluble porphyrins). Preparative separation was performed by column chromatography on alumina (Brockmann Grade II). ^{1}H NMR spectra were recorded on a spectrometer "Mercury Varian 300" (solvents — deuterated chloroform and dimethyl sulfoxide). The electronic spectra of porphyrins and metalloporphyrins were recorded in the wavelength range of 350–800 nm on a "Perkin-Elmer Lambda 800" double-beam UV–visible spectrophotometer (solvents — distilled water and chloroform). The absorption coefficients of bands were determined in 10^{-4}–10^{-6} M porphyrin solutions by the Beer–Lambert law.

2.2. Synthesis of porphyrins and metalloporphyrins

2.2.1. 5-Mono(3′-methoxy-4′-hexadecyloxyphenyl)-10,15,20-tri(4′-N-allylpyridyl)porphine tribromide (H_2TriAll4PyMVanC_{16}P)

The synthesis of H_2TriAll4PyMVanC_{16}P was performed by boiling a mixture of H_2Tri4PyMVanC_{16}P (50 mg, 0.056 mmol) and 3-bromopropene (1.0 mL, 11.6 mmol) in *N*,*N*-dimethylformamide (6 mL) at reflux. The experimental procedures of the synthesis of H_2Tri4PyMVanC_{16}P and its related isomers (H_2TVanC_{16}P, H_2T4PyP,

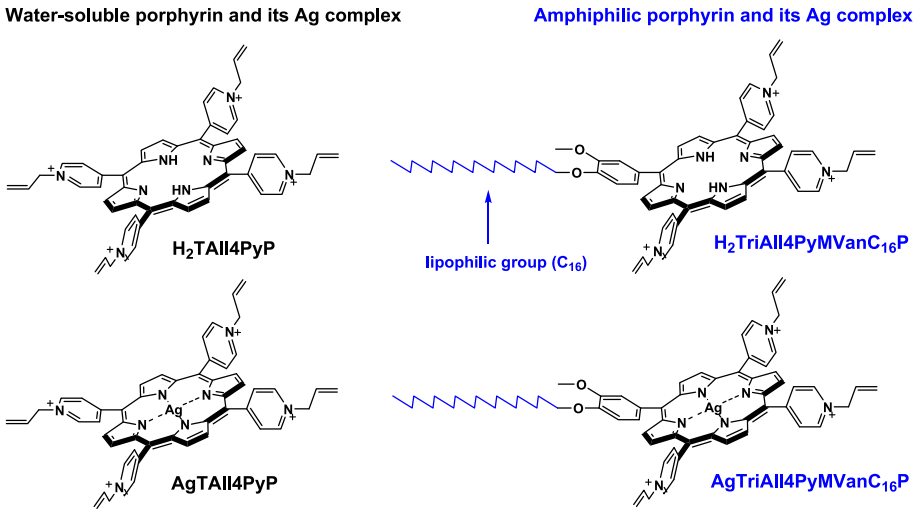

Fig. 1. Chemical structures of porphyrins (H_2TAll4PyP and H_2TriAll4PyMVanC_{16}P) and their Ag(II) complexes (AgTAll4PyP and AgTriAll4PyMVanC_{16}P). Due to the insertion of long alkyl chain and loss of a positive charge (phenyl replacing quaternized pyridyl) the porphyrin synthesized became more lipophilic, thus presumably more bioavailable for the cells.