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Optimal photosensitizers for photodynamic therapy : the preparation and characterization of novel photosensitizers derived from mesoporphyrin

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Summary

Iron and magnesium porphyrins are essential in living organisms (**chapter 1**). The red colored oxygen-carrying part in the blood protein hemoglobin is heme. Heme is the Fe^{2+} complex of protoporphyrin IX. In plants the magnesium containing chlorophyll is responsible for the initial step of photosynthesis: absorption of light followed by the conversion of the energy of this light into redox equivalents. The absorption of light by a porphyrin molecule leads to excitation of the porphyrin to the singlet excited state after which conversion into the triplet state takes place. When oxygen comes into contact with this excited porphyrin molecule the energy is transferred to the oxygen molecule under formation of singlet oxygen, which is a very reactive and toxic species. In heme, the iron prevents formation of singlet oxygen by quenching the triplet excited state and in plants carotenoids are responsible for quenching this triplet state. Persons or animals with genetic defects that lead to the accumulation of free base porphyrins in their bodies are highly light sensitive due to the formation of singlet oxygen in the skin. In water the lifetime of singlet oxygen is very short (3.1 μs), which means that the damage of tissue occurs near the place where it is generated.

If the porphyrin can be localized in tumors, irradiation with light would then selectively destroy the tumor, which is the basis of photodynamic therapy (PDT). The goal of this project is to develop new porphyrin photosensitizers for use in PDT. Hemin, which is easily available and cheap, is the source from which the new photosensitizers are prepared. Photofrin[®] is presently the most popular photosensitizer for PDT and also prepared from hemin but has several drawbacks. It is not a pure single compound, it is not a stable compound and it has weak absorption in the red part of the visible spectrum where light penetration in tissue is far from optimal. The aim in this project is to develop a new photosensitizer, which should be completely non-toxic in the dark, stable in the dark as well as in light, rapidly clear out of the body, easily available and with strong absorption at a wavelength longer than 650 nm.

In **chapter 2** the preparation of new photosensitizers based on mesoporphyrin derived from heme is described. The mesoporphyrin dimethyl ester nickel complex has been formylated via the Vilsmeier-Haack method. The four possible mono meso-formyl derivatives were isolated and characterized. Wadsworth-Emmons coupling with the anion of (diethylphosphono) acetonitrile converted these aldehydes into the four novel meso acrylonitriles. Brief treatment of these acrylonitrile systems with trichloroacetic acid at 180°C resulted in the formation of

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the nickel complexes of four achiral with unprecedented *peri*-condensed quinoline porphyrin structure. Subsequent removal of the nickel gave four quinoporphyrin free bases: quino[4,4a,5,6-*efg*]-annulated 7-demethyl-8-deethylmesoporphyrin dimethyl ester **6a**, 2-(methoxycarbonyl)quino-[4,4a,5,6-*jkl*]-annulated 12-demethyl-13-de[2-(methoxycarbonyl)-ethyl]mesoporphyrin dimethyl ester **6b**, 2-(methoxycarbonyl) quino[4,4a,5,6-*qrs*]-annulated 18-demethyl-17-de(2-methoxy-carbonylethyl)mesoporphyrin dimethyl ester **6c** and quino[4,5,6,7-*abt*]-annulated 2-demethyl-3-deethylmesoporphyrin dimethyl ester **6d**. The structures of these systems were unambiguously determined via mass spectroscopy and a plethora of NMR techniques. In the same way, etioporphyrin and octaethylporphyrin were converted into the corresponding *peri*-condensed quinoporphyrins as products, which shows that the formation of novel *peri*-condensed quinoporphyrins is a general reaction in porphyrin chemistry and will have a wide scope in this field. Also, a plausible reaction mechanism for the formation of the quinoporphyrin systems was derived. As a first test for the use of these systems as sensitizers in far-red phototherapy, the quantum yield of singlet oxygen generation by **6a** in toluene was studied. This quantum yield is 0.77, which is even higher than the singlet oxygen generation by sensitized meso-tetraphenylporphyrin. Secondly, when Chinese Hamster ovary (CHO) cells were incubated in medium, which contained up to 15 $\mu\text{g/ml}$ of **6a**, the survival of the cells in the dark is complete within experimental error, showing that under these conditions, **6a** is not toxic to CHO cells. When CHO cells incubated in medium containing **6a** in concentrations of 1 $\mu\text{g/ml}$ and higher were treated with white light of intensity 30 mW/cm^2 for 15 minutes, complete cell death was observed. Based on these facts, we expect that all four achiral systems will show very promising properties to form the basis of a photodynamic therapy with far-red light. The fact that these systems are achiral is an additional bonus for medical applications.

In **chapter 3** the sterically hindered [5-(2-cyanovinyl)mesoporphyrin dimethyl ester] nickel complex has been treated with *N*-methylformanilide under Vilsmeier conditions. Besides the expected *meso*-formylporphyrin derivatives, 22% of a novel green compound was isolated. After removal of Ni^{2+} by treatment with concentrated sulfuric acid, the structure of the novel compound was elucidated by mass spectrometry and ^1H and ^{13}C NMR spectroscopy, which led to the unambiguous establishment of the unprecedented racemic dimethyl ester of 2'-cyano-8'-formyl-*N'*-methyl-1,1a,5a,6-tetrahydroacrido[4,5,5a,6-*bcd*]-annulated 2,3-dihydro-mesoporphyrin structure **5**. This racemic form was efficiently separated into its two pure enantiomeric forms each of which shows the same electronic spectrum as the racemate mixture. The two optical isomers show the exact opposite circular dichroism spectra. The

structure of this novel product is clearly formed after the initial attack of the carbenium ion formed from *N*-methylformanilide and POCl₃ at the α -carbon atom of the appending *meso*-acrylonitrile function. This process is an unprecedented cyclization process under Vilsmeier conditions. It is clear that in this case the aromatic system of the Vilsmeier reagent is intimately involved in this reaction. In order to further explore the reactivity of the starting material, we treated it with the Vilsmeier reagent prepared from dimethylformamide and POCl₃. In this case we obtained, besides the expected formylation products, a mixture of two *peri*-annulated quinoporphyrins. From the structures of the products and the starting material it could be established that in this case the attack of the Vilsmeier reagent is on the nitrogen atom of the appending acrylonitrile function. For the initial tests of **5** as the basis of a photodynamic therapy we found that in the presence of air and light it is an efficient singlet oxygen generator that is stable during the irradiation time. It shows no toxicity towards lung carcinoma cells in the dark while in the presence of air and light the compound leads to a rapid killing of the cancer cells at concentrations that are an order of magnitude lower than in the case of the quinoporphyrin systems. These are all promising properties for continued studies and further development towards a photodynamic cancer therapy.

In **chapter 4** a ¹H-NMR study towards the properties of porphyrins with appending propionic amide side chains is presented. From the ¹H-NMR spectrum of the zinc(II) complex of *N,N*-bis(2,2-diethoxyethyl) mesoporphyrin bisamide in d₆-dimethylsulfoxide it can be seen that the signals of the diethoxyethyl part in the appending side chains show a large up field shift compared to the diethoxyethylamine. Besides the two appending side chains have different chemical shifts in their ¹H-NMR spectrum. Because the ¹H-NMR-spectrum signals do not change with concentration in dimethylsulfoxide indicating that this upfield shift can only be due to intramolecular interactions. This means that the appending amide side chains are folded back over the porphyrin macrocycle in a dynamic equilibrium. Around the pyrrole rings C and D the structure of the compound is highly symmetric. The fact that the two amide side chains have different chemical shift values can only be explained by the asymmetry that arises from the ordering of methyl and ethyl groups attached to pyrrole rings A and B at the other side of the porphyrin macrocycle. This also indicates that the amide side chains are folded back over the porphyrin macrocycle. The same phenomenon was found for the analogous protoporphyrin and deuteroporphyrin derivatives. In both the protoporphyrin and the deuteroporphyrin derivative the difference in chemical shift values of the two amide side chains is smaller due to that the substituents on positions 3 and 8 of the pyrrole rings A and B have less steric interaction with the ethoxy groups of the amide side chains. Similar results

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were found in the $^1\text{H-NMR}$ -spectra of the corresponding free base porphyrins. In this case the upfield shift is even larger, which can be explained by the absence of coordinating solvent molecules enabling the side chains to come closer to the porphyrin ring system. Modification of the amide side chain led to a whole series of porphyrin derivatives in which the appending side chains fold back to the aromatic porphyrin macrocycle.

A general discussion and future prospects for the work described in this thesis is presented in **chapter 5**. Also the preliminary results obtained from preclinical investigations on the biological properties of two of the new photosensitizers are presented. From this chapter it becomes clear that the chemistry as described in chapters 2 and 3 gives access to a whole series of quino[4,4a,5,6]annulated porphyrins and to tetrahydro acrido annulated dihydro porphyrins. Modification of the starting materials should already give new compounds moreover the products themselves can be modified. Some suggestions for further development of new photosensitizers based on the chemistry described in this thesis are given. E. g. in the case of the *peri*-condensed quinoporphyrins alkylation of the quinoline nitrogen leads to a quinolinium porphyrin with absorption at 755 nm. Also the functional groups in 2'-cyano-8'-formyl-*N*'-methyl-1,1a,5a,6-tetrahydroacrido[4,5,5a,6-*bcd*]-annulated 2,3-dihydromesoporphyrin are very suitable for further modification.

Preclinical investigations in chicken embryos on one hand and on tumors in mice on the other hand of the free carboxylic acids of both quino[4,4a,5,6-*efg*]-8-deethyl-7-demethylmesoporphyrin (PB07) and of 2'-cyano-8'-formyl-*N*'-methyl-1,1a,5a,6-tetrahydroacrido[4,5,5a,6-*bcd*]-annulated 2,3-dihydromesoporphyrin (PB109) make clear that both photosensitizers may be very promising for application in photodynamic therapy.