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Hydroporphyrins as Tumour Photosensitizers: Synthesis and Photophysical Studies of 2,3-Dihydro-5,15-di(3,5-dihydroxyphenyl) Porphyrin

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Abstract—The synthesis and characterization of 2,3-dihydro-5,15-di(3,5-dihydroxyphenyl) porphyrin is reported. The phototoxicity on C6 cell lines and the pharmacokinetics are also reported as preliminary results showing a very high tumor to skin ratio and short retention time in tissues, and thus promising activity in photodynamic therapy.

Recently, promising photosensitizers have been developed for photodynamic therapy.[1] Among these substances, certain hydroxysubstituted *meso*-tetraarylchlorins showed good activity.[2,3] However one problem with these molecules is the long retention time of the drug in normal tissues, more particularly in the skin because after injection and treatment, the patient must be protected against sun during a period up to several days. A possible solution is to use diphenylporphyrins instead of tetraphenylporphyrins as precursors. This class of porphyrins was originally studied by several groups as models of the photosynthetic centre.[4] These compounds are stable and now relatively easy to prepare in high yields. We postulated that (i), the diphenylporphyrin can be reduced in chlorin in order to show absorption in the red and (ii), the presence of two free *meso* positions will make easier the biotransformation in order to decrease prolonged cutaneous photosensitivity, a major adverse effect associated with tetraphenyl porphyrin series and photofrin.

On the basis of these criteria, we now report the synthesis and the biophysical studies of these second generation photosensitizers bearing only two phenyl groups on the *meso* position, a structural change which is accompanied by a short retention time in tissues together with a promising activity. Cell uptake and photodynamic properties are compared to those of the structurally related *meso*-tetrahydroxyphenylchlorin (m-THPC), a drug which is one of the most potent photosensitizers discovered to date.[5]

The condensation of dipyrrylmethane **1** with 3,5-dimethoxybenzaldehyde furnishes, upon oxidation with *o*-chloranil, 5,15-di(3,5-methoxyphenyl)porphyrin **2** in high yield (78%) under reaction conditions that thermodynamically favors the formation of the intermediate porphyrinogen, as previously reported in the Lindsey method.[6] The porphyrin is then demethylated with boron tribromide in dichloromethane to give **3** (yield 75%). In the last step, diimide reduction of the diphenylporphyrin gives a mixture of the corresponding dihydro and tetrahydroporphyrins using the method of Whitlock et al.[7] Selective oxidation with *o*-chloranil removes the bacteriochlorin. Actually, reduction of the porphyrin gave first a mixture of tosylated intermediates **4** and after addition of HCl N/10, the tetrahydroxy product **5** with the desired photophysical properties. Interestingly, the yield obtained for the chlorin is high (75%) and the formation of the corresponding porphyrin in the last step is not observed. Several attempts were made in order to isolate the

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hydroxy form of the bacteriochlorin but it seems quite unstable under acidic conditions. The preparation of the compound **5** is summarized in Figure 1. Whilst this work was in progress, a different diphenylchlorin[8,9] has been reported. The porphyrin was first prepared using phenyldipyrrylmethane and trimethyl orthoformate (yield: 18%). Two hydroxy groups were then added to one pyrrole to give the chlorin.[10,11] However, the present procedure using condensation of aryl aldehyde with unsubstituted dipyrrylmethane leads a better yield than the synthesis using phenyldipyrrylmethane and formaldehyde as precursors.[10,11]

As expected from the presence of four hydroxy groups, the compound is weakly soluble in polar solvent such as methanol and water. The lipophilic and hydrophilic

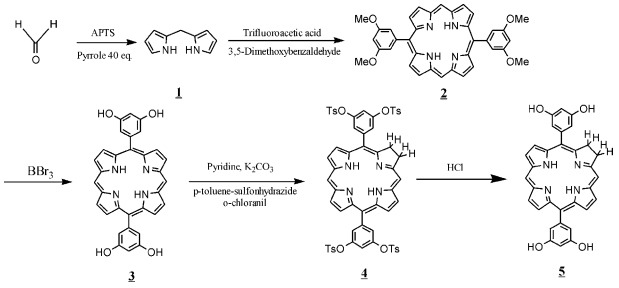

Figure 1. The synthesis of **5**.

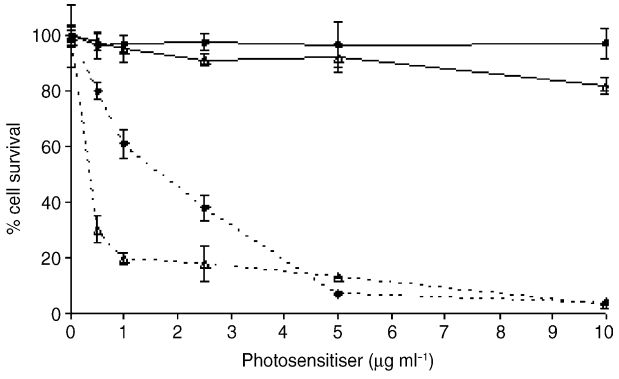

Figure 2. Toxicity and phototoxicity of compound **5** and m=THPC in C6 cells determined by MTT assay. C6 cells were incubated for 5 h with photosensitizers and irradiated at 650 nm and 20 J/cm^2 **5** (···◆···); m-THPC (···△···) or not irradiated **5** (—◆—); m-THPC (—△—).

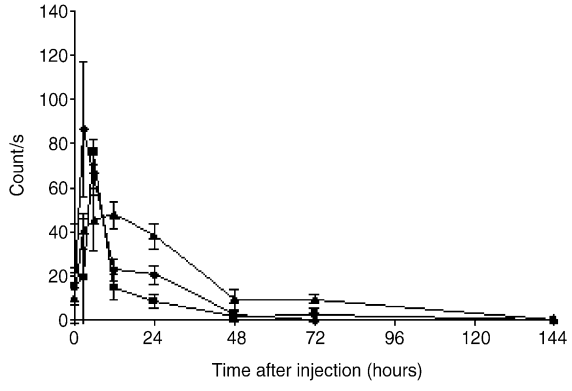

Figure 3. The fluorescence levels recorded in tumour (▲), skin (■) and muscle (◆) of mice detected 3–144 h after 2 mg kg^{-1} of **5**.

properties were characterized by the partition coefficient of the compound between the two non-miscible solvents octanol and water. The octanol/water partition ratios were 26 for compound **5** and 40 for m-THPC indicating that **5** was more hydrophilic than m-THPC. The fluorescence spectrum of compound **5**, in saline isotonic solution (100 μg mL^{-1}), showed a peak at 649 nm with a shoulder between 700 and 750 nm, after excitation at 488 nm. The singlet oxygen quantum yield of **5** and m-THPC was determined using perinaphthenone as reference. As compared to perinaphthenone, the quantum yield was 0.69 for **5** and 0.70 for m-THPC for the highest laser energy in deuterated methanol, indicating a similar efficiency for the two photosensitizers (Fig. 2).

The intracellular localization patterns of the chlorin **5** (10 μg mL^{-1}) were determined by confocal laser microspectrofluorimetry, showing staining in C6 glioma cells as a function of time, with a maximum of fluorescence after 3 h. We employed the MTT assay to evaluate the effect of the concentration on the phototoxic potential of compound **5**.[3] The survival rates ranged from 100% to 97% when cells were incubated for 5 h without light, showing absence of toxicity. The photosensitizer dose inducing a 90% death rate (LD_{90}) for C6 cells was 4.8 μg mL^{-1} for **5** and 6.8 μg mL^{-1} for m-THPC after 5-h incubation and 20 J/cm^2 irradiation (Fig. 1).[12] Thus **5** showed an efficacy similar to that of m-THPC. Compound **5** was also tested for in-vivo photosensitizing activity on human colon adenocarcinoma cell (HT-29) tumour and it was found to show optimal photodynamic response (40% of inhibition growth) for a delay between injection and irradiation of 12 h while for m-THPC optimal values were only observed for a delay of 24 h using in both cases a dye dose of 5 mg kg^{-1} and 300 J cm^{-2} irradiation with laser light (650 nm, 300 mW). This suggests that **5** is rapidly distributed in tissues and rapidly eliminated (vide infra). More importantly, the fluorescence levels recorded in tumour, skin and muscle of mice were detected 3–144 h after 2 mg kg^{-1} of **5** or m-THPC injection. It was found that the maximum concentration is reached quickly and retention time in tissues is shorter than that of m-THPC. For example, the concentration of **5** decreased rapidly in all tissues after 24 h, and no sensitizer was detectable in these tissues after 144 h (Fig. 3). In contrast, m-THPC concentration in skin is about 100 times above what is observed for **5** after 48 h. The shorter retention time of **5** in tissues can be explained by the presence of two free *meso* positions[13] which may facilitate the biotransformation of the drug and thus decrease prolonged cutaneous photosensitivity. Further developments and improvements of this approach are in progress using various diphenylchlorins,[14] and more extensive biological studies will be reported elsewhere.

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14. Selected spectroscopic data:

5,15-Bis(3,5-dimethoxyphenyl)porphyrin (2). ^{1}H NMR (CF_3COOD) ppm: 11.13 (2H-meso), 9.7 and 9.3 (2H, d, $J=6$ Hz, pyrrole), 7.9 (o-H, phenyl), 7.4 (p-phenyl), 4.2 (methoxy); **UV–Vis** (CH_2Cl_2), max (nm): 407 (ε 400 dm^3 $mmol^{-1}$ cm^{-1}), 502 (ε 5.0), 536 (ε 4.9), 574 (ε 4.9), 629 (ε 1.3). FAB-MS: calcd for $C_{36}H_{30}N_4O_4$: 582.2401 found: 582.2432 [M + H +].

5,15-Bis(3,5-dihydroxyphenyl)porphyrin (3). ^{1}H NMR (acetone d_6): 10.55 (H *meso*), 9.6 and 9.3 (2H, d, $J=6$ Hz, pyrrole), 7.3 (o-H, phenyl), 6.9(p-phenyl), −3.1 (NH); UV–Vis (acetone), λ_{max} (nm): 402 (ε 400 dm^3 $mmol^{-1}$ cm^{-1}), 532 (ε 5.0), 572 (ε 4.9), 628 (ε 1.4). FAB-MS: calcd for $C_{32}H_{22}N_4O_4$: 526.1641 found: 526.1657 $[M+H]^+$.

2,3-dihydro-5,15-bis(3,5-dihydroxyphenyl)porphyrin (5). ^{1}H NMR (acetone, d_6) ppm: 10.1 and 9.2 (2H-*meso*), 9.35–8.6 (6H, m, pyrrole), 7.2 and 6.95 (4H, d, o-phenyl), 6.85 and 6.75 (2H, t, p-phenyl), 4.65 and 4.45 (4H, m, pyrrolidine), −1.4 and −1.9 (2H, s, NH); UV–Vis (acetone), λ_{max} (nm): 395 (ε 31.6 dm^3 $mmol^{-1}$ cm^{-1}), 405 (ε 162), 500 (ε 12.6), 645 (ε 40.1). FAB-MS: calcd for $C_{32}H_{24}N_4O_4$: 528.1798 found: 528.1805 $[M^+]$.