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The synthesis of new potential photosensitizers. Part 3. Tetraphenylporphyrin esters of profens

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ABSTRACT

Six tetraphenyl derivatives of arylpropionic acids (profens) were synthesized in search for improved novel photosensitizers that might find application in photodynamic therapy (PDT) of various cancers. Our goal was to alter hydrophobic-hydrophylic characteristics of the tetraphenylporphyrin ring by substitution with propionyl derivatives. As starting materials we used profens known primarily from their widespread use as non-steroidal anti-inflammatory drugs (ibuprofen, naproxen, ketoprofen, fenoprofen, fluorbiprofen) and 2-phenylpropionic acid (model compound). The new compounds were characterized using spectroscopic and photophysical methods with focus given to measurement of molecules' lifetime in excited triplet state, time-resolved singlet oxygen phosphorescence and measurement of photostability. Fluorescence quantum yields were also determined; data revealed no planarity changes in the porphyrin molecule following substitution with profens.

1. Introduction

In our previous reports we described synthesis of a series of tetraphenylporphyrins substituted with alkylcarboxylic fragments [1] and hydroxyphenylporphyrins substituted with alkyl derivatives [2]. Preliminary studies of these alkylcarboxylic derivatives revealed their toxicity to cells cultured *in vitro* and further biological studies were abandoned. Tetraphenylporphyrin derivatives described herein, and containing various profens (used as analgesics and anti-inflammatory drugs) have been the next group of compounds tested for their potential PDT usefulness.

Derivatives of 2-phenylpropionic acid have been in use for many years as effective nonsteroidal anti-inflammatory drugs. They bind to cyclooxygenase (COX), a prostaglandin-endoperoxide synthase (PTGS), an enzyme responsible for formation of prostanoid mediators. Non-steroidal anti-inflammatory drugs, such as aspirin and ibuprofen, exert their effects through inhibition of COX, which leads to relief of symptoms of inflammation and pain, including lowering of fever.

Using racemic mixtures of profenic acids generally does not affect their biological activity; it was shown that inactive isomers of these compounds are transformed *in vivo* into their active counterparts [3–5].

When designing profenic derivatives of porphyrins we considered exploring a possible dual advantage of using such compounds. First, ester bond hydrolysis at the site of delivery ought to lead to the release of antiinflammatory drug and help shield healthy cells adjacent to cancer area. Second, cancer area might be targeted with PDT mediated by the porphyrin fragment of new derivatives. In addition to that, polar structure of the profen moiety (its transport in the body, mode of action and metabolism are well-known) would be also conducive towards improving intracellular transfer of porphyrin moieties.

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In this paper, we describe photochemical properties of these novel porphyrin derivatives. We compared their measured photochemical properties with those of an ideal photosensitizer. Such photosensitizer should have no absorption bands overlapping with absorption bands of endogenic compounds such as hemoglobin, oxyhemoglobin and melatonin. An idealized PDT agent should also have high stability during exposure to light and low value of fluorescence quantum yield. Moreover, compound should not aggregate in solution. Crucial for photodynamic therapy would be a relatively long lifetime of such molecule in the triplet state, as well as high value of singlet oxygen quantum yield generation [6–9].

Profens display certain photosensibility which can exert additional photohemolytic effects [10–12]. In pure form, all of the profens used by us showed such effects, with ketoprofen exerting the strongest ones [13]. At the site of cancer lesion this process would occur only during tissue irradiation and in the presence of profen. It was demonstrated that, during irradiation, profens and their catabolites are capable of generating singlet oxygen species [12]. If only cancer lesion were irradiated during PDT, such effect might augment targeted “cytotoxic” properties of the described derivatized porphyrin compounds.

Photostability of compounds that might act as potential drugs is an important parameter of their usefulness. Such compounds should be photostable in pure form and also in solution so that they would not undergo any conversions upon light exposure during drug preparation or administration. Photostability is often determined for compound solutions that cannot be used directly *in vivo*. This is due to such compounds' poor solubility in water. Porphyrins are a case in point as few of them are water-soluble. This limits their usefulness as photosensitizers, although they do present several advantages for such applications. Various methods have been explored that would allow transport of these compounds in bodily fluids. This requirement is contingent upon many additional studies pertaining to the choice of suitable carriers and examination of biological tolerance of such porphyrin-carrier conjugates.

2. Experimental

All chemical reagents were purchased from Aldrich, Sigma or Acros and were used without further purification. ^{1}H NMR spectra were recorded in $CDCl_3$ using a Varian spectrometer (400 MHz). The peaks were referenced to the residual $CHCl_3$ resonances in ^{1}H and ^{13}C NMR (7.26 and 77.16 ppm, respectively). UV–Vis spectra were recorded in dichloromethane solutions using a Genesys 6 (ThermoSpectronic) spectrophotometer. Fluorescence spectra of the samples were taken on Varian Eclipse Cary fluorescence spectrophotometer. ESI MS spectra were acquired using an LCQ DUO Finingham Thermoquest or Varian MS-500 instruments. Separately, porphyrin solutions were directly injected into the ion sources using a syringe pump. High resolution mass spectra were recorded on Bruker AD-604 spectrometer using electrospray technique. LKS 60 Laser Flash Photolysis Spectrometer (Applied Photophysics) equipped with the third harmonic (355 nm) of a Nd/YAG laser (20 Hz Brilliant, Quantel) was used to obtain transient (triplet–triplet) absorption spectra and kinetic decay curves of the studied compounds' triplet states. Solutions of the compounds were prepared in chloroform at a concentration providing equal absorbance values at the excitation wavelength $A_{355} = 0.20$. Measurements were performed using a quartz microcuvette with optical path length of 1 cm. Experimental logP values were determined by chromatography. Porphyrins were chromatographed on silica gel pre-coated glass RP-18 F_{254} plates (20 × 20 cm, Merck, Darmstadt, Germany) with methanol and methanol-chloroform (8:2, v/v) mixture as mobile phases. Purity of the synthesized compounds was determined by TLC and spectroscopically (NMR, HR-ESI-MS – one molecular peak). Column chromatography on silica gel with dichloromethane as mobile phase was used for purification of all the obtained porphyrins.

2.1. (R,S)-[5-(4-oxaphenylene)-10,15,20-tritolylporphyrin] 2-phenylpropionate ($C_{56}H_{44}N_4O_2 = 804$) ***1***

(**Method A**). 71 mg (~0.11 mmol) 5-(4-hydroxyphenyl)-10,15,20-tritolylporphyrin was dissolved in 5 mL of dichloromethane. Excess (~0.3 mmol) of 2-phenylpropionic acid chloride and 0.2 mL of triethylamine in 3 mL of dichloromethane were added to the solution with stirring (overnight at RT). Next, solvent was evaporated and the residue chromatographed on silica gel using dichloromethane. Yield: 43 mg (~51%).

(**Method B**). 12 mg (0.057 mmol) of DCC, 6 mg of DMAP (0.005 mmol) and 0.01 mL (~0.057 mmol) of 2-phenylpropionic acid and 5 mL of dichloromethane were cooled down in a closed flask. An aliquot of 35 mg (~0.052 mmol) of 5-(4-hydroxyphenyl)-10,15,20-tritolylporphyrin in 5 mL of dichloromethane was refrigerated in a second flask. After cooling both solutions were combined together and the resulting mixture was refrigerated for two days. Subsequently, the mixture was left standing at room temperature for another two days. The product was separated on silica gel column with dichloromethane. Yield: 10 mg (~33%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.80–8.86 (m, 8H), 8.18, 7.57 (dd, 4H, J = 8.4 Hz), 8.09, 7.56 (dd, 12H, J = 8 Hz), 8.47, 7.80 (2 × d, 2H), 7.48, 7.40, (2 × t, 3H), 4.17 (q, 1H), 2.71 (s, 9H), 1.77 (d, 3H), −2.79 (bs, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.3, 150.7, 140.2, 139.9, 139.3, 135.3, 134.6, 129.0, 127.7, 127.6, 127.5, 120.4, 120.3, 119.7, 118.7, 45.9, 21.5, 18.7; HRMS (ES^+): calcd for $[M + H]^+$ 805.3537, found 805.3507.

2.2. (R,S)-[5-(4-oxaphenylene)-10,15,20-tritolylporphyrin] 2-(4-isobutylphenyl)-propionate (ibuprofen derivative) ***2*** *($C_{60}H_{52}N_4O_2 = 860$). Yield: 40% (Method B)*

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.84 (bs, 6H), 8.80 (d, 2H), 8.16 (d, 2H, J = 8.4 Hz), 8.08 (d, 6H, J = 8.0 Hz), 7.54 (d, 6H, J = 8.0 Hz), 7.44 (d, 2H, J = 8.0 Hz), 7.39 (d, 2H, J = 8.4 Hz), 7.23 (d, 2H, J = 8.4 Hz), 4.12 (q, 1H), 2.70 (s, 9H), 2.52 (d, 2H), 1.92 (q, 1H), 1.75 (d, 3H), 0.94 (d, 6H), −2.80 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.5, 167.8, 150.8, 141.0, 139.8, 139.3, 137.3, 135.2, 134.5, 1132.5, 130.9, 129.7, 127.4, 120.3, 119.7, 118.7, 45.5, 45.2, 38.8, 22.5, 21.6, 18.6; HRMS (ES^+): calcd for $[M + H]^+$ 861.4163, found 861.4074.

2.3. (R,S)-[5-(4-oxaphenylene)-10,15,20-tritolylporphyrin] 2-(3-phenyloxyphenyl)propionate (fenoprofen derivative) ***3*** *($C_{62}H_{48}N_4O_3 = 896$). Yield: 26% (Method A)*

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.84–8.90 (m, 8H), 8.22 (d, 2H, J = 8.4 Hz), 8.12 (d, 6H, J = 8 Hz), 7.58 (d, 6H, J = 8 Hz), 7.42 (d, 2H, J = 8.4 Hz), 7.39–7.47 (m, 3H), 7.32 (d, 1H), 7.27 (bs, 1H), 7.12–7.18 (m, 3H), 7.03 (d, 1H), 4.16 (q, 1H, J = 7.2 Hz), 2.74 (s, 9H), 1.78 (d, 3H, J = 7.2 Hz), −2.75 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.9, 157.8, 157.1, 150.7, 142.1, 139.3, 137.4, 134.6, 129.9, 127.5, 123.5, 122.5, 120.3, 119.7, 119.1, 118.6, 118.3, 117.8, 45.8, 21.6, 18.6; HRMS (ES^+): calcd for $[M + H]^+$ 897.3799, found 897.4082.

2.4. (R,S)-[5-(4-oxaphenylene)-10,15,20-tritolylporphyrin] 2-(3-fluor-4-phenylphenyl]propionate (flurbiprofen derivative) ***4*** *($C_{62}H_{47}N_4O_2F = 898$). Yield: 48% (Method A)*

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.85–8.90 (m, 8H), 8.23 (d, 2H, J = 8.4 Hz), 8.13 (d, 6H, J = 8.0 Hz), 8.06 (bs, 1H), 7.93 (d, 2H, J = 8.0 Hz), 7.83 (t, 2H, J = 8.0 Hz), 7.54–7.65 (m, 4H), 7.59 (d, 6H,