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Synthesis of cationic β-vinyl substituted *meso*-tetraphenylporphyrins and their in vitro activity against herpes simplex virus type 1

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Abstract—An easy route to cationic β-vinyl substituted *meso*-tetraphenylporphyrin derivatives is described. Two novel compounds were tested in vitro for their antiviral photoactivity against herpes simplex virus type 1. One of these compounds exhibited a significant activity, reaching 99% of virus inactivation after 15 min of photoactivation.

1. Introduction

For the last two decades, interdisciplinary studies performed with porphyrin macrocycles pointed out the great potential of this type of compound for applications in various fields; indeed porphyrins are used as catalysts, advanced biomimetic models for photosynthesis, new electronic materials, sensors and drugs.[1] A number of porphyrinic compounds concerning biomedical applications are already being used with success for the treatment of several diseases by photodynamic therapy (PDT),[2] a technique which involves oxygen, light and the administration of a photosensitizing agent. Recent studies demonstrated that PDT can be very effective in the selective inactivation of microorganisms and it became a potential alternative tool for the treatment and eradication of microbial infections.[3]

Viruses are responsible for a large number of infectious diseases. In particular, herpes simplex virus (HSV) is among the most common agents responsible for viral infections in human and is often associated with serious clinical symptoms, especially in immunocompromised patients, pregnant women and newborns.[4] In spite of this, up to a decade ago only five drugs were approved and clinically used in the treatment of herpes viruses infections.[5] Acyclovir, developed in the late 1970s, was the first specific and selective antiviral drug for these viruses; acyclovir and its derivatives gave rise to the main class of compounds available for treatment of herpes diseases.[6] The emerging resistance of viruses to the classical antiviral drugs[7] and the drug side effects are the main reasons for further refinement of antiviral drug design and development.[8] The strategies for the design of new antiviral drugs must aim to develop more selective compounds with a broad spectrum of antiviral activity and little or no drug-resistance induction. Although the effectiveness of PDT in virus inactivation was demonstrated over 70 years ago,[9] only has it become the matter of more focused studies.[10]

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Biological evaluation of the activity of porphyrin-like photosensitizers has shown that cationic compounds are more efficient in bacteria and virus inactivation, and that amphiphilic photosensitizers are generally more active than hydrophilic ones.[11] Following our interest in obtaining compounds with adequate physicochemical and biological properties for medicinal applications,[3a,12] we describe here an easy synthetic route to the cationic β-vinyl substituted *meso*-tetraphenylporphyrins **2** and **3**; preliminary studies showing their capacities in the photoinactivation of herpes simplex virus type 1 (HSV-1) are also reported.

2. Results and discussion

2.1. Chemistry

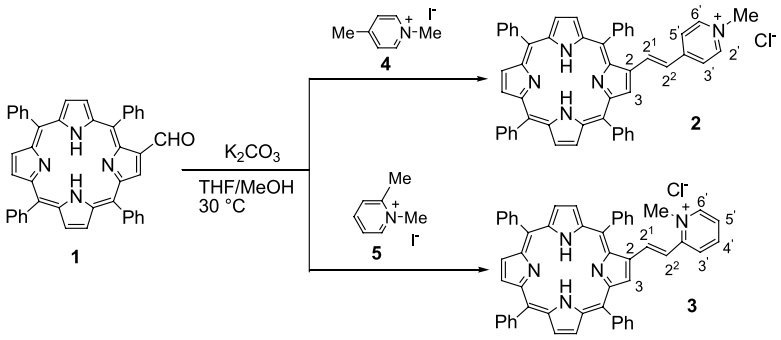
2- and 4-alkylpyridines are known to undergo aldol-like condensations with carbonyl compounds upon treatment with strong bases; the use of *N*-alkylpyridiniums allows the reaction to occur under milder conditions, as a consequence of the electron withdrawing effect exerted on the ring by the presence of the positive charge.[13] We evaluated the possibility of using such aldol-like reaction on 2-formyl-5,10,15,20-tetraphenylporphyrin **1** in order to afford the desired monosubstituted cationic derivatives **2** and **3** in a one-step process (Scheme 1).

Porphyrin **1** was chosen as the starting material because of the well-known versatility of the carbonyl group towards further functionalization and due to the relative synthetic availability of such derivative.[14] Porphyrin **1** was obtained by Vilsmeier formylation of the copper complex of 5,10,15,20-tetraphenylporphyrin, according to the literature procedure.[15] The condensation products **2** and **3** were obtained by reacting porphyrin **1** with either 1,4-dimethylpyridinium iodide **4** or 1,2-dimethylpyridinium iodide **5**[16] in the presence of K_2CO_3 (Scheme 1).[17] The reactions were monitored by TLC and were worked up after 1 h. The new compounds were obtained from the crude material after flash chromatography on silica gel; they were characterized by conventional spectroscopic techniques (NMR, MS, and UV–vis). Compounds **2** and **3** were obtained respectively in 65% and 60% yields. It is worth noting that the synthesis of the neutral analogue of **2**, 2-[2-(4-pyridylvinyl)]-5,10,15,20-tetraphenylporphyrin, has previously been reported in the literature, starting also from **1**.[18] However, the procedure described requires several steps, namely reduction of the carbonyl function, halogenation of the hydroxymethyl group, formation of a porphyrinic triphenylphosphonium salt and its reaction with pyridine-4-carbaldehyde. This method leads to the formation of both *E*- and *Z*-isomers of the 2-(4-pyridylvinyl)-substituted porphyrins, whereas in our case only the *E*-isomer was formed.

The spectroscopic data of the two compounds,[19] namely the ^{1}H NMR spectra, are in agreement with the proposed structures. Unequivocal proton assignments were achieved with COSY, HSQC and HMBC experiments. In the spectra of both compounds **2** and **3**, we observed that the coupling constant between the two vinylic protons 2^1 and 2^2 (see Scheme 1 for numbering) is of about 16 Hz, typical of an *E*-geometry of the double bond. The resonances of the β-pyrrolic protons are observed as multiplets between ca. 8.70 and 8.90 ppm, and a singlet at a lower field (ca. 9.0 ppm) due to the H-3 proton, which is typical of 2-substituted porphyrins. The spectra of both **2** and **3** showed a sharp singlet at ca. 4.70 ppm, corresponding to the CH_3 protons. The chemical shift values of protons H-4′ and H-5′ of compound **3** were identified by bidimensional spectra since their resonances are overlapped, respectively, with the multiplets of the ortho and meta/para protons of the *meso*-phenyl groups.

2.2. Biological assays

2.2.1. Photostability of the compounds. Porphyrins can undergo photobleaching when exposed to UV–vis light and oxygen.[20] The rate of photodegradation exhibited by a photosensitizer after exposure to light is a very important parameter to assess, because a fast photobleaching would cause the concentration of the drug to decrease, thus impairing the effectiveness of the treatment. Studies concerning the photostability of compounds **2** and **3** were performed by irradiating a solution of each porphyrin (1 μM in PBS, air saturated) under the same conditions used for the biological assays, and considering the UV–vis spectra at different times of irradiation. The results of such studies are shown in Table 1, where the residual intensity of the Soret band of each compound is reported as the percentage of the



Scheme 1.

Table 1. Photostability of the photosensitizers **2** and **3** after irradiation with white light (50 mW/cm^2) at different irradiation times

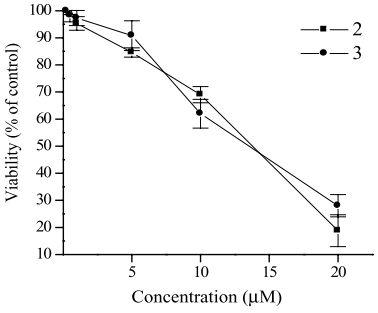
Compound	Irradiation time (total light dose)[a]					
	0 (0)	1 (3)	3 (9)	5 (15)	10 (30)	15 (45)
2	100	100	99	96	93	91
3	100	100	94	93	88	83

[a] The irradiation time and the total light dose are expressed in minutes and joules per square centimetre, respectively. The residual intensity of the Soret band (%) is reported for each compound at a given irradiation time.

initial intensity of the band. Under these conditions, compounds **2** and **3** did not photobleach significantly for the first 15 min of irradiation; such a low rate of photodegradation suggests that the concentration of the photosensitizer would be unaffected by light during the irradiation period required for the photoinactivation of HSV-1.

2.2.2. Photocytotoxicity studies. The photocytotoxicity of compounds **2** and **3** was evaluated on the basis of cell growth inhibition using Vero cells. The cells were incubated for 10 min with different concentrations of photosensitizers **2** and **3** (0–20 μM), and then were irradiated with white light (400–800 nm) for 15 min at a fluence rate of 50 mW/cm^2. After 48 h of incubation, the viability of the cells was determined by the MTT assay.[21] The results of such experiments are reported in Figure 1.

While exhibiting photocytotoxicity under the experimental conditions assayed, both compounds exert no cytotoxic effect at concentrations below 1 μM. When no photoactivation took place, no cytotoxic effect was observed even under the highest concentration assayed (data not shown). Examination by transmission electron microscopy of cells treated with light activated compound **3** at 0.5 μM revealed normal ultrastructural features (Fig. 2) when cells were processed 16 h after photoinactivation.[22] The concentration that renders 50% cellular photocytotoxicity (CC_{50}) was calculated by regression analysis of the dose–response curves generated from the data. The values calculated for both compounds were very similar (13.5 and 13.7 μM for compounds **2** and **3**, respectively).



Conc.(μM)	2 (viability % ± s.d.)	3 (viability % ± s.d.)
0.1	104.5 ± 2.7	104.8 ± 2.3
0.25	99.9 ± 1.3	103.3 ± 4.3
0.5	98.3 ± 2.4	101.0 ± 5.3
1	97.3 ± 2.8	95.3 ± 2.5
5	90.8 ± 5.5	84.6 ± 1.7
10	62.0 ± 5.3	69.0 ± 3.0
20	28.0 ± 4.1	18.8 ± 5.9

Figure 1. Photocytotoxicity profile of compounds **2** and **3** in Vero cells. Each point represents the mean ± standard deviation of three experiments, with two replicates each.

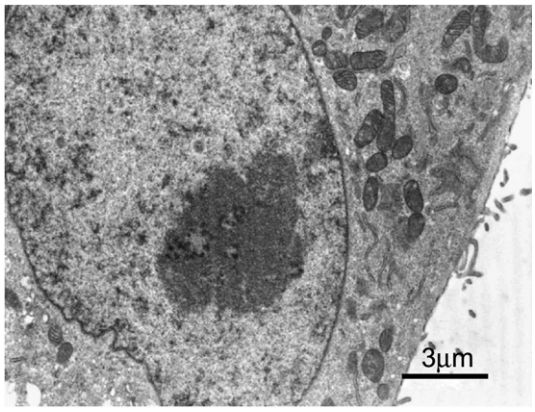


Figure 2. Vero cells treated with compound **3** (0.5 μM) showing normal ultrastructural features 16 h after photoactivation.

2.2.3. Virucidal activity. The virucidal activity tests were performed by exposing HSV-1 suspensions to the highest non-cytotoxic dose previously obtained in the photocytotoxicity tests (0.5 μM). The irradiation time-dependence on virus inactivation was also evaluated. Viral suspensions containing 1×10^7 PFU of HSV-1 were incubated with each compound for 10 min under dark conditions before being irradiated for 5 or 15 min. The controls of these experiments were non-treated viral suspensions either irradiated or non-irradiated and viral suspensions treated but not exposed to light (0 min, in Table 2). Then each sample was subjected to serial dilutions in dark conditions in order to determine its residual infectivity by plaque reduction assay.[23] It was observed that irradiation of viruses in the absence of a photosensitizer did not cause any detectable virus inactivation (data not shown). As shown in Table 2, both compounds exerted inhibitory effect on viral infectivity. However, only compound **3** showed virucidal activity in the absence of light (28%

Table 2. Photovirucidal effect of compounds **2** and **3** on HSV-1

Compound	Irradiation time (min)[a]	Virus inactivation (% of control)[b] ± SD
2	0	0
	5	0
	15	48.99 ± 6.08
3	0	27.80 ± 3.97
	5	96.98 ± 0.39
	15	99.61 ± 0.39

[a] White light, 50 mW/cm^2.
[b] These values represent the mean of three independent experiments.

of viral inactivation). Nevertheless, this activity is strongly enhanced by photoactivation: virus inactivation reaches 97% soon after 5 min of exposure to the light, and 99% when the irradiation time is extended to 15 min. Compound **2** also showed virucidal effect but only when photoactivated, and seems to have a delayed onset, since it is not observed after 5 min of irradiation and after 15 min only half of the virus population was inactivated.

3. Conclusions

Two cationic β-vinyl substituted *meso*-tetraphenylporphyrin derivatives were efficiently synthesized by stereoselective aldol-like condensation of 1,2- or 1,4-dimethylpyridinium iodide with 2-formyl-*meso*-tetraphenylporphyrin. The two compounds displayed similar photocytotoxicity profiles (CC_{50} values 13.5 and 13.7 μM, respectively). However, virus inactivation studies carried out with HSV-1 pointed out a striking difference between them, in spite of their structural resemblance. At a concentration of 0.5 μM, and after 5 min of irradiation, compound **3** was able to photoinactivate 97% of the viral population, while compound **2**, under the same conditions, displayed no virucidal effect. Along with the lack of cellular damage demonstrated by electron microscopy and photocytotoxic tests, these results encourage further studies aiming to elucidate both the mechanism of action and the subcellular target of the compounds.

Acknowledgments

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17. Typical procedure for the synthesis of compound **2**: To a stirred saturated solution of K_2CO_3 (19.8 mg, 0.143 mmol) in THF (3.0 mL) and methanol (1.5 mL) maintained at 30 °C, porphyrin **1** (15.1 mg, 0.0235 mmol) was added. After 15 min, dimethylpyrinium **4** (11.5 mg, 0.0489 mmol) was added to the solution and the resulting mixture was stirred at 30 °C for 1 h. The reaction mixture was allowed to cool to room temperature and the solvents were evaporated. The residue was taken in CH_2Cl_2 and the resulting solution was washed with water and brine. The organic phase was separated, dried (Na_2SO_4) and concentrated. The desired compound was isolated from the crude material by column chromatography (silica gel; eluent: CH_2Cl_2/MeOH = 9:1). The pure compound was washed with a saturated aqueous sodium chloride solution (3×50 mL), dried and crystallized from CH_2Cl_2/hexane (11.2 mg, 65% yield). Porphyrin **1** (2.2 mg, 15%) was also recovered.
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19. Selected data: Compound **2**: mp > 300 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.09 (1H, s, H-3), 9.07 (2H, d, J = 6.4 Hz, H-2′), 8.86–8.83 (3H, m, β-H), 8.79–8.76 (3H, m, β-H), 8.24–8.18 (8H, m, Ph–H_{ortho}), 7.94 (1H, dd, J = 7.5 Hz), 7.86–7.74 (11H, m, Ph–$H_{metalpara}$), 7.54 (2H, d, J = 6.5 Hz, H-3′), 7.49 (1H, d, J = 15.7 Hz, H-2^1), 7.26 (1H, d, J = 15.7 Hz, 2^2), 4.65 (3H, s, CH_3), −2.52 (2H, s, NH). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 159.09, 154.02, 144.68, 144.55, 142.10, 141.99, 141.74, 141.49, 138.42,

134.75, 134.59, 134.54, 129.13, 128.19, 127.96, 127.59, 126.89, 126.83, 123.57, 121.02, 120.65, 119.52, 47.88; UV–vis: ($CHCl_3$), nm (%) 407 (100), 471 (87.2), 529 (21.8), 585 (16.7); HRMS $(FAB)^+$: calcd for $C_{52}H_{38}N_5$ 732.3127. Found: $[M]^{+\cdot}$ 732.3134. Compound **3**: mp > 300 °C; ^1H NMR: (300 MHz, $CDCl_3$) δ: 9.98 (1H, d, J = 10.2 Hz, H-6′), 9.09 (1H, s, H-3), 8.88–8.76 (6H, m, β-H), 8.26–8.18 (10H, m, Ph–H_{ortho} and H-4′), 7.87–7.74 (15H, m, Ph–$H_{meta/para}$ and H-5′), 7.47 (1H, d, J = 15.3 Hz, H-2^1), 7.43 (1H, d, J = 7.0 Hz, H-3′), 7.33 (1H, d, J = 15.6 Hz, H-2^2), 4.67 (3H, s, CH_3), −2.54 (2H, s, NH). ^{13}C NMR (500 MHz, $CDCl_3$) δ: 152.64, 147.85, 143.17, 142.31, 141.61, 141.58, 141.30, 140.89, 134.68, 134.54, 134.45, 133.89, 129.88, 128.52, 128.37, 127.92, 127.42, 126.88, 126.76, 125.07, 123.98, 121.77, 120.74, 120.61, 119.10, 115.43, 108.99, 46.29; UV–vis: ($CHCl_3$), nm (%) 407 (92.2), 445 (100), 529 (27.3), 583 (21.1); HRMS $(FAB)^+$: calcd for $C_{52}H_{38}N_5$ 732.3127. Found: $[M]^{+\cdot}$ 732.3127.

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22. Vero cells were collected for TEM examination 16 h after irradiation during 15 min in the presence or not of compound **3**. Cell monolayers were fixed in situ with 3% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.3. Fixed cells were then pelleted and post-fixed sequentially with 1% osmium tetroxide in the same buffer and 1% uranyl acetate in acetate/acetic acid buffer, pH 5.0. The fixed specimen were dehydrated in ethanol and embedded in an Epon–Araldite mixture. Thin sections were contrasted with 2% aqueous uranyl acetate and lead citrate, observed and photographed.
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