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Synthesis and Antibacterial Study of 10, 15, 20-Triphenyl-5-{4-hydroxy-3-(trimethylammonium)methyl}phenylporphyrin as Models for Combination of Porphyrin and Alkylating Agent

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Abstract—10, 15, 20-Triphenyl-5-{4-hydroxy-3-(trimethylammonium)methyl}phenylporphyrin **4** and its zinc complex **5** have been synthesized and antibacterial activities have been studied for **4** and its derivative. Compound **4** showed stronger inhibition than that of 10, 15, 20-triphenyl-5-{4-hydroxy-3-(dimethylamine)methyl}phenylporphyrin (**2**) and 10, 15, 20-triphenyl-5-{4-methoxy-3-(trimethylammonium)methyl}phenylporphyrin (**6**). It is possible that antibacterial activity of compound **4** involved in photoinducing both *o*-quinone methide intermediate and singlet oxygen formation.

Quinone methide (QMs) chemistry has played an important role in the biological system.[1] As its electrophilic properties, some antibiotic and antitumor drugs were proposed to be involved in quinone methide intermediate mechanism for both alkylation of DNA and amino acids resulting in their toxicological to both normal and cancer cells.[2] One of their derivatives is *o*-QM (*o*-quinone methide) which exists among natural products and it has been found that they are significant in cross-linking two biological useful molecules, for example, nucleic acids and proteins and they were studied for their nucleic acid bases and DNA alkylation by Rokita and other groups.[3]

More recently, Wan's group,[4] Freccero's group (Fig. 1a)[5] and Kresge's group[6] have significantly reported generation of *o*-QMs by photochemical and thermal activation in aqueous solution which could be used in biomolecular applications.

Meanwhile, photodynamic therapy (PDT) has been of long term interest to scientists. It is known that porphyrin as a photosensitizer can localize on tumor cells and phototrigger to produce singlet oxygen to cleave DNA and finally damage the tumor cell.[7]

Among them, cationic porphyrins were found with high photoxicity to tumor cells. The widely known derivative is H_2TMPyP(tetra-4-(*N*-methyl)-pyridyl-porphyrin) which has been considered as a potential PDT candidate because of its very good selectivity to cancer cells and anti-tumor activity by photoexcitation.[8]

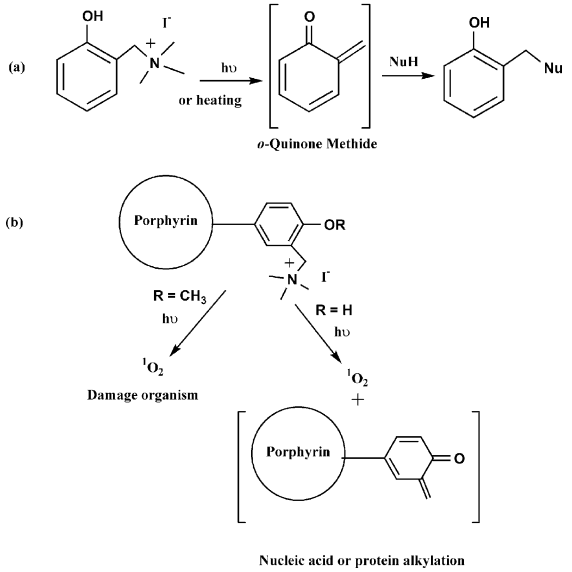

Figure 1. (a) *o*-Quinone methide formation and (b) synthetic strategy.

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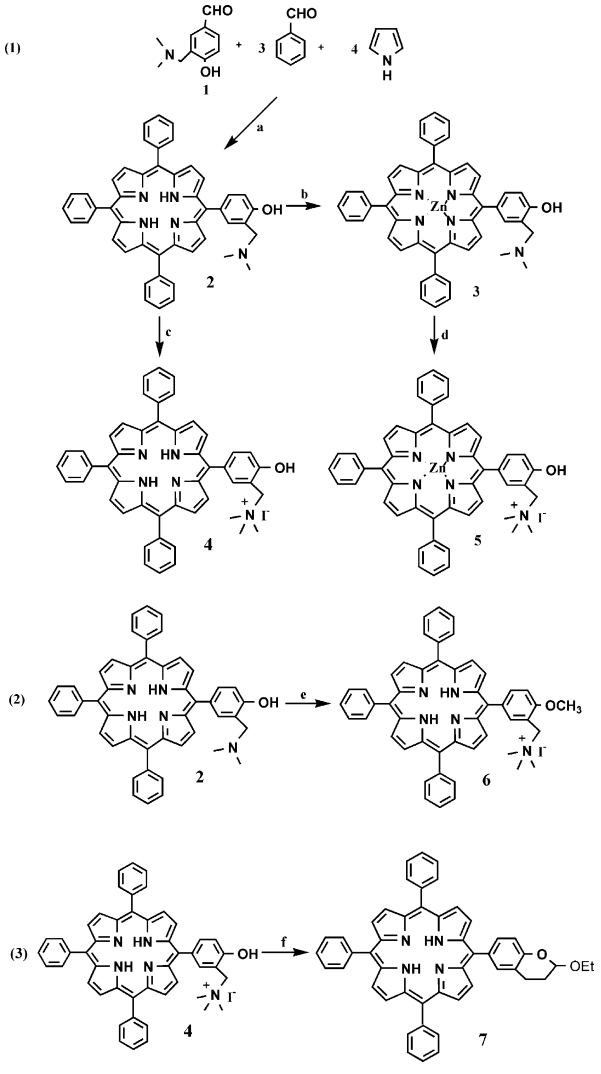
Considering good selectivity and phototoxicity of cationic porphyrins to cancer cells, and also photoinducible for (2-hydroxylbenzyl) trimethyl ammonium to form *o*-QMs which have alkylating function to nucleic acid or protein. We have conjugated both functional units as a new model for combination of porphyrins and alkylation agents (Fig. 1b). We proposed that cation on porphyrin can increase both selective binding affinity to nucleic acid or protein and solubility in aqueous conditions. Furthermore, with photoinducing, *o*-quinone methide intermediate and singlet oxygen could be formed and they may play an important role for both alkylation and oxidative damage to nucleic acids or proteins. Herein, we shall report our preliminary results for this project. The synthetic route is shown in Scheme 1. 4-Formadlehyde-2-[(dimethylamino)methyl]phenol (**1**) was synthesized by modified procedure.[9] After refluxing with **1**, pyrrole and benzenealdehyde in propionic acid, we obtained the desired compound **2** by chromatography purification with silica gel in a solvent of ethanol in 15.2% yield. Metallization was carried out by fluxing with zinc acetate and porphyrin **2** in methanol for 2 h in high yield. For methylation of **2** and **3**, the chosen solvents were very important. CH_2Cl_2 was not a good solvent for their methylation.[10] We finished methylation of porphyrin **2** by methyl iodide in a solvent of acetone.[11] However, we found that acetonitrile and acetone were both good solvents for methylation of **3**. Both methylated porphyrins were purified by successive crystallization with CH_2Cl_2 and a *n*-hexane system in 86.0 and 63.0% yields, respectively. No methylation on inner nitrogen of porphyrin **4** was found when it processed methylation.[12]



Scheme 1. Synthesis of quaternary ammonium porphyrins and their derivatives. (a) Proponic acid, reflux for 0.5 h, (15.2%); (b) $Zn(OAc)_2$, methanol, reflux for 1 h, (87.5%); (c) CH_3I, acetone, rt, 30 min, (86.0%); (d) CH_3I, acetonitrile, rt, 4 h, (63.0%), (e) CH_3I, K_2CO_3, N_2, DMF, overnight, (48.1%), (f) ethyl vinyl ether, DMSO, CH_3CN–H_2O (1:1), hν, (35%).

From proton NMR, multi peaks of pyrroles in porphyrins **2**, **3**, **4** and **5** were found and had more low-shifts in **4** and **5**. Multi peaks in pyrroles of porphyrins were identified when they were functionalized by β-substituting or unsymmetrical formation.[13] For our case, they might be affected by both formation of quaternary ammonium and their unsymmetrical construction. Meanwhile, proton in a hydroxyl group of phenol was found low-shift to 10.93 ppm which also suggested that **4** had strong intermolecular hydrogen bond formation.[14] Proton of phenol disappeared when it was exchanged with deuterium oxide. All the compounds were fully characterized by UV, ^{1}H NMR and HRMS.[15]

Initial testing of their bioactivity, we studied their antimicrobacterial toxicity. Porphyrins **2**, **4** and **6** (dissolved in water–methanol (5%) solution) were tested for their inhibitory activity against *Staphylococcus aureus* (*S. aureus*, ATCC25923). A fresh 3.8×10^7 log phase *S. aureus* was incubated with 0.5 mL different concentrations of porphyrin **2**, **4** and **6** individually. After photoradiation (high pressure mercury lump) for 1 h, each 0.1 mL mixture was spread on 2XYT[19] agar medium. After incubating for 22 h at 37 °C, viable bacterial colonies were counted on each medium plate and results are shown in Table 1 and Figure 2.

When porphyrins are exposed to the light, it is known that oxygen will be excited to form singlet oxygen. In order to clarify their possible mechanism involved in singlet oxygen damage and *o*-quinone methide intermediate, measurement of singlet oxygen production by measuring the decomposition of 1, 3-diphenyliso benzofuran (DPBF)[16] for these porphyrins has been carried out and results were shown in Figure 3. The slopes of the plots of bleached absorption of DPBF versus illumination time were shown in Table 2. The slope of the plot of bleached absorption versus illumination time is proportional to the rate of production of singlet oxygen.[16]

Table 1. Minimium inhibitory concentrations (MIC) in μM for porphyrins **2**, **4** and **6**

Compd	MIC (μM)
2	120
4	60
6	240

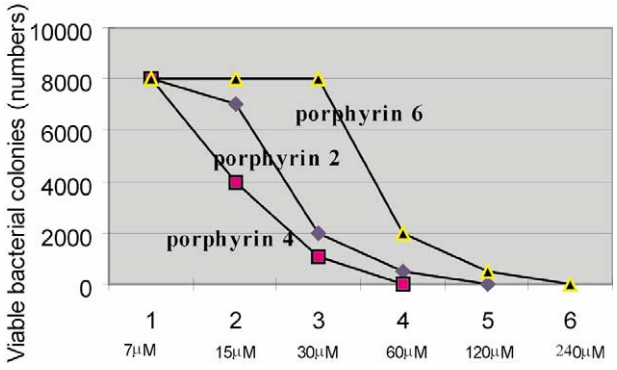


Figure 2. Comparison of antimicrobial ability between porphyrin **2**, **4** and **6**.

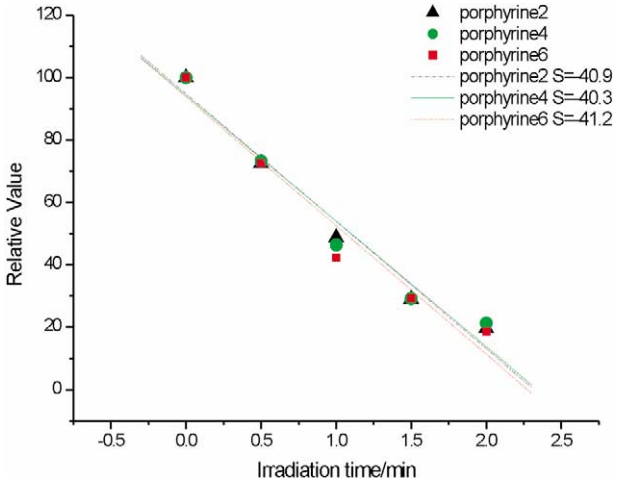


Figure 3. Decomposition of DPBF by compounds **2**, **4** and **6**. Porphyrine (1.0×10^{-6} M) and DPBF(1.0×10^{-4} M) were irradiated in pyridine.

Table 2. The slopes (**S**) of the plots of bleached absorption of DPBF by photosensition of **2**, **4** and **6**

	2	**4**	**6**
S	40.9	40.3	41.2

Their singlet oxygen production was almost the same. According to this result, they should have equal ability to kill bacterials. However, our bioactivity result show that **4** has a stronger antibacterial activity than that of the others. Furthermore, we did another experiment for quenching singlet oxygen by DMSO.[17] When **4** and **6** were mixed with bacterials in the presence of DMSO (100 mM), after illuminating, we found that **4** could still inhibit growth of bacterials. Whereas, no bacterials were killed at all by **6** after illumination. For control experiments, no *S. aureus* was killed when mixed with only methanol (5%) or lighting or DMSO (100 mM) or porphyrins without lighting.

To confirm if **5** was involved in *o*-quinone methide intermediate, we trapped it by ethyl vinyl ether[4,18] and obtained the Diels–Alder adduct **7** in CH_3CN–H_2O (1:1) solution (Fig. 1, eq 3) in 35% yield upon illumination. Meanwhile, DMSO was added to the reaction mixture as a singlet oxygen quencher.

Porphyrin **4** showed a stronger antibacterial activity than that of porphyrins **2** and **4**. Presumably, porphyrin **4** was photochemically activated and induced to form both singlet oxygen and *o*-quonine methide intermediate and these led to both oxidative damage and alkylation of the organism in the bacterial. Meanwhile, only singlet oxygen was formed for activation of porphyrins **2** and **6**. Since the hydroxyl group of compound **6** has been protected by the methyl group [Scheme 1 (2)] and no quaternary ammonium of compound **2** existed, compounds **2** and **6** were impossible to form *o*-quinone methide intermediate when they were photoactivated. Therefore, they showed less toxicity effect to bacterial comparing with **4**. These results suggested that introducing the alkylating group to porphyrin does increase antibacterial activity. Further constructing porphyrins to DNA or protein as cross-linking agents and detailed mechanism are undergoing.

In conclusion, we have synthesized some quaternary ammonium porphyrins which could be photoinduced to produce either singlet oxygen or both singlet oxygen and *o*-quinone methide intermediate to damage organisms. Compared with porphyrins **2** and **6**, **4** showed a stronger photoinduced toxicity to bacterial because of its pending alkylation function group.

Acknowledgements

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15. **2**: ^{1}H NMR (δ $CDCl_3$, 300 MHz), 8.80 (m, 8H), 8.16 (m, 6H), 8.02 (d, 1H, $J=7.0$ Hz), 7.75 (m, 11H), 7.21 (d, 1H, $J=8.4$ Hz), 3.86 (s, 2H), 2.48 (s, 6H), −2.84 (bs, 2H). ^{13}C NMR (δ $CDCl_3$, 75 MHz) 142.4, 135.5, 135.3, 134.7, 133.2, 131.2, 127.9, 126.8, 120.5, 120.3, 120.2, 115.0, 44.76, 62.6; UV–vis (CH_3OH) λ_{max} (nm, log ε), 414 (5.37), 513 (4.04), 549 (3.77), 591 (3.55), 648 (3.61); FAB MS [$M^+ + H$] *m*/*z* 689; HRMS (ESI, *m*/*z*): calcd for $C_{47}H_{38}N_5O[M^+ + H]$ 688.3071, found 688.3068. **4**: ^{1}H NMR (δ [$^2H_6$]DMSO, 300 MHz) 10.93 (s, 1H), 8.98 (m, 2H), 8.81 (bs, 6H), 8.20 (bs, 8H), 7.83 (bs, 9H), 7.35 (d, 1H, $J=8.7$ Hz), 4.71 (s, 2H), 3.22 (s, 9H), −2.93 (s, 2H); ^{13}C NMR (δ [$^2H_6$]DMSO, 75 MHz) 142.4, 135.5, 135.3, 134.7, 133.2, 131.2, 127.9, 126.8, 120.5, 120.3, 120.2, 115.0, 44.76, 62.6; UV–vis (CH_3OH) λ_{max} (nm, log ε), 414 (5.89), 514 (4.26), 548 (3.95), 588 (3.75), 647 (3.68); HRMS (ESI, *m*/*z*): calcd for $C_{48}H_{40}N_5O[M^+ - I]$ 702.3227, found 702.3217.
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