'Porphyrin–Phenothiazine' Hybrid Molecules: Marked Dependence of Light Induced Nuclease Activity on the Linker Moiety

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Porphyrin–phenothiazine hybrid molecules have been synthesized for the first time and their light induced DNA cleavage activity, as a function of linker group, has been evaluated.

There is currently world-wide interest in photodynamic therapy (PDT), a newly introduced modality for cancer treatment in which a photosensitizing drug acts against malignant tumours under the influence of light.[1] Research in this area has mainly centred around the porphyrin nucleus, as the core photosensitizing moiety and much of the present efforts are directed towards crafting new porphyrin-based molecular entities to achieve enhanced tumour localization, better tissue penetration and increased singlet oxygen quantum yield.[1,2] As the precise mechanism of PDT in cancer treatment remains elusive, there is considerable scope and opportunity to explore new structural variations and combinations to amplify the therapeutic efficacy of the porphyrin based sensitizers. We have recently shown that hybrid molecules composed of a porphyrin and an intracellular recognition element, *e.g.* an intercalator or a DNA-crosslinking agent, exhibit enhanced light induced nuclease activity, whilst retaining the photophysical attributes of the porphyrin moiety.[3] As part of the ongoing efforts in the area, we have now synthesized several new hybrid molecules employing 5-(4-hydroxyphenyl)-10,15,20-tri(*p*-tolyl)porphyrin **1**[2c,4] and phenothiazine **2a**, linked through various spacers and studied their light induced DNA cleavage activity. The choice of the phenothiazine ring system, as the partner for the porphyrin in assembling the new hybrid molecules, followed from the broad spectrum biological activity associated with compound **2** and its many derivatives and their known photosensitizing capabilities.[5] Indeed, phenothiazine derivatives, like Methylene Blue, are being scrutinized[1a] in their own right as PDT agents and we felt that a union of compounds **1** and **2a** through a suitable spacer might produce an additive effect.

Readily available ω-bromoalkylated *N*-phenothiazine derivatives **3a–d** appeared well poised for linkage to the porphyrin **1**. Consequently, base catalysed displacement of the bromine in compounds **3a–d** by the phenoxy group of the porphyrin **1** smoothly furnished the desired hybrid molecules **4a–d**, respectively, and were characterized on the basis of their UV–VIS, ^{1}H NMR data and elemental analyses (Scheme 1). Additionally, we ventured to incorporate a piperazine moiety in the linker chain between compounds **1** and **2** as piperazine derivatives of compound **2** are known to exhibit enhanced biological activity.[5,6] Also, the presence of the piperazine moiety offers an opportunity to prepare quaternary salts to induce hydrophilicity and polar interactions at cellular level. Thus, the ω-bromoalkoxyporphyrins **5a–c**[3a] were reacted with the piperazino–phenothiazine **6**[7] to furnish the novel derivatives **7a–c**, Scheme 2. Stirring a solution of compounds **7a–c** with excess methyl iodide led to the precipitation of the quaternary salts formulated as **8a–c** on the basis of their FAB–MS and ^{1}H NMR data.

The UV–VIS and ^{1}H NMR data for compounds **4a–d**, **7a–c** and **8a–c**, showed that, despite a long linker chain, there is minimal ground state interaction between the porphyrin and phenothiazine π-rings and this surmise is further supported by their redox potentials. The fluorescence and singlet oxygen quantum yields of these porphyrin–phenothiazine ensembles are in the same range as those of compound **1**, see Table 1. Thus, neither the appended phenothiazine sub-unit nor the intervening spacer moieties have any adverse effect on the photosensitizing abilities of the porphyrin.

The DNA cleavage reactions of compounds **4a–d**, **7a–c** and **8a–c** were studied using the supercoiled plasmid DNA pBR 322. While control experiments in the absence of light showed no discernible nicking, on irradiation by visible light, compounds **4a–d** relaxed the supercoiled form I to II with compound **4a** exhibiting the highest efficiency, Fig. 1(A). Small amounts of form III (relaxed linear) could also be observed on the gel. However, compounds **7a–c**, unlike compounds **4a–d**, quite unexpectedly displayed very little photocleavage activity, Fig. 1(B). Interestingly, the cationic species **8a–c** under identical conditions showed light induced DNAase activity, possibly due to their hydrophilic nature and additional involvement of Coulombic interactions with the phosphate backbone.

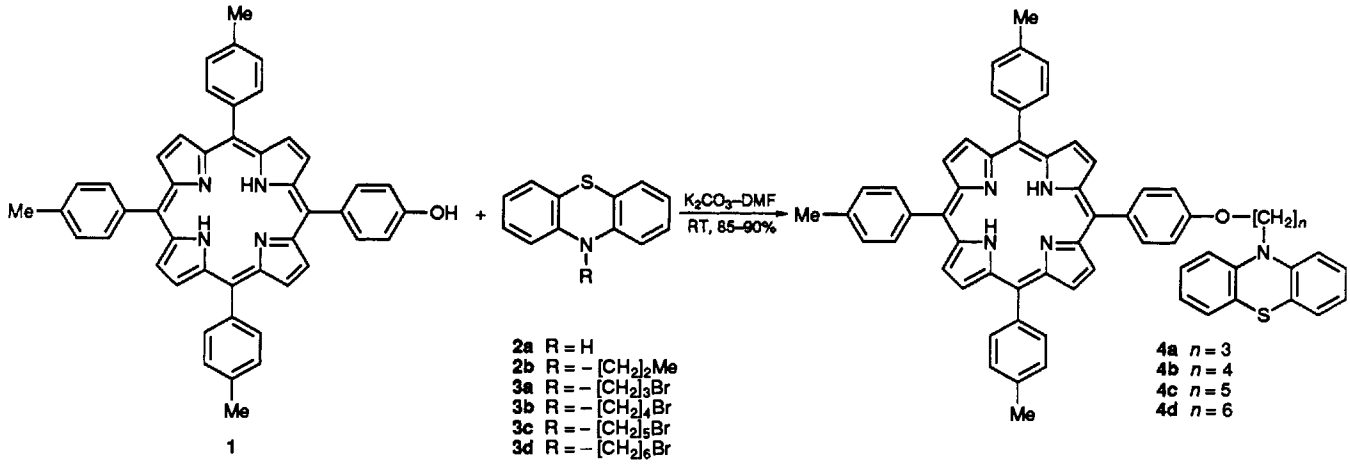
In summary, our findings indicate that while photophysical characteristics in the diverse porphyrin-phenothiazine hybrid molecules remain intact, the nuclease activity is markedly influenced by the nature of the linker moiety.

Experimental

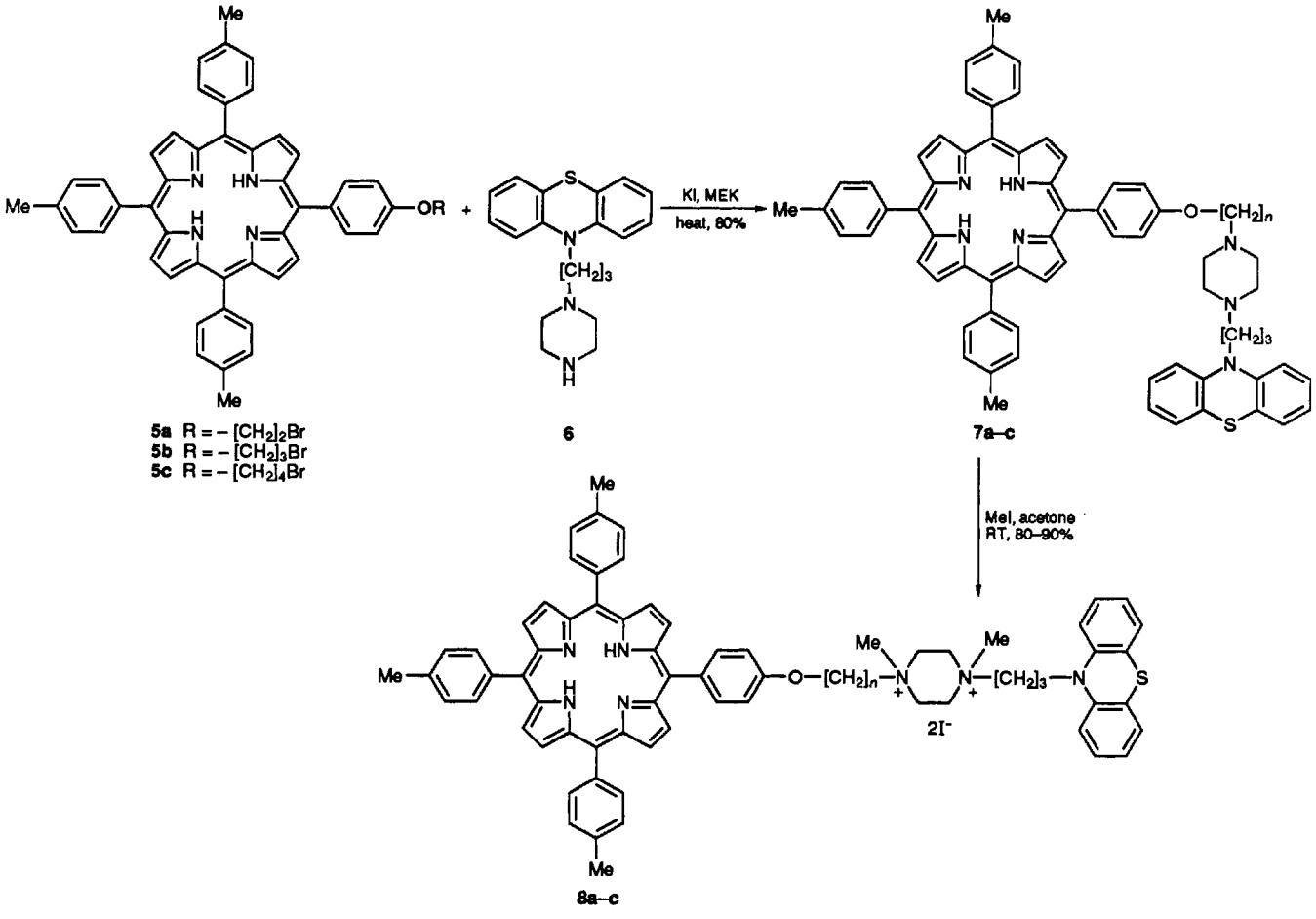
General Procedure for the Preparation of the 'Porphyrin–Phenothiazine' Hybrid Molecules **4a–d** *and* **7a–c**.—A mixture of phenothiazine **2a** (1 g, 5 mmol) and powdered KOH (0.29 g, 5 mmol) in dry DMF (10 cm^3) was stirred under N_2 for 30 min. The requisite α,ω-dibromoalkane (15 mmol) was introduced and the mixture was stirred for 48 h at room temp. The mixture was then poured into water (50 cm^3) and extracted with CH_2Cl_2. After removal of the solvent, the residue was purified by chromatography on silica gel (hexane–ethyl acetate, 2%) to furnish the *N*-alkylated phenothiazines **3a–d** in good yield (60–80%).

A mixture of the porphyrin **1** (0.1 mmol), the alkylated phenothiazine **3a–d** (0.15 mmol) and anhydrous K_2CO_3 (150 mg) in dry DMF (5 cm^3) was stirred under N_2 for 24 h. The mixture was then poured into water (75 cm^3) and filtered. The solid was chromatographed on a silica gel column ($CHCl_3$) to furnish the coupled products **4a–d** in high yield.

A mixture of the bromides **5a–c** (0.076 mmol), the piperazine **6** (0.092 mmol) and KI (100 mg) in methyl ethyl ketone (20 cm^3) was refluxed for 8 h under N_2. The solvent was removed under reduced pressure and the residue was purified on a silica gel column ($CHCl_3$–MeOH, 2%). Recrystallization from CH_2Cl_2–



Scheme 1



Scheme 2

MeOH furnished the coupled products **7a–c** in excellent yield.

All new compounds were characterized on the basis of complementary spectral data and elemental analyses. Selected data are given here. δ_H(200 MHz; $CDCl_3$) **4a**: −2.75 (2 H, s), 2.45 (2 H, q), 2.71 (9 H, s), 4.27 (2 H, t, *J* 6.2), 4.38 (2 H, t, *J* 6.3), 6.97–7.07 (4 H, m), 7.18–7.25 (6 H, m), 7.56 (6 H, d, *J* 7.7), 8.10 (8 H, d, *J* 8.0) and 8.86 (8 H, s); **4b**: −2.74 (2 H, s), 2.13 (4 H, m), 2.70 (9 H, s), 4.07 (2 H, t, *J* 6.2), 4.25 (2 H, t, *J* 6.3), 6.95–6.99 (4 H, m), 7.17–7.25 (6 H, m), 7.55 (6 H, d, *J* 8.0), 8.1 (8 H, d, *J* 1.8) and 8.86 (8 H, s); **4c**: −2.74 (2 H, s), 1.80 (2 H, m), 2.00 (4 H, m), 2.71 (9 H, s), 3.98 (2 H, t, *J* 6.8), 4.19 (2 H, t, *J* 6.2), 6.93–7.00 (4 H, m), 7.16–7.23 (6 H, m), 7.55 (6 H, d, *J* 7.8), 8.1 (8 H, d, *J* 8.0) and 8.87 (8 H, s); **4d**: −2.76 (2 H, s), 1.65 (4 H, m), 1.94 (4 H, m), 2.72 (9 H, s), 3.95 (2 H, t, *J* 6.8), 4.2 (2 H, t, *J* 6.3), 6.86–6.90 (4 H, m), 7.12–7.22 (6 H, m), 7.53 (6 H, d, *J* 7.4), 8.08 (8 H, d, *J* 7.5) and 8.85 (8 H, s); **7a**: −2.75 (2 H, s), 2.03 (2 H, q), 2.62 (10 H, m), 2.72 (9 H, s), 3.00 (2 H, t, *J* 6.2), 3.97 (2 H, t, *J* 6.1), 4.39 (2 H, t, *J* 6.3), 6.91–6.96 (4 H, m), 7.16 (4 H, m), 7.28 (2 H, d, *J* 5.7), 7.56 (6 H, d, *J* 7.1), 8.10 (8 H, d, *J* 7.5) and 8.86 (8 H, s); **7b**: −2.75 (2 H, s), 2.01 (2 H, m), 2.17 (2 H, m), 2.57 (12 H, m), 2.71

Table 1 Fluorescence quantum yield (Φ_f) and singlet oxygen quantum yield [$\Phi(^1O_2)$] data for the porphyrin–phenothiazine hybrids

Compound	**1**	**2b**	**4a**	**4b**	**4c**	**4d**	**6**	**7a**	**7b**	**7c**	**8a**	**8b**	**8c**
$\Phi_f(10^1)^a$	1.1	—	1.0	1.3	1.3	1.2	—	1.3	1.2	1.1	1.3	1.3	1.3
$\Phi(^1O_2)(10^1)^b$	6.5	3.0	6.6	6.4	7.7	7.6	1.3	4.1	6.7	6.0	7.1	8.0	7.9

[a] Fluorescence quantum yield obtained when the compound is excited into an exclusive porphyrin absorption band (420 nm, CH_2Cl_2); error limits ± 10%. [b] Measured in DMF by the steady state photolysis method using diphenyl isobenzofuran as the singlet oxygen scavenger. All the samples were irradiated at 555 nm using a 150 W Xe arc lamp; error limits, ± 15%.

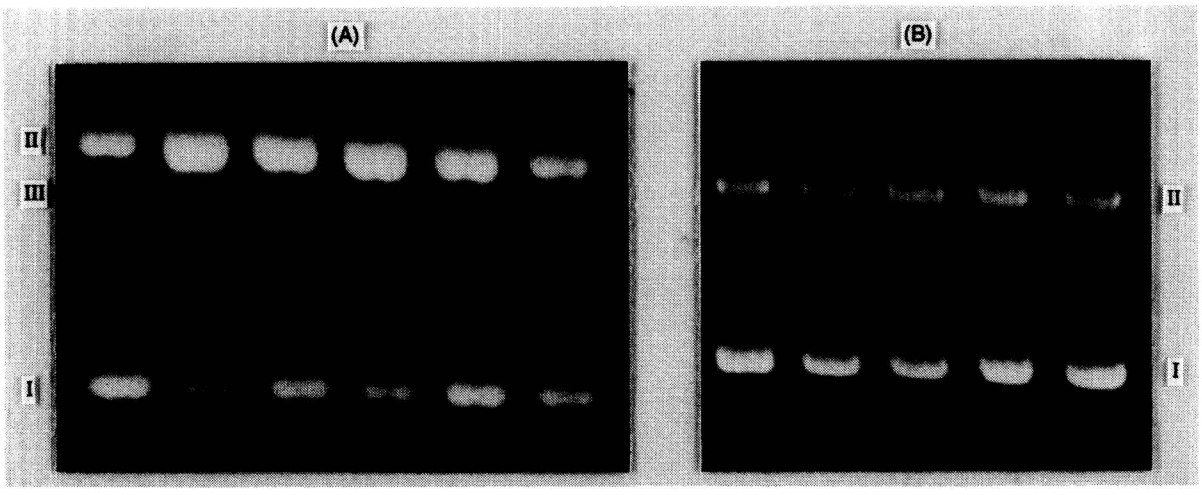


Fig. 1 Light induced nuclease activity of the porphyrin–phenothiazine hybrids with (%) relaxation of form II as measured using UVP gel documentation system GDS 2000: (A) (left to right) Lane 1: Untreated pBR 322 (39); Lanes 2, 3, 4, 5 and 6: pBR 322 + **4a** (82), **4b** (59), **4c** (70), **4d** (49) and **1** (47), respectively. (B) Lane 1: Untreated pBR 322 (22); Lanes 2, 3, 4 and 5: pBR 322 + **7a** (22), **7b** (36), **7c** (29) and **6** (27), respectively. In each case (DNA)/(Drug) = 1 and samples were incubated for 1 h before being irradiated with visible light ($\lambda > 400$ nm) for 2.5 h. Electrophoresis experiments and analysis of the data were carried out as described in ref. 3(*b*). The commercial pBR 322 plasmid DNA used in our experiments contained 32% (A) and 22% (B) of form II, respectively.

(9 H, s), 3.96 (2 H, t, *J* 6.7), 4.3 (2 H, t, *J* 6.3), 6.92–6.95 (4 H, m), 7.15 (4 H, m), 7.28 (2 H, d, *J* 5.6), 7.56 (6 H, d, *J* 8.0), 8.1 (8 H, d, *J* 7.7) and 8.86 (8 H, s); **7c**: −2.74 (2 H, s), 1.85 (2 H, m), 1.99 (4 H, m), 2.53 (12 H, m), 2.72 (9 H, s), 3.94 (2 H, t, *J* 6.6), 4.27 (2 H, t, *J* 6.0), 6.91–6.95 (4 H, m), 7.15 (4 H, m), 7.27 (2 H, d, *J* 5.6), 7.56 (6 H, d, *J* 8.0), 8.11 (8 H, d, *J* 7.7) and 8.87 (8 H, s).

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