Pergamon

Tetrahedron Letters, Vol. 35, No. 24, pp. 4201-4204, 1994
Elsevier Science Ltd
Printed in Great Britain
0040-4039/94 $7.00+0.00

0040-4039(94)E0745-J

Porphyrin–Chlorambucil Conjugates: Synthesis and Light-Induced Nuclease Activity

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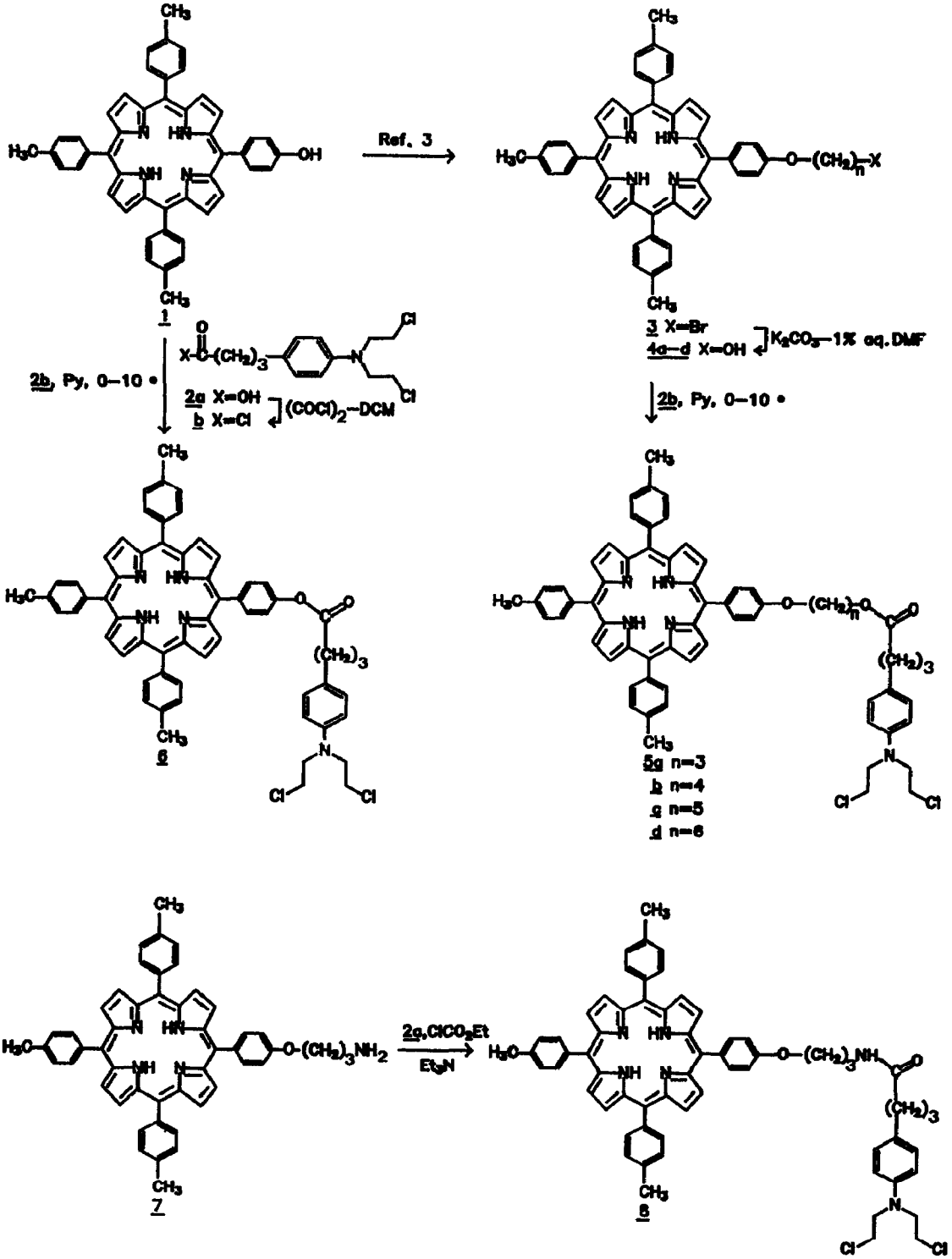
Abstract: Several porphyrin-chlorambucil hybrid molecules have been synthesized and their light induced DNA cleavage activity studied.

Photodynamic therapy (PDT), mediated through a combination of light with a photosensitizing drug, is emerging as a promising alternative to the conventional radio- and chemotherapy in the treatment of malignant tumors.[1] In this context, porphyrins as photosensitizers have acquired a pivotal role and world-wide efforts are underway to modify them to achieve good tumor selectivity, better tissue penetration, increased singlet oxygen quantum yield, etc. and enhance their therapeutic efficacy as PDT agents.[1] Among various modifications, an interesting variation that has been attempted is to combine porphyrin sensitizers with an intracellular recognition element e.g., an intercalator moiety to impart 'dual action' capabilities.[2] In a recent study, we have shown that porphyrin-acridone (intercalator) hybrid molecules exhibit light induced nuclease activity.[3] As an amplification of this dual action theme, we thought of synthesizing new hybrid molecules in which a porphyrin is linked to a clinically used anti-cancer drug chlorambucil, which is known as a DNA cross-linking agent, and is used in the treatment of lymphomas and ovarian carcinoma. We reasoned that such conjugates could function both as a conventional chemotherapeutic agent as well as light-switched PDT agent, thereby enhancing the overall efficacy of the drug. Herein, we describe the synthesis of several hybrid molecules employing 5-(4-hydroxyphenyl)-10,15,20-tris(p-tolyl)porphyrin 1 and chlorambucil 2, joined through flexible $-(CH_2)_n-$ linkers and report on their light induced DNA cleavage abilities.

Previously described ω-bromoalkoxy derivatives 3a-d,[3] obtained from 1 on hydrolysis in K_2CO_3-moist DMF milieu furnished the ω-hydroxyalkoxy porphyrins 4a-d in 70-80% yield, Scheme. Reaction of 4a-d with the acid chloride 2b of chlorambucil 2a in the presence of pyridine readily furnished the hybrid molecules 5a-d in 60-70% yield.[4] Similarly, 6[4] was synthesized from 1 and 2b in good yield with a direct link between the porphyrin 1 and chlorambucil 2a. A hybrid molecule 8[4] having an amide linkage instead of the ester moiety, was synthesized from the ω-aminoalkoxy derivative 7[2c] and chlorambucil via the mixed anhydride method. All porphyrin drug conjugates were characterized through their UV-Vis., ^1H NMR data and elemental analyses.

The fluorescence and singlet oxygen quantum yields of 5a-d, 6 and 8 are presented in the Table. The Φ_f and the $\Phi(^1O_2)$ values of all these compounds are in the same range as that of 1. This implies that there exists no additional pathway(s) for the decay of the porphyrin excited states apart from the usual IC and ISC. In principle,


Ref. 3
1
3 X=Br
4a–d X=OH
K2CO3–1% aq. DMF
2b, Py, 0–10 °
2a X=OH
b X=Cl
(COCl)2–DCM
2b, Py, 0–10 °
6
5a n=3
b n=4
c n=5
d n=6
7
2a, ClCO2Et
Et3N
8

porphyrin excited states can be quenched via electron transfer from the amine functionality of the chlorambucil sub-unit but apparently this does not happen in the present case. There are no major changes observed in either the absorption or electrochemical redox properties of 5a-d, 6 and 8 compared to the precursors 1 and 2a. The foregoing data indicates that porphyrin and chlorambucil moieties in the hybrid molecules retain their intrinsic characteristics that could be exploited in a complementary manner.

Table[a]

Compound	Φ_f[(b)]	$\Phi(^1O_2)$[(c)]
6	0.14	0.65
5a	0.12	0.70
5b	0.12	0.57
5c	0.14	0.64
5d	0.13	0.62
8	0.15	0.57

(a) Error limits for both Φ_f and $\Phi(^1O_2)$ are ± 10%. (b) Excitation wavelength is 420 nm (solvent CH_2Cl_2). (c) Measured in DMF by the steady-state photolysis method using 1,3-diphenylisobenzofuran (DPBF) as the $^1O_2$ acceptor.[3] All samples were irradiated at 555 nm using a 150 W Xe arc lamp as the light source.

The nuclease activity of 5a-d, 6 and 8 was studied using the supercoiled plasmid DNA pBR 322. While no noticeable nicking was observed in the absence of light, on irradiation by visible light the supercoiled form I was relaxed (40-70%)[5] to form II under the experimental conditions employed (Fig.1a, Lanes 2-7).[6] There was no obvious trend in the nicking efficiency as a function of the linker group. The model porphyrins 1, 4a & 7 (Fig.1b) under similar conditions caused ≈20% relaxation.[5] Chlorambucil 2a alone also effects such minor relaxation with or without light (Fig. 1a, Lane 8). The results indicate that combination of a chemotherapeutic drug with a photosensitizer enhances DNA cleavage proclivity under the influence of light. We propose to study the response of these new hybrid molecules at cellular level against human cancer cell lines.

Acknowledgments: TS and MS thank CSIR for the award of fellowships. This research has been supported by Indo-French Centre for the Promotion of Advanced Research/Centre Franco-Indien Pour la Promotion de la Recherche Avancée, New Delhi.

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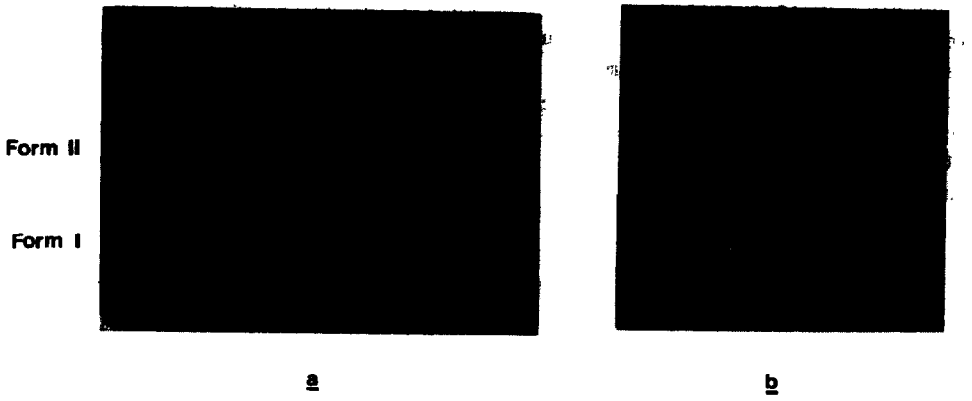

Fig.1a (From left to right) Lane 1: Untreated pBR 322; Lanes 2,3,4,5,6 & 7: pBR 322 + 6, 5a, 5b, 5c, 5d & 8, respectively. Lane 8: pBR 322 + 2a. Lane 9: DNA mol. wt. marker. Fig.1b (From left to right) Lane 1: Untreated pBR 322; Lanes 2,3 & 4: pBR 322 + 1, 7 & 4a.

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4. ^{1}H NMR (200 MHz, $CDCl_3$). 5a: δ -2.77 (2H, s), 1.92 (2H, q), 2.31 (2H, q), 2.42 (2H, t), 2.61 (2H, t), 2.7 (9H, s), 3.55 (8H, m), 4.34 (2H, t), 4.44 (2H, t), 6.59 (2H, d), 7.1 (2H, d), 7.26 (2H, d), 7.55 (6H, d), 8.1 (8H, d), 8.85 (8H, s). 5b: δ -2.78 (2H, s), 1.97 (6H, m), 2.37 (2H, t), 2.59 (2H, t), 2.7 (9H, s), 3.59 (8H, m), 4.27 (4H, t), 6.6 (2H, d), 7.1 (2H, d), 7.26 (2H, d), 7.54 (6H, d), 8.1 (8H, d), 8.85 (8H, s). 5c: δ -2.77 (2H, s), 1.76 (2H, m), 1.99 (6H, m), 2.38 (2H, t), 2.59 (2H, t), 2.7 (9H, s), 3.59 (8H, m), 4.2 (2H, t), 4.25 (2H, t), 6.6 (2H, d), 7.08 (2H, d), 7.25 (2H, d), 7.55 (6H, d), 8.1 (8H, d), 8.85 (8H, s). 5d: δ -2.75 (2H, s), 1.69 (4H, m), 1.95 (6H, m), 2.36 (2H, t), 2.58 (2H, t), 2.7 (9H, s), 3.61 (8H, m), 4.16 (2H, t), 4.23 (2H, t), 6.6 (2H, d), 7.07 (2H, d), 7.25 (2H, d), 7.54 (6H, d), 8.1 (8H, d), 8.85 (8H, s). 6: δ -2.78 (2H, s), 2.16 (2H, q), 2.61-2.81 (4H, m), 2.7 (9H, s), 3.67 (8H, m), 6.65 (2H, d), 7.14 (2H, d), 7.38 (2H, d), 7.54 (6H, d), 8.1 (6H, d), 8.13 (2H, d), 8.85 (8H, s). 8: δ -2.76 (2H, s), 1.99 (2H, q), 2.17 (2H, t), 2.26 (2H, q), 2.65 (2H, t), 2.7 (9H, s), 3.49 (8H, m), 3.63 (2H, m), 4.31 (2H, t), 6.57 (2H, d), 7.1 (2H, d), 7.22 (2H, d), 7.55 (6H, d), 8.1 (8H, d), 8.85 (8H, s).

5. Quantification of DNA cleavage was done using UVP gel documentation system GDS 2000.

6. pBR 322 DNA (0.1 mM) in tris-HCl buffer (pH 8) was treated with an equimolar concentration of the porphyrin dissolved in the same buffer containing 10% (V/V) DMF.[2c] This mixture was incubated for 1h at 25°C and then was irradiated (1h) with visible light (λ> 400 nm) by keeping the sample at a distance of 1m. from a 150 W Xe-arc lamp (UV-light was cut off using filters). The samples were analyzed by 0.8% agarose gel electrophoresis (tris-acetate buffer, pH 8) at a constant voltage. The gel was photographed after staining with ethidium bromide.

(*Received in UK* 2 *February* 1994; *revised* 12 *April* 1994; *accepted* 15 *April* 1994)