Pergamon

Tetrahedron Letters, Vol. 38, No. 40, pp. 7125-7128, 1997

0040-4039/97 $17.00 + 0.00

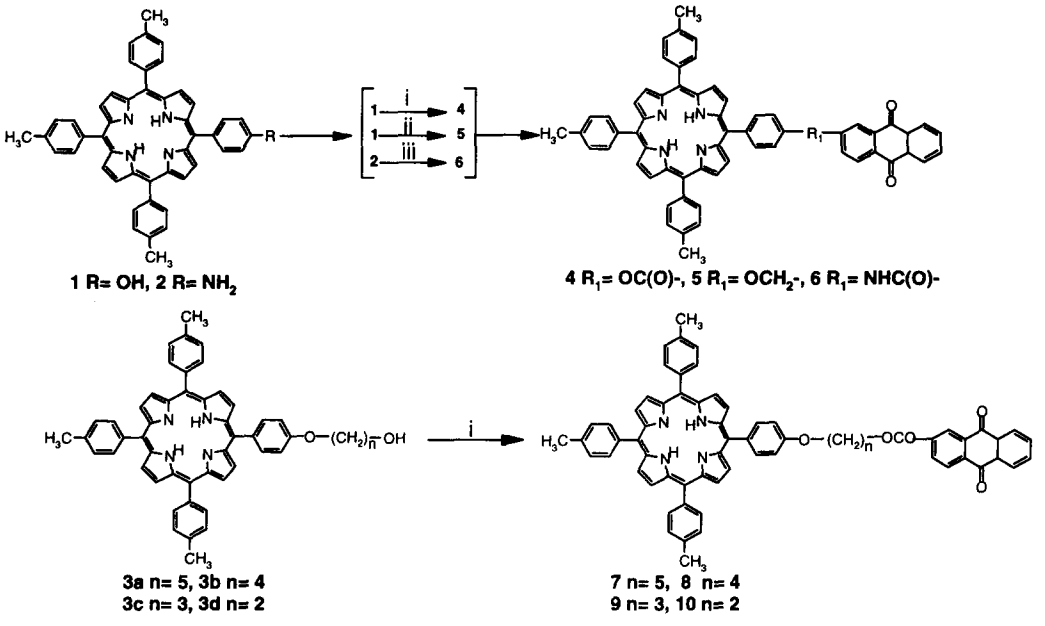
PII: S0040-4039(97)01637-7

Porphyrin-Anthraquinone Hybrids: Wavelength Dependent DNA Photonucleases

Goverdhan Mehta,* Sengodagounder Muthusamy, Bhaskar G. Maiya* and S. Arounaguiri
School of Chemistry, University of Hyderabad, Hyderabad 500 046, India

Abstract: Covalently-linked, bichromophoric systems comprising of a porphyrin donor and an anthraquinone acceptor are shown to exhibit wavelength dependent photonuclease activity. © 1997 Elsevier Science Ltd.

Photodynamic therapy (PDT), in which light activates a photosensitizing drug and elicits the $^1O_2$-mediated cytotoxic action, has recently emerged as a promising modality against cancer and allied diseases.[1] Currently, hematoporphyrin derivative (or its commercial variant Photofrin II®) is the most widely used photosensitizer in PDT. However, application of this drug is known to cause undesirable, post-treatment phototoxic response, probably due to the non-specific subcellular level activity during its photodynamic action.[2] One possible way to circumvent this problem involves the use of targeted drug-delivery approach. We, and also others, have reported the synthesis of photoactive porphyrins which bind selectively to DNA owing to their linkage to either an intercalator[3] (e.g. acridone, phenothiazine), a minor groove binder[4] (e.g. ellipticine) or a cross-linking agent[5] (e.g. chlorambucil). During these studies, it occurred to us that utilization of a photoactive, intercalatable moiety in conjunction with the porphyrin chromophore might accentuate the photochemical activity of the so-derived 'hybrid' molecules leading to an efficient DNA cleavage. Among the various non-porphyrinic chromophores that can be linked to the porphyrin in such new hybrids, anthraquinone seemed to be an ideal candidate because this ubiquitous electron acceptor has been recently established to be an avid binder- and also an efficient photonicking agent of DNA.[6] Thus, conjugates derived from a union of a porphyrin and an anthraquinone subunit are expected not only to absorb light all through in the visible region but also initiate photocleavage of DNA by $^1O_2$ (porphyrin)[1] as well as electron transfer/H-abstraction (anthraquinone)[6] mechanisms. Moreover, the prospect of generation of the anthraquinone anion radical triggered by the visible light irradiation and the subsequent photoinduced electron transfer (PET) reaction from the photoexcited porphyrin donor to the appended anthraquinone acceptor in these hybrids, as previously reported for the covalently-linked, biomimetic porphyrin-quinone donor-acceptor systems[7], can be considered as an attractive attribute of this approach.



Scheme1: (i) AnQ2-COOH, CH_2Cl_2, DCC/DMAP (ii) AnQ2-Br, DMF/ K_2CO_3 (iii) AnQ2-COCl, CH_2Cl_2/py

From a synthetic point of view, porphyrin derivatives **1-3** appeared to be well suited for linkage to the anthraquinone moiety. Thus, compounds **4-10** in which the porphyrin and the anthraquinone subunits are separated by ether, ester and amide linkages as well as flexible -$(CH_2)_n$- spacer moiety were synthesized as outlined in Scheme 1. Reaction of the readily available 2-bromomethyl anthraquinone (AnQ2-Br) with 5-(4-hydroxyphenyl) 10,15,20-tris (4-methylphenyl) porphyrin (**1**)[3] in the K_2CO_3/DMF milieu (stirring, 25 °C, 15 h) furnished **5** in 87% yield. Anthraquinone 2-carboxylic acid (AnQ2-COOH) was esterified with ω-bromoalkoxy derivatives **3a-e**[3] employing classical 1,3-dicyclohexylcarbodimide (DCC)-4-dimethylamino-pyridine (DMAP) procedure (0 °C, dry CH_2Cl_2) to yield hybrids **7-10** (80 -90%).[5] Esterification of AnQ2-COOH under the same DCC/DMAP conditions with porphyrin derivative **1** gave **4** in 85% yield. Finally, reaction of 5-(4-aminophenyl) 10,15,20-tris (4-methylphenyl) porphyrin (**2**) with anthraquinone 2-carbonyl chloride (AnQ2-COCl) provided the amide **6** (53%). Each new hybrid porphyrin synthesized in this study was extensively purified by column chromatography (silica gel, ethyl acetate/hexanes, 3:1, V/V) and characterized by UV-VIS, ^{1}H NMR, IR and mass (FAB) spectroscopic methods.[8]

The UV-VIS and the redox potential data of these new porphyrin-anthraquinone diads were found to be in the same range as those of the corresponding reference compounds **1-3** as well as AnQ2-COOH or AnQ2-Br. However, their fluorescence (Φ_f) and singlet oxygen ($\Phi(^1O_2)$) quantum yields were seen to be drastically reduced in comparison with the corresponding values for the control porphyrins **1-3**, Table 1.

Table 1 Fluorescence (Φ_f) and singlet oxygen ($\Phi(^1O_2)$ quantum yield data [a]

Compound	**1**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Φ_f [b]	0.13	0.037	0.090	0.013	0.053	0.056	0.041	0.047
$(\Phi(^1O_2)$ [c]	0.65	0.43	0.65	0.49	0.49	0.50	0.43	0.46

[a] These values are measured as described in references 3 and 5 [b] CH_2Cl_2, λ_{exc}=515 nm, Error limits, ± 10%
[c] DMF, λ_{exc}=550 nm, Error limits, ± 15%

Quenching of fluorescence in these hybrids can be attributed to the occurrence of a PET reaction from the porphyrin singlet state to the appended quinone, as is true for the covalently-linked, porphyrin-anthraquinone systems reported earlier.[7] The low values of $\Phi(^1O_2)$ are probably a consequence of an inefficient porphyrin triplet state generation in these compounds due, in part, to the PET mentioned above. Nonetheless, the photophysical data presented here collectively suggest that it is possible to photocleave DNA *via* both $^1O_2$ and radical based mechanisms by irradiation into the porphyrin absorption envelope (λ =410-700 nm) of these bichromophoric systems. On the other hand, excitation into the bands corresponding to the absorption due to their anthraquinone subunit (λ < 375 nm) would generate the localized triplet which is known to react with the DNA either directly or *via* the formation of the corresponding anion radical.[6]

The nuclease activity of hybrids **4-10** was investigated using the supercoiled plasmid DNA pBR 322 in the presence and absence of light.[3,5] While no nicking was observed in the absence of light, irradiation at either 550 nm (porphyrin absorption) or 350 nm (anthraquinone absorption) caused nicking and generation of relaxed circular DNA, Fig. 1.[9] Similarly, the reference porphyrins **1** and **2** showed only marginal nicking when irradiated at 550 nm but quinones AnQ2-COOH and AnQ2-Br (or anthraquinone, AnQ, itself) were seen to efficiently photocleave the DNA upon excitation by a 350 nm light. Under the identical experimental conditions of concentration and light-dose, the nicking efficiency was found to be better for irradiation at 350 nm compared to that at 550 nm, thus revealing the superior DNA-photonicking ability of the *intercalated* quinone. On the other hand, although both the $^1O_2$ and the radical based mechanisms can, in principle, participate in the photocleavage induced by excitation of the porphyrin chromophore, the lower efficiency observed during 550 nm irradiation can be rationalized if one considers that porphyrin is situated away from the duplex. The DNA photocleavage efficiency would then be necessarily dictated by (i) diffusion of $^1O_2$, within its lifetime, to the site of action on DNA and (ii) rate of production of the anthraquinone anion radical through an indirect (PET) mechanism. In any case, DNA nicking was found to be extremely efficient for each of these hybrids under the conditions of white-light (λ > 350 nm) irradiation, as expected; complete conversion of form I to Form II DNA could be achieved within half the time-duration used for 550/350 nm excitation.

In summary, these results suggest that the porphyrin-anthraquinone diads investigated here are capable of displaying wavelength dependent DNA cleavage activity. Such novel bichromophoric systems are useful not only in enhancing the photodynamic therapeutic efficacy but also in the design of photo-footprinting agents.

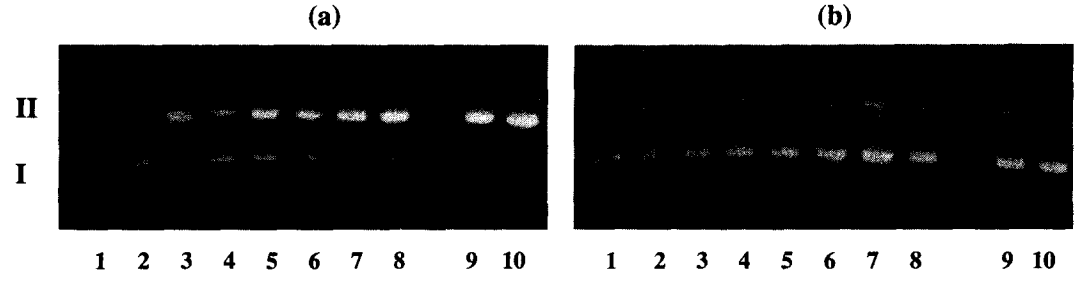


Fig. 1 Light induced nuclease activity of porphyrin-anthraquinone diads. (a) λ_{exc} = 350 nm (1 mW/cm^2; 30 min.); Lane 1: untreated pBR 322, Lanes 2 - 10: pBR 322 + **1**, AnQ, **7**, **8**, **9**, **10**, **5**, **4** or **6**, respectively. (b) λ_{exc} = 550 nm (1 mW/cm^2; 30 min.); Lane 1: untreated pBR 322, Lanes 2 - 10: pBR 322 + **1**, AnQ, **7**, **8**, **9**, **10**, **5**, **4** or **6**, respectively. In each case, the proportion DNA/Drug = 1 and the samples were incubated for 1 h before being irradiated using a 150 W Xe-arc lamp/monochromator assembly. Electrophoresis experiments were carried out as described in references 3 and 5.

Acknowledgments: S. M. and S. A thank CSIR and UGC respectively, for fellowships. This research has been supported by the Indo-French Centre for the Promotion of Advanced Research (Centre Franco-Indien pour la Promotion de la Recherche Avancee), New Delhi.

References

1. Morgan, A. R. *Curr. Med. Chem.*, **1995**, *2*, 604. (b) Bonnett, R., *Chem. Soc. Rev.* **1995**, 19. (c) *Photodynamic Therapy: Basic Principles and Clinical Applications'* , Henderson, R. W.; Dougherty, T. J., Eds.; Marcel Dekker: New York, **1992**.
2. Zhou, C. J. *Photochem. Photobiol. B:Biol.* **1989**, *3*, 299. (and references therein)
3. Mehta, G.; Sambaiah, T.; Maiya, B. G.; Sirish, M.; Chatterjea, D. *J. Chem. Soc. Perkin Trans.* 1. **1993**, 2667. (b) Mehta, G.; Sambaiah, T.; Maiya, B. G.; Sirish, M.; Dattagupta, A. *J. Chem. Soc. Perkin Trans.* 1. **1995**, 295. (c) Mehta, G.; Muthusamy, S.; Maiya, B. G.; Sirish, M., *J. Chem. Soc. Perkin Trans.* 1. **1996**, 2421.
4. Milder, S. J.; Ding, L.; Etemad-Moghadam, G.; Meunier, B.; Paillous, N. *J. Chem. Soc. Chem. Commun.* **1990**, 1131. (b) Sentagne, C.; Meunier, B.; Paillous, N. *J. Photochem. Photobiol B: Biol.* **1992**, *16*, 47.
5. Mehta, G.; Sambaiah, T.; Maiya, B. G.; Sirish, M.; Dattagupta, A. *Tetrahedron. Lett.* **1994**, *35*, 4201.
6. Breslin, D. T.; Coury, J. E.; Anderson, J. R.; Mcfail-Isom, L.; Kan, Y.; Williams, L. D.; Bottomley, L. A.; Schuster, G. B. *J. Am. Chem. Soc.* **1997**, *119*, 5043 (and references therein).
7. Wasielewski, M. R. *Chem. Rev.* **1992**, *92*, 435.
8. Representative data: FAB-MS (m/z): **4**: 907; ^{1}H NMR (200 MHz, $CDCl_3$): δ -2.81 (2H, br s), 2.73 (9H, s), 7.59 (6H, d), 7.70 (2H, d), 7.85(2H, m), 8.12 (6H, d), 8.34 (4H, m), 8.52 (m, 1H), 8.70(m, 1H), 8.90 (m, 8H). **8**: FAB-MS (m/z): 993; ^{1}H NMR (200 MHz, $CDCl_3$): δ -2.79 (2H, br s), 1.87 (2H, m), 2.06 (4H, m), 2.72 (9H, s), 4.32 (2H, t), 4.56 (2H, t), 7.30 (2H, d), 7.57 (6H, d), 7.74 (2H, m), 8.11 (8H, d), 8.26 (2H, m), 8.44 (2H, m), 8.86 (8H, s), 8.99 (1H, br s). **10**: FAB-MS (m/z): 951 ^{1}H NMR (200 MHz, $CDCl_3$): δ -2.81 (2H, br s), 2.72 (9H, s), 4.60 (2H, t), 4.92 (2H, t), 7.33 (2H, d), 7.57 (6H, d), 7.73 (2H, m), 8.12 (6H, d), 8.18 (4H, m), 8.38 (2H, m), 8.87 (8H, s), 8.96 (1H, s).
9. There is <5% competitive absorption by the other chromophore at each of these wavelengths.

(*Received in UK* 22 *July* 1997; *accepted* 8 *August* 1997)