Synthesis and Biological Activities of Phthalocyanine–Estradiol Conjugates

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Abstract—Phthalocyanine-based photosensitizers, coupled via a 17α-ethynyl group to estradiol using Pd(II) as a catalyst, were synthesized and evaluated for their estrogen receptor binding affinity and in vitro photocytotoxicity. The highest receptor binding affinities (RBA = 8-13) were observed with lipophilic conjugates coupled via a relative long spacer group while the sulfonated analogues showed little binding affinities (RBA < 2). The highest photocytotoxicity was observed with the sulfonated conjugates, the nature of the spacer group did not have a pronounced effect.
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Porphyrins and phthalocyanines are being studied extensively as photosensitizer (PS) for the photodynamic therapy (PDT) of various medical conditions.[1] Photofrin^{TM}, a mixture of hematoporphyrin derivatives, is in clinical use and a number of second generation PS are currently in clinical trials for PDT of a variety of oncological and other medical applications.[2] The treatment is based on the localization of the drug in the target tissue followed by excitation of the dye with visible light. Although most second generation PS advanced for PDT offer optimal photophysical properties, their clinical use is limited due to lack of tissue selectivity. Several attempts to improve target selectivity of PS via their attachment to carriers (antibodies, peptides, steroids) have recently been reported.[3] Conjugation of porphyrins[4] and phthalocyanines to cholesterol via a long alkyl chain favors their binding to low density lipoproteins (LDL),[5] which in turn promotes interaction with LDL receptors over expressed on tumor cells.[6] Another approach is the development of steroid receptor-based PS. The attachment of a steroid moiety to the PS molecule could improve uptake by various receptor-rich endocrine tumors. The synthesis of 5,15-diphenylporphyrin linked to estrogen and progesterone deri-

vatives has been reported[7] and recently a tetraphenylporphyrin C11β–estradiol conjugate has also been advanced.[8] In this paper, we describe the synthesis of phthalocyanines conjugated to estrogens, using the palladium catalyzed coupling reaction (Schemes 1 and 2),[9] together with preliminary receptor binding and phototoxicity data (Table 1).

Phthalocyanines have strong red-shifted absorption bands with maxima around 680 nm, that is at wavelengths where tissue transparency is optimal. This allows for the treatment of relative large volumes of diseased tissues, rendering this class of compounds attractive as second generation PS for PDT. The 4-iodo-Pc (**1**) was dissolved in THF and treated with 17α-ethynylestradiol (room temperature, 2–4 h) in triethylamine containing copper(I) iodide and a catalytic amount of bis(triphenylphosphine)Pd(II) chloride. The coupling product **3a** gave a strong Q band at 680 nm and molecular ion at m/z 1040. A bathochromic shift has been reported when substituents are introduced onto the benzene ring of Pc, particularly at site α to the point of fusion to the heterocyclic ring.[10] Accordingly, we also coupled the 17α-ethynylestradiol with the 3-iodo-Pc using the same reaction condition, to yield adduct **4a** linked at the α-position of the Pc. Compound **4a** showed a red-shifted absorption maximum at 691 nm. The Pc–estrogen conjugates were also prepared with

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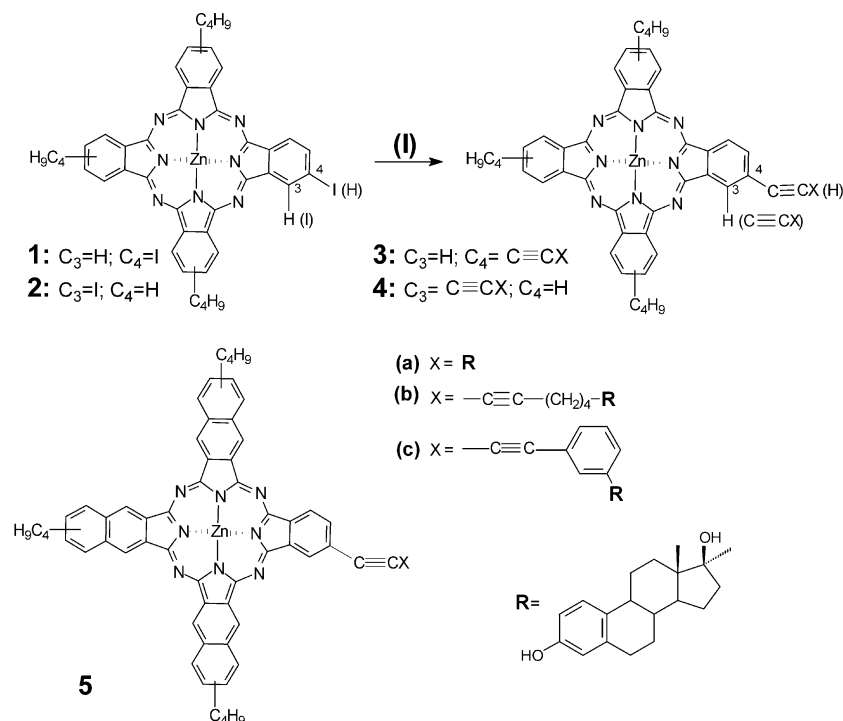
Scheme 1. (I) PdCl$_2$(PPh$_3$)$_2$, CuI, Et$_3$N, THF, rt, 12–24 h, 80–90%; (a) 17α-ethynylestradiol; (b) 17α-(2-buta-1,7-diynyl)estradiol; (c) 17α-(phenyl-1,3-diynyl)estradiol.

both aliphatic and aromatic spacers. Compound **1** was coupled with 17α-(2-buta-1,7-diynyl)estradiol and 17α-(phenyl–1,3-diynyl)estradiol to yield product **3b** and **3c**, respectively. To develop a Pc-steroid adduct with a multiple choice of red-shifted excitation wavelengths, analogues containing both phthalo and naphthalo moieties were also prepared. These composite structures (**5**) feature spectral characteristics of both the Pc and naphthalocyanine parent molecules.[11] Compound **5a** gave a characteristic UV–vis spectrum with peaks at 726.5 and 759.5 nm of almost equal intensity. A series of hydrophobic water-soluble trisulfonated Pc–estradiol conjugates were also prepared in order to compare biological activities with those of the lipophilic conjugates. The protected trisulfonated 4-iodo Pc **6**[12] was reacted with different estradiol alkynes under the above palladium/copper(I)iodide condition to yield Pc conjugates **7a–d** that were readily purified by silica gel column chromatography. After hydrolysis of the protecting group by treatment with lithium methoxide in methanol and THF, the trisulfonated Pc conjugates **8a–d** (λ_{max} = 675 nm) were obtained in high yield. In a similar manner **9a–b** were also synthesized. These compounds gave characteristic absorption spectra with two Q bands at 682 and 702 nm. All products gave a molecular ion in their mass spectrum using the electron-spray technique. Additional spectroscopic data are given as a note at the end of the list of references.

The highest ER binding affinities where measured with the lipophilic conjugates **3–5** (Table 1). Binding affinities increased with increased spacer length, that is **3a–c**, with the position of attachment on the Pc (i.e., **3a** vs **4a**) and

Table 1. Photocytotoxicity against EMT-6 tumor cells and relative binding affinity (RBA) for ER of compounds **3–5** and **8–9**

Conjugate	LD$_{90}$[a] 6 h	LD$_{90}$[a] 24 h	RBA[b]
3a	n.a.	n.a.	1.35
3b	n.a.	n.a.	3.08
3c	n.a.	n.a.	8.54
4a	n.a.	n.a.	12.92
5a	n.a.	n.a.	10.36
8a	7.0	5.1	1.64
8b	7.3	2.9	1.26
8c	10.4	5.3	1.22
8d	10.4	4.0	0.30
9a	12	3.0	1.24
9b	12	4.3	0.56

[a]Values are means of three experiments (n.a., not active). The phototoxicity is expressed as LD$_{90}$, that is the light dose (J/cm^2) required to kill 90% of the EMT-6 cells after incubations of 6 h or 24 h periods with 1 μM conjugate. Survival was measured by the coloremetric MTT test.[13]
[b]The receptor binding affinities (RBA) for estrogen receptors (ER) were measured by a competitive ^{3}H-estradiol binding assay using FlashPlate technology, taking estradiol as 100.[14]

also with the increased overall size of the photosensitizer (i.e., **3a** vs **5a**). The sulfonated, water-soluble conjugates **8–9** showed little binding affinity for the ER. In contrast, the latter showed strong phototoxicity against EMT-6 cells in vitro (Table 1).

The lipophilic conjugates **3–5** where photo-inactive at 1 μM, while at 5 μM they exhibited dark toxicity. The conjugates **8b** and **9a** exhibited good photoactivities, especially after 24-h incubation and their activities are comparable to those reported for the non-conjugated ZnPcS$_3$ (i.e., LD$_{90}$ = 2.6 J/cm^2).[15]
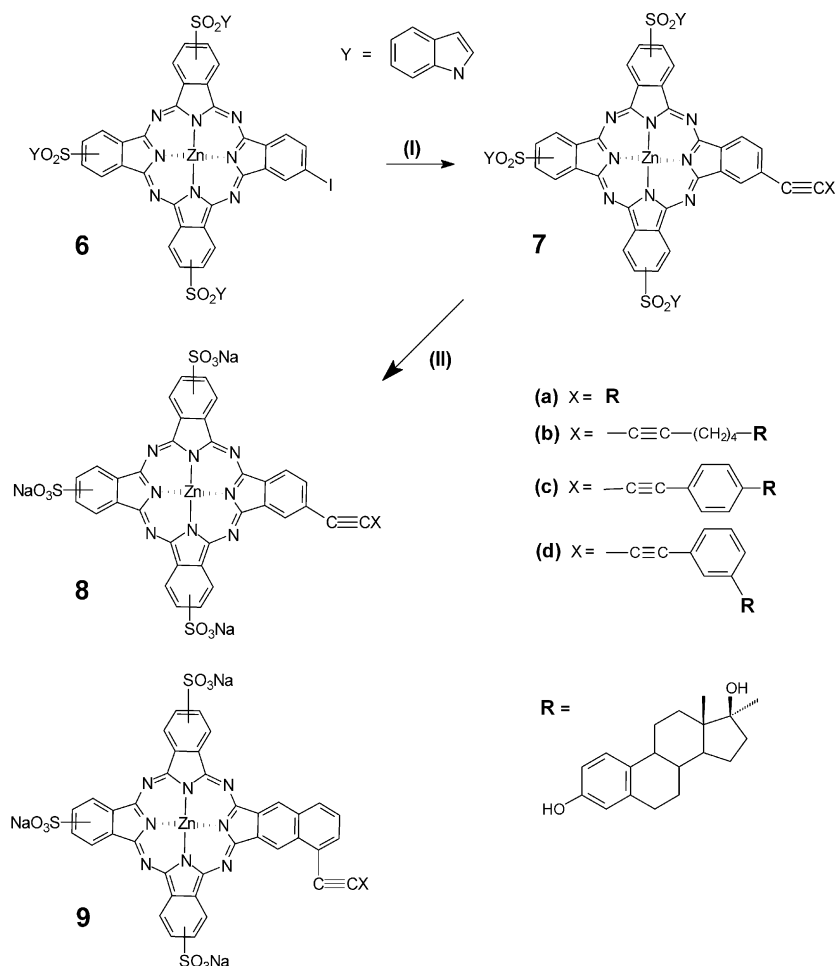
Scheme 2. (I) PdCl$_2$(PPh$_3$)$_2$, CuI, Et$_3$N, THF, rt, 12–24 h, 80–90%; (II) LiOMe, THF, reflux, 24 h, 50–80%; (a) 17α-ethynylestradiol; (b) 17α-(2-buta-1,7-diynyl)estradiol; (c) 17α-(phenyl-1,4-diynyl)estradiol; (d) 17α-(phenyl 1,3-diynyl)estradiol.

Spectroscopic data. **3a**: HRMS (FAB) 1038. 42871 calcd for C$_{64}$H$_{62}$O$_2$N$_8$^{64}Zn. Found 1038. 42,610; λ$_{max}$ (CHCl$_3$) 680 nm. **3b**: MS (FAB) 1119. 5; λ$_{max}$ (DMF) 681 nm. **3c**: MS (FAB) 1139. 4; λ$_{max}$ (DMF) 674, 689 nm. **4a**: HRMS (FAB) 1038. 42871 calcd for C$_{64}$H$_{62}$O$_2$N$_8$^{64}Zn. Found 1038. 42,530; λ$_{max}$ (CHCl$_3$) 691 nm. **5a**: MS (FAB) 1192; λ$_{max}$ (DMF) 726. 5, 759. 2 nm. **8a**: MS (elec. spray) 1110; λ$_{max}$ (MeOH) 674 nm. **8b**: MS (elec. spray) 1190; λ$_{max}$ (MeOH) 675 nm. **8c**: MS (elec. spray) 1210; λ$_{max}$ (MeOH) 675 nm. **8d**: MS (elec. spray) 1210; λ$_{max}$ (MeOH) 675 nm. **9a**: MS (elec. spray) 1128; λ$_{max}$ (MeOH) 694 nm.

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