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Synthesis of Phthalocyanines with Estradiol derivatives evaluated for Estrogen Receptor binding

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Abstract: *Photosensitizers-based phthalocyanine coupled with 17α-ethynyl estradiol group using palladium as 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 sulphonated analogues showed littlebinding affinities (RBA <2). The highest photocytotoxity observed with the sulphonated conjugates the nature of the spacer group did not have a pronounced effect*

Keywords: *Phthalocyanines, Porphyrins, Photosensitizers, Steroid, Photodynamic Therapy*

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I. Introduction

One of the most interesting application of porphyrins and phthalocyanines involve their use asphotosensitizers (PS) in the photodynamic therapy (PDT) of tumor. A number of PS are currently in clinical trials for PDT of a variety of medical conditions [1]. The treatment is based on the localization of the drug in target tissue and treatment with visible light of the affected area. However. The use of most of these compounds is limited due to lack of target selectivity. Efforts have been focused on the synthesis of PS attached to biomolecules (antibodies, peptides, steroids) which could improve target selectivity. Cholesterol conjugates with porphyrins [2] and phthalocyanines [3] have been reported. These lipophilic PS exhibit good solubility in lipophilic media which promotes uptakes by the low-density lipoproteins (LDL) component of blood. Another approach is the development of steroids receptor -based PS. The incorporation of a steroid moiety in the PS molecule could improve the target selectivity for the receptor rich tumors. The palladium catalyzed coupling reaction has been proven an efficient process to modify the porphyrin [4,5,6,7], and phthalocyanine [8] macromolecule. Recently the synthesis of 5,15-diphenyl porphyrin linked to estrogen and progesterone derivative have been reported [9]. Porphyrins and phthalocyanines have been studied well as photosensitizers for the photodynamic therapy of various medical conditions [1,10,11]., Photofrin, 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 [12]. The treatment is based on the localization of the drug in the target tissue followed by excitation of dyes 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 reported13 .Conjugation of porphyrins2 and phthalocyanines to cholesterol via a long alkyl chain favors their binding to low density lipoprotein (LDL) [3,14], which in turn promotes interaction with LDL receptor over expressed on tumor cells [15] Another approach is the development of steroid receptor-based PS. The attachment of the steroids moiety to the PS molecule could improve uptake by various receptor-rich endocrine tumors.

II. Experimental

The synthesis of tetraphenyl porphyrin c11$_{\beta}$-estradiol conjugates has also been advanced [16]. In this paper, we describe the synthesis of phthalocyanines conjugates to estrogens, using the palladium catalyzed coupling reaction. (Scheme I and II) [5,6,8,17,18,19] together with preliminary receptor binding and phototoxicity data (Table 1). Phthalocyanines have strong red shifted absorption bands with maxima around 680 nm, that is wavelengths where tissue transparency is optimal. This allows for the treatment of relatively large volume of diseased tissue, 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-ethynyl estradiol (room temperature, 2-4 h) in triethyl amine containing copper (I)iodide and a catalytic amount of bis (triphenyl phosphine) Pd (II) chloride. The coupling product 3a gave strong Q band at 680 nm and molecular ion at m/z 1040. A bathochromic shift has been reported when substituents are introduced on to benzene ring of Pc, particularly at site α to the point of

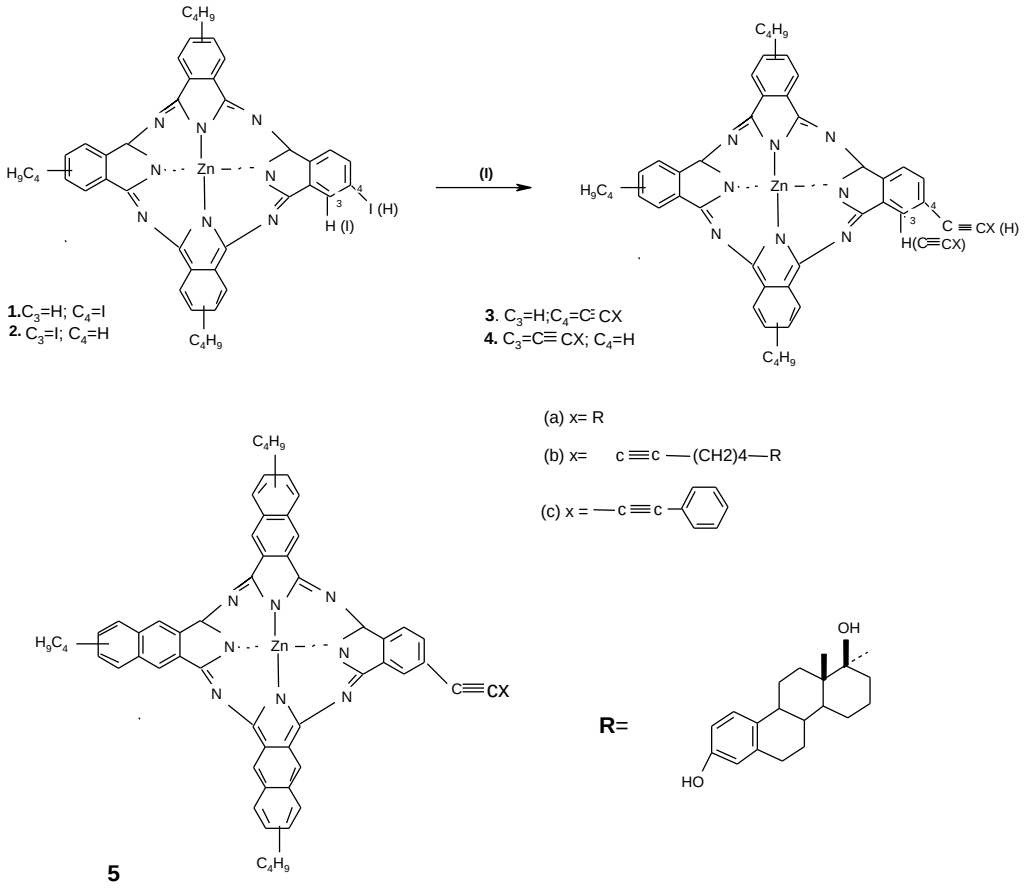
fusion to the heterocyclic ring20,21.

Accordingly, we also coupled the 17α-ethynyl estradiol 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 691nm. The Pc estrogen conjugates were also prepared with 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 redshifted excitation wave lengths, analogues containing both phthalo and naphthalol moieties were also prepared.
These composite structure (5) features spectral characteristics of both Pc and phthalocyanine parent molecule22,23. Compound 5a gave characteristics UV-Vis spectrum with peaks at 726.5 and 759.5nm of almost equal intensity. A series of hydrophobic water soluble trisulphonated Pc-estradiol conjugates were also prepared in order to compare biological activities with these of lipophilic conjugates.

III. Result and Discussion

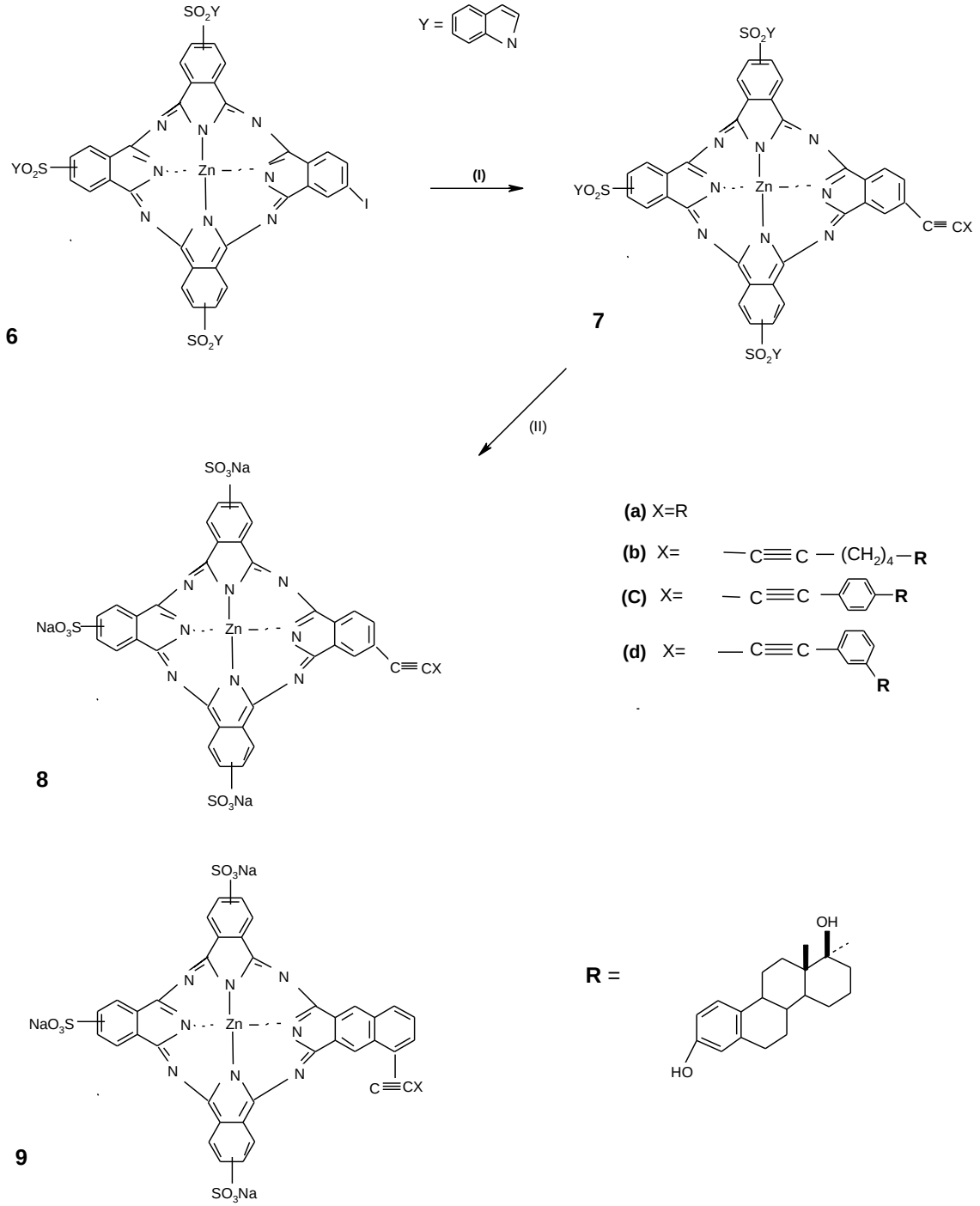
The protected trisulfonated 4-iodo Pc 6[24] was reacted was reacted with different estradiol alkynes under the above palladium / copper(I) iodide condition yield Pc conjugates 7a-d that were readily purified by silica gel column chromatography. After hydrolysis of the protecting group by treatment with lithium methoxide in methanol and THF, the trisulfonated Pc conjugates 8a-d (λmax= 675 nm) were obtained in high yield. In a similar manner 9a-b were also synthesized. These compounds gave characteristics absorption spectra with two Q band 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 anote at the end of the list of reference. The highest ER binding affinities where measured with the hydrophilic conjugates 3-5 (Table 1). Binding affinities increased with increase spacer length, that is 3a-c, with the position of attachment on the Pc(i.e.3a vs 4a) and 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).

Synthesis Scheme I



SchemeI.(1)$PdCl_2(PPh_3)_2$,CuI,Et_3N, THF, rt,12-24h,80-90%:(a)17α-ethynylestradiol;(b)17α-(2-buta-1,7-diynyl) estradiol; (c) 17α-(phenyl-1,3 diynyl estradiol.

Synthesis scheme II



Scheme2.(I)$PdCl_2(PPh_3)_2$,CuI, Et_3N, THF, rt.12-24h,80-90%;(II)LiOMe,THF,reflux,24h,50-80%;(a)17α-

ethynylestradiol;(b)17α-(2-buta-1,7-diynyl) estradiol;(c)17α-(phenyl-1,4-diynylestradiol;(d)17α-phenyl 1,3-diynyl) estradiol.

The lipophilic conjugates 3-5 where photo- inactive at 1µM, while at 5µM 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 ZnPcS3 (i.e., LD90=2.6J/cm2)[27].Spectroscopic data. 3a: HRMS (FAB) 1038. 42871 calculated for C64H62 O2N8 64Zn. Found 1038. 42,610;λmax (CHCl3) 680nm.3b: MS (FAB) 1110.5; λmax (DMF)674, 689 nm. 3c: MS (FAB) 1139.4; λmax (DMF)674, 689 nm. 4a: HRMS(FAB) 1038. 42871 calculated for C64H62O2N8 64Zn. Found 1038, 42,530; λmax (CHCl3) 691 nm.5a: MS (FAB) 1192; λmax (DMF) 726, 5, 759, 2 nm. 8a: MS (elec.spray) 1110; λmax. (MeOH) 4nm 8b: MS(

elec.spray) 1190; λmax. (MeOH) 675nm. 8c : MS (elec.spray) 1210; λmax. (MeOH) 75nm.8d: MS(elec.spray) 1210; λmax. (MeOH) 675nm. 9a: MS (elec.spray) 1128; λmax. (MeOH) 694nm

Table 1. Photocytotoxicity against EMT-6 tumor cells and relative binding affinity (RBA) for ERofcompounds 3-5 and8-9.

Conjugate	LD90[a] 6h	LD90[a] 24h	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 LD90 that is light dose (j/cm2) required to kill 90% of the EMT-6 cells after incubations of 6h or 24 h periods with 1μM conjugates. Survival was measured by the colorimetric MTT test[25]. [b]The receptor binding affinities (RBA) for estrogen receptors (ER) were measured by a competitive ^{3}H-estradiol binding assay using Flash Plate technology, taking estradiol as 100[26].

IV. Conclusion

Photosensitizers based phthalocyanines are conjugated with estradiol to get target selectivity. Photosensitizers-based phthalocyanine coupled with 17α-ethynyl estradiol group using palladium as 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 sulphonated analogues showed little binding affinities (RBA <2). The highest photocytotoxicity was observed with the sulphonated conjugates, the nature of the spacer group did not have a pronounced effect.

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EthicalStatement

There is no such use of animals or humans in this researchwork.

Conflict ofinterest

There is no conflict todeclare.

FundingStatement

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