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# Synthesis and biological evaluation of prodigiosene conjugates of porphyrin, estrone and 4-hydroxytamoxifen

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ABSTRACT

To generate the first series of prodigiosene conjugates, the tripyrrolic skeleton was appended to estrone, tamoxifen and porphyrin frameworks by way of ester linkers and various hydrocarbon chain lengths. The ability of the conjugates to inhibit various types of cancer cells was evaluated in vitro. The porphyrin conjugates did not exhibit significant activity. The estrone conjugates exhibited modest activity, for the most part. However, significantly greater growth inhibition activity against certain breast, colon, lung, leukemia, melanoma and prostate cell lines was noted. This unusual effect for this first generation model class of compound warrants further investigation and comparison to cases where estrogens are linked to prodigiosenes via connection points that do not feature in estrogen receptor binding. The 4-hydroxytamoxifen conjugates exhibit nanomolar range activity against the MCF-7 breast cancer cell line, paving the way to expand the scope and connectivity of prodigiosene–tamoxifen conjugates.



## 1. Introduction

Prodigiosenes constitute a class of tripyrrolic compounds based on the natural product prodigiosin (**a**, Fig. 1) that share a common 4-methoxypyrrolyldipyrrin core unit. They have demonstrated significant immunosuppressive, antimicrobial, antifungal, antiprotozoal, antimalarial and anticancer activities.[1–3] A singular mechanism of action by which prodigiosenes induce apoptosis has not been identified, rather a diverse range of activities have been noted which are thought to collectively account for the cytotoxic effects. For example, prodigiosenes have demonstrated the ability to induce copper-mediated double strand DNA (dsDNA) breakage, inhibit Bcl-2, intercalate DNA and uncouple V-ATPase through promotion of $H^+/Cl^-$ symport.[1,2,4–9]

Multidrug resistance (MDR), a resistance to a range of structurally unrelated compounds, may develop in tumor cells following drug exposure.[10] This may significantly limit the effectiveness of chemotherapeutic treatments. Prodigiosenes are of interest due to their potential to combat MDR for two principal reasons; the number of potential targets affected and their inability to be transported by the MDR transporter.[2] Targeted drug design, i.e. attaching a compound known to accumulate in cancer cells to an anticancer compound, is a strategy that could be used to overcome MDR by increasing uptake into the cancerous cell compared to healthy cells.[11–28]

Conjugating prodigiosenes to a molecule that already possesses selectivity toward the tumor should help to deliver the drug at a specific site. Thus, it should be possible to target estrogen receptor (ER) positive breast cancers by using a ligand of the estrogen receptor.[11–16,20,24–26] Several ligands of the ER are used as a treatment in breast cancer therapy.[29,30] For example the pure antagonist fulvestrant (**b**, Fig. 1)[31,32] is used to treat post-menopausal women with a progressive or metastatic disease. Another example is tamoxifen (**c**, Fig. 1), a selective estrogen receptor modulator (SERM) that has agonist or antagonist activity depending on the tissue.[33] It has been shown that after treatment with tamoxifen therapy 46% of the resistant tumors retain ER expression.[34] This means that the ER is a potential target in resistant tumors. Furthermore, porphyrins are aromatic macrocyclic analogues of the natural hematoporphyrin (**d**, Fig. 1), and have demonstrated an ability to accumulate in cancer cells as compared to normal cells.[35] They are known to localize in subcellular structures such as lysosomes, endoplasmic reticulum, mitochondria and Golgi apparatus.[36] Thus, conjugation of a therapeutic drug to a porphyrin also seems to be an applicable strategy for drug delivery into tumor cells.[28]

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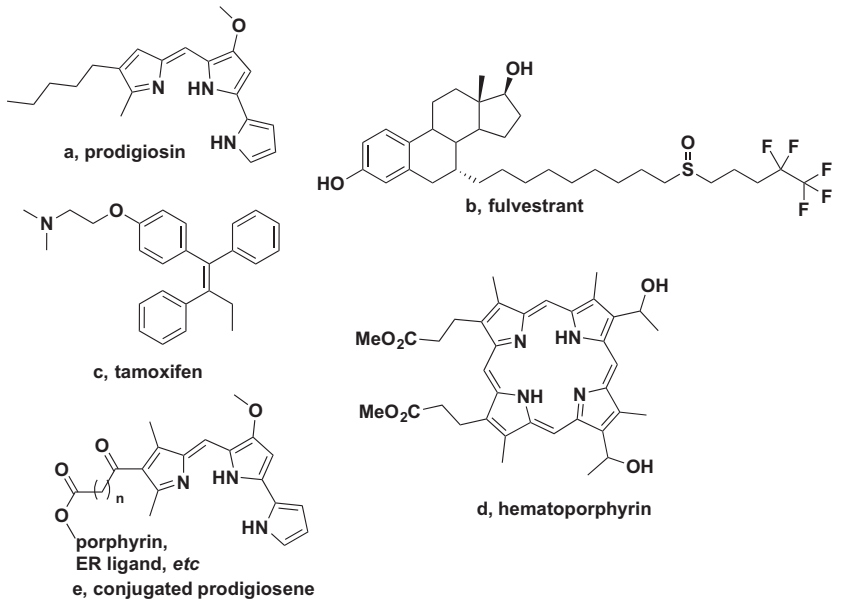


**Figure 1.** Prodigiosin, estrogen receptor ligands, hematoporphyrin and proposed prodigiosene conjugates.

## 2. Results and discussion

We herein report, as part of our efforts to synthesize and identify useful anti-cancer compounds based on the prodigiosene skeleton,[37–41] first steps towards probing the conjugation of prodigiosenes with ER ligands and porphyrins using linkers of various chain lengths as shown in Figure 1, **e**.

We first sought a model set of compounds by which targeting moieties could be appended to prodigiosenes. Towards this goal, the prodigiosene-linker core **1** (Scheme 1) was prepared using a reported procedure.[37,38] The corresponding acids (**2**) were obtained in good yields via saponification using a large excess of potassium hydroxide at 70 °C. We then studied the feasibility of esterification with the commercially available estrone as a model, cognizant that ultimately the phenolic position would need to be available for binding.[42] The use of EDCI as a coupling reagent in the presence of DMAP in $CH_2Cl_2$ emerged as optimal, giving the conjugates **3** in moderate to good yield. This lead us to perform the reaction with the more valuable (*Z*)-4-hydroxytamoxifen (Afimoxifene, 4-OHT), the active metabolite of tamoxifen, that was prepared using a literature process.[43] The corresponding conjugates **4** were obtained in good yields after purification using column chromatography.

To conjugate prodigiosenes with amino-tetraphenylporphyrin ($NH_2$–TPP, **5**, Scheme 2)[44–46] via an amide linkage, two procedures were used: the coupling reagent COMU in the presence of DBU,[47] and a polystyrene supported carbodiimide coupling reagent in the presence of DMAP. Both sets of reaction conditions provided conjugates **6** in moderate yield (Scheme 2).

The porphyrin conjugates were screened at 10 μM by the National Cancer Institute (NCI) against their standard panel of 60 human cell lines, derived from nine cancer cell types. At this concentration significant cytotoxicity was not observed, and the conjugates were not pursued further. Nevertheless, the synthetic methodology could be applied to the coupling of more elaborate porphyrins (i.e., porphyrins substituted with functional groups), including those with structures that match tetrapyrrolic macrocycles used in photodynamic therapy.[48]

A preliminary evaluation of the activity of our first-generation model ER-targeted conjugates was accomplished by treating an ER-positive cell line, MCF-7, with conjugates **3** and **4** at a concentration of 1 μM. The viability of the cells was measured using MTT assays and the results are summarized in Figure 2. The three prodigiosenes conjugated to estrone by means of three different linker lengths (**3a**, $n = 2$; **3b**, $n = 4$; **3c**, $n = 8$) all demonstrated rather poor activity at this concentration against the MCF-7 cell line (Fig. 2), and this is not surprising given that the important phenolic binding site[42] of estrone is essentially blocked by the appended prodigiosene. However, it is interesting to note that the estrone conjugates **3** did not exhibit an estrogenic proliferative effect, as would be expected for estrone alone such as might be the case if hydrolysis were immediate to reveal estrone and the parent prodigiosene carboxylic acid. Rather, the compounds reduced the cell viability of the MCF-7 cells. For each of the conjugates, the longer chain linker ($n = 8$ carbon-chain, **3c** and **4c**) performed relatively poorly as compared to the other two chain lengths within each series.

To determine the effects caused by the presence/absence of estrogen receptors, the activity of conjugates bearing a two-carbon linker (**3a** and **4a**) and the ethyl ester conjugate **1a**, as a control for the parent 'naked' prodigisene versus conjugated analogues, were investigated using the MDA-MB-231 cell line. As this cell line lacks the presence of estrogen receptors we hoped to see a difference in activity for our conjugates against MDA-MB-231 and MCF-7 cell lines. The biological activity of each compound was again determined using MTT assays, again at 1 μM concentration. Results from these studies were compared to results from the MCF-7 cell line and are shown in Figure 3.

At 1 μM there was no notable difference in activity between cell lines for compounds **1a** (ethyl ester prodigiosene) and **4a** ((*Z*)-4-hydroxytamoxifen conjugated at the phenolic position). A lack of selectivity between the MCF-7 and MDA-MB-231 cell lines was similarly reported for the natural product prodigiosin.[49] These results suggest that the conjugates do not exhibit significant ER-mediated selectivity against breast cancer cell lines, as anticipated for this model set of conjugates. However, the estrone–prodigiosene conjugate **3a** exhibited differential activity across the two cell lines used. Indeed, there was a significant difference in cell viability following treatment with 1 μM **3a** (MCF-7: 0.90; MDA-MB-231: 0.25), that is, **3a** was more effective against the ER-negative cell line than against the ER-positive cell line.