Version: Post-print

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

Cassandra L.A. Hawco,[a] Estelle Marchal,[a] Md. Imam Uddin,[a] Alexander E.G. Baker,[a] Dale P. Corkery,[b] Graham Dellaire[b]* and Alison Thompson[a]*

[a]Department of Chemistry, Dalhousie University, PO BOX 15000, Halifax, Nova Scotia, B3H 4R2, Canada
[b]Departments of Pathology and Biochemistry & Molecular Biology, Dalhousie University, PO BOX 15000, Halifax, Nova Scotia, B3H 4R2, Canada
Corresponding authors. Tel.:+1 902-494-3305; Fax +1 902-494-1310 (AT)
E-mail addresses: Alison.Thompson@dal.ca (A. Thompson); Dellaire@dal.ca (G. Dellaire)

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**, **Figure 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 anti-cancer 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**, **Figure 1**)[31, 32] is used to treat post-menopausal women with a progressive or metastatic disease. Another example is tamoxifen (**c**, **Figure 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**, **Figure 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]

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**.
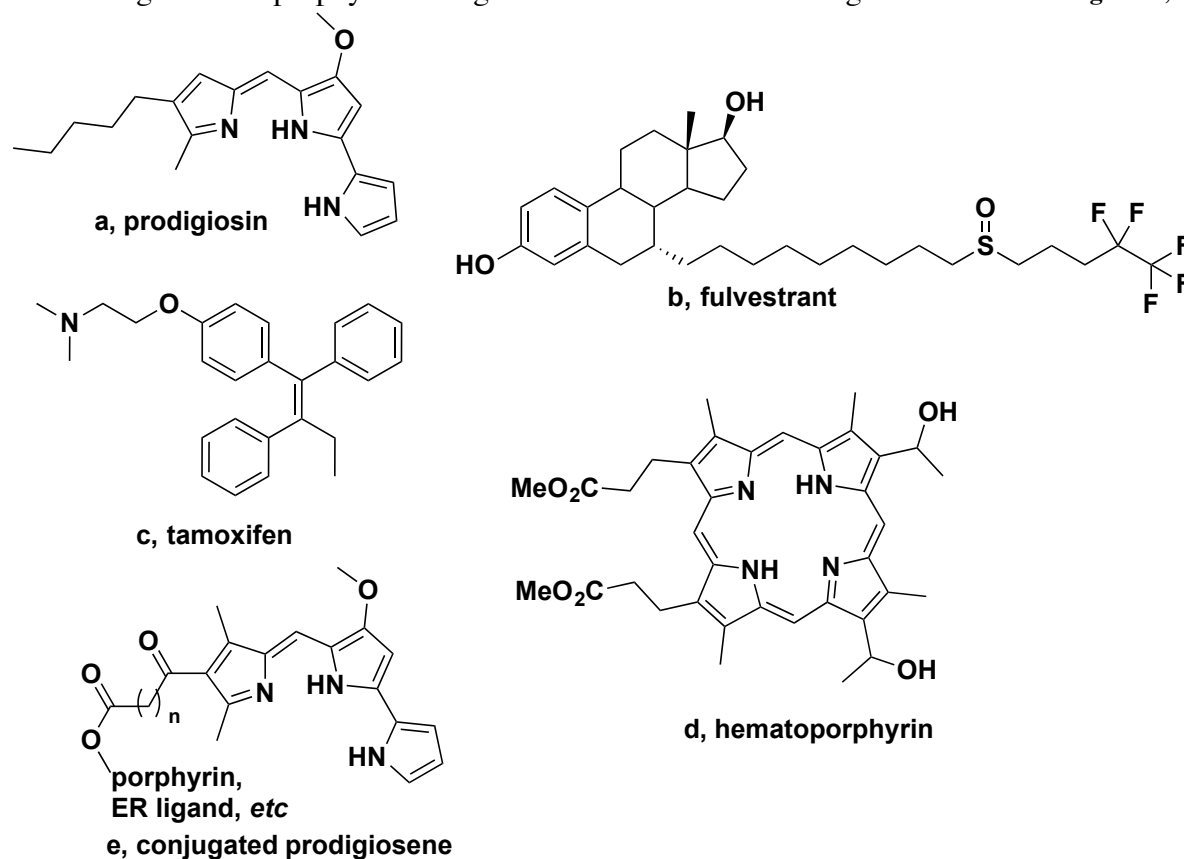

Figure 1: Prodigiosin, estrogen receptor ligands, hematoporphyrin and proposed prodigiosene conjugates.

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$_2$Cl$_2$ 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.
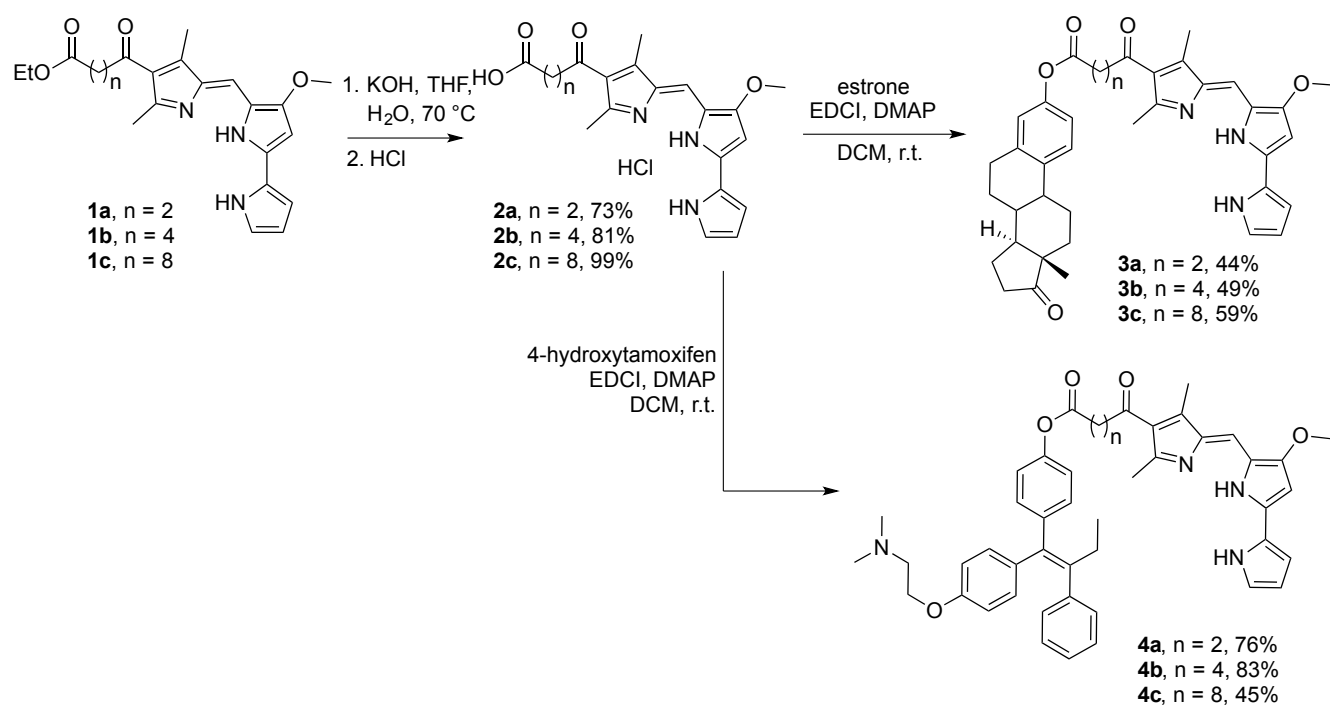
Scheme 1: Synthesis of estrone and tamoxifen prodigiosene conjugates.

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**).
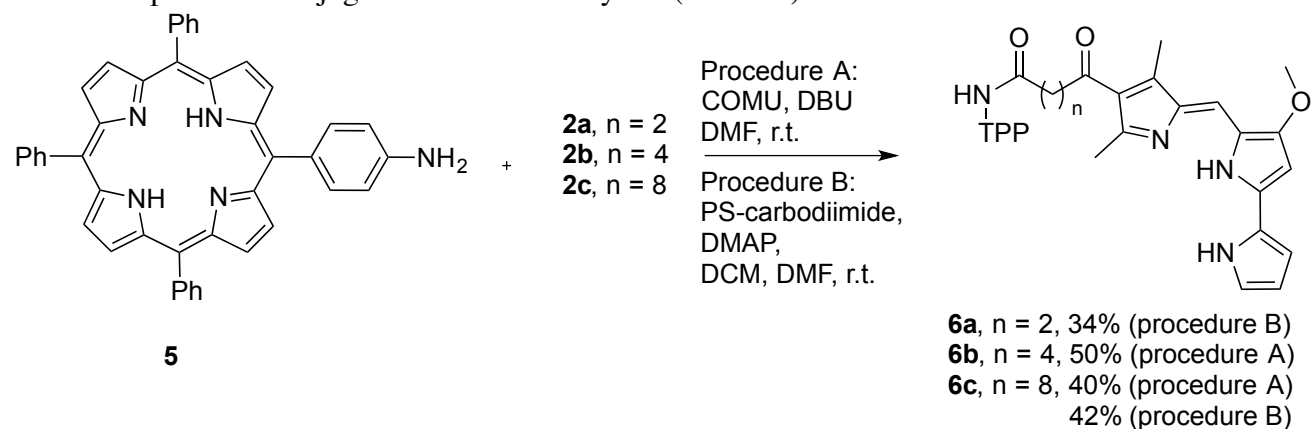
Scheme 2: Synthesis of prodigiosene porphyrin conjugates.

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 (Figure 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.
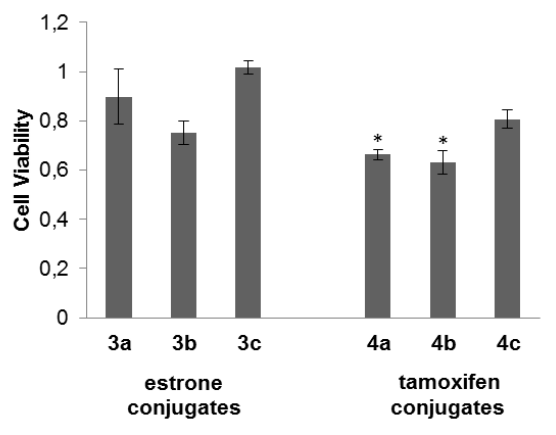

Figure 2: Cell viability following treatment of MCF-7 cell line with conjugates **3** and **4** at 1 μM. MCF-7 cell lines were treated with prodigiosene conjugates for 72 h followed by the MTT dye reduction assay to determine relative viability compared to cells treated with vehicle alone. Average of four replicates. Error bars = SE, *p <0.05.

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 vs. 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.
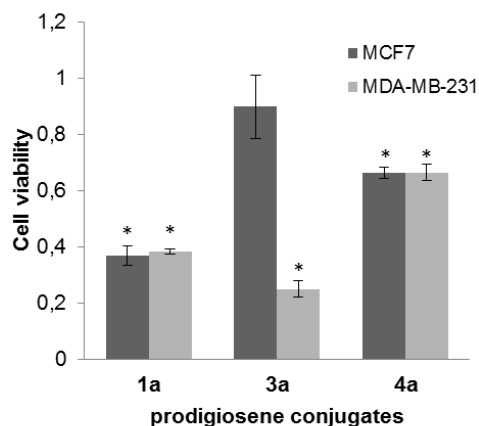
Figure 3: Cell viability following treatment of MCF-7 and MDA-MB-231 breast cancer cell lines with prodigiosene conjugates **1a**, **3a** and **4a** at 1 μM. MCF-7 and MDA-MB-231 cell lines were treated with prodigiosene conjugates for 72 h followed by the MTT dye reduction assay to determine relative viability compared to cells treated with vehicle alone. Average of four replicates. Error bars = SE, *p <0.05.

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), i.e. **3a** was more effective against the ER-negative cell line than against the ER-positive cell line.

Intrigued by the interesting cell viability activity against the ER-negative MDA-MB-231 cell line,[50] **3a** was screened against the NCI60 panel using five different concentration doses. The GI$_{50}$ values thus obtained for estrone conjugate **3a** against six breast cancer cell lines are displayed in Figure 4. A GI$_{50}$ of 1.9 μM was observed against ER-positive MCF-7. However, T-47D is also ER-positive and **3a** exhibited more modest activity against that line (GI$_{50}$ = 15.4 μM), suggesting that ERα status is not a contributing factor to the activity of **3a**, as we had suspected using our 1 μM cell viability MTT assays and given that the estrone derivatives are first generation models whereby a crucial binding position is blocked. As reported above, **3a** is also highly active against the ER-negative MDA-MDAB-231/ATCC cell line (GI$_{50}$ value of 0.3 μM).
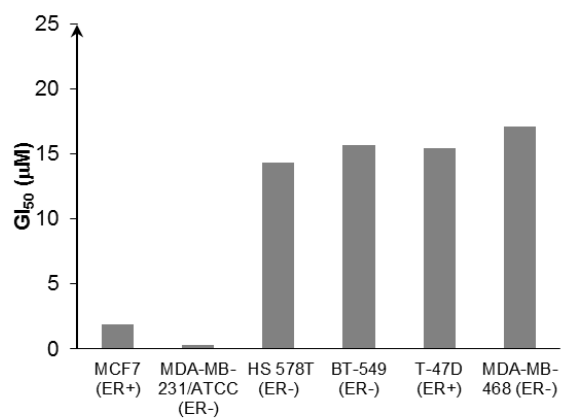
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Figure 4: GI$_{50}$ (half minimal growth inhibition concentration) of estrone conjugate **3a** against the NCI panel of breast cancer cells; average of two replicates. http://dtp.cancer.gov

Previous studies have noted that the natural product prodigiosin triggers apoptosis in hematopoietic cancer cell lines, as well as colon and gastric cell lines.[51] In the NCI screen, compound **3a** was shown to be particularly active against several cell lines, most notably colon cancer (HCT-116, HCT-15, HT29, SW-620) but also leukemia (CCRF-CEM, MOLT-4, SR), melanoma (LOXIMVI), lung cancer (A459/ATCC, HOP92) and prostate cancer (DU-145) cell lines (Figure 5). This data suggests a unifying feature across these cell lines, a feature that results in **3a** inhibiting their growth more dramatically than other cell lines. Prodigiosenes appended with ER ligands via positions not involved in binding to the ER will be used to investigate this hypothesis.
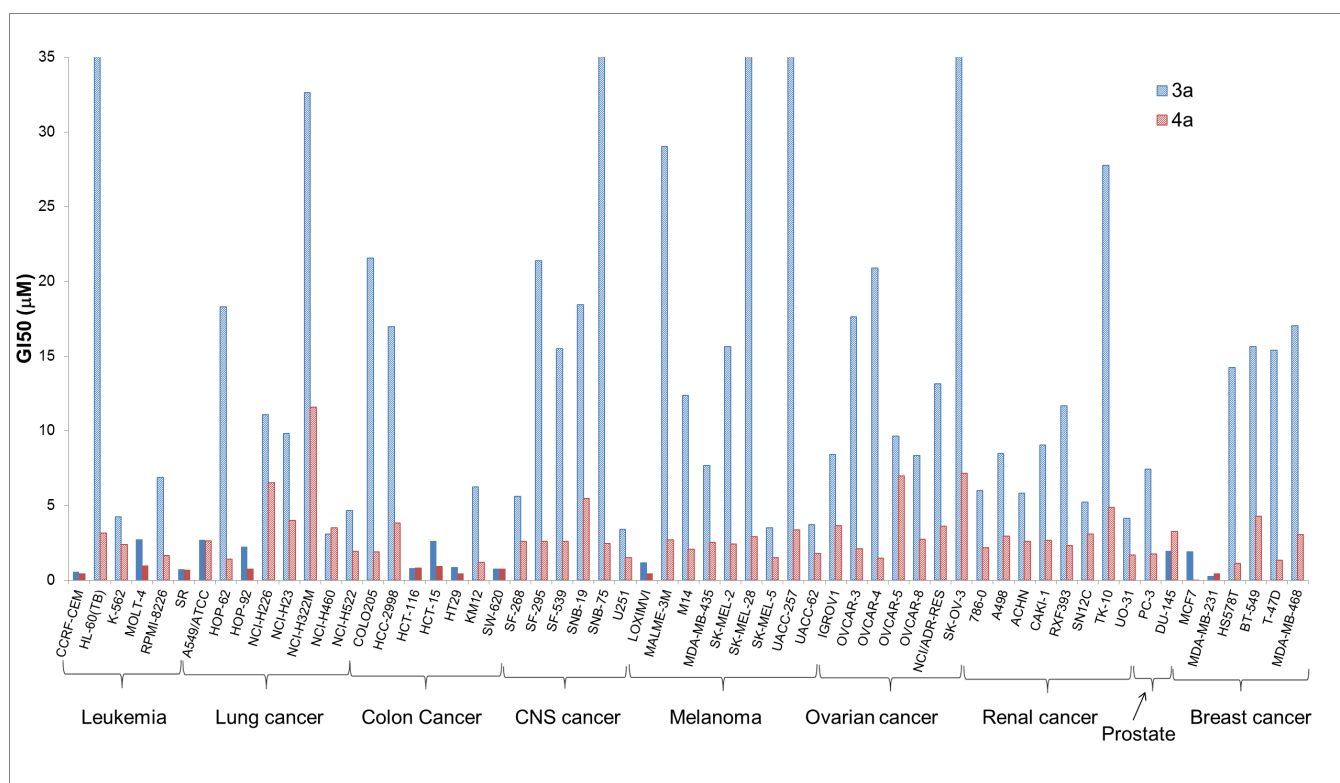
Figure 5: GI$_{50}$ (half minimal growth inhibition concentration) of estrone conjugate **3a** and **4a** against the NCI panel of cancer cells; http://dtp.cancer.gov. Average of two replicates. Darker-coloured bars represents GI$_{50}$ < 3 μM for **3a** and GI$_{50}$ < 1 μM for **4a**.

NOTE for publication: colour figure on-line; black and white figures on paper please.

GI$_{50}$ values of the tamoxifen-prodigiosene conjugates **4** against cancer cell lines in the NCI-60 panel were also measured and compared to values obtained for tamoxifen (**Figure 5** and Figure 6). Again, the conjugate possessing a linker of 8 carbon atoms (**4c**) is the least active. In general, **4a** is more active than **3a**, with excellent activity particularly observed against the MCF-7 cell line (GI50 = 30 nM for **4a**, see Supporting Information). Again, particularly high activity was demonstrated against several cell lines, including breast cancer (MCF-7, MDA-MB-231), colon cancer (HCT-116, HCT-15, HT29, KM12, SW-620), leukemia (CCRF-CEM, MOLT-4, SR), melanoma (LOXIMVI), lung cancer (A459/ATCC, HOP92) and prostate cancer (DU-145) cell lines, as shown in **Figure 5**. This substantial growth inhibition paves the way for investigations regarding other conjugate models for prodigiosenes and targeting moieties designed so as to maximise binding to receptors.
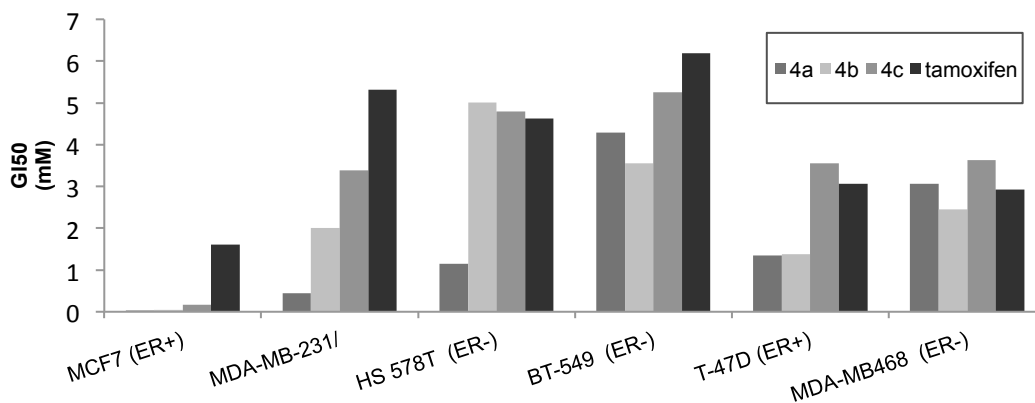
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Figure 6: GI$_{50}$ (half minimal growth inhibition concentration) of tamoxifen conjugates **4** and tamoxifen (NSC 180973, average of three replicates) against the NCI panel of breast cancer cells. http://dtp.cancer.gov

The NCI COMPARE software calculates correlation coefficients that can be interpreted as an indication of similarities in differential cellular sensitivities, courtesy of characteristic NCI60 activity fingerprints for each compound.[52] Given the nanomolar activity of **4a** against MCF-7, we used the COMPARE software to compare the GI$_{50}$ values of the tamoxifen conjugate **4a** versus the NCI standard-agent database. The best match for the activity fingerprint profile of **4a** compared to those of the 171 standard agents was found to be tamoxifen, with a correlation coefficient of 0.54. A correlation coefficient of only 0.35 was obtained when comparing **4a** and the natural product prodigiosin. This suggests that the mode of activity of compound **4a** most closely parallels tamoxifen.

3.   Conclusions

To summarize, although the porphyrin-prodigiosene conjugates showed no activity against the NCI cancer cell panel, the estrone conjugate **3a** demonstrated growth inhibition against the ER-negative cell line MDA-MB-231/ATCC (GI$_{50}$ = 0.3 μM) cf. the ER-positive cell line MCF-7 (GI$_{50}$ = 1.9 μM). The tamoxifen conjugates **4a** and **4b** showed excellent growth inhibition against MCF-7 (GI$_{50}$ = 30 nM for **4a**; GI$_{50}$ = 30 nM for **4b**), with better activity than tamoxifen itself against this cell line. These results bode well as we delve into prodigiosene-ER conjugates featuring optimum binding characteristics. Furthermore, from these experiments it transpires that conjugates with shorter chain length (linker with 2 and 4 carbon atoms) are more active than conjugates with longer chain length (linker with 8 carbon atoms). The peculiar observations as regards the growth inhibition effects of **3a** and **4a** against certain cell lines warrant further study. Indeed, these effects will be investigated using prodigiosene-estrogen conjugates linked via other positions on the estrogen, thereby maximizing binding to the ER.

4.   Experimental

Synthesis general procedures: All chemicals were purchased and used as received unless otherwise indicated. Moisture sensitive reactions were performed in flame-dried glassware under a positive pressure of nitrogen or argon. Air- and moisture-sensitive compounds were introduced via syringe or cannula through a rubber septum. Flash chromatography was performed using ultra pure silica (230-400 mm) or 150 mesh Brockmann III activated neutral alumina oxide as indicated. The NMR spectra were recorded using a 500 MHz spectrometer instrument using CDCl$_3$, DMSO-d$_6$ or MeOD as solvent and are reported in parts per million (δ) using the solvent signals at 7.26 ppm for ^{1}H and at 71.16 ppm for ^{13}C when CDCl$_3$ was used, at 2.50 ppm for ^{1}H and at 39.52 ppm for ^{13}C when DMSO-d$_6$ was used and at 3.31 ppm for ^{1}H and at 49.00 ppm for ^{13}C when MeOD was used, as internal reference with *J* values given in Hertz. Mass spectra were obtained using TOF and LCQ Duo ion trap instruments

operating in ESI+ mode. UV-visible spectra were recorded using a Varian CARY 100 bio spectrophotometer. Analytical HPLC was carried out using a Varian Prostar instrument. The purity of all tested compounds was ≥95%, as determined by analytical HPLC. Melting points are uncorrected. Compounds **2a-c**,[37, 38] (*Z*)-4-hydroxytamoxifen[43] and NH$_2$-TPP[44-46] were prepared according to literature procedures.

MTT Testing: Human breast cancer cell lines MCF-7 and MDA-MB-231 were maintained in DMEM (Sigma Aldrich) medium supplemented with 10% fetal bovine serum (Sigma Aldrich) as well as 1% penicillin and streptomycin (Sigma Aldich). The cells were incubated at 37 ℃ and 5% CO$_2$. MTT analyses followed standard protocol,[17] using a treatment time of 72 h. Viability was then expressed as a mean percentage of MTT reduced to purple formazan by treated versus mock treated (0.1% DMSO) cells. All (non-NCI) experiments were performed in quadruplicate with error analysis representing the standard error and significance determined by the "Student's" t-Test.

NCI Testing: The Developmental Therapeutics Program (DTP) of the National Cancer Institute (NCI) employs the NCI60 cell line screen as an early stage of drug discovery and development. The NCI60 cell line screen consists of 60 human tumor cell lines, each chosen for their ability to perform consistently and provided appropriate representation of a variety of tumor types: 59 cell lines were available for screening when this work was performed.[53] Each cell line used has been extensively characterized.[53] The multi-dose drug screen involves treatment of each cell line with compounds over a 5-log mol/L concentration range for 2 days.[53, 54] The cells are then fixed and stained with sulphorhodamine B and optical densities are measured.[53, 54] Growth inhibition is calculated relative to cells at the time zero control and those without drug treatment.[53, 54] http://dtp.cancer.gov.

(*Z*)-10-(2-((4-Methoxy-1*H*,1'*H*-2,2'-bipyrrol-5-yl)methylene)-3,5-dimethyl-2*H*-pyrrol-4-yl)-10-oxodecanoic acid hydrochloride (2c•HCl): A solution of prodigiosene **1c** (100 mg, 0.21 mmol) in THF (21 mL) under N$_2$ was treated with aq. KOH (21 mL, 5 M) and stirred at 70 °C under N$_2$ for 24 h. The reaction mixture was then cooled to room temperature. THF was removed *in vacuo* and the aqueous solution was adjusted to pH 2 with conc. HCl. The resulting precipitate was isolated via filtration, washed with H$_2$O (3 × 3 mL) and acetone (3 × 3 mL) and then dried *in vacuo* to give the desired prodigiosene **2c•HCl** (84 mg, 99%) as a dark red solid. ^{1}H NMR (CDCl$_3$, 500 MHz) 1.31-1.37 (m, 8 H), 1.59-1.69 (m, 4 H), 2.34 (t, *J* = 7.5 Hz, 2 H), 2.50 (s, 3H), 2.73 (t, *J* = 7.5 Hz, 2 H), 2.83 (s, 3 H), 4.01 (s, 3 H), 6.11 (s, 1 H), 6.39-6.40 (1H, m), 7.01-7.02 (m, 1 H), 7.12 (s, 1 H), 7.30 (s, 1 H), 12.66 (bs, 2 H), 12.94 (bs, 1 H). HR-MS (ESI): [M-Cl]$^+$ calcd. For C$_{26}$H$_{34}$N$_3$O$_4$: 452.2544; found 452.2547.

General procedure for ester coupling: Prodigiosene **2** (1.0 eq), the alcohol (1.0 eq.), EDC (1.1 eq.) and DMAP (1.1 eq.) were dissolved in DCM under N$_2$. After stirring at room temperature water was added and the crude mixture was extracted with DCM three times. The combined organic layers were washed with brine, and then dried (Na$_2$SO$_4$). After evaporation of the solvent under *vacuum* the crude solid was purified using column chromatography.

(13*S*,14*S*)-13-Methyl-17-oxo-7,8,9,11,12,13,14,15,16,17-decahydro-6*H*-cyclopenta[a]phenanthren-3-yl 4-((*Z*)-2-((4-methoxy-1*H*,1'*H*-[2,2'-bipyrrol]-5-yl)methylene)-3,5-dimethyl-2*H*-pyrrol-4-yl)-4-oxobutanoate (3a): Using the general ester coupling procedure, prodigiosene **2a** (100 mg, 0.25 mmol) and estrone (100 mg, 0.38 mmol) were reacted for three days in DCM (25 mL). The crude solid was purified using column chromatography (Al$_2$O$_3$ neutral type III, EtOAc/hexane 5/5) to give the title compound as a red solid (35 mg, 49%). M.p. 157 ℃. ^{1}H NMR (CDCl$_3$, 500 MHz) 0.90 (s, 3 H), 1.41-1.64 (m, 7 H), 1.94-2.08 (m, 4 H), 2.10-2.16 (m, 1 H), 2.25-2.30 (m, 1 H), 2.32-2.41 (m, 4 H), 2.45 (s, 3 H), 2.47-2.53 (m, 1 H), 2.88-2.89 (m, 4 H), 3.12 (t, *J* = 6.5 Hz, 2 H), 3.96 (s, 3 H), 6.01 (s, 1 H), 6.26 (s, 1 H), 6.74 (d, *J* = 3.0 Hz, 1 H), 6.83-6.88 (m, 3 H), 6.87-6.88 (m, 1 H), 6.90 (s, 1 H), 7.25-7.27 (m, 1 H). ^{13}C NMR (CDCl$_3$, 125 MHz) 12.6, 14.0, 15.1, 21.7, 25.9, 26.5, 28.7, 29.5, 31.7, 36.0, 37.5, 38.1, 44.3, 48.1, 50.6, 58.7, 95.9, 111.0, 112.0, 113.8, 118.9, 121.7, 122.8, 126.4, 126.6, 137.3, 138.0, 148.8,

168.8, 172.4, 194.8, 7 ^{13}C signals missing. HR-MS (ESI): [M+H]$^+$ calcd. for $C_{38}H_{42}N_3O_5$: 620.3124; found 620.3119.

(13S,14S)-13-Methyl-17-oxo-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthren-3-yl 6-((Z)-2-((4-methoxy-1H,1'H-[2,2'-bipyrrol]-5-yl)methylene)-3,5-dimethyl-2H-pyrrol-4-yl)-6-oxohexanoate (3b): Using the general ester coupling procedure, prodigiosene **2b** (50 mg, 0.11 mmol) and estrone (30 mg, 0.11 mmol) were reacted for two days in DCM (12 mL). The crude solid was purified using column chromatography (Al$_2$O$_3$ neutral type III, EtOAc/hexane 5/5) to give the title compound as a red solid (35 mg, 49%). Rf = 0.28 (EtOAc/hexane 5/5). M.p. 146 ºC. ^{1}H NMR (CDCl$_3$, 500 MHz) 0.90 (s, 3 H), 1.39-1.64 (m, 6 H), 1.78-1.79 (m, 4 H), 1.94-2.07 (m, 3 H), 2.10-2.18 (m, 1 H), 2.24-2.26 (m, 1 H), 2.30 (s, 3 H), 2.37-2.40 (m, 1 H), 2.42 (s, 3 H), 2.50 (dd, J = 9.0, 19.5 Hz, 1 H), 2.26-2.59 (m, 2 H), 2.73-2.75 (m, 2 H), 2.87-2.90 (m, 2 H), 3.98 (s, 3 H), 6.05 (s, 1 H), 6.24-625 (m, 1 H), 6.75-6.85 (m, 4 H), 6.93 (s, 1 H), 7.26 (d, J = 8.0 Hz, 1 H). ^{13}C NMR (CDCl$_3$, 125 MHz) 12.5, 13.9, 14.5, 21.7, 23.7, 24.8, 25.8, 26.4, 29.5, 31.6, 34.4, 36.0, 38.1, 42.4, 44.2, 48.1, 50.5, 58.7, 95.9, 110.9, 112.1, 114.5, 118.9, 121.7, 123.5, 124.0, 126.1, 126.5, 127.6, 130.4, 137.4, 138.1, 142.7, 148.7, 168.9, 172.4, 197.2, 221.0, 2 ^{13}C signals missing. UV (DMSO) λ_{max} (ε): 460 (41 000). HR-MS (ESI): [M+H]$^+$ calcd. for $C_{40}H_{46}N_3O_5$: 648.3432; found 648.3410.

(8R,9S,13S,14S)-13-Methyl-17-oxo-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthren-3-yl-10-((Z)-2-((4-methoxy-1H,1'H-2,2'-bipyrrol-5-yl)methylene)-3,5-dimethyl-2H-pyrrol-4-yl)-10-oxodecanoate (3c): Using the general ester coupling procedure, prodigiosene **2c** (35 mg, 0.072 mmol) and estrone (20 mg, 0.072 mmol) were reacted for two days in DCM (7 mL). The crude solid was purified using column chromatography (SiO$_2$, EtOAc/hexane 10/90 to 40/60) to give the title compound as a red film (30 mg, 50%). Rf = 0.15 (EtOAc/hexane 2/1). ^{1}H NMR (CDCl$_3$, 500 MHz) 0.90 (s, 3 H), 1.36-1.74 (m, 18 H), 1.93-2.18 (m, 6 H), 2.24-2.30 (m, 1 H), 2.44-2.46 (m, 6 H), 2.52 (t, J = 7.5 Hz, 2 H), 2.70 (t, J = 7.5 Hz, 2 H), 2.88-2.91 (m, 2 H), 3.99 (s, 3 H), 6.04 (bs, 1 H), 6.30 (bs, 1 H), 6.79-6.85 (m, 3 H), 6.96 (bs, 1 H), 7.02 (bs, 1 H), 7.28 (d, J = 8.5 Hz, 1 H). ^{13}C NMR (CDCl$_3$, 125 MHz) 12.6, 13.9, 21.7, 24.2, 25.0, 25.1, 25.9, 26.4, 29.1, 29.2, 29.5, 31.6, 34.5, 35.9, 38.1, 41.4, 42.9, 44.2, 48.1, 50.5, 58.8, 60.5, 95.2, 111.4, 112.1, 115.9, 118.9, 121.7, 123.9, 124.0, 125.5, 125.6, 126.5, 137.3, 138.1, 143.7, 143.8, 148.7, 168.1, 171.3, 172.7, 179.3, 197.9, 220.9. HR-MS (ESI): [M+H]$^+$ calcd. for $C_{44}H_{54}N_3O_5$: 704.4058; found 704.4056. UV (DMSO) λ_{max} (ε): 461 (30 000).

4-((E)-1-(4-(2-(Dimethylamino)ethoxy)phenyl)-2-phenylbut-1-en-1-yl)phenyl 4-((Z)-2-((4-methoxy-1H,1'H-[2,2'-bipyrrol]-5-yl)methylene)-3,5-dimethyl-2H-pyrrol-4-yl)-4-oxobutanoate (4a): Using the general ester coupling procedure, prodigiosene **2a** (34 mg, 0.08 mmol) and (Z)-4-hydroxtamoxifen (34 mg, 0.08 mmol) were reacted for two days in DCM (10 mL). The crude solid was purified using column chromatography (Al$_2$O$_3$ neutral type III, DCM/MeOH 9.5/0.5) to give the title commpound as an orange-red solid (45 mg, 76%): ^{1}H NMR (CD$_2$Cl$_2$, 500 MHz) 0.88-0.91 (m, 3 H), 1.25 (s, 4 H), 2.22 (s, 6 H), 2.44 (t, J = 3.7 Hz, 4 H), 2.58 (t, J = 5.8 Hz, 2 H), 2.87 (t, J = 6.4 Hz, 2 H), 3.13 (t, J = 6.4 Hz, 2 H), 3.89 (t, J = 4.8 Hz, 2 H), 3.95 (s, 3 H), 6.05 (s, 1 H), 6.26 (dd, J = 4.8, 2.6 Hz, 2 H), 6.53-6.56 (m, 2 H), 6.75-6.77 (m, 2 H), 6.78 (t, J = 2.6 Hz, 1 H), 6.87 (s, 1 H), 6.93 (s, 1 H), 7.06-7.09 (m, 2 H), 7.11-7.13 (m, 3 H), 7.16-7.19 (m, 2 H), 7.21-7.24 (m, 2 H). ^{13}C NMR (CD$_2$Cl$_2$, 125 MHz) 12.6, 13.6, 29.0, 29.3, 37.8, 45.9, 58.5, 58.9, 66.2, 66.3, 66.4, 96.0, 111.1, 112.1, 113.6, 113.9, 116.6, 121.6, 123.2, 126.4, 128.1, 130.0, 130.6, 132.1, 135.7, 137.8, 141.6, 142.2, 142.7, 149.9, 157.3, 172.2, 194.8, 6 ^{13}C signals missing. HR-MS (ESI): [M+H]$^+$ calcd. for $C_{46}H_{49}N_4O_5$: 737.3703; found 737.3697.

4-((Z)-1-(4-(2-(Dimethylamino)ethoxy)phenyl)-2-phenylbut-1-en-1-yl)phenyl 6-((Z)-2-((4-methoxy-1H,1'H-[2,2'-bipyrrol]-5-yl)methylene)-3,5-dimethyl-2H-pyrrol-4-yl)-6-oxohexanoate (4b): Using the general ester coupling procedure, prodigiosene **2b** (50 mg, 0.11 mmol) and (Z)-4-hydroxtamoxifen (43 mg, 0.11 mmol) were reacted for one day in DCM (12 mL). The crude solid was

purified using column chromatography (Al$_2$O$_3$ neutral type III, DCM/MeOH 9.5/0.5) to give the title compound as an orange-red solid (51 mg, 85%). Rf = 0.39 (CH$_2$Cl$_2$/MeOH 19/1). ^{1}H NMR (MeOD, 500 MHz) 0.88 (t, J = 7.5 Hz, 3 H), 1.82-1.85 (m, 4 H), 2.30 (s, 6 H), 2.42 (s, 3 H), 2.43-2.45 (m, 2 H), 2.64-2.65 (m, 2 H), 2.68 (s, 3 H), 2.68-2.71 (m, 2 H), 2.88-2.89 (m, 2 H), 3.92 (s, 3 H), 3.95 (t, J = 5.5 Hz, 2 H), 6.1 (s, 1 H), 6.27 (dd, J = 3.0, 2.5 Hz, 1 H), 6.55 (d, J = 9.0 Hz, 2 H), 6.74 (d, J = 9.0, 1.0 Hz, 2 H), 6.77-6.78 (m, 1 H), 6.79 (s, 1 H), 6.99 (d, J = 8.0 Hz, 2 H), 7.03 (bs, 1 H), 7.08-7.10 (m, 3 H), 7.14-7.20 (m, 4 H). ^{13}C NMR (MeOD, 125 MHz) 11.8, 13.2, 15.4, 23.1, 24.1, 28.5, 33.5, 41.4, 45.4, 57.6, 58.4, 65.4, 96.3, 110.2, 121.6, 122.3, 122.9, 126.4, 127.5, 127.9, 128.2, 129.3, 129.9, 131.3, 134.7, 137.1, 140.6, 140.8, 141.1, 141.7, 142.2, 149.1, 156.4, 159.4, 167.3, 171.7, 196.3, 4 ^{13}C signals missing. UV (DMSO) λ_{max} (ε): 460 (48 000). HR-MS (ESI): [M+H]$^+$ calcd. for C$_{48}$H$_{53}$N$_4$O$_5$: 765.4035; found 765.4010.

4-((*E*)-1-(4-(2-(Dimethylamino)ethoxy)phenyl)-2-phenylbut-1-enyl)phenyl-10-((*Z*)-2-((4-methoxy-1*H*,1'*H*-2,2'-bipyrrol-5-yl)methylene)-3,5-dimethyl-2*H*-pyrrol-4-yl)-10-oxodecanoate (4c): Using the general ester coupling procedure, prodigiosene **2c** (44 mg, 0.09 mmol) and (*Z*)-4-hydroxtamoxifen (35 mg, 0.09 mmol) were reacted for one day in DCM (9 mL). The crude solid was purified using column chromatography (Al$_2$O$_3$ neutral type III, DCM/MeOH 99/1 to 90/10) to give the title compound as a deep red solid (33 mg, 45%). Rf = 0.40 (DCM/MeOH 9/1). ^{1}H NMR (CD$_2$Cl$_2$, 500 MHz) 0.90 (t, J = 7.5 Hz, 3 H), 1.33-1.43 (m, 8 H), 1.62-1.66 (m, 2 H), 1.71-1.77 (m, 2 H), 2.23 (s, 6 H), 2.42 (s, 3 H), 2.43-2.47 (m, 2 H), 2.55 (t, J = 7.5 Hz, 2 H), 2.59-2.61 (m, 5 H), 2.69 (t, J = 7.5 Hz, 2 H), 3.90 (t, J = 7.5 Hz, 2 H), 3.98 (s, 3 H), 6.10 (s, 1 H), 6.34 (q, J = 4.0, 2.5 Hz, 1 H), 6.55 (dt, J = 9.0, 2.5 Hz, 2 H), 6.78 (dt, J = 9.0, 2.5 Hz, 2 H), 6.91 (dd, J = 4.0, 1.0 Hz, 1 H), 7.01 (s, 1 H), 7.06 (dt, J = 8.5, 2.5 Hz, 2 H), 7.11-7.14 (m, 4 H), 7.15-7.19 (m, 2 H), 7.23 (dt, J = 8.5, 2.5 Hz, 2 H), 11.93 (bs, 1 H) ppm. ^{13}C NMR (CD$_2$Cl$_2$, 125 MHz) 12.5, 13.6, 15.4, 24.4, 25.3, 29.3, 29.4, 29.5, 29.7, 29.8, 34.6, 43.3, 45.9, 58.5, 59.2, 66.1, 94.4, 112.2, 112.7, 113.6, 117.4, 121.6, 124.7, 124.8, 126.4, 126.7, 128.1, 130.0, 130.6, 132.1, 135.8, 137.7, 141.6, 142.2, 142.7, 146.4, 149.8, 153.6, 157.3, 167.7, 172.6, 197.5, 3 ^{13}C signals missing. HR-MS (ESI): [M+H]$^+$ calcd. for C$_{52}$H$_{61}$N$_4$O$_5$: 821.4636; found: 821.4649. UV (in DMSO) λ_{max} (ε): 460 (30 000).

Procedure A: To a solution of prodigiosene **2** (50 mg) in DMF (6 mL) was added COMU (1.5 eq.) and DBU (3 eq.) at room temperature under N$_2$. After 30 min, TPP-NH$_2$ (1.2 eq.) was added and the reaction mixture was stirred for 14 h. Water (20 mL) was added and the mixture was extracted with ethyl acetate (3 × 20 mL). The combined organic layers were washed with water (2 × 40 mL) and brine (50 mL) and then dried (Na$_2$SO$_4$). After removal of the solvent under reduced pressure the crude product was purified using column chromatography (Al$_2$O$_3$ type III basic, DCM 100% then EtOAc/hexane 5/5). After removal of the solvent under reduced pressure, the purple solid was triturated with methanol then filtered using Millipore® apparatus and then washed with methanol to give a purple solid.

Procedure B: To a suspension of the PS-carbodiimide resin (2 eq.) in DMF (0.30 mL) was added prodigiosene **2** (50 mg) under N$_2$. The mixture was stirred for 10 minutes, then TPP-NH$_2$ (1 eq.) and DMAP (3 eq.) were added and the reaction mixture was stirred for 24 h. Analysis of the reaction mixture using TLC showed incompletion so DMF (0.25 mL) was added. After 24 h further stirring, the reaction mixture was filtered and the polymer was washed with DCM. Water (20 mL) was added to the filtrate and the mixture was extracted with ethyl acetate (3 × 20 mL). The combined organic layers were washed with water (3 × 40 mL) and brine (50 mL) and then dried (Na$_2$SO$_4$). The crude solid was purified using column chromatography (Al$_2$O$_3$ type III basic, DCM/hexanes 5/5, then MeOH/DCM /hexane 5/47.5/47.5). After removal of the solvent under reduced pressure, the purple solid was triturated with methanol then filtered using Millipore® apparatus and then washed with methanol to give a purple solid.

Prodigiosene-NH-TPP conjugate 6a: According to procedure B and using **2a** (40 mg, 0.099) the title compound was obtained as a purple solid (30 mg, 34% yield). ^{1}H NMR (DMSO, 500 MHz) -2.92 (s, 2H), 2.36 (s, 3H), 2.45 (t, J = 6.0 Hz, 2H), 2.64 (t, J = 6.0 Hz, 2H), 2.80 (s, 3H), 3.89 (s, 3H), 6.21 (s, 1H), 6.26 (s, 1H), 6.75 (s, 1H), 6.81 (s, 1H), 7.16 (s, 1H), 7.82-7.85 (bs, 7H), 8.10 (d, J = 8.7 Hz, 2H), 8.16 (d, J = 8.7 Hz, 2H), 8.22-8.24 (bs, 5H), 8.83-8.91 (m, 9H), 10.49 (s, 1H), 11.87 (s, 1H). Considering the poor solubilty of this compound ^{13}C NMR data is not provided. HR-MS (ESI): [M+H][+] calcd. for $C_{64}H_{51}N_8O_3$: 979.4079; found 979.4043.

Prodigiosene-NH-TPP conjugate 6b: According to procedure A, the title compound was obtained as a purple solid (55 mg, 50%). Mp: 206 °C. ^{1}H NMR (CD$_2$Cl$_2$, 500 MHz) -2.85 (s, 2 H), 1.89-1.90 (m, 4 H), 2.48 (s, 3 H), 2.57-2.60 (m, 5 H), 2.91 (t, J = 6.0 Hz, 2 H), 3.89 (s, 3 H), 5.97 (s, 1 H), 6.29 (dd, J = 3.5, 2.7 Hz, 1 H), 6.73 (dd, J = 3.5, 1.5 Hz, 1 H), 6.89 (s, 1 H), 6.96 (bs, 1 H), 7.74-7.81 (m, 9 H), 8.08 (d, J = 6.5 Hz, 2 H), 8.17 (d, J = 6.5 Hz, 2 H), 8.20-8.22 (m, 6 H), 8.51 (bs, 1 H), 8.85 (m, 5 H), 8.91 (d, J = 5.0 Hz, 2 H). ^{13}C NMR (CD$_2$Cl$_2$, 125 MHz) 12.6, 12.4, 23.6, 25.4, 37.9, 42.5, 58.9, 95.9, 111.1, 112.0, 113.8, 118.2, 120.2, 120.5, 123.1, 123.4, 125.2, 126.9, 127.0, 128.0, 128.4, 131.5, 134.9, 135.3, 137.7, 138.9, 142.2, 142.4, 169.0, 171.8, 197.9. HR-Ms (ESI): [M+H][+] calcd. for $C_{66}H_{55}N_8O_3$: 1007.4392; found: 1007.4424.

Prodigiosene-NH-TPP conjugate 6c: This compound was obtained according to both procedure A (41 mg, 40 % yield) and procedure B (43 mg, 42% yield). ^{1}H NMR (DMSO, 500 MHz) -2.92 (s, 2H), 1.35 (m, 8H), 1.59 (m, 2H), 1.73 (m, 2H), 2.31 (s, 3H), 2.48 (t, J = 7.5 Hz, 2H), 2.67 (s, 3H), 2.71 (t, 2H), 3.82 (s, 3H), 6.18 (s, 1H), 6.24 (s, 1H), 6.67 (s, 1H) 6.79 (s. 1H), 7.13 (s, 1H), 7.81-7.82 (m, 7H), 8.08 (d, J = 8.0 Hz, 2H), 8.15 (d, J = 8.5 Hz, 2H), 8.21-8.22 (m, 5 H), 8.82-8.89 (m, 9H), 10.34 (s, 1H), 11.84 (s, 1H). ^{13}C NMR (DMSO, 125 MHz) 11.8, 15.4, 23.8, 24.5, 25.3, 28.7, 28.8, 28.9, 29.0, 36.7, 41.9, 58.4, 96.3, 110.0, 113.2, 117.4, 119.9, 120.0, 120.1, 122.4, 126.3, 127.0, 128.1, 131.6, 132.7, 134.2, 134.7, 135.6, 139.4, 141.2, 159.4, 167.3, 171.8, 196.6. HR-MS (ESI): [M+H][+] calcd. for $C_{70}H_{63}N_8O_3$: 1063.5018; found 1063.5013.

Acknowledgements

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Graphical abstract

X = O, NH
R = porphyrin, estrogen, tamoxifen