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Synthesis and antiproliferative activity of coumarin-estrogen conjugates against breast cancer cell lines

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Abstract

The syntheses and cytotoxic activity of coumarin-estrogen conjugates are described. *In vitro* results indicated that conjugates **10, 11** and **13** show growth inhibitory activities at 5-dose concentration (100, 10, 1, 0.1, 0.01 μM) against the following NCI-7- human breast cancer cell lines: BT-549, HS 578T, MCF 7, MDA-MB-231/ATCC, MDA-MB-435, NCI/ADR-RES, and thus serve as new leads for further development of antibreast cancer agent.

Keywords

coumarin; 17β-estradiol; conjugates; cytotoxicity; antiproliferative activity and breast cancer

Introduction

Breast cancer is the second leading cause of cancer death in American women behind lung cancer [1]. Breast, ovary and gonads produce an abundance of estrogens *via* the aromatase and sulphatase pathways [2]. Estrogens stimulate the proliferation of normal and malignant cells in these organs through “estrogen receptor (ER)” *via* the induction of nucleic acid synthesis and activation of growth regulatory genes. Post-menopausal women, whose production of ovarian estrogen has ceased with estrogens originating in extra-glandular tissues, accounted for approximately 80% of all breast cancer cases [3]. It has been reported that about one-third of all postmenopausal breast cancer cases are hormone-dependent, which involve the stimulation of cancer cell proliferation by estrogens [4,5]. Thus, the new approach for the successful treatment of postmenopausal breast cancers (hormone-dependent) involves the use of therapeutic agents that prevent the biosynthesis or physiological action of estrogen on tumor cells [6].

There is an over-expressed ER in breast tumor cell in the earlier stage of breast cancer and during hormonal treatment [7]. The non-selectivity and acute toxicity of many antitumor agents have been the major deterrent in their usage for treating human cancer [8]. Among the current cancer therapy focusing on the improvement of drug selectivity, conjugation of cytotoxic drug components to a carrier with either selectivity toward the tumors or the tumor tissues has proven

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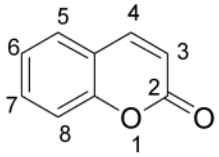
to be an effective strategy in the development of efficient antitumor drugs with high therapeutic indices [9-12]. Estradiol (E2) has served as a framework for the attachment of various substituents; e.g. cytotoxic moieties, radioisotopes, dietary antioxidants, affinity and photoaffinity-labeling groups, of which several E2 conjugates have advanced as synthetic ligands for therapeutic applications targeting the ER [13-15]. Studies have shown that conjugated groups provide biomolecules with novel properties, such as catalytic activity, altered hydrophobicity or bioaffinity [16]. During the past decades, the application of bioconjugates (i.e. biomolecules bearing unnatural organic structures) in molecular and cell biology has significantly increased [17]. Furthermore, studies involving the concept of ER-targeting have generated ligands with high affinity for calf-uterine ER and selective toxicity for the ER-positive cell line MCF-7.

Coumarins, naturally occurring benzofurans, exhibit useful and diverse biological activities. In recent years, there has been a growing interest in their synthesis and possible applications for drug discovery. Coumarin derivatives have been found to be useful in photochemotherapy, antitumor and anti-HIV therapy [18,19], and as stimulants for the central nervous system (CNS) [20], antibacterial [21,22], anti-inflammatory [23], anti-coagulants [24], and dyes [25]. Among the many pharmacological actions of coumarins, antioxidant and antiproliferative actions are the most extensively examined. The antiproliferative coumarins display both cytostatic and cytotoxic activities [26,27]. For example, coumarin (**1**) and its active metabolite, 7-hydroxycoumarin (**2**), demonstrated growth-inhibitory (cytostatic) in human malignant cell lines, such as A549 (lung), ACHN (renal), H727 (lung), MCF-7 (breast) and HL-60 (leukemia) [28,29], and have also demonstrated activity against prostate cancer, malignant melanoma, and metastatic renal cell carcinoma in clinical trials [30-32].



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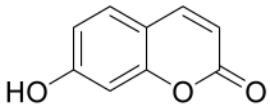

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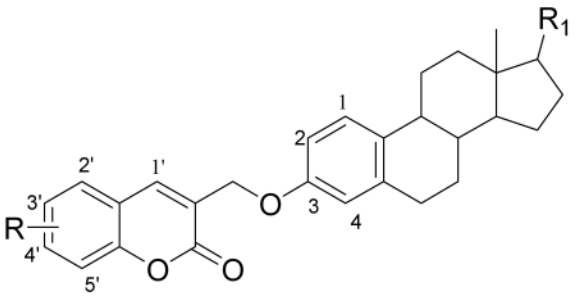
In the present study, we hypothesize based on the novel concept of biconjugation that the conjugation of antitumor coumarin to estrogen would result in potential agents with selective antiproliferative effect in the breast tissues. The combination of antitumor coumarin to estrogen, a carrier with strong affinity for the ER, would mediate selective delivery of the coumarin to the cells or tissues bearing a high concentration of ERs. This in turn would result in better penetration and selective cytotoxicity of coumarin towards ER enriched breast tumor cells. Previous studies had shown that coupling of cytotoxic porphyrins with 17β-estradiol resulted in improved antitumor activity in the target tissues as the result of sufficient binding to the ER, allowing selective accumulation of the conjugates in ER-rich cells [33-36].

Results and Discussion

The coumarin quaternary salts (**4-6**) were prepared following literature methods [37,38] *via* the intramolecular Baylis-Hillmann reaction using 1,4-diazabicyclo [2.2.2] octane (DABCO) as a catalyst, which were then coupled to 17β-estradiol (7) and estrone oxime (**9**) to afford conjugates **10-13** (Scheme 1).

The 17β-estradiol (7) was dissolved in acetonitrile and treated with K_2CO_3. The mixture was stirred for 45 mins; followed by addition of coumarin *quaternary salt* (**4**) and then subsequently refluxed overnight to afford product, **10** (60% yield) with a molecular ion at m/z 431.27 (M^+ +1) corresponding to the coupled conjugate. The ^{1}H NMR spectrum of conjugate **10** shows a

singlet at δ 5.00 ppm corresponding to OCH_2- linkage involving the coumarin and the hydroxyl group at the C-3 of the A ring of 17β-estradiol. Other characteristic peaks between δ 6.75- 7.92 ppm correspond to the coumarin five aromatic protons (C-1′ - C-5′) and the C-1, C-2 and C-4 protons of the A ring of 17β-estradiol. None of the conjugates showed any C-17 hydroxy peak when the spectra were recorded in $CDCl_3$ as NMR solvent has indicated in the experimental section. However, the ^{1}H NMR spectrum of conjugate 12 in acetone-d_6 showed the presence of the C-17 hydroxyl peak at δ 3.50ppm, which disappeared upon the addition of D_2O.



10, R = H R_1 = -OH

Using the same reaction condition we coupled the 17β-estradiol (7) with coumarin *quaternary salts* (**5** & **6**) to yield the corresponding conjugates (**11** & **12**). Furthermore, conjugate **13** was prepared by coupling coumarin *quaternary salt* 5 with estrone oxime (**9**), generated by reacting estrone (**8**) with hydroxylamine in THF.

These conjugates were purified using preparative plate chromatography and recrystallized from acetone (in overall 65% isolated yields), characterized using ^{1}H NMR, ^{13}C NMR, and elemental and MS analyses and then tested for antiproliferative activities.

Cytotoxicity

The initial tests conducted by our research group using MTT assay [39,40] at concentration range 100 μm-0.1 nm with conjugate **11** in the presence of 10 nm concentration of estradiol demonstrated significant antiproliferative activity in the human breast cancer MCF-7 cells (GI_{50} = 9.55 μM), comparable to that of 4-hydroxytamoxifen (GI_{50} = 9.33 μM) (Figure 1). Estradiol was used to do competitive growth inhibitory studies mimicking physiological estradiol concentration. This initial result intrigued us to conduct further studies against series of cell lines in the National Cancer Institute Developmental Therapeutics (NCIDT) program to determine three dose-dependent parameters (GI_{50}, TGI, and LC_{50}) for conjugates **10-13**. GI_{50} is the concentration of the drug resulting in 50% growth reduction in comparison to the untreated control, LC_{50} is concentration of the compound leading to the 50% of net cell death following treatment, and TGI is the drug concentration resulting in total growth inhibition.

The *in-vitro* testing results obtained from NCIDT program demonstrated that the conjugates **10-13** possess antiproliferative activities against the following full NCI-7 cell panels: (BT-549, HS 578T, MCF 7, MDA-MB-231/ATCC, MDA-MB-435, NCI/ADR-RES and T-47D) at single dose tested (10 μM). These cell lines differ in term of their pathology, and all are classified as invasive ductal carcinoma cell lines, except NCI/ADR-RES and MDA-MB-231/ATCC, which are adenocarcinoma cell lines. This classification is primarily based on the observed gene expression corresponding to the tumor from which the cell lines were derived. All of the above conjugates, except conjugate **12**, were selected for 5-dose testing since they satisfied the pre-determined threshold inhibition criteria (single dose, 10 μM), which were

designed to efficiently capture compounds with anti-proliferative activity. The data for the 5-dose testing (100, 10, 1, 0.1, 0.01 μM) is reported in (Table 1).

Comparisons of the GI_{50} values among the conjugates **10, 11** and **13** showed that conjugate **10** displayed the highest antiproliferative activity against MDA-MB-435, conjugate **11** against **ER**-enriched MCF-7, and conjugates **11** and **13** against MDA-MB-231/ATCC breast cancer cell lines. As far as the distinction between noninvasive and invasive breast cancer cell lines, overall conjugates **11 and 13** appear to be active against both cell types while conjugate 10 was surprisingly inactive against MDA-MB-231/ATCC (noninvasive), MCF-7 (Invasive) and T-47D (Invasive) breast cancer cell lines. In general, it was shown that cytotoxicity occurred at around 100 μM for all of the conjugates. Furthermore, conjugate **11** displayed the most cytostatic properties based upon GI_{50} values being less than LC_{50} values.

Conclusion

The synthesis and cytotoxic activities of a new group of coumarin-estrogen conjugates as antiproliferative agents have been described. Initial preliminary activity results demonstrated that conjugate **11** possesses significant antiproliferative and growth inhibitory activities in MCF-7 cell at 100 μm-0.1 nm concentration range comparable to that of the active metabolite of the clinically used drug, 4-hydroxytamoxifen. The *in vitro* results obtained from the NCI have validated this result by showing that conjugates **10**, **11** and **13** possess significant growth inhibitory activities against panel of breast cancer cell lines. On the basis of these results, conjugates **11** and **13** could be considered as attractive leads for the further development of anti-breast cancer agents.

Experimental

General

Commercial grade solvents and reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA) or Alfa Aesar (Ward Hill, MA, USA) and used without further purification. NMR spectra were recorded on Varian 300 MHz spectrometer. The appropriate deuterated solvents are indicated in the procedure, and line positions recorded in *ppm* from the reference signal. Infrared (IR) spectra were recorded on Fourier Transformation Infrared (FTIR-8400S) spectrometer (SHIMADZU, Japan). Elemental analyses were carried out by Atlantic Microlab, Inc., Norcross. GA. ESI-TOF Mass Spectrometer was recorded on Agilent 6210 TOF with 1200 HPLC using fast atom bombardment (FAB), (TOF H^+). Melting points were determined on a Gallenkamp (UK) apparatus and are uncorrected. The purification of the conjugates was carried out using preparative plate chromatography obtained from Analtech (Newark, DE, USA).

Synthesis of coumarins quaternary ammonium salt (6)

The preparation and characterization of coumarins quaternary ammonium salt (**4** & **5**) have been reported previously [37,38] and the same method was utilized in the present experiment to synthesize the compound (**6**).

To a suspension of NaH (50% dispersion in oil; 0.86 g, 18 mmol) in dry THF (15 ml) under nitrogen was added 3-hydroxynaphthalene-2-carbaldehyde (2.36 g, 13.7 mmol) and the resulting mixture was boiled under reflux for 1h to generate the anion. To the cooled solution of the anion, acryloyl chloride (1.45 ml, 17.9 mmol) in dry THF (15 ml) was added dropwise with stirring, and the reaction mixture was boiled under reflux for 3 h. The reaction was quenched by the addition of water (20 ml) and the resulting mixture extracted with diethyl ether. The organic layer was washed with saturated brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo* to afford, as yellow oil, 2-formylnaphthalen-3-yl acrylate (2.04 g, 66%).

To a solution of 2-formylnaphthalen-3-yl acrylate (2.48 g, 11 mmol) in dry dichloromethane (23 ml) at −10 °C was added DABCO (1.27 g, 11 mmol), and the stirred solution was allowed to warm to rt over several hours. The resulting precipitate was filtered off, washed with dichloromethane and recrystallized from methanol and dichloromethane to afford, as a pale yellow solid, coumarin quaternary ammonium salt (**6**)

Compound (6)—(2.01 g, 51%), m.p. < 283.8 °C (dec.); ^{1}H-NMR (300 MHz, CD_3OD) 3.18-3.30 [m, 6H, $(CH_2)_3N^+$], 3.50-3.61 [m, 6H, $(CH_2)_3N^+$], 4.55 (2H, s, CH_2), 7.46 (d, *J* 9 Hz, 1H), 7.61(t, *J* 7.5 Hz, 1H), 7.75(t, *J* 7.4 Hz, 1H), 7.97(d, *J* 8.1 Hz, 1H), 8.16 (d, *J* 9.3 Hz, 1H), 8.53 (d, *J* 8.7 Hz, 1H), 9.26 (s, 1H); ^{13}C-NMR (75MHz, CD_3OD) 45.1 ($NCH_2CH_2N^+$), 52.7 ($^+NCH_2CH_2N$), 63.1 (CH_2N^+), 113.1, 114.1, 116.3, 122.1, 126.6, 129.0, 129.4, 130.7, 135.6, 147.4 and 155.0 (Ar-C) and 161.9 (C=O); HR-MS (ESI-MS) (m/z): calcd for $C_{20}H_{21}O_2N_2^+$, 321.1603: found, 321.1601; analysis (% calcd, % found for $C_{20}H_{21}O_2N_2Cl$): C (67.32, 77.42), H (5.93, 6.66).

Synthesis of Coumarin-17β-estradiol conjugates (10-12)

To a mixture of estradiol (7) (0.554 mmol, 150 mg) and K_2CO_3 (2.215 mmol, 300 mg) in acetonitrile (10 ml) was added the coumarin quaternary ammonium salt **(4)** (0.665 mmol, 198.8 mg). The mixture was refluxed overnight, allowed to cool to rt and the solid was removed by filtration. The filtrate was collected and acetonitrile removed *in vacuo* to afford clear yellow oil residue that was purified by preparative plate chromatography [elution with Dichloromethane / Ethyl acetate (5:1)] and then recrystallized using acetone to afford the conjugate **10**. The quaternary ammonium salts, **5** and **6**, were used to afford conjugates **11** and **12**, respectively, following this methodology.

Conjugate 10—(120 mg, 60%) as white solid, m.p. = 234-236 °C. γ_{max} (KBr)/cm^{-1} 1712.7 (C=O), and 2923.9 (CH_2); ^{1}H-NMR (300 MHz, $CDCl_3$) 0.80 (s, 3H, 13-CH_3), 1.18-2.40 (m, 13H), 2.84-2.88 (m, 2H, 6-C*H*$_2$-), 3.70-3.76 (t, *J* 7.8 and 9 Hz, 1H, 17α-CH), 5.00 (s, 2H, OC*H*$_2$-), 6.75(d, *J* 2.7 Hz, 1H), 6.80-6.84 (dd, *J* 2.7 Hz, 1H), 7.22-7.38 (overlapping signals, 3H), 7.50-7.60 (overlapping signal, 2H), and 7.92 (s, 1H); ^{13}C-NMR (75MHz, $CDCl_3$) 11.27 (*C*H_3), 23.36, 26.55, 27.43, 30.00, 30.85, 36.97, 39.05, 64.68 (-CH_2), 43.50, 44.21, 50.34, 82.11 (*C*H-), 112.50, 114.87, 116.83, 119.35, 124.76, 125.40, 126.76, 128.13, 131.51, 133.86, 138.55, 138.68, 153.38, 156.19, 160.47 (Ar-C and C=O); HR-MS (ESI-MS) (m/z): $[M+H]^+$ calcd for $C_{28}H_{31}O_4^+$, 431.2222: found, 431.2217; analysis (% calcd, % found for $C_{28}H_{30}O_4$): C (78.11,74.24), H (7.02, 6.76).

Conjugate 11—(158.2 mg, 62%) as white solid, m.p. = 211-212 °C. γ_{max} (KBr)/cm^{-1} 1713.09 (C=O) and 2930.41(CH_2); ^{1}H-NMR (300 MHz, $CDCl_3$) 0.78 (s, 3H, 13-C*H*$_3$), 1.18-2.34 (m, 13H), 2.85-2.88 (m, 2H, 6-C*H*$_2$-), 3.37 (d, *J* 5.7 Hz, 1H, 17α-CH), 3.98 (s, 3H, -OCH_3), 5.01 (2H, s, OCH_2-), 6.75 (d, *J* 2.4 Hz, 1H), 6.79 (dd, *J* 2.4 and 3 Hz, 1H), 7.06-7.11 (t, *J* 7.5 Hz, 2H), 7.20-7.26 (2H), and 7.90 (s, 1H); ^{13}C-NMR (75MHz, $CDCl_3$) 11.6 (-CH_3), 56.24 (OCH_3), 23.11, 26.29, 27.18, 29.80, 30.58, 36.67, 64.31 (-CH_2), 38.76, 42.25, 43.95, 50.0, 81.90 (*C*H-), 112.23, 113.14, 114.52, 119.30, 119.70, 124.45, 125.31, 126.57, 133.53, 138.32, 138.51, 142.72, 147.13, 155.87, 159.76 (Ar-C and C=O); HR-MS (ESI-MS) (m/z): $[M+H]^+$ calcd for $C_{29}H_{33}O_5^+$, 461.2328: found, 461.2325; analysis (% calcd, % found for $C_{29}H_{32}O_5$): C (75.63, 75.11), H (7.00, 7.05).

Conjugate 12—(160 mg, 60%) as white solid, m.p. = 198-200 °C. γ_{max}(KBr)/cm^{-1} 1712.67 (C=O) and 2908.45(CH_2); ^{1}H-NMR (300 MHz, $CDCl_3$) 0.78 (s, 3H, 13-CH_3), 1.36-2.36 (m, 13H), 2.85-2.91 (m, 2H, 6-CH_2-), 3.71-3.76 (t, *J* 8.1 Hz, 1H, 17α-CH), 5.01 (2H, s, OCH_2-), 6.87 (s, 1H), 6.88-6.91 (d, *J* 4.8 Hz, 1H), 7.26-7.28 (t, *J* 4.8 and 3.6 Hz 1H), 7.511(d, *J* 9 Hz, 1H), 7.55-7.60 (t, *J* 7.5 and 7.8 Hz, 1H), 7.68-7.73 (t, *J* 6.9 and 8.4 Hz, 1H,), 7.92 (d, *J* 19.2

Hz, 2H), 7.99 (d, *J* 9.0 Hz, 2H), 8.33 (d, *J* 8.4, 1H) and 9.71 (s, 1H,); ^{13}C-NMR (75MHz, $CDCl_3$) 11.06 (-CH_3), 23.11, 26.29, 27.19, 29.81, 30.55, 36.67, 64.58 (-CH_2), 38.74, 43.95, 49.99, 81.90 (*C*H-), 112.35, 113.31, 114.66, 116.76, 121.81, 124.09, 126.10, 126.61, 128.16, 128.96, 129.02, 130.37, 132.67, 133.63, 134.38, 138.35, 152.64, 155.93, 160.43 (Ar-C and C=O); HR-MS (ESI-MS) (m/z): $[M+H]^+$ calcd for $C_{32}H_{33}O_4^+$, 481.2379: found, 481.2361; analysis (% calcd, % found for $C_{32}H_{32}O_4$): C (79.97, 77.42), H (6.71, 6.66).

Synthesis of Coumarin-17-oximino-estradiol conjugates (Conjugate 13)

A solution of estrone (1.0 mmol), hydroxylamine hydrochloride (0.14 g, 2.0 mmol) and pyridine (0.16 ml, 2.0 mmol) in 10 ml absolute methanol was refluxed for 3.5 h. After cooling, the solvent was removed, following by addition of 10 ml water to afford a white precipitate, **9** that was collected using vacuum filtration (60% yield). To a mixture of 9 (0.554 mmol, 158 mg) and K_2CO_3 (2.215 mmol, 300 mg) in acetonitrile (10 ml) was added coumarin quaternary ammonium salt **4** (0.665 mmol, 198.8 mg). The mixture was refluxed overnight. Upon cooling the solid was removed by filtration. The filtrate was collected and acetonitrile removed *in vacuo* to a afford clear yellow oil residue that was purified by preparative plate chromatography [elution with DCM / EtOAc (5:1) and then recrystallization using acetone to afford the conjugate 13 (64% yield).

Conjugate 13—(168 mg, 64%) as white solid, m.p. = 216-217 °C. γ_{max}(KBr)/cm^{-1} 1712.67 (C=O) and 2923.88 (CH_2); ^{1}H-NMR (300 MHz, $CDCl_3$) 0.96 (s, 3H, 13-C*H*$_3$), 1.26-2.58 (m, 13H), 2.88 (m, 2H, 6-C*H*$_2$-), 3.98 (s, 3H, -OCH_3), 5.01 (2H, s, OCH_2-), 6.75 (d, *J* 2.7 Hz, 1H), 6.82 (dd, *J* 2.4 Hz, 1H), 7.06-7.10 (1H), 7.20-7.26 (overlapping signals, 3H), and 7.88 (s, 1H); ^{13}C-NMR (75MHz, $CDCl_3$) 17.43 (-CH_3), 56.52 (OCH_3), 23.13, 25.24, 26.42, 27.42, 29.91, 34.31, 38.35, 64.63 (-CH_2), 44.26, 44.47, 53.17, (*C*H-), 112.64, 113.57, 114.83, 119.60, 120.01, 124.62, 125.61, 126.72, 133.48, 138.33, 138.76, 147.45, 156.27, 159.94, 162.0 and 171.59 (Ar-C and C=O); HR-MS (ESI-MS) (m/z): $[M+H]^+$ calcd for $C_{29}H_{32}NO_5^+$, 474.2280: found, 474.2238; analysis (% calcd, % found for $C_{29}H_{31}NO_5$): C (73.55, 71.99), H (6.60, 6.49).

Cytotoxicity Studies [41,42]—MCF-7 (purchased from ATCC) human breast cancer cells were cultured in phenol red-free MEM (500 mL) supplemented with L-glutamine (5 mL), sodium pyruvate (5 mL), MEM non-essential amino acids (5 mL), pen/strep (5 mL), human insulin (1.25 mL) and 10% fetal bovine serum (50 mL). The MCF-7 cells were propagated in 75-cm^2 flask under 37 °C air with 5% CO_2 with media change every 3-4 days. Once confluent, the media was removed and cells washed with phosphate buffer solution (PBS). The cells were then detached using 2 mL of 0.25% trypsin/EDTA solution (incubation 5–10 minutes) followed by the addition of growth media containing estradiol at the concentration of 10 nM. To each well of 96-well microplate, 100 μl of cell suspension was added at final density of 10^4 cells per well. After the cells were allowed to attach and grow overnight, the growth media was removed and different drugs treatments (conjugate **11** and 4-hydroxytamoxifen) ranging from 100 nM to 0.1 nM in the growth media were added. Incubation lasted for 4 days at 37 °C air with 5% CO_2 and GI_{50} values determined using MTS assay and GraphPad Prism 3.0

National Cancer Institute (NCI) screening procedures [41-46]—The cytotoxic activity of the conjugates 10, 11 and 13 was evaluated at the National Cancer Institute (NCI, Bethesda, Maryland, USA). The following breast cancer lines: BT-549, HS 578T, MCF 7, MDA-MB-231/ATCC, MDA-MB-435, NCI/ADR-RES and T-47D were used as a pre-screen for the large 7 cell line panel. Cell suspensions were diluted according to particular cell type, follow by addition of the expected target cell density (5000-40,000 cells per well based on cell growth characteristics) into 96-well microtiter plates using a 100 μL pipet. In order to inoculate, a preincubation period of 24 h at 37 °C for stabilization was permitted. The test concentrations were diluted twice in 100 μL aliquots and added to the microtiter plate wells at time zero. The

tested conjugates **10, 11** and **13** were evaluated at five concentrations (100, 10, 1, 0.1and 0.01 μM) in the NCI's screening panel. The cells were incubated for 48 h in 5% CO_2 atmosphere and 100% humidity and assayed using the sulforhodamine B assay (SRB), a protein-binding dye. The special concentration parameters were determined using plate reader and microcomputer. The cytotoxic effects of each conjugate was expressed as the molar drug concentrations required for 50% growth inhibition (GI_{50}), total growth inhibition (TGI), and 50% cell kill (LC_{50}).

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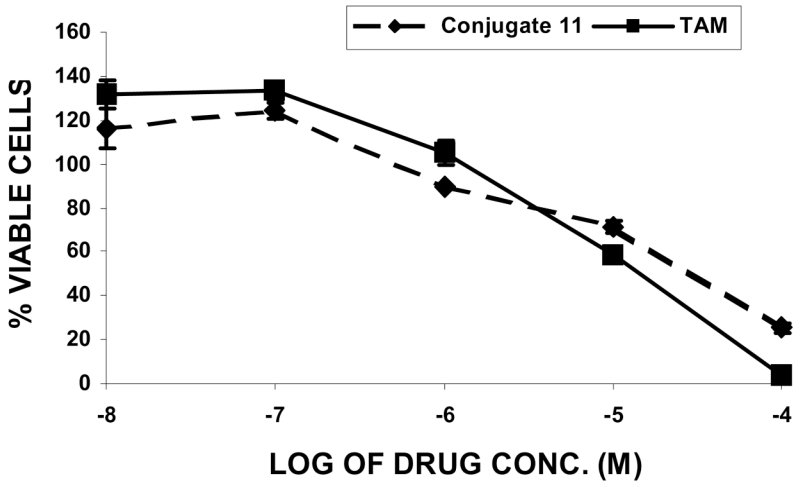
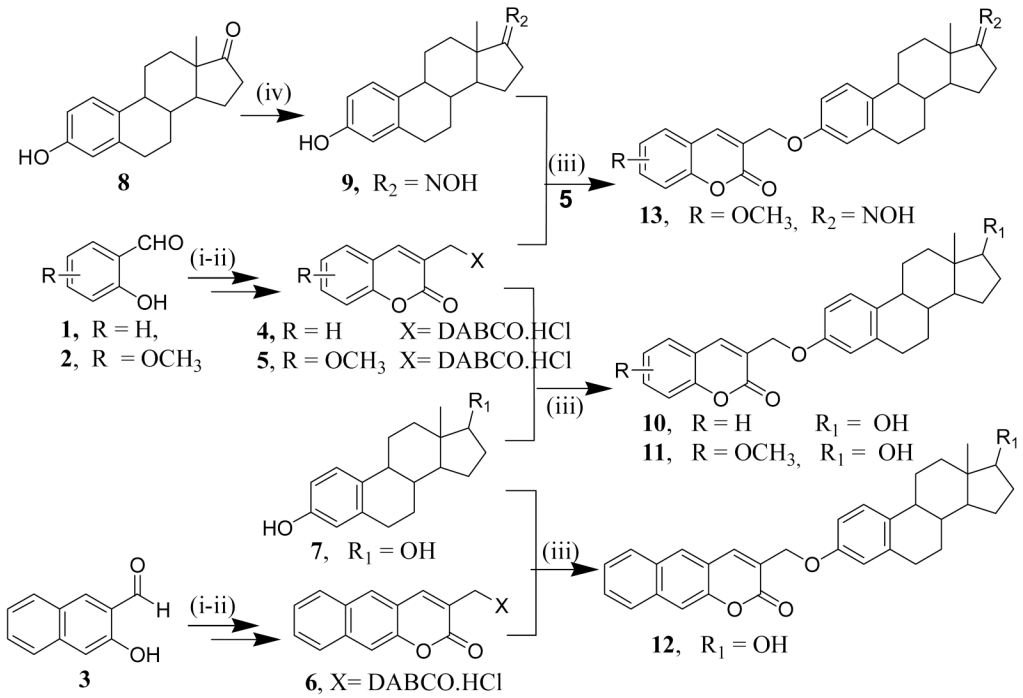


Figure 1.
In vitro cytotoxicity study in MCF-7 human breast cancer cell line as assessed by MTT assay.



Scheme 1.

Reagents: i) Acryloychloride, NaH, reflux; ii) DABCO, CH2Cl2, rt, iii) K2CO3, CH3CN, reflux, overnight, iv) NH2OH, THF, reflux

Table 1

GI_{50}, TGI and LC_{50} values (μM) for conjugates **10, 11** and **13** for 5-dose assay in the full NCI-7-cell line panels (Data obtained from NCI's *in vitro* tumor-cell screen).

Breast cancer cell (Type)	GI_{50} (μM) of Conjugate			TGI (μM) of conjugate			LC_{50} (μM) of conjugate		
	10	**11**	**13**	**10**	**11**	**13**	**10**	**11**	**13**
MCF-7 (Invasive ductal carcinoma)	100	3.63	12.6	100	15.0	42.5	100	100	100
NCI/ADR-RES (Adenocarcinoma)	29.5	26.2	12.4	100	100	100	100	100	100
MDA-MB-231/ATCC (Adenocarcinoma)	100	2.80	4.85	100	14.4	18.4	80.5	80.5	48.1
HS 578T (Invasive ductal carcinoma)	27.6	17.7	29.4	100	100	87.5	100	100	100
MDA-MB-435 (Invasive ductal carcinoma)	1.22	5.40	11.4	100	100	100	100	100	100
BT-549 (Invasive ductal carcinoma)	4.53	27.0	12.1	100	100	41.2	100	100	100
T-47D (Invasive ductal carcinoma)	100	32.0	8.45	100	100	38.2	100	100	100

GI_{50} = growth inhibition by 50%; TGI = 100% (total) growth inhibition, signifying a cytostatic effect; LC_{50} = 50% cell kill or lethal concentration, signifying a cytotoxic effect.