Carboxy derivatives of isoflavones as affinity carriers for cytotoxic drug targeting in adrenocortical H295R carcinoma cells

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Abstract

Carboxy derivatives of isoflavones that exhibit oestrogenic/anti-oestrogenic properties were used as carriers for affinity drug targeting to H295R adrenocortical carcinoma cells that express transcripts of oestrogen receptor (ER)\textsubscript{\alpha} and \textsubscript{\beta}. These derivatives were prepared by introducing a carboxymethyl group at the 6-position of genistein and of biochanin A, yielding 6CG and 6CB respectively. In transactivation assays, 6CG displayed mixed agonist/antagonist activity for ER\textsubscript{\alpha}, whereas 6CB displayed only weak antagonist activity. Low concentrations of oestrogen, 6CG and 6CB were capable of inducing proliferation in H295R cells and of stimulating creatine kinase (CK) specific activity, suggesting that these cells were sensitive to oestrogenic compounds. In in vivo experiments, both 6CG and 6CB were capable of inhibiting oestrogen-induced CK specific activity in rat tissues. For affinity drug targeting, the cytotoxic drug daunomycin was coupled to 6CB and 6CG, yielding 6CB–Dau and 6CG–Dau respectively. These conjugates were tested for their antiproliferative ability to inhibit DNA synthesis as assessed by incorporation of [\textsuperscript{3}H]thymidine in H295R cells. A dose-dependent cytotoxicity was observed with both conjugates. At 0·3–3 nM, both conjugates were 10 to 30 times more potent than daunomycin. At 30 nM these conjugates were two to three times more potent than daunomycin. At concentrations ranging between 300 and 3000 nM, no difference in cytotoxicity was observed between the conjugates and daunomycin. When the cells were treated over a wide range of concentrations with a combination of 6CG plus daunomycin, the observed cytotoxicity was less than with daunomycin alone. When non-transformed rat enterocytes, which do not express ER, were treated with 6CG–Dau or daunomycin, the antiproliferative effect of 6CG–Dau was the same as that of daunomycin over the concentration range tested. These pilot studies suggest that the ready availability of oestrogenic binding sites in H295R cells can be exploited for site-directed chemotherapy.


Introduction

The human H295R adrenocortical carcinoma cell line possesses all the major adrenocortical enzyme systems (e.g. P450\textsuperscript{scc}, 450c17, P450c21, aromatase etc) (Gazdar et al. 1990, Staels et al. 1993). In recent years this cell line has been used as a model system to study the induction and inhibition of steroidogenic enzymes by a variety of compounds such as oestrogen (Gell et al. 1998), phytoestrogens (Ohno et al. 2002) and pesticides (Sanderson et al. 2002). In these cells, oestrogen at high concentrations (1–10 \textmu mol/l) inhibited cortisol synthesis by specifically inhibiting the activity of the 3\textbeta hydroxysteroid dehydrogenase enzyme (Gell et al. 1998). In addition, high concentrations of the phytoestrogens, daidzein and genistein, stimulated the production of dehydroepiandrosterone sulphate and decreased cortisol synthesis by suppressing P450c21 enzymatic activity (Mesiano et al. 1999).

Several studies have indicated that oestrogens and phytoestrogens (Kurzer & Xu 1997) can act as growth modulators in various cell types. To date, no studies on the effects of low doses of either oestrogen or phytoestrogen derivatives on the oestrogenic response of these cells in terms of DNA synthesis and creatine kinase (CK) specific activity have been reported. It was therefore of interest to explore the sensitivity of these cells to low concentrations of oestrogenic compounds.

In a previous study, we have described the properties of 6-carboxymethyl genistein (6CG) (Somjen et al. 2002), synthesized in our laboratory. Interestingly, in human vascular smooth muscle cells, 6CG and oestrogen
stimulated DNA synthesis at a low concentration (0.3 nmol/l). Moreover, 6CG displayed antagonist activities when oestrogen was bound to oestrogen receptor (ER) α but not ERβ. Biochanin A is a derivative of genistein having a methoxy group at position 4′. In several cell systems, biochanin A can be converted to genistein (Peterson et al. 1998). As an extension of our previous work, we describe here the synthesis and evaluation of 6-carboxymethyl biochanin A (6CB) as an agonist/antagonist of oestrogen action in vitro and in vivo. In addition, we evaluate the effects of oestrogen, 6CG and 6CB on [3H]thymidine synthesis and CK specific activity in H295R cells; in pilot studies we have observed that these cells were sensitive to oestrogenic stimulation. Moreover, H295R cells expressed transcripts of ERα and ERβ. Taking these facts into consideration, we prepared cytotoxic isoflavone–daunomycin conjugates of 6CG and 6CB for the selective delivery of cytotoxic drugs to H295R cells that express ER. Our results indicate that the isoflavone–daunomycin conjugates in H295R cells were more potent than daunomycin alone, and caused inhibition of cell proliferation at concentrations much lower than that required with the free daunomycin.

Materials and Methods

Reagents

All reagents were of analytical grade. Chemicals and kits for CK determination were purchased from Sigma (St Louis, MI, USA). Biochanin A was purchased from Indofine Chemical company (Somerville, NJ, USA) and purified by alumina column chromatography before use. Methyl-[3H]thymidine (5 Ci/mmol) and [3H]oestradiol (85 Ci/mmol) were obtained from New England Nuclear (Boston, MA, USA). Baculovirus-expressed recombinant ERα and ERβ preparations were from Panvera (Madison, WI, USA). Streptavidin–coated plates were from Wallac (Turku, Finland). Cy3·5 Bis reactive dye was purchased from Amersham Pharmacia Biotech (Buckinghamshire, UK).

Cells

Human adrenocortical cancer cells (NCI-H295R) were from ATTC (Rockville, MD, USA) and were grown according to the instructions of ATTC. Cells were grown to subconfluence and then treated with various hormones or agents as indicated. Non-tranformed rat enterocytes (IEC cells), used as control, were obtained from Professor N Arber, Ichilov Hospital, Tel-Aviv, and grown as described previously (Arber et al. 1996).

Preparation of carboxy derivatives of isoflavones

6-CG (Kohen et al. 1999) (compound I, Fig. 1) was prepared as previously described. 6-CB (compound II, Fig. 1) was prepared as follows: sodium (0.31 g), cut into small pieces, was added under nitrogen to a three-necked flask containing n-propanol (8 ml). After dissolution of sodium, purified biochanin A (100 mg) in 6 ml n-propanol was added. The reaction mixture was stirred for 15 min and bromoacetic acid (0.377 g) in 2 ml n-propanol was added. A precipitate was formed immediately, and the colour of the reaction changed gradually from yellow to green. The reaction mixture was stirred for 2 h at 60°C and then cooled to room temperature. Water was added and the solvent was evaporated. The residue was acidified with 5N HCl to pH3 and extracted with ether. The organic phase was washed with water, separated, dried with anhydrous magnesium sulphate, evaporated and chromatographed on Silica gel 60. Elution of Silica gel 60 with methanol:chloroform:acetic acid (5:94.7:0.3) yielded the desired mono-addition product (20 mg) with an Rf of 0.5 in the solvent system chloroform:methanol:acetic acid (89:7:10:0.3); biochanin A showed an Rf of 0.8. The [1H]NMR spectrum of this carboxy derivative of biochanin A (compound II, Fig. 1) in deuterated dimethyl sulphoxide showed the following signals: δ: 8.3 (1H, 2-H), 3.9 (1H, 3-H), 3.6 (1H, 4-H), 3.0 (1H, 5-H), 2.5 (1H, 6-H), 1.0 (3H, 7-H), 0.9 (3H, 8-H), 0.8 (3H, 9-H), 0.7 (3H, 10-H), and 0.6 (3H, 11-H).
7.46 (2H, d, J=2Hz, 2’H and 6’H), 6.97 (2H, d, J=2Hz, 3’H and 5’H), 6.28 (1H, s, 8-H), 3.6 (2H, s, –CH₂–COOH) and 3.74 (3H, s, OMe). The NMR spectrum of 3 formed. In the same solvent system, 6CG showed an Rf of 0.95 was obtained, indicating that the active ester of 6CG was formed. In the same solvent system, 6CG showed an Rf of 0.4.

Preparation of total RNA
Total RNA from H295R cells was extracted using the TRizol reagent (Gibco Life Technologies) according to the manufacturer’s instructions.

RT-PCR
Total RNA (1 µg) was subjected to reverse transcription using the BD Advantage One-Step RT-for PCR kit from BD Biosciences Clontech (Palo Alto, CA, USA). For ERα, we used 5 µl cDNA in the reaction mixture with the primers 5’-TTTCTGACATCGCCGACGCGC-3’ (forward) and 5’-TGCTCTGACATCGCCGACGCGC-3’ (reverse), for 30 cycles at 94 °C for 30 s, at 57 °C for 30 s and at 72 °C for 1 min. For ERβ, the same amount of cDNA was used with the primers 5’-TTTCTGACATCGCCGACGCGC-3’ (forward) and 5’-TTTCTGACATCGCCGACGCGC-3’ (reverse) for 30 cycles at 94 °C for 30 s, at 58 °C for 30 s and at 72 °C for 1 min. Plasmid pRST7ERα and pRST7ERβ (kindly provided by Dr D McDonnell, Durham, NC, USA) served as positive controls in these reactions.

Transfection and luciferase assay
The transcriptional activity of the carboxy isoflavone derivatives relative to oestradiol was determined in vitro as described previously (Somjen et al. 2002). Briefly, MCF7 human breast cancer cells (ATTC) were transfected with a 3 xERE-TATA-Luc reporter plasmid, from Dr B van der Burg (Utrecht, the Netherlands) and Renilla phRL (Promega). After transfection and incubation with the various stimulants was completed, the luciferase activity of each well was determined using the dual Luciferase system (Stop and Glow; Promega) and a luminometer (M2010; Lumac, Landgraaf, Holland).

Assessment of DNA synthesis
Cells were grown until subconfluence using conditions described previously (Somjen et al. 1998) and then treated with various hormones or agents for 24 or 48 h as indicated. At the end of incubation, [3H]thymidine was added for 2 h. Cells were then treated with 10% ice-cold trichloroacetic acid (TCA) for 5 min and washed twice with 5% TCA and then with cold ethanol. The cellular layer was dissolved in 0.3 ml 0.3 M NaOH, aliquots were taken for counting radioactivity, and [3H]thymidine incorporation into DNA was calculated.

Creatine kinase extraction and assay

In vitro studies
Cells were treated for 24 h with various hormones and agents as specified. CK specific activity was determined as described previously (Somjen et al. 1998).
In vivo studies Changes in CK specific activity in the epiphyseal cartilage, diaphyseal bone, uterus, aorta and left ventricle of the heart, induced by the 24 h treatment with oestrogen (5 µg), biochanin A (0·5 mg), 6CB (250 µg), biochanin A (0·5 mg) plus oestrogen (5 µg) or 6CB (250 µg) plus oestrogen (5 µg) were studied in 25-day-old Wistar-derived immature female rats, using five animals in a group. Rats injected intraperitoneally (i.p.) with 0·05% DMSO in PBS served as controls. The dose of oestrogen used for each rat was 5 µg, because this dose is optimal for skeletal tissue. The rats were killed by decapitation 24 h after i.p. injection. The various organs were removed and stored at −20 °C until processed for CK activity as described previously (Somjen et al. 1998). All experiments were carried out according to the regulations of the committee for experimental animals of the Weizmann Institute of Science.

Statistical analysis

The significance of differences between the mean values obtained from experimental groups and controls was evaluated by the unpaired two-tailed Student’s t-test and by analysis of variance.

Results

Oestrogen receptor binding studies

In binding assays, carried out as described previously (Somjen et al. 2002), the relative binding affinity of 6CG, relative to oestradiol which was set arbitrarily to 100%, to ERα was 0·1%; that to ERβ was <0·01%. In contrast, 6CB at concentrations of 1 nM to 1 µM did not inhibit the binding of [3H]oestradiol to ERα or to ERβ.

Transcriptional activity of carboxy isoflavone analogues

The transactivation activities of 6CG and 6CB were measured after incubation of transfected MCF7 cells with biochanin A (1 µM) with or without carboxy isoflavone analogues (1 µM) in the absence or presence of oestrogen (1 nM). Only 6CG showed moderate agonistic activity, and 6CB had no effect in these cells. In the presence of oestrogen, both compounds displayed moderate antagonist activity for ERβ (Fig. 2). Biochanin A showed agonistic activity and did not stimulate or inhibit the agonistic effect of oestradiol (Fig. 2). Tamoxifen (1 µM) had negligible

![Figure 2](image-url)

**Figure 2** Transcriptional activity of ERα with oestrogen (E2), biochanin A (Bio), 6-carboxymethyl biochanin A (6CB) and 6-carboxymethyl genistein (6CG). MCF7 human breast cancer cells were transfected with two plasmids consisting of 3 xERE-TATA-Luc reporter and Renilla luciferase. The cells were then treated with oestrogen (10⁻⁹ M), Bio (1 µM), 6CG (1 µM), 6CB (1 µM) or combinations of Bio-E2, E2+6CG or E2+6CB. Firefly luciferase activity was measured 24 h later. Luciferase activity was standardized to the internal co-transfected Renilla activity and expressed as the ratio of induced activity to the activity in the absence of ligand. Each data point represents the mean ± s.d. of triplicates of three different experiments.

![Figure 3](image-url)

**Figure 3** Expression of mRNAs encoding (a) ERα and (b) ERβ in H295R cells using reverse transcriptase PCR. (a) Amplified fragments: lane 2, positive control (ERα 344 bp); lane 3, H295R cells. (b) Amplified fragments: lane 2, positive control (ERβ 203 bp); lane 3, H295R cells.


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transcriptional activity. In the presence of oestrogen, tamoxifen blocked the transcriptional activity of oestrogen to basal levels (data not shown).

Expression of oestrogen receptor isoforms ERα and ERβ in H295R cells

Figure 3 depicts RT-PCR products of mRNA extracted from cultured H295R cells with specific primers for either ERα and ERβ. As shown, both oestrogen receptor isoforms are expressed in cultured H295R cells.

Effects of oestrogenic compounds on [3H]thymidine incorporation and CK specific activity in vitro in H295R cells

Oestrogen at very low concentrations (0.03–3 nmol/l) stimulated [3H]thymidine incorporation in these cells. Greater concentrations of oestrogen (> 30 nmol/l) had no
significant effect on DNA synthesis (Fig. 4a). Dose-dependent stimulation of DNA synthesis was also observed with the carboxy isoflavones 6CB and 6CG (Fig. 4b).

Oestrogen stimulated CK activity in a dose-related manner, reaching a plateau at 30 nmol/l (Table 1). Raloxifene (3 µM) showed no effect on either DNA synthesis or CK specific activity in these cells, and also did not block oestrogen-, 6CB- or 6CG-driven stimulation (data not shown). CK specific activity was increased by 6CG (30 nmol/l: 148 ± 12%; 300 nmol/l: 174 ± 10%) and by 6CB (100 nmol/l: 240 ± 5%). The combination of oestrogen (30 nmol/l) and 6CB (100 nmol/l) did not have an additive or inhibitory effect on CK activity in these cells (oestrogen alone: 264 ± 24%; 6CB+oestrogen: 236 ± 34%). Control, unstimulated cells were arbitrarily set at 100 ± 12%.

Table 1 Dose-dependent effects of oestrogen on creatine kinase (CK) specific activity in H295R cells. Results are means ± S.E.M. of 8–16 incubates from 2–4 experiments and are expressed as the ratio between the specific activities of creatine kinase in hormone-treated and control cells.

<table>
<thead>
<tr>
<th>Dose of oestrogen (nM)</th>
<th>CK specific activity ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.00 ± 0.16</td>
</tr>
<tr>
<td>0.03</td>
<td>1.09 ± 0.11</td>
</tr>
<tr>
<td>0.3</td>
<td>1.27 ± 0.13</td>
</tr>
<tr>
<td>3</td>
<td>1.43 ± 0.15**</td>
</tr>
<tr>
<td>30</td>
<td>1.68 ± 0.19*</td>
</tr>
<tr>
<td>300</td>
<td>2.03 ± 0.12**</td>
</tr>
<tr>
<td>3000</td>
<td>1.88 ± 0.22*</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01 compared with no oestrogen.

Stimulation of the specific activity of CK by biochanin A analogues in vivo

In immature female rats, oestradiol (5 µg/rat) and biochanin A (0.5 mg/rat) stimulated the CK specific activity in all the rat tissues that were examined (uterus, pituitary, epiphysis, diaphysis, aorta and left ventricle of the heart; Table 2), whereas 6CB (250 µg/rat) increased the CK specific activity in all the rat tissues with the exception of the uterus. The stimulatory response of oestrogen to CK specific activity was inhibited in all the tissues when rats were treated with a combination of oestrogen plus 6CB, suggesting that 6CB acts like a selective oestrogen receptor modulator (SERM) in these tissues (Table 2).

Cytotoxicity studies of isoflavone–daunomycin conjugates in cultured cells

The cytotoxicity of isoflavone–daunomycin conjugates in H295R cells was tested after 48 h of incubation using incorporation of [3H]thymidine as a marker of proliferation. At low concentrations (0.3–3 nM), both conjugates (i.e. 6CG–Dau and 6CB–Dau) were 10–30-fold more toxic than daunomycin. At 30 nM, the conjugates were still two to three times more toxic than daunomycin. At high concentrations (300–3000 nM), these conjugates showed the same toxicity as daunomycin. Moreover, when H295R cells were treated with a combination of 6CG plus daunomycin, the net effect was less than that seen with daunomycin alone, thus suggesting that 6CG partially prevented the cytotoxic effects of daunomycin at the entire range of concentrations tested (0.3–3000 nM; Fig. 5a). In contrast, when IEC cells, which are devoid of functional ER, were treated with daunomycin or 6CG–Dau conjugate, no difference in cytotoxicity was observed between the free daunomycin and the cytotoxic isoflavone conjugate over a wide range of concentrations (Fig. 5b).

Table 2 Stimulation of the specific activity of creatine kinase by oestrogen and isoflavone derivatives in rat tissues in vivo. Immature female rats were injected with 0.5 ml saline containing 0.5% DMSO (C, control) oestradiol (E2, 5 µg/rat), biochanin A (Bio, 0.5 mg/rat), 6-carboxymethyl biochanin A (6CB, 0.25 mg/rat), oestradiol (5 µg/rat) plus Bio (0.5 mg/rat) (Bio+E2) or oestradiol (5 µg/rat) plus 6CB (0.25 mg/rat) (6CB+E2). Organs were assayed for creatine kinase activity 24 h after treatment. The results are expressed as means ± S.D. for n = 5 rats and further expressed as experimental over control where the control is given a value of 1.0.

<table>
<thead>
<tr>
<th>Organ</th>
<th>C</th>
<th>E2</th>
<th>Bio</th>
<th>6CB</th>
<th>Bio+E2</th>
<th>6CB+E2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epiphysis</td>
<td>1 ± 0.09</td>
<td>1.85 ± 0.16*</td>
<td>2.38 ± 0.18**</td>
<td>1.61 ± 0.17*</td>
<td>2.09 ± 0.19**</td>
<td>1.02 ± 0.29</td>
</tr>
<tr>
<td>Diaphysis</td>
<td>1 ± 0.16</td>
<td>2.75 ± 0.23**</td>
<td>1.9 ± 0.24*</td>
<td>1.51 ± 0.05*</td>
<td>2.78 ± 0.13**</td>
<td>0.84 ± 0.07</td>
</tr>
<tr>
<td>Uterus</td>
<td>1 ± 0.11</td>
<td>1.49 ± 0.13*</td>
<td>1.42 ± 0.13*</td>
<td>0.89 ± 0.12</td>
<td>1.48 ± 0.11*</td>
<td>1.02 ± 0.22</td>
</tr>
<tr>
<td>Aorta</td>
<td>1 ± 0.1</td>
<td>2.43 ± 0.06**</td>
<td>2 ± 0.18*</td>
<td>1.63 ± 0.11*</td>
<td>2.38 ± 0.06**</td>
<td>1.33 ± 0.15</td>
</tr>
<tr>
<td>Left ventricle</td>
<td>1 ± 0.09</td>
<td>1.53 ± 0.13*</td>
<td>1.42 ± 0.04*</td>
<td>1.91 ± 0.16*</td>
<td>1.6 ± 0.09*</td>
<td>1.1 ± 0.12</td>
</tr>
<tr>
<td>Pituitary</td>
<td>1 ± 0.14</td>
<td>1.45 ± 0.05*</td>
<td>1.58 ± 0.05*</td>
<td>1.54 ± 0.05*</td>
<td>1.66 ± 0.14*</td>
<td>1.16 ± 0.08</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01, treated compared with control.
Our main goal in this study was to use H295R adrenocortical carcinoma cells as a model system for affinity targeting of cytotoxic drugs (e.g. daunomycin) linked to a carrier that could be preferentially recognized by membranal or nuclear steroid receptors present in these cells. For this purpose, we first established that H295R cells express nuclear ERα and ERβ (Fig. 3) and most probably membranal ER (unpublished results). Moreover, these cells are sensitive to low doses of oestrogen in terms of proliferation (Fig. 4a) and in terms of stimulating CK.
activity (Table 1). We then chose a carrier that exhibits oestrogenic and anti-oestrogenic properties and contains a reactive group (carboxy) that is able to react with the primary amino group of the sugar moiety of daunomycin. A derivative of genistein, 6-carboxymethyl genistein (6CG), synthesized in our laboratory fulfilled these requirements. In addition to 6CG, we explored the possibility of using another carboxy isoflavone as carrier.

Because biochanin A can be converted to genistein in certain tumour cells (Peterson et al. 1998), we synthesized a novel carboxy derivative of biochanin A, which can serve as a prodrug, and evaluated the oestrogenic and anti-oestrogenic properties of the resulting 6-carboxymethyl biochanin A (6CB). 6CB acted similarly to 6CG (Somjen et al. 2002) in vitro and in vivo. 6CB was not capable of inducing transcription via an oestrogen receptor element in MCF7 cells that possess endogenous ERα, but in the presence of oestrogen, 6CB displayed weak antagonist activity (Fig. 2), in common with 6CG. In addition, 6CG and 6CB, in common with oestrogen, were capable of stimulating DNA synthesis in H295R cells (Fig. 4) and of increasing CK specific activity in these cells. Interestingly, the combination of oestrogen with 6CB did not have additive or stimulatory effects on CK activity in these cells. Moreover, raloxifene exhibited no stimulatory effect on either DNA synthesis or CK specific activity and did not attenuate oestrogen- or 6CB- or 6CG-driven CK stimulation in H295R cells, suggesting that the response profile of the SERM raloxifene is different in this cell type.

In in vivo experiments, 6CB increased CK activity in rat tissues derived from immature female rats, with the exception of the uterus. Moreover, 6CB, like 6CG and raloxifene, was capable of inhibiting oestrogen-induced CK stimulation in rat tissues (Table 2, 6CB). Collectively, the results suggest that these carboxy derivatives of isoflavones appear to possess a mixed agonistic/antagonistic character: they increase CK specific activity in several sites in rat tissues in vitro and in vivo, but antagonize oestrogen-induced CK activity in vivo, and partially block the transcriptional activity of oestrogen in MCF7 cells transfected with ERE-luciferase, but not in H295R cells in vivo. This is by no means an unprecedented profile of a SERM. Thus a response profile in one cell type cannot be extrapolated to other cell types.

The presence of a reactive carboxy group in the isoflavone molecule permitted the synthesis of cytotoxic isoflavones. The cytotoxic isoflavone conjugates were prepared by coupling daunomycin via the amino group of the sugar moiety to carboxy derivatives of isoflavones (6CG and 6CB; Fig. 1). The cytotoxicity of these conjugates was tested in H295R cells (Fig. 5a). At low concentrations (0.3–3 nM), both conjugates were 10 to 30 times more potent than daunomycin. At 30 nM, both conjugates were two to three times more potent than daunomycin. No difference in toxicity was observed between daunomycin and these conjugates at high concentrations (300–3000 nM; Fig. 5a). In addition, when H295R cells were treated with a combination of 6CG plus daunomycin, the observed cytotoxicity was less than that of daunomycin alone over a wide range of concentrations. In contrast, when IEC cells devoid of ER, used as control, were treated with 6CG–Dau conjugate or daunomycin alone, no difference in cytotoxicity was observed between the drug and the conjugate over the range of concentrations tested (0.3–3000 nM; Fig. 5b). Whether or not these conjugates recognize a putative membranal ER (unpublished results) and exert part of their effects via a putative membranal ER is currently under investigation.

The clinical management of malignant adrenal tumours (Ng & Libertino 2003) is difficult at present. Our results suggest that the presence of specific ER recognition sites in some tumour types may be exploited for site-directed chemotherapy using the cytotoxic isoflavone conjugates described in this paper.

Acknowledgements

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References


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