Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 22, pp. 2683-2686, 1996

0960-894X/96 $15.00 + 0.00

PII: S0960-894X(96)00502-1

CONVERSION OF THE PHYTOESTROGEN COUMESTROL INTO A SELECTIVE ESTROGEN RECEPTOR MODULATOR (SERM) BY ATTACHMENT OF AN AMINE-CONTAINING SIDECHAIN

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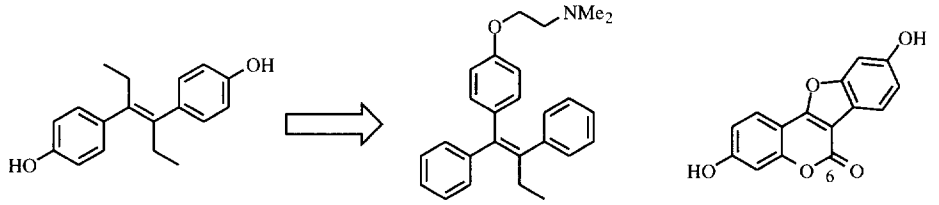
Abstract: The naturally occurring estrogen mimetic coumestrol has been shown to stimulate proliferation of MCF-7 mammary tumor cells and to cause uterotrophic effects in ovariectomized (OVX) rats. Attachment of a basic amine-containing sidechain to C-6 of coumestrol converts this estrogen agonist into an antagonist in breast and uterine tissue, while maintaining its estrogen-like activity as a hypocholesterolemic agent.

A frequently used strategy for the design of estrogen antagonists has been the attachment of a basic amine-containing sidechain to molecules that interact with the estrogen receptor (ER), such as 17β-estradiol or diethylstilbestrol (DES).[1] The generation of tamoxifen (**2**) from DES (**1**) is a prime example of the utility of this strategy.



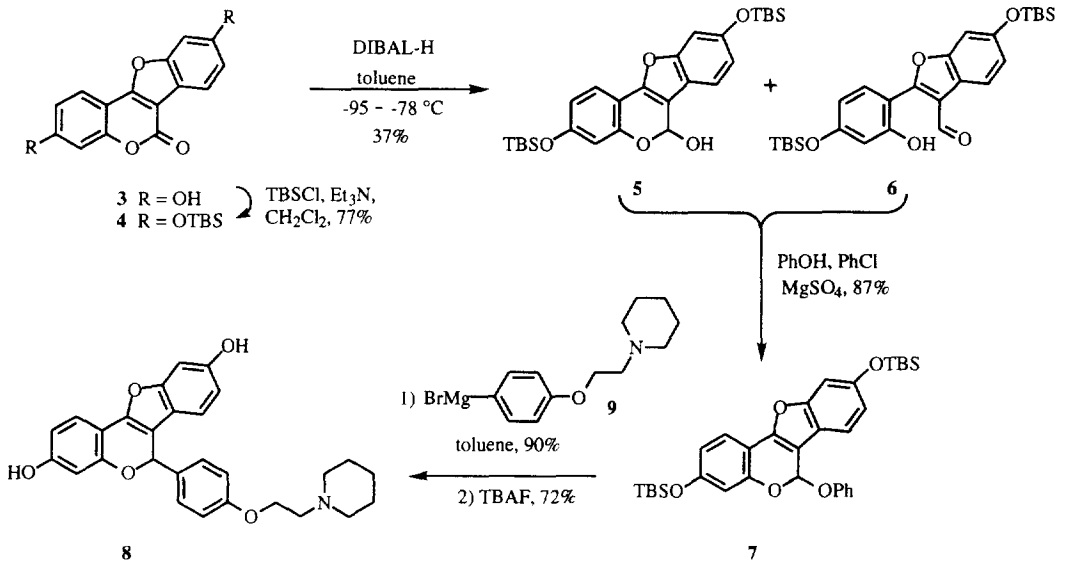
1 **2** **3**

Phytoestrogens are naturally occurring, plant-derived substances that interact with the ER and have been demonstrated to produce estrogen-like effects in a number of in vitro and in vivo models.[2] The effects of long-term exposure to dietary phytoestrogens has recently been the subject of substantial research and debate.[3] Many of these compounds are flavanoids or isoflavanoids and bear structural homology to known, basic amine-containing antiestrogens and selective estrogen receptor modulators (SERMs).[4,5] Therefore, we speculated that the attachment of a basic amine-containing sidechain to the appropriate position of a phytoestrogen would confer estrogen antagonist properties to the compound. We selected coumestrol **3** as a representative example, and chose to attach the sidechain at the 6-position in order to utilize chemical methodology that we have recently developed in a related program.[6]

As shown in Scheme 1, coumestrol was protected as its bissilyl ether and subjected to DiBAL-H reduction under the conditions we have previously described.[6] In this instance, the reduction was carried out at -95 °C due to the facility of overreduction. Even under these conditions, only a modest yield of the desired lactol **5** could be isolated. Proton NMR analysis of purified material indicated that **5** was in equilibrium with its aldehyde tautomer **6**. Nevertheless, condensation of this mixture with phenol provided the desired phenyl

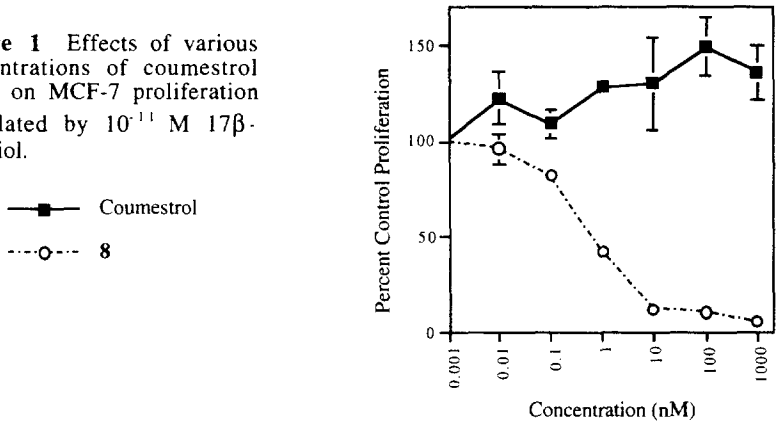
acetal **7** in good yield and displacement of the phenoxy group with 4-[2-(1-piperidino)ethoxy]-phenylmagnesium bromide (**9**) proceeded uneventfully. Desilylation then revealed the fully elaborated coumestrol analog **8** with a basic amine-containing sidechain incorporated at C-6.

Scheme 1



Compound **8** maintained affinity for the estrogen receptor (ER) in MCF-7 cell lysate, albeit with reduced activity (binding affinity relative to ^{3}H-estradiol (RBA) = 7.2%) compared to coumestrol (RBA = 13.2%).[7] The ability of **8** to antagonize estrogen action in mammary tissue was assayed as its inhibition of MCF-7 cell proliferation (Figure 1).[8] In this assay, coumestrol stimulated proliferation with an EC_{50} of 40 nM (not shown), and was unable to reduce the stimulation caused by 10^{-11} M 17β-estradiol. Conversely, **8** functioned as a potent antagonist of 17β-estradiol with an IC_{50} of 0.7 nM. As expected, attachment of the sidechain at C-6 resulted in an alteration of the compound profile, converting an estrogen agonist into an antagonist.

Figure 1 Effects of various concentrations of coumestrol and **8** on MCF-7 proliferation stimulated by 10^{-11} M 17β-estradiol.



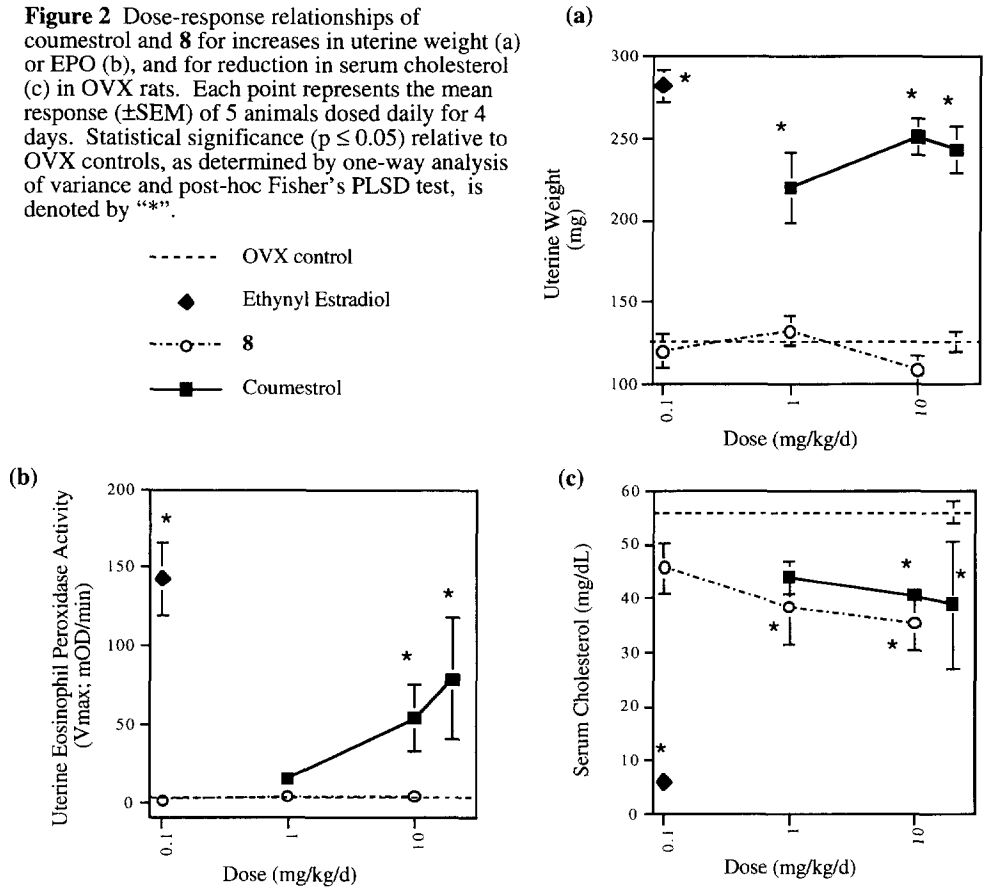


Figure 2 Dose-response relationships of coumestrol and **8** for increases in uterine weight (a) or EPO (b), and for reduction in serum cholesterol (c) in OVX rats. Each point represents the mean response (±SEM) of 5 animals dosed daily for 4 days. Statistical significance ($p \leq 0.05$) relative to OVX controls, as determined by one-way analysis of variance and post-hoc Fisher's PLSD test, is denoted by "*".

The estrogen agonist effects of compound **8** on uterine parameters and on serum cholesterol were evaluated in an ovariectomized (OVX) rat model utilizing uterine weight, uterine eosinophil peroxidase (EPO) activity, and serum cholesterol levels as endpoints (Figure 2).[9] In this assay coumestrol had little effect when dosed orally; however, upon subcutaneous administration it demonstrated potent estrogen-like effects on uterine weight and EPO, and significantly lowered serum cholesterol, albeit with reduced efficacy relative to ethynyl estradiol. Conversely, compound **8** (oral gavage, 20% β-hydroxycyclodextrin (CDX) solution) had no effect on uterine weight or EPO, while it maintained the ability to reduce serum cholesterol at a level similar to that of coumestrol. Apparently, the attachment of the amine-containing sidechain eliminated uterotrophic effects without reducing effects on serum cholesterol, thereby inducing a measure of tissue-selectivity.[10] Furthermore, compound **8** was able to significantly inhibit the effects of estrogen in the immature rat uterus.[11] In this assay, 21-day old female Sprague-Dawley rats were dosed by oral gavage with a maximally stimulatory dose of ethynyl estradiol (0.1 mg/kg) for three consecutive days. Test groups were also administered various doses of compound **8**, by oral gavage (CDX solution) 15 min prior to ethynyl estradiol dosing, for three days. On the fourth day the rats were sacrificed and uterine wet weight was determined. Compound **8** inhibited the uterine stimulation induced by ethynyl estradiol in dose-dependent fashion, as shown in Figure 3.

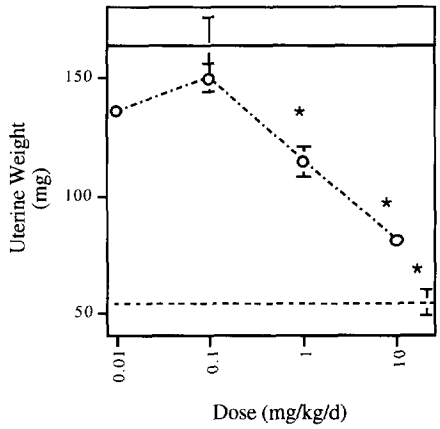


Figure 3 Effect of **8** on estrogen-induced uterine weight increase in immature rats. Each point represents the mean uterine weight (±SEM) for 6 animals. Statistical significance ($p \leq 0.05$) relative to ethynyl estradiol-treated controls, as determined by one-way analysis of variance and post-hoc Fisher's PLSD test, is denoted by "*".

In conclusion, we have demonstrated that the appropriate attachment of a basic amine-containing sidechain is an effective strategy for the conversion of a phytoestrogen into an antiestrogen or SERM. Further elaborations of this strategy and the novel structural class represented by **8** will be published in due course.

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12. All new compounds exhibited appropriate ^{1}H NMR, ^{13}C NMR, IR, and MS or elemental analysis.

(*Received in USA* 9 *September* 1996; *accepted* 11 *October* 1996)