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ORIGINAL PAPER

Screening of synthetic and plant-derived compounds for (anti)estrogenic and (anti)androgenic activities

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Abstract Recently we constructed yeast cells that either express the human estrogen receptor α or the human androgen receptor in combination with a consensus ERE or ARE repeat in the promoter region of a green fluorescent protein (yEGFP) read-out system. These bioassays were proven to be highly specific for their cognate agonistic compounds. In this study the value of these yeast bioassays was assessed for analysis of compounds with antagonistic properties. Several pure antagonists, selective estrogen receptor modulators (SERMs) and plant-derived compounds were tested. The pure antiestrogens ICI 182,780 and RU 58668 were also classified as pure ER antagonists in the yeast estrogen bioassay and the pure antiandrogen flutamide was also a pure AR antagonist in the yeast androgen bioassay. The plant-derived compounds flavone and guggulsterone displayed both antiestrogenic and antiandrogenic activities, while 3,3′-diindolylmethane (DIM) and equol combined an estrogenic mode of action with an antiandrogenic activity. Indol-3-carbinol (I3C) only showed an antiandrogenic activity. Coumestrol, genistein, naringenin and 8-prenylnaringenin were estrogenic and acted additively, while the plant sterols failed to show any effect. Although hormonally inactive, in vitro and in vivo metabolism of the aforementioned plant sterols may still lead to the formation of active metabolites in other test systems.

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Introduction

For drug design, specific antiestrogens and antiandrogens are important to treat breast and prostate tumours via mediation by the human estrogen receptor α (hERα) or the human androgen receptor (hAR), respectively [1, 2]. Specific agonists for hERα might be used to treat climacteric complaints in females, while specific agonists for hAR might be used to treat libido loss, impotence or muscle weakness in males. Synthetic compounds like tamoxifen, 4-hydroxytamoxifen, ICI 182,780, ICI 164,384, raloxifen, RU 58668 and nafoxiden were designed and their antiestrogenic activity in breast cells was proven to be useful to treat patients with breast cancer. Tamoxifen and its more active metabolite 4-hydroxytamoxifen are routinely used for the treatment of estrogen-dependent breast cancers [3]. However, after 5 years of treatment, most breast carcinoma become resistant to tamoxifen. Moreover, tamoxifen is a selective estrogen receptor modulator (SERM) showing antagonistic effects in breast tissue, agonistic effects on bone and either agonistic or antagonistic effects on the endometrium and vaginal epithelium, depending on the intrinsic 17β-estradiol (E2) serum levels. Prolonged use of tamoxifen is associated with an increased risk of endometrial cancer, but has beneficial effects on the bone and serum lipid profile [4, 5]. The pure antiestrogens ICI 182,780 and ICI 164,384 are more potent than tamoxifen in inhibiting the growth of breast cancer cells, but lack the beneficial effects of tamoxifen. However, they are also antiestrogenic on the endometrium and are not associated

with an increased risk of endometrial cancer [6, 7]. ICI 182,780 is often administered by deep intramuscular injections in cases of tamoxifen resistance [8]. Raloxifen, on the other hand, acts as an antagonist on the breast, showed beneficial effects on the bone, but displayed no estrogenic side effects on the endometrium. Therefore, raloxifen is a promising alternative for tamoxifen. Moreover, raloxifen is currently being tested for chemoprevention in women whose risk at breast cancer is moderately increased [9]. RU 58668 is a strong pure antagonist, just like ICI 182,780 and ICI 164,384. RU 58668 is poorly water-soluble compound and has a weak oral bioavailability, but RU 58668-loaded liposomes are currently being evaluated to treat multiple myeloma [10]. Nafoxiden is a derivative of tamoxifen that has an antiproliferative effect on glioma cells and cultured pulmonary artery endothelial cells [11, 12]. Moreover, an antiangiogenic effect is observed for the latter cell type.

The fact that some of these compounds can act both as estrogens and antiestrogens is rather confusing when dealing with screening assays for testing these types of properties. Many researchers assumed first that tamoxifen and 4-hydroxytamoxifen were classical and pure antiestrogens. As a result they decided in favour of transcription activation assays based on mammalian cell lines, most often a breast cancer cell, because yeast screens were unable to show the antagonistic activities of tamoxifen and 4-hydroxytamoxifen. In this aspect the outcomes using yeast assays were odd as both compounds behaved as ER agonists instead of antagonists [13–15]. Later, more specific observations showed that both compounds are partial agonists with respect to transcription activation assays based on different mammalian cell lines, as well as in cellular proliferation tests based on breast tumour cell lines [16]. Thus, as tamoxifen and 4-hydroxytamoxifen are not pure antagonists, but selective estrogen receptor modulators (SERMs), the observed agonistic activities as determined in yeast-based estrogen bioassays correlate relatively well with the growth response of endometrial cells and of breast cells in an estradiol-free environment [17]. Since the uterotrophic assay, measuring the uterus weight gain in the rat, is still the gold standard for testing estrogenicity, one might just as well say that yeast-based assays as well as the well-developed in vitro reporter assays using human breast cancer, Chinese hamster ovary (CHO) or human U2-OS bone cells are suitable tests to determine the estrogenic potency of a given compound [18–20].

In the present study we demonstrate that the yeast estrogen and androgen bioassays are easy-to-handle tools to identify the possible agonistic and antagonistic properties of man-made chemicals. In addition, several plant-derived compounds like coumestrol, campesterol, 3,3′-diindolylmethane (DIM), equol, flavone, genistein, guggulsterone, indol-3-carbinol (I3C), ipri-flavone, naringenin, 8-prenylnaringenin, resveratrol and β-sitosterol were tested. The antagonistic activities were examined with coadministration of 17β-estradiol (E2) or testosterone (T) that caused a half-maximal or near maximal response in the yeast estrogen or androgen bioassay, respectively. The outcome of yeast-based testing together with results obtained with different mammalian cell lines will help researchers to elicit the mechanism of action of agonists, partial agonists and pure antagonists.

Materials and methods

Chemicals

Chlomiphene citrate, 17β-estradiol, equol, genistein, flavone, flutamide, *Z*-guggulsterone, indole-3-carbinol, 8-prenylnaringenin, resveratrol, tamoxifen and 4-hydroxytamoxifen were obtained from Sigma. ICI 182,780 was obtained from Tocris. Campesterol, β-sitosterol and 17β-testosterone were obtained from Steraloids; dimethyl sulfoxide (DMSO) from Merck; 3,3′-diindolylmethane from LKT Lab. Inc.; ipri-flavone from Aldrich; and ICI 164,384, nafoxiden, raloxifen and RU 58668 were a gift of NV Organon (Oss, The Netherlands). Naringenin was purchased from Apin Chemical Limited and coumestrol from Fluka Biochemika. Chemicals to prepare the growth media and the preparation of the growth media for yeast cells were as described previously [21].

Yeast estrogen and androgen bioassays with fluorescence measurement

Cultures of the yeast estrogen and androgen biosensor were grown overnight at 30 °C with vigorous orbital shaking [21, 22]. At the late log phase, the cultures of both cytosensors were diluted in the selective MM/L medium to an OD value at 630 nm between 0.04 and 0.06. For exposure, 200-μL aliquots of this diluted yeast culture were pipetted into each well of a 96-well plate and 2 μL of a stock solution of compound in DMSO was added to test the agonistic properties of these compounds. To test the antagonistic properties of the compounds, 1-μL amounts of the compound stock solutions were coexposed with 1 μL of E2 or T stock solutions giving about a half-maximal and a near maximal response (i.e. 80–100% of the maximal response). DMSO and control samples containing only E2 or T were included in each experiment and each sample concentration was assayed in triplicate. Exposure was performed for 24 h at 30 °C and orbital shaking with 125 rpm. Fluorescence and OD were measured at 0 and 24 h directly in a SynergyTM HT Multi-Detection Micro-

plate Reader (BioTek Instruments Inc., USA) using excitation at 485 nm and measuring emission at 530 nm. The fluorescence signal was corrected with the signals obtained with the MM/L medium containing DMSO solvent only. In order to check whether a sample was toxic for the yeast cells, densities of the yeast culture were determined by measuring the OD at 630 nm.

Results

The estrogen, antiestrogen, androgen and antiandrogen activities of several compounds were tested with the in-house-developed yeast hormonal bioassays [21, 22]. In order to study the agonist activities, pure compounds were added. The antiestrogenic and antiandrogenic properties of the compounds were tested by coadministration of E2 and T, respectively. Figure 1 shows the responses of the known pure antiestrogens ICI 182,780 and RU 58668 in the yeast estrogen bioassay. These pure antiestrogens did not show any agonistic activity, but with respect to ICI 182,780 an inhibition of the response caused by E2 was observed at 100 nM. In combination with E2 a partial inhibition of only 25% was identified and the inhibiting activity of ICI 182,780 was only observed in combination with a dose of E2 that gave a near maximal response (1.5 nM). The inhibiting activity of RU 58668 was more pronounced when tested in combination with a dose of E2 that gives a near maximal response then when tested in combination with a dose of E2 that gives about a half-maximal response (0.6 nM), 70% versus 40%, respectively (data of the latter not shown). Table 1 gives an overview of all the determined responses. Remarkably, the pure antiestrogen ICI 164,384 did not show an estrogenic or antiestrogenic activity (Table 1).

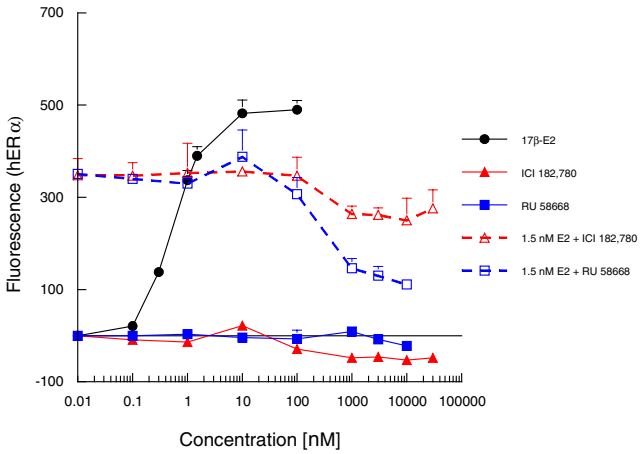

Fig. 1 Responses of the pure estrogen receptor antagonists ICI 182,780 and RU 58668 in the yeast estrogen bioassay without 17β-estradiol (*closed symbols*) and by coexposure with 1.5 nM 17β-estradiol (*open symbols*)

Figure 2a shows the responses of the SERMs tamoxifen, 4-hydroxytamoxifen, raloxifen and chlomiphene citrate in the yeast estrogen bioassay. Figure 2b shows the responses of these compounds when tested in combination with a dose of E2 that gives about a half-maximal response (0.6 nM). These SERMs all show an estrogenic activity from 3 μM onwards. When tested in combination with 0.6 nM E2, these compounds turn out to be additive in the yeast system. Similar results were obtained with the SERM nafoxiden (Table 1).

In the yeast androgen bioassay, using T as a positive agonist reference and flutamide as a positive antagonist reference, all the pure antiestrogens and SERMs turned out to be completely inactive (Table 1).

Figure 3a shows the responses of the plant-derived compound I3C and its major digestive product DIM in the yeast estrogen bioassay. I3C does not show an estrogenic or antiestrogenic activity, but DIM displays an estrogenic activity and when tested in combination with E2, DIM acted additively. Figure 3b shows the responses of I3C and DIM in the yeast androgen bioassay. Neither compound shows an androgen agonist activity, but both inhibit the response caused by T. I3C is thought to be the major active compound in cruciferous vegetables like broccoli, Brussels sprouts and cauliflower, and together with its derivative DIM, exhibit promising cancer-protective properties in vitro and in vivo [23–25]. DIM was shown to be an ER agonist and considered as the first example of a plant-derived pure AR antagonist. DIM suppressed cell proliferation of androgen-dependent LNCaP cells and inhibited dihydrotestosterone-stimulated DNA synthesis [24]. Similar effects of DIM were observed in PC-3 cells, but only when these cells were cotransfected with a wild-type androgen receptor expression plasmid [24]. Thus, the activity found in the yeast estrogen bioassay (Fig. 3a) corresponds with the known agonistic effect of DIM via the estrogen receptor. The activities as measured in the yeast androgen bioassay (Fig. 3b) are in agreement and confirm the antiandrogenic activities of I3C and DIM on prostate cancer cells.

The estrogenic activity of equol, the major active metabolite of the phytoestrogen daidzein, one of the main isoflavones found abundantly in soy, is well known. The present study shows that equol is also active as an ER agonist in the yeast estrogen bioassay (Table 1). Its relative estrogenic potency (REP) compared with E2 is about 10,000 times lower (data not shown). Recent studies demonstrate that equol can modulate reproductive function and is a novel antiandrogen that inhibits prostate growth [26, 27]. That equol might be a potent in vivo AR antagonist is reflected by the fact that equol is patented

Table 1 Responses of compounds in the yeast estrogen and yeast androgen bioassay expressing yEGFP in response to estrogens and androgens respectively

Compound	CAS no.	Yeast estrogen bioassay				Yeast androgen bioassay			
		Agonist	0.6 nM E2 antagonist[a]	1.5 nM E2 antagonist[a]	Comments	Agonist	70 nM 17β-T antagonist[b]	1 μM 17β-T antagonist[b]	Comments
17β-Estradiol	50-28-2	+	NA	NA	Strong agonist	+	+	+	Agonist and antagonist
17β-Testosterone	58-22-0	–	–	–		+	NA	NA	Strong agonist
ICI 164,384		–	–	–		–	–	–	
ICI 182,780	129453-61-8	–	–	+	Weak antagonist	–	–	–	
RU 58668		–	+	+	Weak antagonist	–	–	–	
Flutamide	13311-84-7	–	–	–		–	+	+	Strong antagonist
Tamoxifen	10540-29-1	+	–	–	Additive	–	–	–	
Hydroxytamoxifen	68047-06-3	+	–	–	Additive	–	–	–	
Raloxifen	82640-04-8	+	–	–	Additive	–	–	–	
Chlomiphene citrate	50-41-9	+	–	–	Additive	–	–	–	
Nafoxiden		+	–	–	Additive	–	–	–	
Indol-3-carbinol	700-06-01	–	–	–		–	+	+	Weak antagonist
Diindolylmethane		+	–	–	Additive	–	+	+	Strong antagonist
Flavone	525-82-6	–	+	+	Strong antagonist	–	+	+	Strong antagonist
Ipriflavone	35212-22-7	–	–	–		–	–	–	
Guggulsterone	39025-23-5	–	+	+	Strong antagonist	–	+	+	Strong antagonist
β-Sitosterol	000083-46-5	–	–	–		–	–	–	
Campesterol	474-62-4	–	–	–		–	–	–	
Resveratrol	501-36-0	–	–	–		–	–	–	
Genistein	446-72-0	+	–	–	Additive	–	–	–	
Equol	94105-90-5	+	–	–	Additive	–	+	+	Antagonist
Naringenin	480-41-1	+	–	–	Additive	–	–	–	
8-Prenylnaringenin	53846-50-7	+	–	–	Additive	–	–	–	
Coumestrol	479-13-0	+	–	–	Additive	–	–	–	

NA not applicable, + positive response as an agonist or antagonist, − negative response as an agonist or antagonist
[a] Antagonism tested in the yeast estrogen bioassay by coadministration of 0.6 or 1.5 nM 17β-estradiol
[b] Antagonism tested in the yeast androgen bioassay by coadministration of 70 or 1,000 nM testosterone

for treating androgen-mediated diseases [28]. According to a study by Lund et al., equol does not bind to prostate hAR, but specifically binds to 5α-dihydrotestosterone (5α-DHT), but not T, with high affinity, thus preventing 5α-DHT from binding to the hAR [26]. The present study demonstrates that equol can inhibit the response caused by T in the yeast androgen bioassay, thus acting as an AR antagonist. If excluding the binding to T, this antiandrogenic effect in yeast is most likely caused by its ability to either bind to or inactivate the hAR. Overall, the observed activities in the yeast estrogen and androgen bioassays correlate well with the known hormonal effects of equol in mammalian systems.

Furthermore, a number of compounds belonging to one of the major classes of the phytoestrogens, the flavonoids, were tested. Flavonoids are widely distributed throughout the plant kingdom and are of interest owing to their biological, pharmacological and therapeutic potential [29]. Genistein, the main representative chemical of the flavonoids, has been shown to have a relative binding affinity for hERα of 0.45 (E2=100) and agonist activities in transcription activation assays with ERα and ERβ [21, 30–32]. The estrogenic properties of genistein, naringenin and 8-prenylnaringenin were similar as described before [21]. These compounds are ER agonists and act additively when tested in combination with E2 (Table 1). They turned out to be

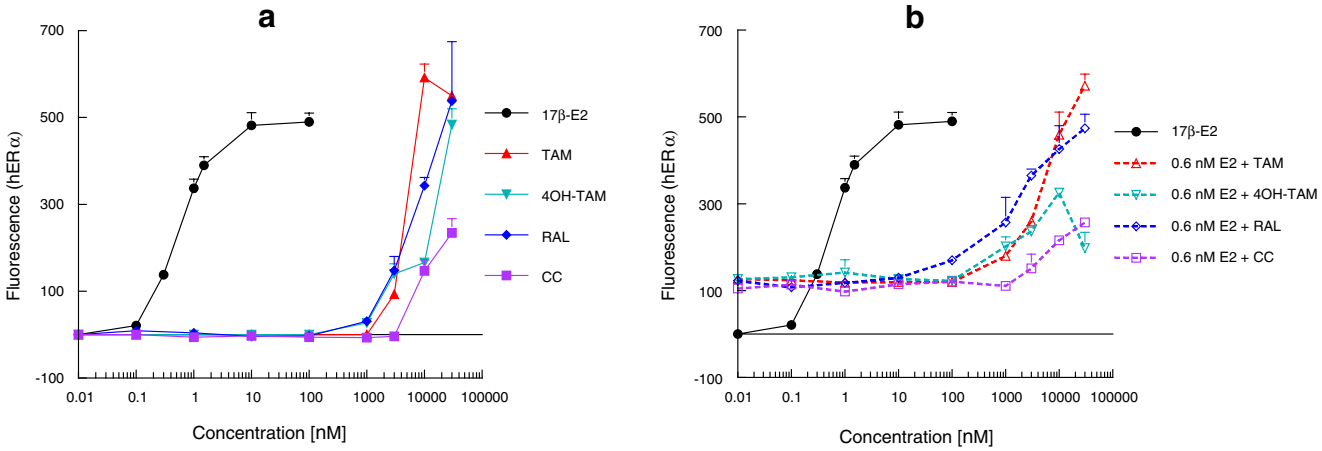

Fig. 2 Testing the agonistic and antagonistic properties of selective estrogen receptor modulators (SERMs) in the yeast estrogen bioassay: **a** shows the responses of tamoxifen (TAM), 4-hydroxytamoxifen (4OH-TAM), raloxifene (RAL) and chlomiphene citrate (CC); **b** shows the responses of these compounds when tested in combination with 0.6 nM 17β-estradiol

inactive as AR agonists or antagonists (Table 1). The flavonoid coumestrol belongs to the coumestrans and showed the same activity profile as genistein, naringenin and 8-prenylnaringenin (Table 1).

The flavonoid flavone and the plant sterol guggulsterone did not show estrogenic activity, but both compounds inhibited the signal caused by E2 (Fig. 4a). This inhibiting activity was more pronounced when tested in combination with a dose of E2, giving a near maximal response. Figure 4b shows the responses of flavone and guggulsterone in the yeast androgen bioassay. Neither compound showed androgenic activity, but both were able to inhibit the signal caused by T.

Flavone has already been demonstrated to lack binding affinity for the ER and is known as a weak ER antagonist [14, 30]. Besides that this antiestrogenic effect was confirmed by our results (Fig. 4a), the present work shows that flavone also acts as an AR antagonist in a yeast androgen bioassay (Fig. 4b). Guggulsterone is an active substance in guggulipid, an extract of the guggul tree, *Commiphora mukul*. Guggulipid is used to treat a variety of disorders in man, including dyslipidemia, obesity, arthritis and inflammation. In cell-based functional cotransfection assays, guggulsterone was shown to be an antagonist on the androgen, glucocorticoid and mineralocorticoid receptor (AR, GR and MR), but an agonist on the progesterone and estrogen receptor (PR and ER) [32]. However, guggulsterone displayed agonist activity in functional assays with mammalian cell lines expressing endogenous AR, GR, PR and ER [33]. In the present work, using yeast-based estrogen and androgen bioassays, we show that guggulsterone not only acts as an AR antagonist, but also acts as an ER antagonist.

The plant sterols campesterol and β-sitosterol did not show an effect and also ipri-flavone and resveratrol were

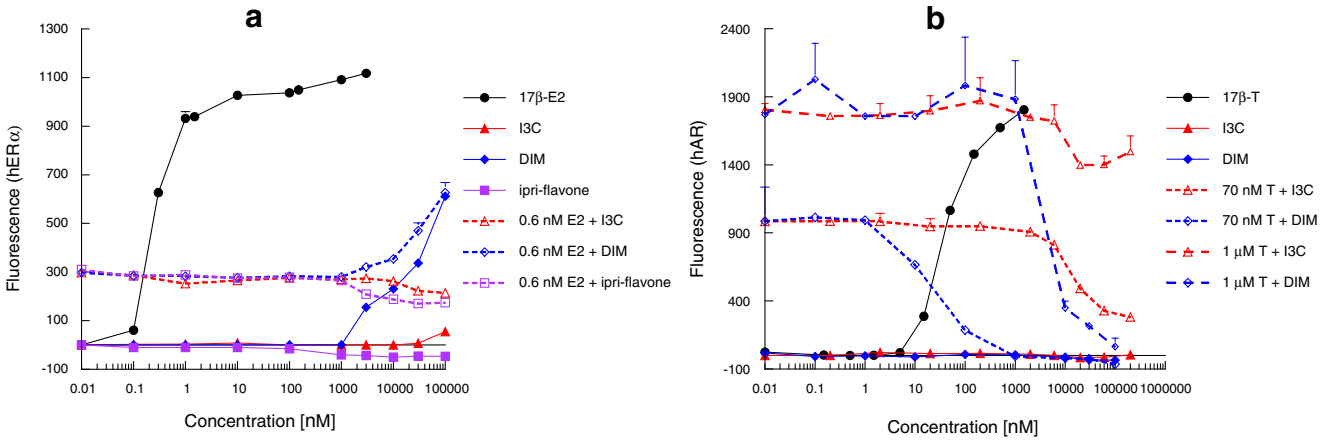

Fig. 3 Responses of the plant-derived compounds ipri-flavone, indol-3-carbinol (I3C) and diindolylmethane (DIM) in the yeast estrogen and androgen bioassays: **a** shows the responses of these compounds in the yeast estrogen bioassay without 17β-estradiol (*closed symbols*) and by coexposure with 0.6 nM 17β-estradiol (*open symbols*); **b** shows the responses of these compounds in the yeast androgen bioassay without testosterone (*closed symbols*) and by coexposure with 70 and 1,000 nM testosterone (*open symbols*)

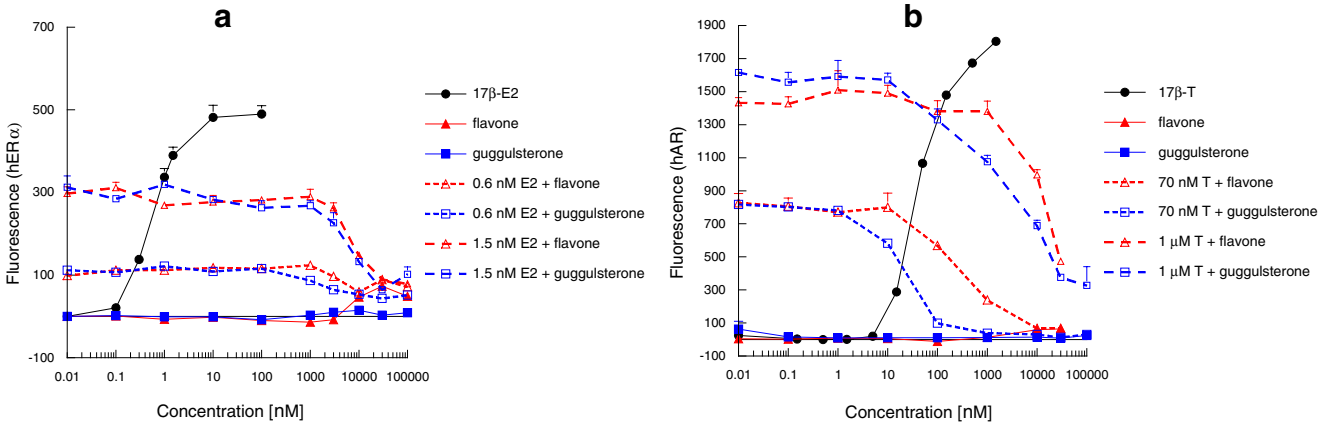

Fig. 4 Responses of flavone and guggulsterone in the yeast estrogen and androgen bioassays: **a** shows the responses of these compounds in the yeast estrogen bioassay without 17β-estradiol (*closed symbols*) and by coexposure with 0.6 and 1.5 nM 17β-estradiol (*open symbols*); **b** shows the responses of these compounds in the yeast androgen bioassay without testosterone (*closed symbols*) and by coexposure with 70 and 1,000 nM testosterone (*open symbols*)

unable to show a response. Although it is considered to be proven that β-sitosterol is an endocrine disruptor in fish, its mechanism of action is mainly unknown [34]. Resveratrol is a natural product found in red wine and is thought to have cancer preventive properties owing to its AhR antagonistic activity [35]. Resveratrol is classified as a phytoestrogen, but hormonal activities of this compound are not reported and neither were they found in this study.

In the present work relatively few compounds were tested that were able to show an effect in the yeast androgen bioassay. Previously, however, a large number of androgenic compounds were tested and used to perform a quantitative structure–activity relationship (QSAR) approach [36]. That yeast-based assays are extremely useful for QSAR model approaches is further demonstrated by Fig. 5, which shows the response of E2 in the yeast androgen bioassay and its effect in combination with a dose of T that gives a half-maximal and a near maximal response. Together with the data from Table 1, these dose–response curves demonstrate that E2 combines three activities: it is an ER agonist, a weak AR agonist and an AR antagonist. The dip in the 17β-estradiol curve in combination with the dose of T that gives a half-maximal response is expected, reflecting that at this concentration E2 starts to act as an AR antagonist, while at slightly higher concentrations it starts to act as an AR agonist too.

Discussion

The antiestrogens and SERMs tested in this study on estrogenic, antiestrogenic, androgenic and antiandrogenic activities in yeast-based bioassays show an activity profile that corresponds with the activities and effects that these compounds have on the uterus in animals and endometrial cells in vitro. Only the pure antiestrogen ICI 164,384 did not show its antiestrogenic activity in the yeast estrogen bioassay. Various explanations can be envisaged for this discrepancy, such as the absence of cell-specific factors (coactivators and corepressors), the presence of endogenous estrogen binding proteins, and the selective uptake of estrogenic compounds in yeast cells [37]. Although the long apolar 7α side chain in combination with its very polar tail ending might prevent ICI 164,384 uptake by diffusion or accelerate active excretion, the last two explanations seem unlikely as all compounds tested so far in our yeast-based bioassays, ranging from polar compounds like estriol and equol to the more apolar compounds like diethylstilbestrol (DES) and BDE-39, were able to show their specific activity and all within 6 h after exposure [21, 22, 36]. Moreover, the predominant mechanism of antiestrogen action of ICI 164,384 in both uterus and breast involves

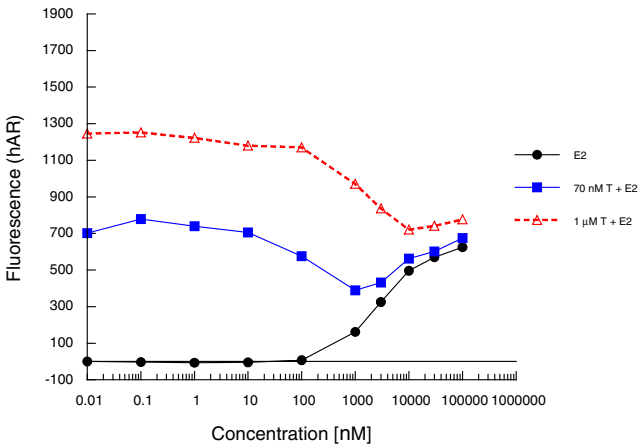

Fig. 5 Response of 17β-estradiol in the yeast androgen bioassay (standard deviations/error bars are not visible because they are smaller than the size of the symbols)

the rapid loss and turnover of estrogen receptor content [7, 38]. The absence of cell-specific factors is thus probably also not a valid explanation for the inactivity of ICI 164,384 in yeast. A possible explanation could be that rapid formation of active hERα (the hERα is continuously expressed in our yeast estrogen bioassay by use of the strong and constitutive GPD promoter) exceeds the ICI 164,384-induced inactivation of hERα. This might also explain the relative insensitivity of the yeast estrogen bioassay to detect the antiestrogenic activity of ICI 182,780. The mechanism of action of this compound partially overlaps that of ICI 164,384, but the overall antiestrogenic potency is much stronger. The antiuterotrophic potency of ICI 182,780 is more than tenfold greater than that of ICI 164,384 and ICI 182,780 binds five times stronger to ERα than ICI 164,384 [39–41], possibly explaining why the antiestrogenic effect of ICI 182,780 is just visible in yeast.

However, the mechanisms of antiestrogenic action of ICI 164,384 and ICI 182,780 are rather complex and not fully understood and the possible explanations for the relative insensitivity of yeast to detect their antiestrogenic activity as described above, including the selective uptake of estrogenic compounds, cannot be excluded by the current data. In addition, it is also possible that ICI 164,384 is unable to inactivate hERα in yeast at all or that ICI 164,384 and ICI 182,780 are both poorly soluble compounds in yeast growth media, but dissolve in cell culture media due to the presence of 5–15% serum. The different in vitro and in vivo mechanisms involved in the antiestrogenic effects of pure antiestrogens and SERMs are reviewed by Jordan [1] and it is illustrated that there is no unifying theory that can explain the target site-specific actions of SERMs. There are many inconsistent data and until recently the complexity of (anti)hormone action was not anticipated [1].

The determined activities of the plant-derived compounds, e.g. I3C, DIM, equol, flavone and guggulsterone, correlate well with their known properties. Here we show for the first time that flavone is not only an ER antagonist, but also acts as an AR antagonist in a yeast androgen bioassay and that guggulsterone is not only an AR antagonist, but also acts as an ER antagonist in a yeast estrogen bioassay. When tested in the yeast androgen bioassay, there is about a 100-fold difference between the inhibiting concentrations needed to inhibit the half-maximal and near maximal response. This is due to the fact that there is a 14 times difference in the T concentration, 70 and 1,000 nM, respectively. In the yeast estrogen assay there is only a 2.5 difference in the E2 concentration needed for a half-maximal and near maximal response, 0.6 and 1.5 nM, respectively. The observed differences in the yeast androgen bioassay are thus due to common receptor kinetics.

Results show that the agonistic and additive effects of compounds can be observed best in combination with a dose of E2 or T that gives a half-maximal response. It is advisable to study the antagonistic effects both with a dose of an agonist that gives a half-maximal response and a dose that gives a near maximal response.

Yeast estrogen and androgen bioassays are relatively insensitive compared with their mammalian counterparts and the relatively high EC_{50} values of the receptor agonists might, from a physiological point of view, be less relevant. However, in a physiological context, 1 nM E2 or the circulating level in premenopausal women, 272 pg mL^{-1}, will maximally activate an ERα estrogen response element (ERE) in vivo [1]. The maximal response in yeast estrogen bioassays is obtained at E2 concentrations between 1 and 2 nM. Thus one might just as well argue that yeast-based assays are at least as relevant as their mammalian counterparts, showing a maximal response in the low picomolar range. The relatively low EC_{50} values for agonists obtained with human cell lines most likely reflect the hormone dependence of the tumour cell lines for growth and their sensitivity for antihormone treatment. In addition, in general the relative estrogenic and androgenic potencies (REPs and RAPs) determined in the yeast estrogen and androgen bioassay, respectively, show a good correlation with those determined in vitro with mammalian cell-based assays and the in vivo Allen and Doisy test for estrogenic activity and the in vivo Herschberger test for androgenic activity [20–22]. Thus, yeast cells are less sensitive, but the determined effects and relative potencies are very specific. The same is valid for the antagonistic responses of the compounds described in this study. Only the differences in the relative potencies of tamoxifen and both ICI compounds between yeast and mammalian cell systems are relatively high.

Differences between data obtained with endometrial, prostate, breast and yeast cells may be explained by different mechanisms that underlie endocrine response (e.g. as in the case of ICI 164,384), different organ and species sensitivities (e.g. assembling and presence of both coactivators and corepressors, amount of receptors), limitations of the assay techniques (e.g. selective uptake, solubility), differences in metabolism and many more. These differences not only occur between yeast and mammalian cells, but also between different mammalian cell types [1].

The effects observed with tamoxifen have been intensively investigated and it was shown that different breast cell lines show different responses, agonistic or antagonistic, with tamoxifen and other compounds [42–44]. Moreover, breast cells often not only express hERα, but also hERβ [1, 42]. This coexpression of hERβ has a great influence on how the cells respond to estrogens and other hormones. In general the hERβ decreases the response of

the hERα and acts as a kind of a regulator (repressor) of hERα [21, 45]. Of course this may have physiological importance, but it complicates the interpretation of the results.

The importance of the metabolism and the differences in the metabolism of compounds has been underestimated for a long time. Differences in metabolism were often responsible for the differences found between different cell lines and between cell lines and yeast-based bioassays [36, 46, 47]. Estrone for instance showed a REP of 0.2 in a proliferation test based on human breast cancer cells (E-screen) and the yeast estrogen bioassay, but was equally potent to E2 in a transcription activation assay based on human T47-D breast cancer cells [16, 21, 46]. In the ER-CALUX test with the T47-D breast cancer cells, the 17β-hydroxysteroid dehydrogenase 3 enzyme was responsible for the conversion of estrone into E2 and vice versa [46]. Another example is benzo[*a*]pyrene (B[a]P). This compound was shown to be estrogenic in different mammalian cell reporter gene (MCRG) systems and in this aspect the outcomes using yeast assays were odd as B[a]P is completely inactive in yeast estrogen screens [14]. However, more specific observations showed that the hydroxylated benzo[*a*]pyrene metabolites are responsible for the estrogen receptor-mediated gene expression induced by benzo[*a*]pyrene in the in vitro mammalian systems, but do not elicit uterotrophic effects in vivo [47]. Yeast generally lacks the metabolic capacity of mammalian cells. Agonists or antagonists that require metabolic activation for conversion to a bioactive state may not be detected in yeast. In contrast, compounds that are metabolically inactivated by mammalian cells may persist and be especially potent in yeast assays.

Conclusion

Yeast cells might by definition not necessarily be relevant for the higher vertebrates, but nevertheless the relative simplicity of yeast versus mammalian cells can be very helpful in studying the mechanism of action of different cellular processes. Overall, it can be concluded that in order to investigate the hormonal properties of different compounds and their mechanism of action, there is no superior assay system. The only way to elicit the mechanism of action of compounds is to be aware of the limitations of the different assay techniques, to fully understand the mechanism in the assay itself (including the metabolism and possible cross talk from other nuclear receptors) and to compare the outcomes from the different assay types. Different assay types are thus complementary and are needed together to be able to elicit the mechanism of actions of compounds and their metabolites [48].

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