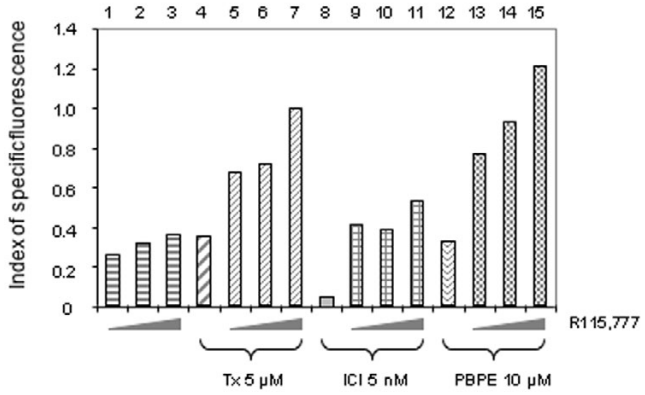
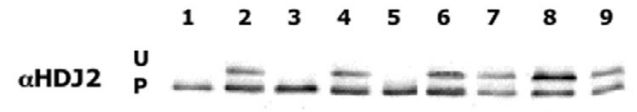
Figure 4



Effects of anti-estrogens and R115,777 on the caspase cleavage product of cytokeratin 18. Determined by FACS on MCF-7 cells incubated for 5 days with either 5 µM tamoxifen (Tx), 1 nM ICI182,780 (ICI) or 10 µM N-pyrrolidine(-phenylmethyl-phenoxy)-ethanamine-HCl (PBPE) combined with 5, 10 or 25 nM R115,777. Floating and adherent cells were harvested, fixed and stained with the fluorescein conjugated monoclonal antibody M30 CytoDeath and analysed using flow cytometry. Results are expressed as an index of specific fluorescence, as described in Materials and methods. Data are representative of one to three independent experiments in duplicate; variations were <1%.

Figure 5



R115,777	-	+	-	+	-	+	-	+	-
Tamoxifen	-	-	-	-	+	+	-	-	-
ICI182780	-	-	+	+	-	-	-	-	-
PBPE	-	-	-	-	-	-	+	+	+
Estradiol	-	-	-	-	-	-	-	-	+

Effects of anti-estrogens and R115,777 on isoprenylated proteins in MCF-7 cells. Cells were incubated for 2 days with R115,777 (1 nM) combined with either tamoxifen (5 µM), ICI182777 (5 nM), N-pyrrolidine(-phenylmethyl-phenoxy)-ethanamine-HCl (PBPE; 10 µM) or estradiol (1 nM). Floating and adherent cell lysates were resolved by SDS-PAGE and blotted onto PVDF membranes and 5 µg protein were loaded for HDJ 2 detection, as described in Materials and methods. U, unprenylated; P, prenylated.

target of the other. To examine these proposals we first used bindings assays and verified that R115,777, like FTI 277, does not interact either with ERs or with AEBS (data not shown).

R115,777 does not affect the cellular uptake of [^{3}H]tamoxifen

We next examined whether R115,777 was able to increase the cellular uptake of Tam. Exposure to increasing concentrations of R115,777 (from 2 to 50 nM) did not modify the cellular level of tritiated Tam in MCF-7 cells (data not shown). Thus, the effects of combining R115,777 with Tam were not attributable to any increase of cellular Tam concentrations by the FTI.

With the unavailability of an assay for R115,777 or a radiolabelled product we were unable to determine the effects of Tam addition on the intracellular concentration of R115,777. To circumvent this problem, we looked for any farnesylation inhibitory activity of R115,777 in the presence or absence tamoxifen.

Effects of R115,777 and anti-estrogens on isoprenylated proteins

We have previously shown that AEBS is a multiprotein complex consisting of enzymes involved in sterol biosynthesis [8], suggesting that they possibly interfer with the mevalonate pathway. For this reason we examined the capacity of Tam to act on the protein farnesylation process. HDJ2 farnesylation, a surrogate clinical marker for farnesyl transferase inhibitor activity, was used here as a reporter to define any potential inhibitory effect on the mevalonate pathway. Fig. 5 shows western blots of MCF-7 cells incubated with the three 'anti-estrogens'. As a control, untreated cells exhibited complete prenylation of HDJ2 (P, lane 1), while treatment with R115,777 (1 nM) resulted in an accumulation of unprenylated HDJ2, seen as a more slowly migrating band (U, lane 2). Furthermore, addition of neither ICI182,780 (5 nM) nor Tam (5 µM) affected the farnesylation of HDJ2, either alone or in combination with R115,777, which inhibits farnesylation of HDJ2 (lanes 3 to 6), even though the HMG CoA reductase promoter has been shown to be estrogen regulated [24]. The selective AEBS ligand PBPE (10 µM), however, was able to regulate HDJ2 farnesylation itself in the absence of R115,777 (lane 7) and further increased the effect of R115,777 (lane 8). Finally, estradiol did not have any effect, either alone or in association with R115,777, ICI182,780 or Tam (data not shown), or indeed in the presence of PBPE (lane 9).

In this series of experiments, a low 1 nM concentration of R115,777 was used so as to evaluate any increase of the unfarnesylated protein resulting from exposure to the various combinations. Among the three 'anti-estrogens' tested, it is clear that PBPE interferes with the farnesylation of HDJ 2 (Fig. 5), as previously suggested in an earlier study with another AEBS selective ligand in the farnesylation of H-ras [25]. Tam exhibits only a slight or negligible effect on the same protein at concentrations that cause apoptosis and growth inhibition of MCF-7 cells; however, higher concentrations of Tam (50 µM) that induce a high level of cell mortality are able to inhibit HDJ2 prenylation (data not shown). These observations therefore suggest that, although Tam and PBPE bind AEBS with equivalent affinity, they appear to have variable effects on the enzymatic activities of AEBS [5,20].

Any direct or indirect action of the AEBS ligands on the mevalonate pathway were not examined in this study, but the fact