Fig. S1. The ER inhibitor ICI 182,780, decreases PR-mediated gene expression in T47D cells. In panel A before plating the cells were maintained in full serum (5% FBS) that contains some estrogen. In panel B the cells were depleted of estrogen by 4 days in medium containing 5%1X CD-FBS. A. T47D cells were maintained in MEM containing 10 mM HEPES pH 7.4 and 5% FBS. 10,000 cells/well were plated in 96 well plates in 100 µl of medium containing 5% CD-FBS. B. The cells were maintained for 4 days in medium containing 5% 1X CD-FBS before plating. A. After 24 h 2.5 nM or 500 nM progesterone (P₄) was added with or without 1 µM ICI 182,780. 500nM progesterone was used to be certain that ICI 182,780 was not competing with P₄ for binding to PR. B. After 24 h cells were maintained in medium with or without 5 nM progesterone (P₄) and 1 µM ICI 182,780. Alkaline phosphatase assay. After 24 h in the indicated treatment, the cells were washed once with PBS, lysed in 100 µl of buffer (20 mM potassium phosphate, pH 7.8, 5 mM MgCl₂, 0.5% Triton X-100, frozen. Lysate was stored at –70°. 5 µl of supernatant was removed and assayed in a 96 well plate in 25 µl of assay buffer (100 mM diethanolamine, pH 9.5, 1 mM MgCl₂, 0.4 mM CSPD (substrate, Applied Biosystems/Tropix) and 1X Emerald ll enhancer (Applied Biosystems/Tropix). After 1 h at room temp., luminescence in the visible (emission max. 542 nM) was read for 1 sec. Data were the average of 3 experiments ± SEM
Fig. S2. TPSF does not exhibit nonspecific toxic effects in ERα negative MDA-MB-231 cells. Cell maintenance, growth and plating were as described in the Fig. 4 legend. The indicated concentrations of OHT (filled triangles) and TPSF (filled circles) and 1 pM E2 were added and cell number was determined using MTS and a standard curve (Fig. 4 legend). Cell number in the absence of TPSF or OHT was set as 100%. Data were the average of 3 experiments ± SEM.
Supplemental Figure 3

Fig S3. TPSF but not OHT inhibits E₂-ERα-dependent growth of MCF7ERαHA cells.
MCF7ERαHA cells were maintained for 4 days in 6x CD-FBS (22,37), seeded into 12 well plates with or without 0.5 μg/ml Dox, 1 pM E₂, 5 μM OHT and 5 μM TPSF, grown for 4 days and assayed using MTS and a standard curve for cell number (see Fig. 4 legend). Cells in ethanol and DMSO vehicle alone were set to 100%. Data represent the average of 3 experiments ± SEM. The difference between the cells incubated with TPSF + E₂ + Dox and cells in OHT + E₂ + DOX was significant (P <0.05 using Student’s t test).
Supplemental Figure 4

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**Fig. S4. TPSF has little or no effect on levels of AR and GR.** To test the selectivity of TPSF for down-regulation of ERα, we evaluated the effect of 5 μM and 10 μM TPSF on the levels of AR and GR. The cell lines used and cell growth conditions were those employed in the dose-response studies evaluating effects of TPSF on gene expression (Fig. 2D). Cells were maintained in medium containing or lacking dexamethasone (panel A) or DHT (panel B) and 0, 5 or 10 μM TPSF for 24 hours. Extracts were prepared and analyzed on separate gels for AR and GR levels by western blotting. Total protein (12 μg for AR and 40 μg for GR) was fractionated on 10% polyacrylamide gels and AR and GR levels determined by western blot. The antibody used to detect AR was AR441 monoclonal antibody used at 1:1000 dilution (NeoMarkers, Freemont, CA). The GR antibody was MA1-510 monoclonal antibody (Thermo Scientific, Rockford, IL) used at 1:1000 dilution. Data is representative of at least 2 gels for each sample.
**Supplemental Figure 5**

![MUC1 and beta-actin expression levels](image)

**Fig. S5. TPSF does not down-regulate the level of Muc1.** MCF-7 cells were maintained and plated as described in Materials and Methods. After 4 days in MEM + 5% 1X CD-FBS, the medium was replaced with medium with or without 10 nM E₂ and with or without 10 µM TPSF. Cells were harvested at 24 h extracts prepared and Muc1 detected using Muc1 specific antibody (60). The actin samples were run on a separate gel.