Supplementary Materials and Methods

Cell Cycle Analysis

Cells were cultured under steroid-free conditions for 3 days prior to seeding 1 million cells per 15 cm dish. Following seeding of cells, cells were serum-starved for 24 h, and then treated with media containing the test compounds for 24 h. Cells were lifted from the dish using Versene (Invitrogen). Cells were stained for DNA content by first fixing cells in 75% EtOH, and then incubating the cells in a solution of 50 ng/ml propidium iodide, 0.1% Triton X-100, and 200 µg/ml RNase A at 37° C for 30 minutes. Cell cycle analysis was conducted by measuring DNA content per cell in 30,000 cells per sample using a fluorescence-activated cell sorter (FACS) flow cytometer (Becton Dickinson, San Jose, CA) and fitting the data to G0/G1-phase, S-phase, and G2/M-phases using Modfit (Verity Software House, version 5.2).

Supplementary Results

Cell cycle progression responses to 17-H-EXE

To investigate 17-H-EXE effects on cell cycle progression, MCF-7 and T47D cells were treated with test compounds for 24 h, followed by and staining with propidium iodide and flow cytometric analysis. In both cell lines, 10^{-10} M E_2 stimulated cell cycle progression by increasing the percentage of cells in S-phase 2.6 to 3-fold over control (all *P* values < 0.001). E_2's effect was completely blocked by FUL, confirming E_2-stimulated cell cycle progression was mediated by ER (Supplementary Fig. 1A-B; both *P* values < 0.001). In T47D cells but not MCF-7 cells, either a low 10^{-9} M or high 10^{-6} M concentration of R1881 stimulated both S- and G2/M-phase entry by ~ 2-fold compared to CON treatment (Supplementary Fig. 1B; both *P* values < 0.001). In T47D cells, R1881 at 10^{-9} M acted via AR, since its effects were blocked by co-treatment with

BIC ($P < 0.001$), whereas R1881 at 10^{-6} M was too high a concentration to have been out-competed by BIC for binding to AR. FUL did attenuate the ability of 10^{-6} M R1881 to stimulate S-phase entry by 25% ($P < 0.001$), indicating a similar degree of involvement of ER , but this also indicated that the majority of effect of 10^{-6} M R1881 was through AR (Supplementary Fig. 1B). In MCF-7 (Supplementary Fig. 1A), only a high 10^{-6} M concentration of R1881 stimulated S-phase entry by 2.5-fold ($P < 0.001$) and 2.2-fold ($P < 0.001$), respectively, compared to CON-treatments. These effects of 10^{-6} M R1881 in MCF-7 were mediated by ER since they were blocked by co-treatment with FUL (both P values < 0.001). It is unclear why R1881 stimulated both S-phase and G2/M-phase entry to equivalent levels in T47D cells, and only S-phase entry in MCF-7 cells, but may reflect a difference in AR- versus ER-mediated cell cycle progression. The effects of 17-H-EXE on cell cycle progression were similar to those of R1881 in each respective cell line. In T47D cells, either a low 10^{-8} M or high 5×10^{-6} M concentration of 17-H-EXE stimulated S-phase entry by 1.9-fold ($P < 0.001$) and 2.4-fold ($P < 0.001$), respectively, with BIC blocking the effect of 10^{-8} M 17-H-EXE ($P < 0.001$), whereas the effect of 5×10^{-6} M 17-H-EXE was not blocked by FUL (Supplementary Fig. 1B). In MCF-7 (Supplementary Fig. 1A), only the high 5×10^{-6} M concentration of 17-H-EXE induced S-phase entry by 2.2-fold ($P < 0.001$), and this effect was blocked by co-treatment with FUL. Therefore, 17-H-EXE acted through AR to stimulate cell cycle progression at a low concentration in T47D cells, but through ER at a high concentration in MCF-7 cells.