Estrogen receptor α inhibitor activates the unfolded protein response, blocks protein synthesis, and induces tumor regression

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Recurrent estrogen receptor α (ERα)-positive breast and ovarian cancers are often therapy resistant. Using screening and functional validation, we identified BHPI, a potent noncompetitive small molecule ERα biomodulator that selectively blocks proliferation of drug-resistant ERα-positive breast and ovarian cancer cells. In a mouse xenograft model of breast cancer, BHPI induced rapid and substantial tumor regression. Whereas BHPI potently inhibits nuclear estrogen–ERα-regulated gene expression, BHPI is effective because it elicits sustained ERα-dependent activation of the endoplasmic reticulum (EnR) stress sensor, the unfolded protein response (UPR), and persistent inhibition of protein synthesis. BHPI distorts a newly described action of estrogen–ERα: mild and transient UPR activation. In contrast, BHPI elicits massive and sustained UPR activation, converting the UPR from protective to toxic. In ERα+ cancer cells, BHPI rapidly hyperactivates plasma membrane PLCγ2, generating inositol 1,4,5-triphosphate (IP3), which opens EnR IP3R calcium channels, rapidly depleting EnR Ca2+ stores. This leads to activation of all three arms of the UPR. Activation of the PERK arm stimulates phosphorylation of eukaryotic initiation factor 2α (eIF2α), resulting in rapid inhibition of protein synthesis. The cell attempts to restore EnR Ca2+-levels, but the open EnR IP3R calcium channel leads to an ATP-depleting futile cycle, resulting in activation of the energy sensor AMP-activated protein kinase and phosphorylation of eukaryotic elongation factor 2 (eEF2). eEF2 phosphorylation inhibits protein synthesis at a second site. BHPI’s novel mode of action, high potency, and effectiveness in therapy-resistant tumor cells make it an exceptional candidate for further mechanistic and therapeutic exploration.

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estrogens, acting via estrogen receptor α (ERα), stimulate tumor growth (1–3). Approximately 70% of breast cancers are ERα-positive and most deaths due to breast cancer are in patients with ERα+ tumors (2, 4). Endocrine therapy using aromatase inhibitors to block estrogen production, or tamoxifen and other competitor antiestrogens, often results in selection and outgrowth of resistant tumors. Although 30–70% of epithelial ovarian tumors are ERα-positive (1), endocrine therapy is largely ineffective (5–7). After several cycles of chemotherapy, tumors recur as resistant ovarian cancer (5), and most patients die within 5 years (8).

Noncompetitive ERα inhibitors targeting this unmet therapeutic need, including DIBA, TPBM, TPSF, and LRH-1 inhibitors that reduce ERα levels, show limited specificity, require high concentrations (>5 μM), and usually have not advanced through preclinical development (9–12). These noncompetitive ERα inhibitors and competitor antiestrogens are primarily cytostatic and act by preventing estrogen–ERα action; therefore, they are largely ineffective in therapy-resistant ERα containing cancer cells that no longer require estrogens and ERα for growth.

To target the estrogen–ERα axis in therapy-resistant cancer cells, we developed (13) and implemented an unbiased pathway-directed screen of ~150,000 small molecules. We identified ~2,000 small molecule biomodulators of 17β-estradiol (E2)–ERα-induced gene expression, evaluated these biomodulators for inhibition of E2–ERα-induced cell proliferation, and performed simple follow-on assays to identify inhibitors with a novel mode of action. Here, we describe 3,3-bis(4-hydroxyphenyl)-7-methyl-1,3-dihydro-2H-indol-2-one (BHPI), our most promising small molecule ERα biomodulator.

In response to stress, cancer cells often activate the endoplasmic reticulum (EnR) stress sensor, the unfolded protein response (UPR). We recently showed that as an essential component of the E2–ERα proliferation program, estrogen induces a different mode of UPR activation, a weak anticipatory activation of the UPR before increased protein folding loads that accompany cell proliferation. This weak and transient E2–ERα-mediated UPR activation is protective (14). BHPI distorts this normal action of E2–ERα and induces a massive and sustained ERα-dependent activation of the UPR, converting UPR activation from cytoprotective to cytotoxic. Moreover, independent of its effect on the UPR and protein synthesis, BHPI rapidly suppresses E2–ERα-regulated gene expression.

Results

BHPI Is Effective in Drug-Resistant ERα+ Breast and Ovarian Cancer Cells.

We investigated BHPI’s effect on proliferation in therapy-sensitive and therapy-resistant cancer cells. BHPI (SI Appendix, Fig. S1 A

Significance

Late-stage estrogen receptor α (ERα)-positive breast and ovarian cancers exhibit many regulatory alterations and therefore resist therapy. Our novel ERα inhibitor, BHPI, stops growth and often kills drug-resistant ERα+ cancer cells and induces rapid and substantial tumor regression in a mouse model of human breast cancer. BHPI distorts a normally protective estrogen–ERα-mediated activation of the unfolded protein response (UPR) and elicits sustained UPR activation. The UPR cannot be deactivated because BHPI, acting at a second site, inhibits production of proteins that normally help turn it off. This persistent activation converts the UPR from protective to lethal. Targeting therapy-resistant ERα-positive cancer cells by converting the UPR from cytoprotective to cytotoxic may hold significant therapeutic promise.


Conflict of interest statement: The authors have filed a patent application on BHPI. This article is a PNAS Direct Submission. R.H. is a guest editor invited by the Editorial Board.

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BHPI Induces Tumor Regression. We next evaluated BHPI in a mouse xenograft model using MCF-7 cell tumors (19). For each tumor, cross-sectional area at day 0 (~45 mm²) was set to 0%. Control (vehicle injected) and BHPI-treated mice were continuously exposed to estrogen. After daily i.p. injections for 10 d, the tumors in the vehicle-treated mice exhibited robust growth (Fig. 2, red bars). Whereas BHPI at 1 mg/kg every other day was ineffective (SI Appendix, Fig. S4), initiation of 15 mg/kg daily BHPI treatment resulted in rapid regression of 48/52 tumors (Fig. 2, blue bars). BHPI easily exceeded the goal of >60% tumor growth inhibition proposed as a benchmark more likely to lead to clinical response (20). Furthermore, BHPI, at 10 mg/kg every other day, ultimately stopped tumor growth and final tumor weight was reduced ~60% compared with controls (SI Appendix, Fig. S4 A and B). BHPI was well tolerated; BHPI-treated and control mice exhibited similar food intake and weight gain (SI Appendix, Fig. S4 C and D).

BHPI Is an ERα-Dependent Inhibitor of Protein Synthesis. Surprisingly, BHPI greatly reduced protein synthesis in ERα+ cancer cells (Fig. 3A and SI Appendix, Fig. S5). If BHPI inhibits protein synthesis through ERα, it should only work in ERα+ cells, and ERα overexpression should increase its effectiveness. BHPI inhibited protein synthesis in all 14 ERα+ cell lines, with no effect on protein synthesis in all 12 ERα− cell lines (Fig. 3A and SI Appendix, Fig. S5 A and B). BHPI does not inhibit protein synthesis in ERα-negative MCF-10A breast cells, but gains the ability to inhibit protein synthesis when ERα is stably expressed in isogenic MCF10AER IN9 cells (Fig. 3B) (21). Notably, BHPI loses the ability to inhibit protein synthesis when ERα is knocked down in the stably transfected cells after siRNA (Fig. 3C and SI Appendix, Fig. S6 A) or is degraded by ICI (Fig. 3D). Furthermore, increasing the ERα level in MCF7ERαHA cells (22), stably transfected to express doxycycline-inducible ERα, progressively increased BHPI inhibition of protein synthesis (Fig. 3E). BHPI does not work by activating the estrogen binding protein GPR30. BHPI has no effect on cell proliferation (SI Appendix, Fig. S2) or protein synthesis (SI Appendix, Fig. S5A) in HepG2 cells that contain functional GPR30 (23), and activating GPR30 with G1 did not inhibit protein synthesis (SI Appendix, Fig. S6 B and C). Thus, ERα is necessary and sufficient for BHPI to inhibit protein synthesis.

BHPI Rapidly Inhibits Protein Synthesis by a PLCγ-Mediated Opening of the Insoluble Triphosphate Receptor (IP₃,R) Ca²⁺ Channel, Activating the PERK Arm of the UPR. Inhibiting mechanistic target of rapamycin (mTOR) signaling did not strongly inhibit protein synthesis (SI Appendix, Fig. S6D), suggesting BHPI is unlikely to work through mTOR. We next investigated whether initial inhibition of protein synthesis by BHPI is due to activation of the UPR. There are three UPR arms. The transmembrane kinase PERK is activated by autophosphorylation, p-PERK phosphor-ylates eukaryotic initiation factor 2α (eIF2α), inhibiting translation of most mRNAs (SI Appendix, Fig. S7A) (24, 25). The other arms of the UPR initiate with ATF6 activation (SI Appendix, Fig. S7B), leading to increased protein folding capacity and activation of IRE1α, which alternatively splices XBP1, producing active spliced (sp)-XBP1 (SI Appendix, Fig. S7C) (24, 25). In ERα+ MCF-7 and T47D cells, but not in ERα− MDA-MB-231 cells, BHPI rapidly inhibited protein synthesis (SI Appendix, Fig. S8A) and in parallel increased eIF2α phosphorylation (Fig. 3F).
and SI Appendix, Fig. S8 B and C). Downstream readouts of eIF2α phosphorylation, CHOP, and GADD34 mRNAs, were rapidly induced by BHPI (SI Appendix, Fig. S8 D and E). Consistent with BHPI inhibiting protein synthesis through eIF2α-Ser51 phosphorylation, transfecting cells with a dominant-negative eIF2α-S51A mutant largely prevented BHPI from inhibiting protein synthesis (SI Appendix, Fig. S8F). We next evaluated whether increases in eIF2α phosphorylation and rapid inhibition of protein synthesis occur through activation of PERK. p-PERK was increased 30 min after BHPI treatment (Fig. 3F and SI Appendix, Fig. S8G), and pretreating cells with a PERK inhibitor (PERKi) abolished rapid BHPI inhibition of protein synthesis (SI Appendix, Fig. S9A). RNAi knockdown of PERK abolished BHPI inhibition of protein synthesis at 30 min and strongly inhibited BHPI-stimulated eIF2α phosphorylation (Fig. 3G and SI Appendix, Fig. S9B). Because PERK knockdown blocks rapid eIF2α phosphorylation, BHPI is not inhibiting translation by activating other upstream kinases that phosphorylate eIF2α. Furthermore, BHPI rapidly activates the ATF6α and IRE1α arms of the UPR, as shown by increased cleaved p50-ATF6α and sp-XBP1 (Fig. 3H).

To explore how BHPI activates the UPR, we examined inhibition of protein synthesis by known UPR activators. Thapsigargin (THG) and ionomycin, which activate the UPR by release of Ca^{2+} from the lumen of the ER into the cytosol (24, 25), but not UPR activators that work by other mechanisms, elicited the rapid and near quantitative inhibition of protein synthesis seen with BHPI (SI Appendix, Fig. S10A). To test whether BHPI alters intracellular Ca^{2+}, we monitored intracellular Ca^{2+} levels following treatment of MCF-7 cells with either 50 nM noncoding (NC) siRNA or PERK siRNA, followed by treatment with BHPI (n = 4). IPγR SmartPool and PLCγ Knockdown contained equal amounts of three individual SmartPools directed against each isoform of IPγR and PLCγ (Fig. S10B). IPγR SmartPool contained equal amounts of three individual SmartPools directed against each isoform of IPγR. (L) Effects of BHPI on protein synthesis in MCF-7 cells treated with either 100 nM NC siRNA, pan IPγR siRNA, or PLCγ siRNA SmartPool (n = 4). (M) Quantitation of intracellular IPγR levels following treatment of MCF-7 cells for 10 min with E2 or BHPI (n = 3). (N) Model of BHPI acting through the UPR, eIF2α, and AMPK to kill ERα+ cancer cells. Data are mean ± SEM. Different letters indicate a significant difference among groups (P < 0.05) using one-way ANOVA followed by Tukey’s post hoc test. *P < 0.05, **P < 0.01. n.s., not significant.
Treatment with 2-APB, which locks the IP_3R Ca^{2+} channels closed, but not closing the RyR Ca^{2+} channels with high concentration ryanodine (Ry), abolished the rapid BHPI–ERα-mediated increase in cytosol Ca^{2+} and inhibition of protein synthesis (Fig. 3 K and L). Furthermore, RNAi knockdown of IP_3R (SI Appendix, Fig. S11A) abolished the BHPI-mediated increase in cytosol Ca^{2+} and inhibition of protein synthesis (Fig. 3 K and L). IP_3R Ca^{2+} channels are also modulated through protein kinase A (PKA), but BHPI did not induce PKA-dependent IP_3R-Ser_1756 phosphorylation (26) (SI Appendix, Fig. S11B).

BHPI Strongly Activates Phospholipase Cγ, Producing Inositol 1,4,5-Triphosphate. Inositol 1,4,5-triphosphate (IP_3) is produced when the activated phosphorylated plasma membrane enzyme, phospholipase Cγ (PLCγ), hydrolyzes PIP_2 to diacylglycerol (DAG) and IP_3. Supporting a role for PLCγ, siRNA knockdown of PLCγ (SI Appendix, Fig. S11C) abolished the BHPI-mediated increase in cytosol Ca^{2+} (SI Appendix, Fig. S11C) and BHPI inhibition of protein synthesis (Fig. 3L), and the PLCγ inhibitor U73122 abolished the BHPI–ERα increase in cytosol Ca^{2+} (SI Appendix, Fig. S11C). Confirming PLCγ’s role, BHPI induces rapid PLCγ-Tyr^783 phosphorylation (SI Appendix, Fig. S11D), and strongly increased IP_3 levels (Fig. 3M). Supporting the idea that BHPI acts by distorting the newly described weak E2–ERα activation of the TBP (14), BHPI induced a much larger increase in IP_3 levels than E2 (Fig. 3M).

Rapid BHPI activation of plasma membrane PLCγ indicates UPR activation is an extranuclear action of BHPI–ERα. PLCγ and ERα communcoprecipitate (27), and overexpression of ERα in MCF7ERα cells further increased IP_3 levels in response to BHPI (SI Appendix, Fig. S11E). Consistent with extranuclear ERα-dependent activation of the UPR, an estrogen-dendrimer conjugate (EDC) that cannot enter the nucleus (28), induced sp-XBPI, but not nuclear estrogen-regulated genes (SI Appendix, Fig. S12). A model depicting BHPI action is presented in Fig. 3N.

BHPI Inhibits E2–ERα–Regulated Gene Expression and Likely Interacts with ERα. Consistent with BHPI binding to E2–ERα, BHPI, but not an inactive close relative, compound 8 (SI Appendix, Fig. S1B), significantly altered the fluorescence emission spectrum of purified ERα (Fig. 4A). We also tested whether BHPI alters the sensitivity of purified ERα ligand-binding domain (LBD) to protease digestion. Addition of BHPI followed by cleavage with proteinase K revealed a 15-kDa band in BHPI-treated ERα LBD that was nearly absent in the LBD treated with DMSO or compound 8 (Fig. 4B).

Because BHPI interacts with ERα and distorts an extranuclear action of E2–ERα, we tested whether, independent of its ability to inhibit protein synthesis and activate the UPR, BHPI would also modulate nuclear E2–ERα-regulated gene expression. At early times when BHPI inhibited E2–ERα induction of pS2 mRNA, neither inhibiting protein synthesis with cycloheximide (CHX), nor activating the UPR with tunicamycin (TUN) or THG (SI Appendix, Fig. S13A), inhibited induction of pS2 mRNA (Fig. 4C). BHPI inhibited E2–ERα induction of pS2, GREB1, XBPI, CXCL12, and ERE-luciferase in ERα MC57F-7, and T47D cells (SI Appendix, Fig. S13 B–F) and blocked E2–ERα down-regulation of IL1-R1 and EFNA1 mRNA (SI Appendix, Fig. S13 E and G). BHPI is not a competitive ERα inhibitor. Increasing the concentration of E2 by 1,000-fold had no effect on BHPI inhibition of E2 induction of pS2 mRNA (Fig. 4D). Moreover, BHPI did not compete with E2 for binding to ERα (SI Appendix, Fig. S14A). Because BHPI inhibits E2–ERα induction and repression of gene expression, BHPI acts at the level of ERα and not by a general inhibition or activation of transcription. BHPI did not alter ERα protein levels or nuclear localization (SI Appendix, Fig. S14 B and C). Chromatin immunoprecipitation (ChIP) showed that BHPI strongly inhibited E2-stimulated recruitment of ERα and RNA polymerase II to the pS2 and GREB1 promoter regions (Fig. 4E and SI Appendix, Fig. S14D). Consistent with BHPI inducing an ERα conformation exhibiting reduced affinity for gene regulatory regions, 10-fold overexpression of ERα in MCF7ERα cells abolished BHPI inhibition of induction of GREB1 mRNA (Fig. 4F). BHPI still killed 500,000 cells because ERα overexpression enhances BHPI inhibition of protein synthesis (Fig. 3E). Taken together, our data provide compelling evidence that BHPI is a new type of modulator, altering both nuclear and extranuclear actions of ERα.

BHPI Rapidly Depletes Intracellular ATP Stores and Activates AMPK. BHPI treatment results in rapid depletion of EnR Ca^{2+}. To restore EnR Ca^{2+}, the cell activates sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) pumps, which catalyze ATP-dependent transfer of Ca^{2+} from the cytosol into the lumen of the EnR. Because BHPI opens the IP_3R Ca^{2+} channel, Ca^{2+} pumped back into the EnR lumen by SERCA flows back into the cytosol (model in Fig. 3N). This futile cycle rapidly depletes intracellular ATP, resulting in activation of AMP-activated protein kinase (AMPK) by AMPKα-Thr^172 phosphorylation (Fig. 5A and B). Moreover, the AMPK target, acetyl CoA-carboxylase (ACC) is rapidly phosphorylated (Fig. 5B). Because thapsigargin, which depletes EnR Ca^{2+} by inhibiting SERCA pumps, had no effect on ATP levels (Fig. 5A) and did not increase levels of p-AMPKα and p-ACC (SI Appendix, Fig. S15A), ATP depletion, rather than increased cytosol Ca^{2+}, is responsible for AMPK activation. Importantly, preblocking SERCA pumps with thapsigargin abolished the BHPI-induced decline in ATP levels and phosphorylation of AMPKα (Fig. 5A).

BHPI Blocks UPR Inactivation by Targeting a Second Site of Protein Synthesis Inhibition. In ERα−/−, but not ERα−/− cells, after ~2 h, BHPI phosphorylates and inactivates eukaryotic elongation
factor 2, (eEF2) (Fig. 5C and SI Appendix, Fig. S15 B and C). eEF2 phosphorylation is regulated by a single Ca\(^{2+}\)-calmodulin-dependent kinase, eukaryotic elongation factor 2 kinase (CAMKIII/eEF2K). eEF2K is inhibited by mTORC1-p70S6K and ERK-p90RSK through eEF2K-Ser\(^{366}\)-phosphorylation and activated by Ca\(^{2+}\)-calmodulin and AMPK (29, 30). BHPI increases cytosol Ca\(^{2+}\) and activates AMPK, but inhibiting AMPK did not inhibit eEF2 phosphorylation (SI Appendix, Fig. S15D). BHPI also rapidly induces a transient increase in ERK1/2 activation (SI Appendix, Fig. S15 E and F), which stimulates ERK-p90RSK and mTORC1-p70S6K activation (31). Together, these pathways induce eEF2K-Ser\(^{366}\)-phosphorylation (Fig. 5D) and prevent increases in p-eEF2 for -1 h after BHPI treatment (Fig. 5C and SI Appendix, Fig. S15G). Consistent with this mechanism, blocking ERK activation with U0126 prevented BHPI from producing transient declines in eEF2 phosphorylation through inactivation of eEF2K (SI Appendix, Fig. S15G).

UPR activation with conventional UPR activators produces transient eEF2 phosphorylation and inhibition of protein synthesis (SI Appendix, Figs. S15A and S16 A and B) in part because they induce BiP and p58\(^{IPK}\) chaperones (SI Appendix, Fig. S16 C and D). The chaperones help resolve UPR stress and inactivate the UPR. In contrast, BHPI blocks induction and reduces levels of BiP and p58\(^{IPK}\) protein (Fig. 5E), leading to sustained eEF2 phosphorylation and inhibition of protein synthesis (SI Appendix, Figs. S5 and S8B). BHPI failed to increase p58 protein despite inducing p58 mRNA (Fig. 5E), and at later times PERK inhibition failed to prevent BHPI from inhibiting protein synthesis (SI Appendix, Fig. S9A). This is consistent with BHPI targeting protein synthesis at a second site at later times.

Discussion

BHPI and estrogen share the same ER\(\alpha\)-dependent pathway for UPR activation: activation of PLC\(\gamma\) producing IP\(_3\), opening of the IP\(_3-R\) Ca\(^{2+}\) channels, release of EnR Ca\(^{2+}\), and activation of the PERK, IRE1\(\alpha\), and ATF6\(\alpha\) arms of the UPR (model in Fig. 3N). We recently reported that as an early component of the proliferation program, E\(_2\)-ER\(\alpha\) weakly and transiently activates the UPR. We showed that E\(_2\)-ER\(\alpha\) elicits a mild and transient activation of the PERK arm of the UPR, while simultaneously increasing chaperone levels and protein folding capacity by activating the IRE1\(\alpha\) and ATF6\(\alpha\) arms of the UPR (14). BHPI distorts this normal action of E\(_2\)-ER\(\alpha\) by increasing the amplitude and duration of UPR activation. Compared with E\(_2\), BHPI hyperactivates PLC\(\gamma\), producing much higher IP\(_3\) levels, Ca\(^{2+}\) release from the EnR, and UPR activation. BHPI initially inhibits protein synthesis by strongly activating the PERK arm of the UPR. Knockdown of ER\(\alpha\), PLC\(\gamma\), IP\(_3-R\), and PERK blocked rapid BHPI inhibition of protein synthesis. Whereas BHPI activates the IRE1\(\alpha\) and ATF6\(\alpha\) UPR arms, by acting at later times to inhibit protein synthesis at a second site, BHPI prevents the synthesis of chaperones required to inactivate the UPR. Because the cell attempts to restore EnR Ca\(^{2+}\) while the IP\(_3-R\) Ca\(^{2+}\) channels remain open, BHPI rapidly depletes ATP (Fig. 3N), resulting in activation of AMPK. Several actions of BHPI, including strong elevation of intracellular calcium, sustained UPR activation, long-term inhibition of protein synthesis, ATP depletion, and AMPK activation can potentially contribute to BHPI’s ability to block cell proliferation. How the cascade of events initiated by BHPI enables BHPI to block cell proliferation, and often kill, ER\(\alpha\) cancer cells requires further exploration. Supporting BHPI targeting PLC\(\gamma\) and the UPR through ER\(\alpha\), independent of its effects on the UPR, BHPI inhibits E\(_2\)-ER\(\alpha\)-mediated induction and repression of gene expression.

BHPI and E\(_2\) activation of plasma membrane-bound PLC\(\gamma\), resulting in increased IP\(_3\), is an extranuclear action of ER\(\alpha\). Increasing the level of ER\(\alpha\) increased IP\(_3\) levels. Consistent with ER\(\alpha\) and PLC\(\gamma\) interaction, they coimmunoprecipitate (27). BHPI and E\(_2\) induce Ca\(^{2+}\) release in 1 min, too rapidly for action by regulating nuclear gene expression (14). Furthermore, a membrane-impermeable estrogen-dendrimer induces the UPR marker sp-XBP1, but not nuclear E\(_2\)--ER\(\alpha\)-regulated genes. The UPR plays important roles in tumorigenesis, therapy resistance, and cancer progression (14, 32). Moderate and transient UPR activation by E\(_2\) and other activators promotes an adaptive stress response, which increases UPR expression and confers protection from subsequent exposure to higher levels of cell stress (14, 33). In contrast, sustained UPR activation triggers cell death. Because most current anticancer drugs inhibit a pathway or protein important for tumor growth or metastases, most UPR targeting efforts focus on inactivating a protective stress response by inhibiting UPR components (34). UPR overexpression in cancer is associated with a poor prognosis (14), suggesting that sustained lethal hyperactivation of the UPR by BHPI represents a novel alternative anticancer strategy.

BHPI can selectively target cancer cells, because its targets, ER\(\alpha\) and the UPR, are both overexpressed in breast and ovarian cancers (14, 22, 32, 35). Cells expressing low levels of ER\(\alpha\), more typical of nontransformed ER\(\alpha\)-containing cells, such as PC-3 prostate cancer cells, were less sensitive to BHPI inhibition of protein synthesis (SI Appendix, Fig. S5), whereas doxycycline-treated MCF7ER\(\alpha\)HA cells expressing very high levels of ER\(\alpha\) exhibited near complete inhibition of protein synthesis (Fig. 3E). Consistent with low toxicity, in the xenograft study, BHPI-treated mice showed no evidence of gross toxicity.

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Most gynecological cancers show little dependence on estrogens for growth, and other noncompetitive ERα inhibitors have not demonstrated effectiveness in these cells. BHPI is highly effective in several breast and ovarian cancer drug-resistance models and extends the reach of ERα biomodulators to gynecologic cancers that do not respond to current endocrine therapies. BHPI’s effectiveness in ERα-containing breast, ovarian, and endometrial cancer cells is consistent with the finding that female reproductive cancers exhibit common genetic alterations and might respond to the same drugs (36) and with our finding that E2–ERα weakly activates the UPR in breast and ovarian cancer cells (14).

With its submicromolar potency, effectiveness in a broad range of therapy-resistant cancer cells, ability to induce substantial tumor regression, and unique mode of action, BHPI is promising small molecule for therapeutic evaluation and mechanistic studies.

Materials and Methods

Additional methods are in SI Appendix, SI Materials and Methods.

Cell Culture and Reagents, Chemical Libraries, Screening, IP, Assays, Luciferase Assays, qRT-PCR, ChIP, Transfections, and In Vitro Binding Assays. Techniques are further described in SI Appendix, SI Materials and Methods.

Calcium Imaging. Cytoplasmic Ca2+ concentrations were measured using the calcium-sensitive dye, Fluo-4 AM (SI Appendix, SI Materials and Methods).

Protein Synthesis. Protein synthesis rates were evaluated by measuring incorporation of 35S-methionine into newly synthesized protein (SI Appendix, SI Materials and Methods).

Mouse Xenograft. All experiments were approved by the Institutional Animal Care Committee of the University of Illinois at Urbana–Champaign. The MCF-7 cell mouse xenograft model has been described previously (19), and studies were performed as described in SI Appendix, SI Materials and Methods.

Statistical Analysis. Calcium measurements are reported as mean ± SE. All other pooled measurements are represented as mean ± SEM. Two-tailed Student t tests or one-way ANOVA with post hoc Fisher’s least significant difference tests were used for statistical significance (P < 0.05).

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