Supplementary Information

Bruceantin targets HSP90 to overcome resistance to hormone therapy in castration-resistant prostate cancer

This PDF file includes: Supplementary Materials and Methods, Supplementary References, Supplementary Figures S1 to S13, and Supplementary Tables S1 to S4.
Supplementary Materials and Methods

**Transient transfection and plasmids.** Cells were transiently transfected with MMTV-LUC reporter or expression plasmids for AR-FL, AR-V7, HSP90, HSP70, or HSP40 using Lipofectamine 3000 (ThermoFisher Scientific). HSP90, HSP70, and HSP40 cDNAs were obtained from Addgene (HSP90 HA, #22487) or PCR amplified from 22RV1 cDNA and cloned into pSG5.HA (pSG5.HA-HSP70, pSG5.HA-HSP40), pGEX-4T-1 (pGEX-4T-1-HSP90, pGEX-4T-1-HSP70), or pETDuet-1 (pETDuet-1-HSP90). The following plasmids were described previously1, 2: MMTV-LUC, pcDNA3.1-3xFLAG-AR-FL, pcDNA3.1-3xFLAG-AR-V7, pcDNA3.1-3xFLAG-AR-V7-1-360, pcDNA3.1-3xFLAG-AR-V7-361-645, pSG5.HA-AR-FL, pSG5.HA-AR-1-600, pSG5.HA-AR-591-919, pSG5.HA-AR-V7, pSG5.HA-CHIP, pGEX-4T-1-AR-FL, pGEX-4T-1-AR-V7, and pETDuet-1-CHIP.

**Cell growth inhibition, colony formation, migration, invasion, and sphere formation assays.** PCa cells and normal prostate epithelial cells were seeded in 96-well plates (1x10^4 cells/well) and then treated next day with compounds at the indicated concentrations in the figure for 3 days. Cell growth inhibition was determined by MTT assays (Promega). IC50 values were determined by non-linear regression using PRISM v5.0 (GraphPad). For colony formation assays, cells were seeded into 6 well plates (1x10^3 cells/well) and treated with compounds at the indicated concentrations in the figure for 14 days. Crystal violet-stained colonies were solubilized in 10% SDS, and absorbance was measured at 570 nm. Two-chamber cell migration and invasion assays were performed in 24-well plates using Transwell inserts (Costar) coated with fibronectin (Sigma-Aldrich) and with Matrigel (BD Biosciences)/fibronectin, respectively. 22RV1 cells (1x10^5 cells/well) in serum-free medium were pretreated with 5 μg/ml mitomycin C (Sigma-Aldrich) for 2 h to avoid the effect of BCT on cell proliferation and then loaded into the upper chamber and treated with DMSO or BCT (10 nM or 20 nM). Following 18 hr incubation, migrated or invaded cells in the lower chamber with medium supplemented with 10% FBS as chemoattractant were fixed with methanol and then stained with hematoxylin and eosin (H&E). Images of the cells on the lower layer of the chamber were captured and counted in four random fields. Each assay was performed in triplicate. For sphere formation assays, 22RV1 cells (2x10^2 cells/well) were cultured in suspension to form spheres in 96-well ultralow attachment plates (Costar) with serum-free DMEM-F12 (1:1) supplemented with 20 ng/μl hEGF, 10 ng/μl bFGF, 1x B27, and 1x N-2 (Life technologies). After 14 days, the number of spheres with a diameter of >100 μm was counted.
**Cell apoptosis analysis.** 22RV1 cells were treated with 10 nM BCT for the indicated time in the figure, trypsinized, and washed with phosphate-buffered saline (PBS). Apoptotic cells were stained with propidium iodide (PI) and Annexin V-FITC following the manufacturer's instructions (BD Biosciences) and detected by flow cytometry analysis using a BD FACSVersa flow cytometer (BD Biosciences).

**Gene set enrichment analysis (GSEA).** GSEA was performed using the Java GSEA software package (http://www.broadinstitute.org/gsea) and run in pre-ranked mode to identify enriched signatures. We used the gene sets in the Molecular Signatures Database (MSigDB) and added Androgen_UP, Androgen_DOWN (genes upregulated and downregulated in response to androgen)³ and ARV_Activated, ARV_Repressed (genes activated and repressed by AR-Vs)⁴. The GSEA plot, normalized enrichment score (NES), nominal p values (NOM p-val), and false discovery rate q-values (FDR q-val) were derived from GSEA output.

**Real-time quantitative reverse transcription PCR (qRT-PCR).** Total RNA was extracted using TRIzol Reagent (ThermoFisher Scientific), and qRT–PCR was performed with One-step PrimeScript RT-PCR (Perfect Real Time) Kit (Takara Bio). qRT-PCR was performed in triplicate using gene-specific primers on ABI Prism 7900HT Fast real-time PCR system (Applied Biosystems). Each reaction was normalized to GAPDH or β-actin mRNA levels. The primer sequences used are listed in Table S3.

**Histological analysis.** Freshly isolated mouse organs and prostate tumors were fixed in 10% neutral-buffered formalin (Sigma-Aldrich), progressively dehydrated in increasing concentrations of ethanol, embedded into paraffin blocks, and sectioned at a thickness of 5 µm. Slide-mounted sections were deparaffinized by xylene, rehydrated in serial dilutions of ethanol, and stained with H&E. For immunohistochemistry, sections of patient-matched PCa tissues (primary HSPC and recurrent CRPC following androgen deprivation therapy) from our previous study⁵ were deparaffinized with xylene, rehydrated in serial dilutions of alcohol, subjected to heat-induced antigen retrieval (for 18 min at 800 W microwave; for AR-V7 staining, Tris-EDTA buffer pH8.0; for AR and HSP90 staining, in citrate buffer pH6.0), and blocked with blocking solution (Dako). Sections were incubated with anti-AR-V7 antibody (clone RM7, 1:500), anti-AR antibody (N-20, 1:1,000), or anti-HSP90 antibody (clone F-8, 1:800) for 1 hr. After washes, reactions were visualized using EnVision Detection System (Dako) and DAB chromogen. Sections were counterstained with hematoxylin. Digital images of sections were captured using the Aperio ScanScope XT slide scanner (Aperio Technologies). Cases were scored by
a pathologist (G.Y.K.) blinded to clinical data using the modified H score (HS) method. All procedures involving human subjects were approved by the Institutional Review Board of the Samsung Medical Center.

**Immunoblot, coimmunoprecipitation (CoIP), and GST pull-down.** For immunoblot, cells were lysed in RIPA buffer (50 mM Tris–HCl, pH 7.5, 150 mM NaCl, 1% NP-40, 0.5% deoxycholate and 0.1% SDS) or FLAG lysis buffer (50 mM Tris–HCl, pH 8.0, 137 mM NaCl, 1 mM EDTA, 1% Triton-X 100, 0.2% Sarkosyl, 10% glycerol) with protease inhibitor cocktail (Roche). Whole cell extracts were resolved by SDS-PAGE, transferred to PVDF (Millipore), and probed with specific antibodies as indicated in figures. AR antibody (N-20 or 441) was used to detect both AR-FL and AR-Vs. For CoIP, cell lysates were immunoprecipitated with specific antibodies or control IgG and protein A/G-agarose beads (Santa Cruz Biotechnology), and immunoprecipitates were analyzed by immunoblot as indicated in figures. For GST pull-down assays, purified recombinant or in vitro translated proteins were incubated with either immobilized GST or GST-fusion proteins, and bound proteins were detected with specific antibodies as indicated in figures.

**Antibodies.** The following antibodies were used in this study: AR (441), AR (N-20), AR (C-19), HSP90 (F-8), HSP70 (H-2), HSP70 (3A3), ubiquitin (P4D1), c-Myc (9E10), GR (M-20), His-probe (H-3), and luciferase (C-12) (Santa Cruz Biotechnology); HSP40 (ab69402) (Abcam); AR-V7 [RM7] (GTX33604) (GeneTex); AR-V7 (AG10008) (Precision Antibody); CHIP (C3B6), AKT2 (D6G4) (Cell Signaling Technology); HA (3F10) (Roche); FLAG M2 and FLAG M2-agarose (Sigma-Aldrich); GAPDH (LF-PA0018) (AbFrontier).

**Limited proteolysis assays.** Partial proteolytic digests were performed at 30°C for 15 min by adding trypsin (0.4, 0.8, 1.2, 1.6, 2.0, 2.4 μg/ml) to recombinant N-terminal His-tagged HSP90 protein in PBS. Proteolytic fragments were separated on SDS-PAGE gels, and digested fragments were detected by immunoblot with anti-His antibody.

**ATPase activity assays.** ATPase assays were performed for 3 hr at 37°C with recombinant HSP90 or HSP70 protein in reaction buffer (100 mM Tris-HCl, pH 7.4, 20 mM KCl, 6 mM MgCl2, and 10 μM ATP) in the presence or absence of BCT (0.05, 0.2, 0.5, 1, 2, 20 μM). ATP depletion and ADP detection were performed using the ADP-Glo Kinase/ATPase assay kit (Promega), and luminescence was measured using the Mithras LB940 multimode plate reader (Berthold).
**Cell-based luciferase refolding assays.** 22RV1-LUC cells were heat-denatured at 50ºC for 7 min, which was determined as the optimal time and temperature for luciferase denaturation without affecting the viability of cells. Cells were seeded into 96-well plates (5x10⁴ cells/well), treated with DMSO or BCT (1, 10, 100, 500 nM), and incubated at 37ºC for 1 hr to allow luciferase refolding. Luciferase activity was measured using Firefly Luciferase Assay kit (Promega).

**Data availability.** All RNA-seq data have been deposited in the GEO database under accession number GSE145790. The hyperlink of the dataset is: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE145790. All data are available from the authors upon reasonable request.

**Supplementary References**

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Figure S1. Luciferase assay-based screening of compounds for inhibitory activity against AR-V7 (DHT-) and AR-FL (DHT+) transcriptional activities in 22RV1 cells. (A-C) 22RV1 cells transfected with MMTV-LUC were treated with indicated compounds at 10 μM (A), 1 μM (B), and 0.1 μM (C) with or without 20 nM DHT for 24 hr, and luciferase activities were measured. (D) Identification of four quassinoids as inhibitors of the transcriptional activity of AR-FL and AR-V7.
Figure S2. **BCT inhibits colony formation and induces apoptosis in 22RV1 cells.** (A) Prostate cancer cells were plated and treated with indicated concentrations of BCT for 7 days. Viable colonies were stained with crystal violet, and the dye was extracted and quantified by spectrophotometry (Fig. 1F). (B) BCT induces apoptosis in CRPC cells. 22RV1 cells were treated with 10 nM BCT for the indicated times. To quantify apoptotic cells, cells were stained with propidium iodide (PI) and Annexin V-FITC and subjected to flow cytometry analysis.
A

B

DMSO_1  DMSO_2  DMSO_3  BCT_1  BCT_2  BCT_3

PMEPA1
TMPRSS2
CCNA2
NKX3.1
KLK2
KLK3
CDH2
FKBP5

DMSO_1  DMSO_2  DMSO_3  BCT_1  BCT_2  BCT_3

INSIG1
SCD
HMGCOS1
DBI
DHCR24
AZGP1
KLK3
IDI1
FADS1
SPCS3
NKX3.1
SMS
HMGCGR
ELOVL5
KLK2
PMEPA1
TMPRSS2
NDRG1
FKBP5
SORB
CAMKK2
GSR
HPGD
E2M
HOMER2
GFPDL
ELL2
UAP1
PPAP2A
AKT1
CCND3
TARP
RAS4B
TPD52
TMEM50A
ACSL3
TNFAIP8
ANKH
PTK2B
CENPN
ACTN1
ABHD2
SLC26A2
LMAN1
INPP4B
HERC3
B4GALT1
PA2G4
ITGAV
XRCC5
UBE2B
SPDEF
SEPP1
ZBTB10
HSD17B14
PCMS
CDK6
SRF
ABCC4
ALDH1A3
**Figure S3. BCT-regulated genes in 22RV1 cells.** (A) Hierarchical clustering of the differentially expressed genes between vehicle (DMSO) and 10 nM BCT treatment in 22RV1 cells with fold change (FC) > 1.8 and FDR < 0.05. (B) Heat map of mRNA levels of BCT-repressed androgen target genes listed in the Hallmark_androgen response gene set. (C,D) Validation of BCT-regulated AR-FL target genes by qRT-PCR in 22RV1 cells cultured in hormone-depleted medium in the absence or presence of 10 nM DHT. qRT-PCR data are presented as means ± s.d. (n=3) (C), and the visualized heatmap was generated using R package based on the qRT-PCR data (D).
Figure S4. 22RV1 xenograft tumors are resistant to ENZ. 22RV1 cells were injected into the right flank of BALB/c nude mice. Mice bearing 22RV1 xenograft tumor were treated i.p. with DMSO or ENZ (10 mg/kg) everyday for 18 days (n= 5). Tumor growth curves are shown (A), and mouse body weight was measured (B).

Figure S5. BCT inhibits in vivo expression of AR-FL/AR-V7 target genes. qRT-PCR analysis of TMPRSS2, PMEPA1, and CDH2 expression in 22RV1 xenograft tumors treated with DMSO or BCT (2 mg/kg). Data are means ± s.d. (n = 3). **p < 0.01, *p < 0.05.
Figure S6. BCT does not have apparent in vivo toxicity but downregulates protein levels of AR-FL and AR-V7. (A) No significant differences were observed in the levels of plasma enzymes including alanine transaminase (ALT), aspartate transaminase (AST), lactate dehydrogenase (LDH), creatinine (CR), and creatine phosphokinase (CPK) between the control and BCT treatment groups. (B, C) BCT does not affect the body weight of mice. Mice bearing 22RV1 xenograft tumor were treated i.p. (B) or p.o. (C) with BCT, and mouse body weight was measured every 3 days (i.p.) or every the other day (p.o.). (D, E) Mice livers, kidneys, and spleens from the same experiment as Fig. 3E were anatomically (D) and histologically (E, H&E staining) evaluated. (F) Immunoblot analyses of AR-FL, AR-V7, and HSPs in 22RV1 xenograft tumors treated i.p. with DMSO or BCT. (G, H) Effect of BCT and first- (17-AAG) and second-generation (onalespib and AUY-922) HSP90 inhibitors on the expression of HSP70 and HSP40 and stability of AR-FL/AR-V7 in 22RV1 cells. The protein levels of AR-FL, AR-V7, HSP70, HSP40, and GAPDH were determined by immunoblot analysis. BCT decreased protein levels of AR-FL and AR-V7 more effectively than 17-AAG, onalespib, and AUY-922, without affecting the expression of inducible HSPs, HSP70 and HSP40. The ranges of concentrations of HSP90 inhibitors tested: 2~20 nM or 20~300 nM for BCT; 100~1000 nM for 17-AAG and onalespib; 20~300 nM for AUY-922 (luminespib). (I) 22RV1 cells were treated with different concentrations of indicated compounds. Cell proliferation was detected by MTT assays, and IC50 values were determined. Data are means ± s.d. (n=6).
**Figure S7.** BCT inhibits the growth and metastasis of CRPC cells in vivo. 22RV1-LUC-T cells were intracardially injected in male nude mice, and the mice were treated with vehicle or BCT (2 mg/kg) by oral gavage every other day for 7 weeks (n= 8). Bioluminescence images were taken from both the dorsal and ventral sides of the mice every week. (A) Bioluminescence images of mice bearing metastatic tumors and numbers of metastatic sites. (B) Bioluminescence images of non-metastatic mice at day 35. (C) Normalized bioluminescence of metastatic tumors of the control and BCT groups. BCT treatment induced 72% reduction in bioluminescence signals of metastatic tumors at the end of treatment. *p < 0.05.
Figure S8. BCT negatively regulates protein stability of AR-FL and AR-V7. (A) 293T cells transfected with 3xFLAG-AR-FL expression vector were treated with indicated concentrations of BCT for 24 hr, and cell lysates were analyzed by immunoblot with indicated antibodies. (B) 22RV1 and LNCaP cells were treated with 20nM DHT and indicated concentrations of BCT as indicated for 24 hr. Cell lysates were analyzed by immunoblot with indicated antibodies. (C) LNCaP and C4-2B cells were treated with 10nM BCT and 10 μM MG132 as indicated for 24 hr, and cell lysates were analyzed by immunoblot with indicated antibodies. (D, E) 22RV1 cells transfected with 3xFLAG-AR-FL (D) or 3xFLAG-AR-V7 (E) expression vector were treated with 0.1 nM BCT and 10 μM MG132 as indicated for 8 hr. Cell lysates were immunoprecipitated with anti-FLAG M2 agarose and immunoblotted with indicated antibodies.
Figure S9. BCT inhibits interactions between AR-FL/AR-V7 and HSPs and increases CHIP-mediated ubiquitination of AR-FL and AR-V7. (A) LNCaP, C4-2B-MDV, and VCaP cells were treated with indicated concentrations of BCT for 24 hr, and cell lysates were analyzed by immunoblot using the indicated antibodies. (B, C) 293T cells transfected with HA-tagged HSP expression vectors and 3xFLAG-AR-FL (B) or 3xFLAG-AR-V7 (C) expression vector were treated with 10 μM MG132 and DMSO or 10 nM BCT for 12 hr. Cell lysates were immunoprecipitated with anti-FLAG M2 agarose and immunoblotted with indicated antibodies. (D) 22RV1 cells transfected with HA-tagged HSP90 expression vector were treated with 10 μM MG132 and DMSO or BCT for 4 hr, and cell lysates were immunoprecipitated with anti-HA agarose and immunoblotted with indicated antibodies. (E, F) 293T cells transfected with HA-CHIP and 3xFLAG-AR-FL (E) or 3xFLAG-AR-V7 (F) expression vectors were treated with 10 μM MG132 and DMSO or BCT for 8 hr. Cell lysates were immunoprecipitated with anti-FLAG M2 agarose and immunoblotted with indicated antibodies.
Figure S10. BCT binds to HSP90. (A) 293T cell extracts expressing HA-HSP90, HA-HSP70, and HA-HSP40 were incubated with BCT-immobilized Unosphere beads or control Unosphere beads, and bound proteins were analyzed by immunoblot with anti-HA antibody. (B) Molecular docking model of BCT bound to HSP90. BCT (shown in ball-and-stick representation) fits into the ATP-binding pocket of HSP90. Amino acid residues involved in BCT binding in HSP90 are labelled. (C) Docked conformation of BCT (green carbon) in comparison with that of ADP (yellow carbon).

Figure S11. HSP90 expression is upregulated in PCa tumors. (A) Analysis of HSP90AA1 and HSP90AB1 mRNA expression in human PCa using databases at Oncomine (http://www.oncomine.org). P-value was determined by Student’s t-test. (B) Meta-analyses of HSP90AA1 and HSP90AB1 gene expression from three Oncomine datasets where colored squares indicate the median rank for HSP90AA1 and HSP90AB1 (vs. Normal tissue) across 3 analyses. Oncomine datasets: 1. Singh Prostate; 2. Vanaja Prostate; 3. Welsh Prostate.
Figure S12. HSP90 expression is correlated with PCa progression and with levels of AR/AR-V7. (A) HSP90 level is correlated with PCa progression. HSP90 gene (HSP90AA1 and HSP90AB1) expression levels were determined in benign prostate (BP), primary PCa (PCaP), and metastatic CRPC (mCRPC). Statistical analysis was performed using one-way ANOVA. ***p < 0.001. (B) Protein expression (H-score, HS) of HSP90 and nuclear ARs and AR-V7 in matched HSPC and CRPC tissues.

Figure S13. BCT downregulates protein levels of MYC, AKT, and GR in CRPC cells. 22RV1 cells were treated with indicated concentrations of BCT for 24h, and cell lysates were analyzed by immunoblot with indicated antibodies.
### Table S1. List of compounds screened in this study.

| No | Compounds                        | Manufacturer | Cat.   |
|----|----------------------------------|--------------|--------|
| 1  | Ailanthone                       | TOCRIS       | 6161   |
| 2  | Enzalutamide                     | Biovision    | 9479-10|
| 3  | Artesunate                       | abcam        | ab142829|
| 4  | Artemisinin                      | abcam        | ab141308|
| 5  | Dihydroartemisinin               | abcam        | ab142690|
| 6  | Artemether                       | abcam        | ab142689|
| 7  | Chloroquine diphosphate          | Santa cruz   | sc-205629|
| 8  | Niclosamide                      | abcam        | ab120868|
| 9  | Primaquine diphosphate           | abcam        | ab142355|
| 10 | Plumbagin                        | abcam        | ab145184|
| 11 | Pyrimethamine                    | abcam        | ab143106|
| 12 | Sulfadoxine                      | abcam        | ab143199|
| 13 | Hydroxychloroquine sulfate       | Santa cruz   | sc-215157|
| 14 | Amodiaquine dihydrochloride dihydrate | Selleckchem  | S4589  |
| 15 | Lumefantrine                      | Santa cruz   | sc-207836|
| 16 | Mefloquine Hydrochloride         | Santa cruz   | sc-211784|
| 17 | 10,11-Dehydrocurvularin          | abcam        | ab144855|
| 18 | Astemizole                       | Santa cruz   | sc-201088|
| 19 | Licochalcone A                   | Selleckchem  | S7828  |
| 20 | Quinine hydrochloride            | TOCRIS       | 4114   |
| 21 | Magnolol                         | Selleckchem  | S2321  |
| 22 | Andrographolide                  | Santa cruz   | sc-205594|
| 23 | Phlorizin                        | TOCRIS       | 4627   |
| 24 | Ganoderic acid A                 | Selleckchem  | S4753  |
| 25 | Brucine A                        | ChemNorm     | TRY0101|
| 26 | 1,8-Dihydroxynaphthalene         | ChemNorm     | TPC0344|
| 27 | 1,8-Diacetoxy-3-carboxynaphthalene| ChemNorm     | TPC0250|
| 28 | Eurycomanone                     | ChemNorm     | TBZ1340|
| 29 | Aurantio-obtusin                 | ChemNorm     | TB0101-0100|
| 30 | Mollugin                         | ChemNorm     | TB0623-0500|
| 31 | β,β-Dimethylacrylalkannin        | ChemNorm     | TB0635-0100|
| 32 | Acetylshikonin                   | ChemNorm     | TB0586-0100|
| 33 | Aloin A                          | ChemNorm     | TB0786-0025|
| 34 | Sennoside A                      | ChemNorm     | TB0027-1000|
| 35 | Cryptotanshinone                 | ChemNorm     | TB0061 |
| 36 | Rhein                            | ChemNorm     | TB0043 |
| 37 | Brusatol                         | SIGMA        | SML1868|
| 38 | Quassin                          | ChemFacs     | CFN97246-1MG|
| 39 | Curvularin                       | ENZO         | ALX-380-305-M001|
|    |                                  | TRC          | B6893109|
| 40 | Brucetin                         | ChemNorm     | TRY0213|
| 41 | Yadanzioside I                   | ChemFacs     | CFN96421-1MG|
| 42 | Yadanzioside F                   | ChemFacs     | CFN96423-1MG|
| 43 | Yadanzioside C                   | ChemFacs     | CFN96424-1MG|
| 44 | Brucetinol                       | ChemFaces    | CFN89336-1MG|
| 45 | Brucanic acid C                  | ChemFaces    | CFN91975-1MG|
| 46 | Brucine D                        | ChemFaces    | CFN90771-10-MG|
| 47 | Dehydrobrucine A                 | ChemFaces    | CFN9341-1MG|
| 48 | Brucetinoside A                  | ChemFaces    | CFN-89343-1MG|
| 49 | (+)-Glaucarubinon                | ChemFaces    | CFN92979-1MG|
| 50 | Yadanziolide A                   | ChemFaces    | CFN96428-1MG|
| 51 | Yadanziolide B                   | ChemFaces    | CFN96427-MG|
| 52 | Yadanziolide C                   | ChemFaces    | CFN96426-1MG|
### Table S2. Screening of compounds for inhibitory activity against AR-V7 (DHT-) and AR-FL (DHT+) transcriptional activities in 22RV1 cells.

#### Compound screening at 10 μM

| Conc. | Compound | MMTV-LUC activity (%) | DHT- | DHT+ |
|-------|----------|------------------------|------|------|
|       |          |                        |      |      |
|       | -        | 22.3±5.2               | 100.0±8.7 |
|       | Enzalutamide | 17.0±7.4               | 51.8±0.1 |
|       | Ailanthone    | 1.9±0.5                | 2.2±0.4  |
|       | Artesunate    | 23.1±0.2               | 117.5±9.3 |
|       | Artemisinin   | 22.9±0.9               | 156.1±17.6 |
|       | Dihydroartemisinin | 24.7±2.2               | 137.5±7.0 |
|       | Artemether    | 20.9±4.2               | 138.2±8.8 |
|       | Chloroquine diphasate | 18.5±0.8               | 106.9±6.0 |
|       | Niclosamide   | 2.0±0.1                | 2.3±0.5  |
|       | Primusquinate diphasate | 24.4±3.8               | 115.6±6.7 |
|       | Plumbagin     | 5.6±1.6                | 7.1±0.1  |
|       | Pyriforminamide | 28.0±0.0               | 149.2±2.4 |
|       | Saffodioxine  | 24.9±0.9               | 170.3±11.2 |
|       | Hydroxychloroquine sulfate | 19.8±0.4               | 122.1±0.7 |
|       | Amiodaquine dihydrochloride dihydate | 19.6±0.6               | 102.2±4.3 |
|       | Lumefantrine   | 29.5±2.0               | 160.7±18.2 |
|       | Mefloquine Hydrochloride | 24.5±4.3               | 109.0±6.3 |
|       | 10,11-Dehydrocurvularin | 3.5±0.5                | 5.5±0.6  |
|       | Astemizole    | 5.1±1.1                | 39.5±15.5 |
|       | Licochalcone A | 3.5±1.5                | 235.6±0.2 |
|       | Quinine hydrochloride | 3.1±1.4                | 159.9±2.3 |
|       | Magnolol       | 27.8±2.6               | 151.9±8.6 |
|       | andrographolide | 33.7±0.9               | 196.2±5.0 |
|       | Phlorizin      | 33.1±1.4               | 184.4±12.6 |
|       | Gnanosperic acid A | 1.0±0.8                | 148.9±2.8 |
|       | Bruceine A     | 1.0±0.1                | 1.0±0.1  |
|       | 1,8-Dihydroxyanthraquinone | 21.9±2.6               | 168.1±32.4 |
|       | 1,8-Diacetoxy-3-carboxyanthraquinone | 27.2±1.2               | 114.5±13.7 |
|       | Eurycomanone   | 3.2±0.3                | 4.2±0.7  |
|       | Auranto-obusin | 44.6±7.1               | 128.6±13.6 |
|       | Moffugin       | 22.0±2.1               | 146.2±19.7 |
|       | β,β-Dimethylcarylalkamin | 3.0±0.6                | 8.1±1.0  |
|       | Acetylshikonin | 2.1±0.1                | 3.5±0.1  |
|       | Aloin A        | 26.5±5.3               | 123.0±12.7 |
|       | Semioside A    | 30.8±5.7               | 136.5±8.8 |
|       | Cryptotanshinone | 13.1±4.1               | 67.2±11.9 |
|       | Rhein          | 25.6±4.5               | 122.7±5.7 |
|       | Brusatol       | 1.0±0.2                | 1.2±0.2  |
|       | Quassin        | 31.0±6.9               | 102.7±13.4 |
|       | Curvularin     | 18.0±0.7               | 91.6±9.4 |
|       | Bruceantin     | 4.9±0.7                | 3.9±0.4  |
|       | Yadananzoide C | 32.7±3.4               | 5.8±0.7  |
|       | Yadananzoide F | 39.6±6.4               | 139.5±11.0 |
|       | Yadananzoide I | 36.5±3.1               | 136.0±7.1 |
|       | Bruceantinol   | 13.0±10.0              | 44.5±2.1 |
|       | Bruceamic acid C | 11.0±1.0               | 85.8±14.2 |
|       | Bruceine D     | 1.2±0.2                | 1.1±0.1  |
|       | Bruceantinoside A | 1.0±0.1               | 3.8±1.1  |
|       | Dehydrobrucene A | 20.1±2.2               | 164.2±16.0 |
|       | (+)-Glaucarubinone | 18.3±4.5               | 117.7±3.5 |
|       | Yadananzoide A | 1.4±0.1                | 4.1±0.2  |
|       | Yadananzoide B | 1.4±0.2                | 4.0±0.5  |
|       | Yadananzoide C | 0.9±0.1                | 1.1±0.1  |

#### Compound screening at 1 μM

| Conc. | Compound | MMTV-LUC activity (%) | DHT- | DHT+ |
|-------|----------|------------------------|------|------|
|       | -        | 25.0±5.0               | 100.0±11.4 |
|       | Enzalutamide | 20.5±2.7               | 96.7±1.7  |
|       | Ailanthone    | 4.2±0.6                | 4.5±0.9  |
|       | Niclosamide   | 27.9±2.5               | 56.7±5.3  |
|       | Plumbagin     | 59.9±4.5               | 142.9±27.5 |
|       | 10,11-Dehydrocurvularin | 106.8±6.9             | 206.9±15.0 |
|       | Astemizole    | 32.0±1.5               | 126.4±3.6 |
|       | Bruceine A    | 3.7±0.5                | 3.6±0.2  |
|       | Eurycomanone   | 16.0±2.1               | 51.3±4.6  |
|       | β,β-Dimethylcarylalkamin | 27.1±3.1              | 107.9±7.0 |
|       | Acetylshikonin | 27.8±2.1               | 113.0±16.6 |
|       | Brusatol       | 3.8±0.4                | 4.0±0.5  |
|       | Bruceantin     | 4.5±0.5                | 4.4±0.3  |
|       | Yadananzoide C | 35.3±5.4               | 55.6±0.7  |
|       | Bruceantinol   | 11.8±1.9               | 118.9±21.8 |
|       | Bruceine D     | 1.0±0.2                | 2.0±0.2  |
|       | Bruceantinoside A | 1.7±0.8               | 8.0±0.2  |
|       | Yadananzoide A | 9.2±2.2                | 58.3±10.9 |
|       | Yadananzoide B | 1.2±0.1                | 4.2±0.4  |
|       | Yadananzoide C | 2.6±0.5                | 12.1±2.0 |

#### Compound screening at 0.1 μM

| Conc. | Compound | MMTV-LUC activity (%) | DHT- | DHT+ |
|-------|----------|------------------------|------|------|
|       | -        | 25.7±3.1               | 100.0±3.0 |
|       | Ailanthone    | 7.3±0.5                | 9.8±0.4  |
|       | Bruceine A    | 5.6±0.5                | 5.7±1.0  |
|       | Eurycomanone   | 34.6±3.2               | 107.7±4.7 |
|       | Brusatol       | 5.5±0.9                | 5.6±0.6  |
|       | Bruceantin     | 4.0±0.7                | 4.0±0.5  |
|       | Bruceine D     | 6.9±1.0                | 32.6±2.2 |
|       | Bruceantinoside A | 6.7±0.8               | 61.0±7.3 |
|       | Yadananzoide B | 3.9±0.9                | 32.8±9.0 |
|       | Yadananzoide C | 12.1±0.4               | 92.5±9.0 |

22RV1 cells transfected with MMTV-LUC were treated with indicated compounds at indicated concentrations with or without 20 nM DHT for 24 hr. Luciferase activities were measured, and results were expressed as the ratio of luciferase activity. Data are means±s.d. (n=3).
Table S3. qRT-PCR primer sets used in this study.

| Gene   | Forward                                      | Reverse                                      |
|--------|----------------------------------------------|----------------------------------------------|
| β-actin| Forward: CCA CAC TGT GCC CAT CTA CG          | Reverse: AGG ATC TTC ATG AGG TAG TCA GTC AG  |
|        | GAPDH Forward: TCT GGT AAA GTG GAT ATT GTT GCC | Reverse: GAA GAT GGT GAT GGG ATT TCC         |
|        | KLK2 Forward: GCT GCC CAT TGC CTA AAG AAG     | Reverse: TGG GAA GCT GTG GCT GAC A           |
|        | KLK3 (PSA) Forward: TGT GTG CTG GAC GCT GGA  | Reverse: CAC TGC CCC ATG ACG TGA T           |
|        | TMPRSS2 Forward: CCT GCA GGG ACA TGG GCT ATA  | Reverse: CCG GCA CTT GTG TTC AGT TTC         |
|        | FKBP5 Forward: AGG CTG CAA GAC TGC AGA TC     | Reverse: CTT GCC CAT TGC TTT ATT GG          |
|        | NKX3.1 Forward: CCG AGC CAG AAA GGC ACT TGG G | Reverse: AGC GCT TCT GCG GCT GCT TA          |
|        | CDH2 Forward: GGA GAT CCT ACT GGA CGG TTC G   | Reverse: CCC TTG GCT AAT GGC ACT TGA         |
|        | CCNA2 Forward: CGC TGG CGG TAC TGA AGT C      | Reverse: GAG GAA CGG TGA CAT GCT CAT         |
|        | AR-FL Forward: ATC TGT GGA GAT GAA GCT TCT    | Reverse: GGG CTG ACA TTC ATA GCC TTC AAT GTG TGA C |
|        | AR-V7 Forward: ATC TGT GGA GAT GAA GCT TCT    | Reverse: TGC CAA CCC GGA ATT TTT CTC CC      |
|        | PMEPA1 Forward: TGC AAC TGC AAA CGC TCT TT    | Reverse: CCA CCA CCA TCA CCA TCA TC          |
|        | KLK4 Forward: GGC ACT GGT CAT GGA AAA CGA     | Reverse: TCA AGA CTG TGC AGG CCC AGC C       |
|        | LDLR Forward: CAA TGT CTC ACC AAG CTC TGG     | Reverse: CAT CTG TCT CGA GGG GTA GCT         |
|        | METTL7A Forward: CAG AGT GCT GAG ACC GGG A    | Reverse: CTG GTC AGG TTG CAC CCA TC          |
|        | NDRG1 Forward: ACA ACC CTG AGA TGG TGG AG     | Reverse: TGT GGA CCA CTT CCA CGT TA          |
|        | FASN Forward: CTG GCT CAG CAC CTC TAT CCC     | Reverse: CAG GTT GTC CCT GTG ATC             |
|        | SREBF1 Forward: GCT TCT CTA CAG GAA GCC CTC   | Reverse: TCT GCC TTT ATG AAG TGG GGC         |
|        | GNMT Forward: AGT ACA AGG CAT GGC TGC TT      | Reverse: CTT TGC AGT CTG GCA AGT GA          |
| Gene    | Forward                      | Reverse                      |
|---------|------------------------------|------------------------------|
| GREB1   | CAA AGA ATA ACC TGT TGG CCC TGC | GAC ATG CCT CCG CTC TCA TAC TTA |
| ZWINT   | CCC TGA CTC AGA TGG AGG AA | AAA CCT CCG CCA GAT GCT |
| PGC     | ACA GGC ACC TCT CTG CTC ACT | AGT AGC GTG TGT TAC TGA GGA T |
| EDN2    | TCT GGG TGA ACA CTC CTG AAC A | GAA GGC AGA AGG TGG CAC AG |
| MYC     | CCT CAA CGT TAG CTT CAC CAA | CTC CTC GTC GCA GTA GAA ATA |
| CDC6    | AAA GAG AAT GGT CCC CCT CAC TC | AGT TTT TCC AGT TCC AGG AGC AC |
| hnRNPA1 | TTG GAG GTG GTG GAA GCT ACA | ATA GCT ACT GCT GCT GCT GGA |
| INSIG1  | ATT TTT CTC AGG AGG CGT CA | CTG CGG GTT GTT AAT TGA GT |
| DHCR24  | GCC GCT CTC GCT TAT CTT CG | GTC TTG CTA CCC TGC TCC TT |
| MCCC2   | TTT GTC CAG TTA TGC TGC CAA AGA | GGC ACC ATC CTT GGC AAT TC |
| ATAD2   | CAC CGA GTA CTC CTG TGG CTT | TCT AGC TCG AGT CAT TCG CAG |
| PPAP2A  | CCA CAC TGC AAT TTG GTC TTG | CCC TGA ATG AGT CCA GTC AAC |
| ACSL3   | GGG CCA AAG TGT GAC AAT GG | CGG GTT CAA ACT CTC CAA TAT CC |
| HMGCR   | GCC TGA CAC AGG AAC CTG AA | AGT GCT GTC AAA TGC CTC CTZ |
| SCD     | CGA CGT GGC TTT TTC TCC TC | CCT TCT CTT TGA CAG CTG GG |
| SQLE    | CAG CAA GCT TCC TCT CTC CTT | CTC CAC CAG TAA GTG GAT GCC |
| DBI     | AAG CAA CTG TGG GCG ACA TAA | CTC ATT CCA GGC ATC CCA CTT |
| EBP     | GGT TGT CCC ATT GGG GAC TTG | GGC AAG CTG TGA TGG TTT CCA |
| FDFT1   | GCA ACG CAG TGT GCA TAT TTT | CGC CAG TCT GGT TGG TAA AGG |
Table S3. qRT-PCR primer sets used in this study (continued).

| Gene   | Forward                   | Reverse                   |
|--------|---------------------------|---------------------------|
| EMP2   | TCT TCG TGC TCC AGC TCT TCC | GAG TAG CCG TAG CTG CCT TCT |
| CAMKK2 | TCC AGA CCA GCC CCG ACA TAG | CAG GGG TGC AGC TTG ATT TC |
| ACAT2  | TGG GCA GAA TCC TGT TAG ACA | TGC AAG GCA CAC AGC TTT TAG |
| ELOVL5 | ATT GCT AGG CCC TCG AGA TAC | AAA TCC CCC GGC AAG AGA ATG |
| SGK3   | CCA GGA GTG AGT CTT ACA G  | CCA GCC ACA TTA GGA TTA    |
| SORD   | TGA GAT GAC CAC CGT ACC CC | GAA ACA CGC CCT TGA TAT CCA |
| IDH1   | TGC AAA AAT ATC CCC CGG CTT | TTT GGG TTC CGT CAC TTG GTG |
| SLC29A2| CAA CAT CAT GGA CTG GCT GGG | CAT GAA GAG GGG CAC GAA CAG |
| AKAP1  | GGA GTT GTC AGA CTT GGG GGC | CTG CCT GGG CAT TTT GGA AGC |
| NCAPD3 | GCA TCT GCA GAG ACA CCA GCA | GAG AAC GAT GTT GGA GAG GCG |
| PDIA5  | TGG AGT GGC TGA AGA ATC CGC | TCA GGT GAT AAA CGG AGC CGC |
| RAB3B  | TAC CGG ACC ATC ACA ACA ACA GCC | GTC ACA CTG GTT CCC CAC CAG |
| VLDLR  | TCA GTG TAT CCC AGT GTC C  | ATA CAA AGT TCC TGG AGA TGC |
| HPGD   | TGG TCA ATA ATG CTG GAG TGA | GGT TCC ACT GAT AAC AGA AAC CA |
| TXNIP  | TCG GCT TTG AGC TTC CTC AG | GTC TCT TGA GTT GGC TGG CT |
| GDF15  | GTT GCA CTC CGA AGA CTC CAG | GAG AGA TAC GCA GGT GCA GGT |
| TLE1   | TGC TGT CCC TGA AAT TTG CT | TGA CGA GGA CTC TTT GGA CT |
| ENC1   | CAT CTC CCA CCT TGG TCC ACT | CCA TGG CTC TGC TGA ACT GAA |
| RBM5   | TAT GAT CCG ACA ACA GGG CTC | AGC TGG CAC GTC GGT CTC TTT |
Table S3. qRT-PCR primer sets used in this study (continued).

|     | Forward                                                   | Reverse                                                   |
|-----|----------------------------------------------------------|-----------------------------------------------------------|
| UGT2B15 | GTG TTG GGA ATA TTA TGA CTA CAG TAA C                  | GGG TAT GTT AAA TAG TTC AGC CAG T                       |
| UGT2B17 | TGA CTT TTG GTT TCA AGC ATA                              | TTC CAT TTC CTT AGG CAA GGG                              |

Table S4. siRNA sequences used in this study.

|     | Sense: AGU UUG AGA ACC UCU GCA AGC UCdA dT                  | Antisense: AUG AGC UUG CAG AGG UUC UCA AAC UUU            |
|-----|----------------------------------------------------------|-----------------------------------------------------------|
| HSP90α/β | AGU UUG AGA ACC UCU GCA AGC UCdA dT                              | AUG AGC UUG CAG AGG UUC UCA AAC UUU                       |
| Non-specific (NS) | GUA CGG UUA UAC CGU GAC CdTdT                                 | GGU CAC GGU AUA ACC GUA CdTdT                              |