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Synthesis and Antitumor Activity of Brominated-Ormeloxifene (Br-ORM) against Cervical Cancer

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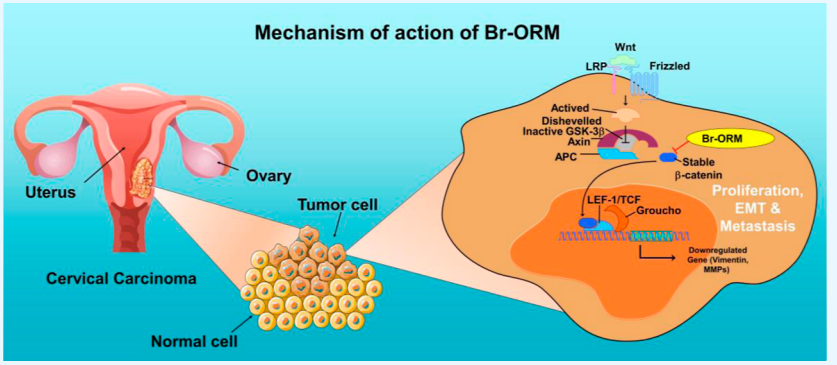

ABSTRACT: Aberrant regulation of β-catenin signaling is strongly linked with cancer proliferation, invasion, migration, and metastasis, thus, small molecules that can inhibit this pathway might have great clinical significance. Our molecular modeling studies suggest that ormeloxifene (ORM), a triphenylethylene molecule that docks with β-catenin, and its brominated analogue (Br-ORM) bind more effectively with relatively less energy (−7.6 kcal/mol) to the active site of β-catenin as compared to parent ORM. Herein, we report the synthesis and characterization of a Br-ORM by NMR and FTIR, as well as its anticancer activity in cervical cancer models. Br-ORM treatment effectively inhibited tumorigenic features (cell proliferation and colony-forming ability, *etc.*) and induced apoptotic death, as evident by pronounced PARP cleavage. Furthermore, Br-ORM treatment caused cell cycle arrest at the G1-S phase. Mechanistic investigation revealed that Br-ORM targets the key proteins involved in promoting epithelial−mesenchymal transition (EMT), as demonstrated by upregulation of E-cadherin and repression of N-cadherin, Vimentin, Snail, MMP-2, and MMP-9 expression. Br-ORM also represses the expression and nuclear subcellular localization of β-catenin. Consequently, Br-ORM treatment effectively inhibited tumor growth in an orthotopic cervical cancer xenograft mouse model along with EMT associated changes as compared to vehicle control-treated mice. Altogether, experimental findings suggest that Br-ORM is a novel, promising β-catenin inhibitor and therefore can be harnessed as a potent anticancer small molecule for cervical cancer treatment.

1. INTRODUCTION

Cervical cancer starts in the cervix and is the fourth most prevalent cancer among women, with approximately 604,127 new cases and 341,831 reported deaths worldwide.[1−4] Prolonged infection with human papillomavirus (HPV) is closely associated with the advancement of this disease.[5,6] Approximately 99.97% of cervical cancer cases are associated with HPV infection.[7−9] The oncogenic HPV protein that is produced by high-risk human papillomavirus (HR-HPV) has been linked to cervical cancer. Continuous annual reduction of ∼2% in death levels is attributed to effective interventions such as HPV vaccinations and Pap screening.[10] The HPV is a common sexually transmitted disease that affects almost all women at least once in their life time. However, only fewer women get cancer due to HPV.[8,11] The female immune system can frequently eliminate HPV infection without assistance. However, if the infection is left untreated, it may lead to the development of cancer.[12,13] Although HPV infection is the main cause of cervical cancer, other factors, such as genetic and epigenetic changes that work together, are also involved in cervical cancer progression. Recent research indicates that HPV oncoproteins may modulate key cancer-associated pathways such as Wnt/β-catenin signaling.[14,15] Aberrant

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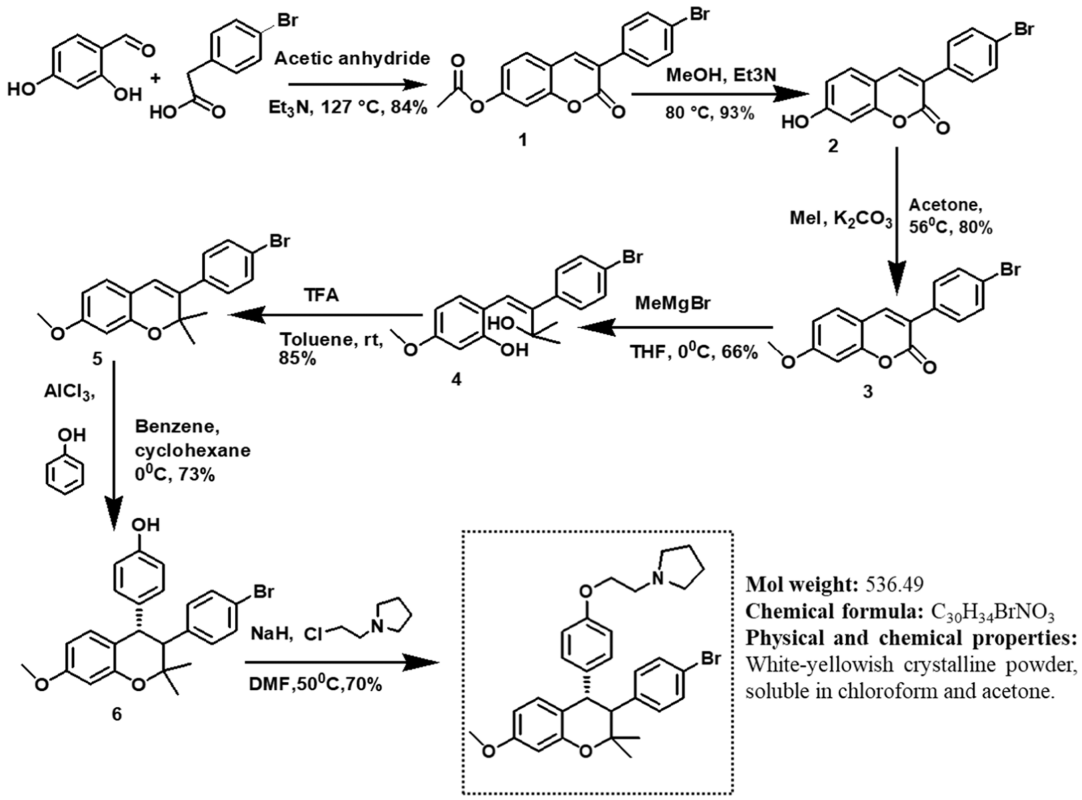

Figure 1. Schematic representation of the multistep chemical synthesis of Br-ORM.

activation of the Wnt/β-catenin signaling pathway has been involved in chemotherapy resistance.[16−21]

Additionally, a recent study showed that Wnt/β-catenin signaling also contributed to HPV-negative or low E6/E7-expressed cervical cancer progression.[22] Studies also suggest that the apoptosis induction, cell motility invasion angiogenesis, and tumor growth suppression can be achieved in cervical cancer through the repression of Wnt signaling.[23,24] These studies demonstrate that Wnt/β-catenin signaling plays a significant role in the development of cervical cancer, independent of HPV.[20,25] Accumulating evidence also suggests that the epithelial−mesenchymal transition (EMT) process, characterized by loss of E-cadherin and overexpression of N-cadherin, is responsible for the aggressive and metastatic characteristics of cervical cancer.[26,27] Additionally, β-catenin expression/subcellular localization also plays a significant role in the EMT process in a variety of malignancies, including cervical cancer.[28] Notably, studies have shown that higher tumor grades and tumor burden are associated with higher nuclear β-catenin expression as compared to adjacent normal tissues.[27,29−32] Stabilization of the transcriptional coactivator β-catenin modulates the expression of a number of genes involved in survival, metastasis, and EMT.[33] This evidence suggests that β-catenin could be a molecular target of significant importance. Targeting it effectively could potentially result in successful cancer treatment, including cervical cancer. Consequently, there is a dire need to develop nontoxic pharmacological inhibitors for targeting this important signaling pathway and treatment of Wnt/β-catenin-driven cancers alone or in combination with conventional chemotherapy.

Ormeloxifene (ORM) is a synthetic and nontoxic compound with well-defined pharmacokinetics and pharmacodynamics in humans. In addition to its widespread usage as an oral contraceptive, a number of studies have demonstrated its potential anticancer activities.[34−38] ORM is known as a selective estrogen receptor modulator (SERM) and it works by blocking estrogen receptors in the cervix, uterus, and breast.[39,40] Research conducted in the past by our and other groups have demonstrated that ORM has excellent anticancer activity on several cancers, including ovarian, prostate, pancreas, breast, and head and neck cancers.[30,35,40−44] ORM's remarkable therapeutic index and lack of systemic toxicity upon prolonged administration serve as important criteria for its selection for cancer therapy.[39] The potential anticancer activity of ORM laid the foundation for the design and synthesis of novel analogues of ORM, *i.e.*, bromo-ormeloxifene (Br-ORM), that could potentially be useful for the effective targeted suppression of Wnt/β-catenin signaling. Our study determined Br-ORM as a potential inhibitor of Wnt/β-catenin, EMT-associated signaling pathways, and cervical cancer growth.

2. MATERIALS AND METHODS

2.1. Chemistry. All of the chemicals and reagents used in this study were obtained from Sigma-Aldrich and Fisher Scientific. Halogenated ORM analogue, Br-ORM, was synthesized through a multistep organic synthetic approach, as depicted in Figure 1. Detailed chemical synthesis of Br-ORM is described in the Supporting Information Figure S1). Standard spectroscopic, analytical, and chromatographic techniques were employed to confirm the formation of Br-ORM. The melting points were determined using the Mel-Temp apparatus. Br-ORM is crystalline and stable and its chemical characterization was confirmed through NMR and HR-MS (Figure S2).

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2.2. Cell Lines and Growth Condition. Cervical cancer (CaSki and SiHa) cells were procured from American Type Cell Culture (Manassas, VA) and cultured in RPMI-1640 and DMEM media (HyClone Laboratories, Inc., Logan, UT) supplemented with 10% heat-inactive FBS (Atlanta Biologicals, Norcross, GA), 1% penicillin, and streptomycin (Gibco BRL, Grand Island, NY). Cells were maintained in a humidified atmosphere containing 5% CO_2 at 37 °C.

2.3. Chemicals and Antibodies. We acquired β-actin (cat. no. 3700), Histone H3 (cat. no. 4499), N-cadherin (cat. no. 4061), Slug (cat. no. 9585), Snail (cat. no. 3879), Vimentin (cat. no. 5741), PARP (cat. no. 9532), MMP2 (cat. no. 4022), and MMP9 (cat. no. 13667) from Cell Signaling Technology Inc. β-catenin (cat. no. SC-7199) and E-cadherin (cat. no. SC-7870) antibodies were procured from Santa Cruz Biotechnology. Anti-mouse (cat. no. 4201) and anti-rabbit (cat. no. 4011) antibodies coupled with horseradish peroxidase (HRP) were obtained from Promega (Madison, WI, USA).

2.4. Cell Proliferation Assay. The effect of Br-ORM on cell viability was determined by the MTS assay. Briefly, a 96-well plate was seeded with 5000 cells per well, and the cells were allowed to grow in a 37 °C, 5% CO_2 incubator overnight. The next day, the cells were exposed to Br-ORM at 1, 5, 10, and 20 μM concentrations. After 48 h, 20 μL of MTS reagent was added to each well, followed by 2 h of incubation in a CO_2 incubator, and the absorbance at 490 nm was measured (SpectraMax M2 spectrophotometer, Molecular Devices, Sunnyvale, CA, USA).

2.5. Colony Formation Assay. A colony formation assay was performed to examine the effect of Br-ORM on the clonogenic potential of cervical cancer cells. Briefly, a 6-well plate was seeded with 500 cells per well and allowed to incubate for 3 days. The cells were exposed to Br-ORM (0, 2.5, 5, and 7.5 μM) for 7 days, followed by normal growth media. The cells in the control group were treated with DMSO. The colonies were methanol-fixed, hematoxylin-stained, and counted using UVP 810 software.

2.6. Apoptosis Analysis. Annexin V-FLUOS Staining Kit (Roche Diagnostic Corp, Indianapolis, IN) was used to examine the apoptosis-inducing effect of Br-ORM on cervical cancer cells. Cells were grown to 60% confluence and then treated with Br-ORM for 24 h. Following the treatment, cells were incubated in an Annexin-V solution for 20 min. Images were captured using a fluorescent microscope.

2.7. Flow Cytometry. Flow cytometry was carried out to investigate the effect of Br-ORM on the cell cycle progression. Cervical cancer cells (80% confluent) were treated with Br-ORM (10 and 20 μM) for 24 h, and cell cycle analysis was performed as described earlier.[45]

2.8. Migration and Invasion Assay. Cell motility was performed *in vitro* scratch wound assay and in a 96-well format HTS transwell plate from Corning using the same procedure as previously reported.[45] Migration assay was carried out by agarose bead assay as described earlier.[46] A cell invasion assay was conducted using a cell invasion kit (BD Biosciences, San Jose, CA).[32]

2.9. Immunoblotting and Immunofluorescence Assay. The effect of Br-ORM on protein expression in cervical cancer cells was determined by immunoblotting techniques by using specific antibodies.[30] β-catenin localization was determined in nuclear and cytoplasmic lysates as reported previously.[30] Immunofluorescence was performed as described earlier.[33]

2.10. TCF Luciferase Assay. CaSki cells (1.5×10^5 cells/well) were plated in triplicate in 12-well plates. TCF-firefly luciferase reporter constructs pTOP-FLASH, pFOP-FLASH, and Renilla luciferase were transiently cotransfected into cells. The β-catenin/TCF transcription activity was determined as described previously.[30]

2.11. Molecular Docking. Protein Data Bank (www.rcsb.org) was used to get the atomic coordinates for the crystal structure of β-catenin (PDB ID: 1JDH), whereas PubChem was used to recover the 2D and 3D structures for Br-ORM. Following our established method, further computations and file preparations were performed.[47] Docking was performed using the AutoDock 4 package.[48] Docked complexes were verified and explored using Discover Studio 4.0.[49] The molecular docking interactions were visualized by using PyMOL.

2.12. RNA Isolation and PCR. mRNA in Br-ORM-treated cervical cancer cells was extracted using a Qiagen kit and analyzed with a NanoDrop 2000. E-cadherin and N-cadherin levels were analyzed by using real-time PCR.

2.13. Xenograft Study. Female athymic nude mice (Jackson Laboratory, Bar Harbor, ME, USA), aged 6−8 weeks, were used in an orthotopic mouse model of cervical cancer to test the anticancer efficacy of Br-ORM. All operations performed on mice were approved by the UTHSC Institutional Animal Care and Use Committee (IACUC), and mice were kept in a pathogen-free environment. We injected a combination of 100 μL of matrigel (BD Biosciences) and 2.5×10^6 CaSki cells into their cervix. Mice received an intraperitoneal injection of Br-ORM (250 μg/mouse) or a vehicle control (1× PBS) three times weekly. To track the growth of the tumor over time, we used the ellipsoid volume formula: tumor volume (mm^3) = $0.5 \times L \times W \times H$, where L, W, and H are the tumor's length, width, and height, respectively. As the tumor volume of control mice reached 1000 mm^3, the animals were euthanized, and their tumors were removed and utilized for tissue sectioning for histopathological analysis.

2.14. Immunohistochemistry. Immunohistochemistry using Biocare kits (Biocare Medical, Concord, CA, USA) was used to assess the effect of Br-ORM on E-cadherin, β-catenin, Vimentin, Snail, Slug, and PCNA proteins in excised tumors as described previously.[30]

2.15. Statistical Analysis. The data were analyzed by a two-tailed Student *t*-test and employed to assess the statistical significance between the control and Br-ORM-treated groups.

3. RESULTS

3.1. Synthesis of Analogue Br-ORM. Novel analogue of ORM, Br-ORM was synthesized using the method of Ji *et al.* (1998) with slight modification.[50] Commercially available 2, 4-hydroxybenzaldehyde and various derivatives of phenylacetic acid undergo nucleophilic addition−elimination in the presence of triethylamine and acetic anhydride to give compound (1). Hydrolysis of (1) with triethylamine using methanol as a solvent gave compound (2), followed by alkylation using methyl iodide in the presence of K_2CO_3 and acetone to produce (3). The Grignard reaction of (3) with methyl-magnesium bromide in the presence of THF produced (4), followed by intramolecular dehydration with TFA and $NaHCO_3$ to give (5).[51] Hydroarylation of phenol with (5) in benzene and cyclohexane afforded a 1:1 (w/w) racemic mixture of D- and L-enantiomers (6). The presence of halogens

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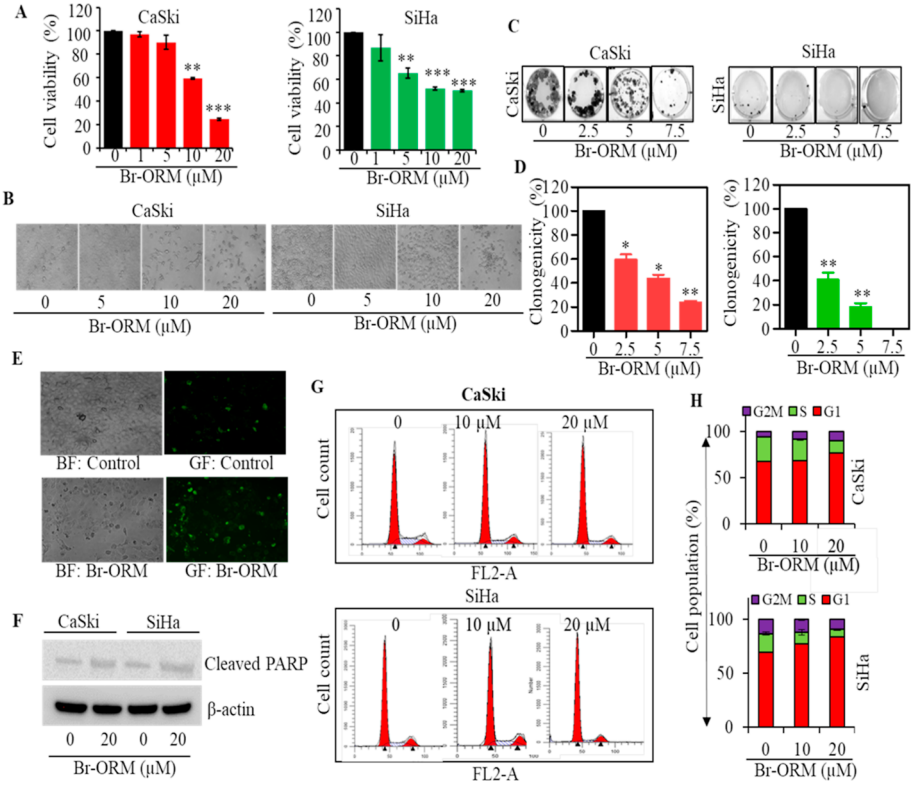

Figure 2. Br-ORM decreases proliferation and colony formation of cervical cancer cells. (A) Effect of Br-ORM on cell viability of CaSki and SiHa cells. Cell viability was assessed by the MTS assay. (B) Representative images of cells after Br-ORM treatment using a phase contrast microscope. (C) Effect of Br-ORM on the clonogenic potential of CaSki and SiHa. (D) Bar graphs indicating quantification of colony formation with respect to vehicle control. (E) Effect of Br-ORM on apoptosis-inducing ability in cervical cancer cells as analyzed by Annexin V staining. (F) Western blot analysis of PARP cleavage following Br-ORM treatment in cervical cancer cells. (G) Effect of Br-ORM on the progression of the cell cycle in cervical cancer cells as analyzed by flow cytometry. (H) Percentage of cells in each cell cycle phase. (*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$).

para to the aromatic ring at C-3 changed the confirmation of ORM analogues. Condensation of (6) with 1-(2-chloroethyl)-pyrrolidine. HCl (responsible for antagonistic activity) in the presence of NaH and DMF finally gave the title compound Br-ORM in approximately 70% yield. The multistep total chemical synthesis of Br-ORM is depicted in Figure 1.

3.2. Br-ORM Inhibits the Growth of Cervical Cancer Cells. To assess the antiproliferative effect of Br-ORM in cervical cancer (CaSki and SiHa) cells, an MTS assay was performed. As shown in Figure 2A, Br-ORM treatment suppressed the cell viability in a dose-dependent manner. The results demonstrated an IC50 of 15−20 μM in CaSki and SiHa cells, which indicates the enhanced efficacy of synthetically modified ORM due to the attachment of a bromo functional group. Previous published studies from our group have reported the IC50 of ORM more than 20 μM in cervical cancer cells,[52,53] which further highlights the significance of an additional functional group as bromo to ORM. Using a phase contrast microscope, the morphology of Br-ORM-treated CasKi and SiHa cells was compared with the control group.

Results indicated a disrupted morphology of cells treated with Br-ORM in contrast with controls, which represented normal morphology (Figure 2B). Next, we examined the long-term effect of Br-ORM on the clonogenic potential of cervical cancer cells. As shown in Figure 2C,D, Br-ORM treatment significantly ($p < 0.01$) decreased the number of colonies in both cells. These results indicate that treatment with Br-ORM reduces the proliferation and clonogenicity of cervical cancer cells.

3.3. Br-ORM Induces Apoptosis in Cervical Cancer Cells and Arrest Cells in G1/S Phase. Next, we investigated whether Br-ORM induces apoptosis in cervical cancer cells. The apoptosis-inducing effect of Br-ORM was determined by Annexin V staining and Western blot analysis for PARP cleavage in cells treated with 20 μM Br-ORM for 24 h. As shown in Figure 2E, Br-ORM treatment led to the induction of apoptosis in cells, as determined by Annexin V staining. Furthermore, following Br-ORM treatment, there was increased PARP protein cleavage in both cells (Figure 2F). The arrest of the cell cycle is an attractive therapeutic target for the treatment of a wide range of cancers; hence, medicines and small molecules that are able to halt the progression of the cell cycle are highly desirable.[54] In order to assess the effect on the cell cycle activity, cervical cancer cells were treated with Br-ORM at concentrations of 10 and 20 μM and evaluated by flow cytometry. Br-ORM treatment arrested the cell cycle transition at the G1/S phase in a dose-dependent manner compared to vehicle control (Figure 2G,H). Taken together, these findings suggest that Br-ORM can effectively induce apoptosis and arrest cell cycle progression in cervical cancer cells.

3.4. Br-ORM Inhibits Migratory and Invasive Potential of Cervical Cancer Cells. The agarose beads, scratch wound healing, and Boyden chamber assays were performed to confirm whether Br-ORM therapy decreases the migratory capability of cervical cancer cells. As revealed by the agarose bead assay (Figure 3A), Br-ORM treatment suppressed the migratory potential of cervical cancer cells as compared to the

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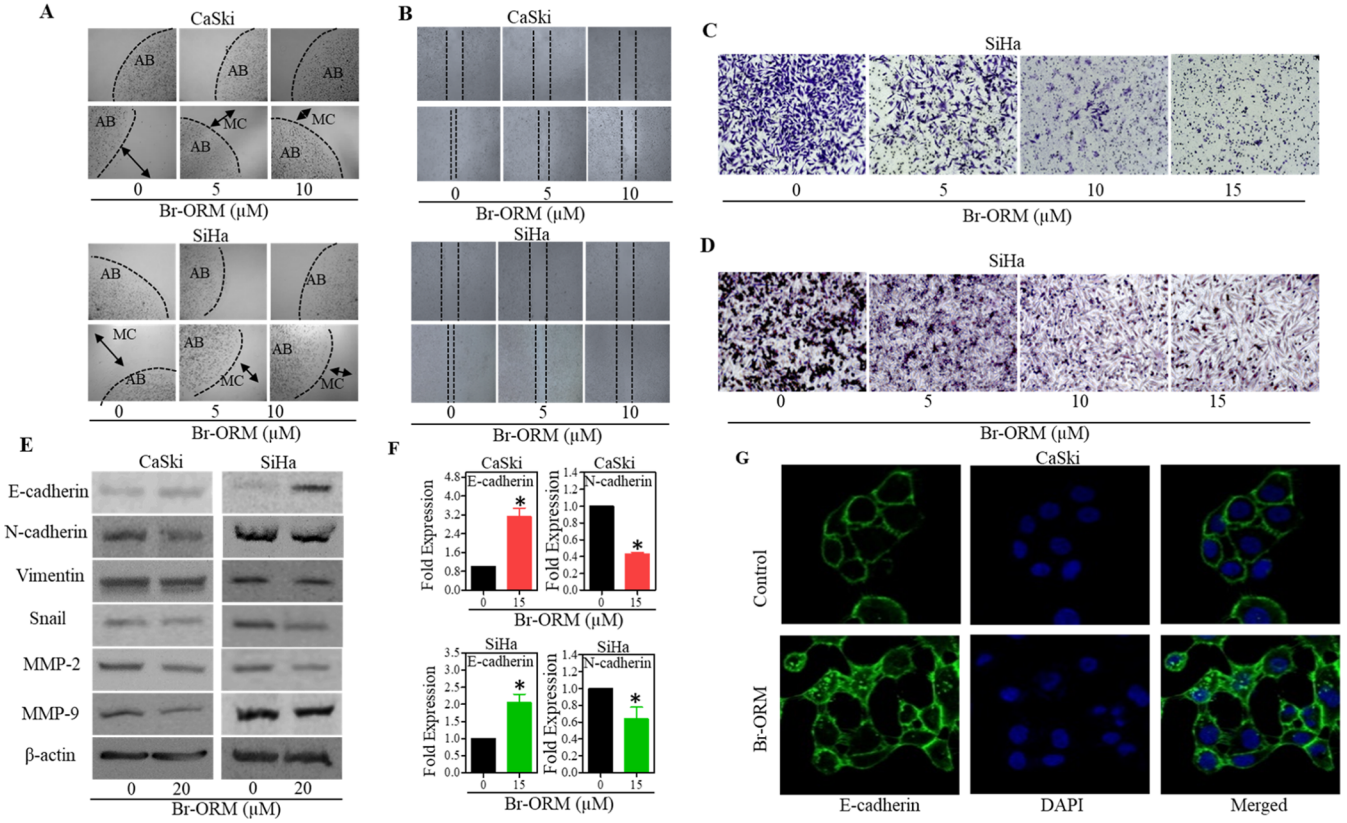

Figure 3. Br-ORM decreases migration and invasion potential and attenuates EMT in cervical cancer cells. (A) Effect of Br-ORM on agarose bead assay. AB represents agarose bead, while MC represents migratory cells. (B) Representative images from a scratch wound healing assay demonstrating cervical cancer cell migration following the Br-ORM. (C) Effect of Br-ORM on the migratory potential of SiHa cells using the Boyden chamber assay. (D) Effect of Br-ORM treatment on invasion of SiHa cells as determined by a commercially available kit. (E) Western blot analysis of various EMT and associated effector protein following Br-ORM treatment in cervical cancer cells. (F) Effect of Br-ORM on mRNA levels of E-cadherin and N-cadherin as determined by qPCR (*$p < 0.05$). (G) Effect of Br-ORM on E-cadherin (green) in cervical cancer cells.

control group. Reductions in wound healing and transmembrane cellular movement under chemotactic drive were also observed in Br-ORM-treated cells compared to control cells, as determined by scratch wound healing and the Boyden chamber assay, respectively (Figure 3B,C). Next, we investigated whether or not Br-ORM treatment also inhibits cancer cell invasion. Interestingly, Br-ORM inhibited invasion of cervical cancer cells (Figure 3D), as determined by a commercially available cell invasion kit. These findings suggest that Br-ORM inhibits the migration and invasive abilities of cervical cancer cells.

3.5. Br-ORM Treatment Effectively Modulates the Expression of EMT-Associated Proteins/Genes in Cervical Cancer Cells. Cancer cells are characterized by EMT, a process in which epithelial cells undergo morphologic and molecular changes that ultimately result in a mesenchymal, highly metastatic (invasive and motile), and drug-resistant phenotype.[55] Hence, targeting the EMT reduces cancer cell metastasis and chemoresistance. Herein, we investigated the effect of Br-ORM treatment on the EMT and associated markers in cervical cancer cells. Br-ORM treatment downregulated N-cadherin, Vimentin, Snail, MMP-2, and MMP-9 expression in CaSki and SiHa, but it upregulated E-cadherin expression as shown in Western blots (Figure 3E). Furthermore, qPCR analysis revealed a similar trend of decreased N-cadherin and increased E-cadherin following Br-ORM treatment (Figure 3F). E-cadherin restoration was further confirmed by immunofluorescence analysis (Figure 3G). These findings clearly showed that Br-ORM inhibits EMT processes in cervical cancer cells.

3.6. Br-ORM Represses Nuclear Translocation of β-Catenin. As Br-ORM modulated the expression of EMT-associated proteins/genes, we next investigated its effect on β-catenin nuclear trafficking, where it interacts with TCF-4 to promote oncogenic and EMT-associated signaling pathways involved in cancer and metastasis.[56−59] Western blotting analysis of cytoplasmic and nuclear lysates confirmed that Br-ORM treatment (20 μM) prevented the nuclear translocation of β-catenin expression in CaSki and SiHa cells (Figure 4A). Appropriate internal controls for cytoplasmic (β-actin) and nuclear (Histone H3) lysates were used in this analysis to ascertain the quality and purity of cytoplasmic and nuclear lysates. Additionally, confocal microscopy analysis also demonstrated that Br-ORM prevented nuclear localization of β-catenin in cervical cancer cells compared to that in control cells (Figure 4B). In addition, we determined if Br-ORM-mediated reduced levels of nuclear β-catenin repress the transcriptional activity of TCF-4 using the TCF-4 transcriptional reporter assay, as described in our previous study.[60] This study also suggests a marked repression of TCF-4 activity in Br-ORM-treated cervical cancer cells.

Molecular docking studies were performed to see the different types of interactions that exist between amino acid residues of selected target proteins and Br-ORM. The crystal structure of β-catenin (PDB ID: 1JDH) was downloaded from Protein Data Bank (www.rcsb.org), while the 2D and 3D

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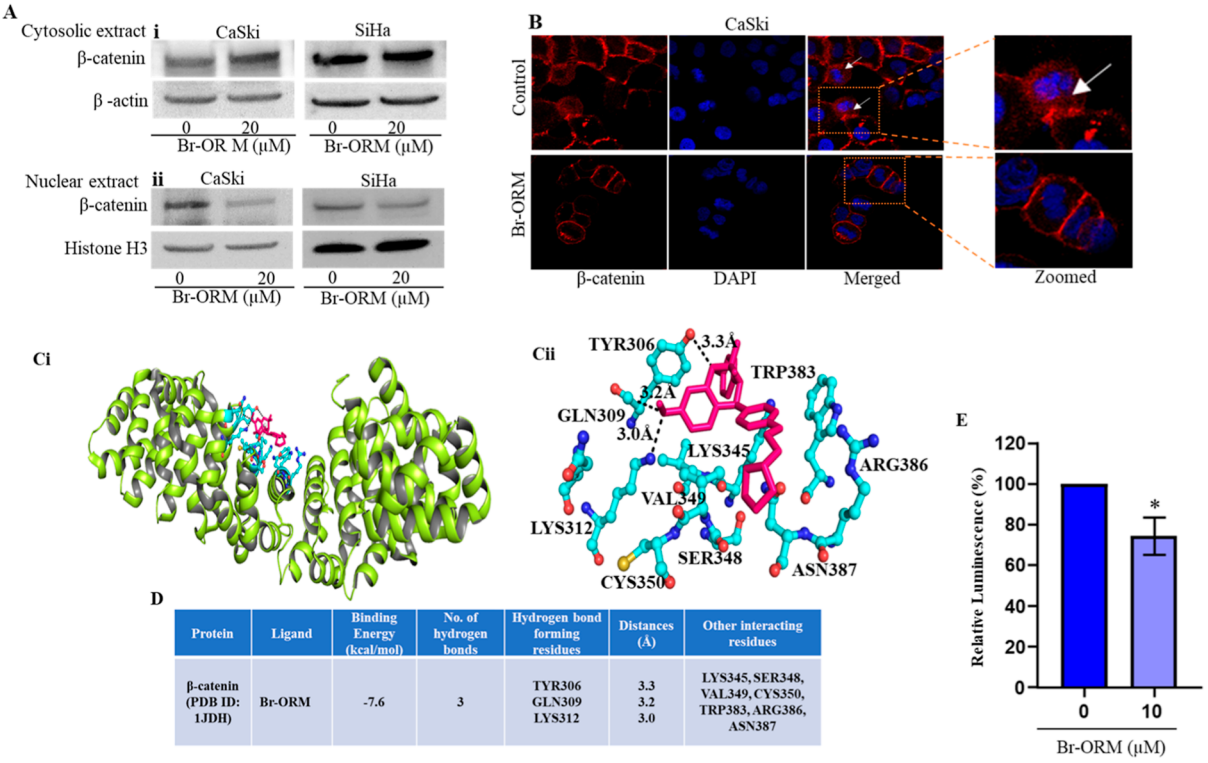

Protein	Ligand	Binding Energy (kcal/mol)	No. of hydrogen bonds	Hydrogen bond forming residues	Distances (Å)	Other interacting residues
β-catenin (PDB ID: 1JDH)	Br-ORM	-7.6	3	TYR306 GLN309 LYS312	3.3 3.2 3.0	LYS345, SER348, VAL349, CYS350, TRP383, ARG386, ASN387

Figure 4. Effect of Br-ORM on β-catenin signaling pathway in cervical cancer cells. (A) Western blot analysis of cytosolic (i) and nuclear (ii) localization of β-catenin in CaSki and SiHa cells. (B) Effect of Br-ORM on β-catenin localization in CaSki cells as determined by confocal microscopy. White arrows indicate localization of β-catenin in control and Br-ORM-treated cells after 18 h. (C). Molecular docking of Br-ORM with β-catenin. Cartoon representation of Br-ORM in-complexed with β-catenin (i). Three-dimensional view of different interacting residues of β-catenin with Br-ORM. Br-ORM is shown in the stick model (pink color), and residues participating in polar contacts and other interactions are shown in ball and stick (ii). (D) Table depicting Br-ORM score with β-catenin as well as the hydrogen bond formation of the binding site residues of this target protein offers numerous van der Waals interactions with Br-ORM. (E) Effect of Br-ORM on TCF4 promoter activity followed Br-ORM treatment. The values in the bar graph represent the mean ± SE of three well readings in each group. (*$p < 0.05$).

structures of Br-ORM were obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/compound/5281318#section=2D-Structure/3D-Conformer). Additional computations and file preparations were performed according to our previously described protocol.[45−47] After preparing the coordinate files of the selected targets and ligand (Br-ORM), they were subjected to docking using the AutoDock 4 package.[48] Docking results clearly signifies that Br-ORM binds into the binding site region of some known inhibitors of β-catenin [Figure 4C(i,ii)]. The complex formed between the ligand and protein is stabilized by different noncovalent interactions. The binding energy (ΔG) of Br-ORM with β-catenin is 7.6 kcal mol^{-1}. It is inference from Figure 4C,D that Br-ORM binds significantly with β-catenin. Docking research with LigPlot reveals several van der Waals contacts between β-catenin and the ligand Br-ORM. In the binding region, the following residues of β-catenin LYS345, SER348, VAL349, CYS350, TRP383, ARG386, and ASN387 show van der Waals interactions with ligand. Along with that, TYR306, GLN309, and LYS312 form three hydrogen bonds with Br-ORM (Figure 4D). These bonds provide stability to the protein−ligand complex (Supporting Information, Figure S3). Based on these findings, Br-ORM appears to be an effective inhibitor of the β-catenin signaling pathway in cervical cancer cells.

3.7. Br-ORM Inhibits Cervical Cancer Cell-Derived Orthotopic Xenograft Tumors in Athymic Nude Mice. Next, we developed an orthotopic xenograft mouse model using CaSki cells to examine whether Br-ORM treatment reduces cervical tumor growth *in vivo*. We treated mice with Br-ORM (250 μg/mice, three times per week) intraperitoneally. Our results demonstrate that Br-ORM treatment inhibited cervical tumor growth as compared to the vehicle-treated group (Figure 5A). As shown in Figure 5B,C, Br-ORM significantly decreased tumor volume and tumor weight as compared to the control groups. In addition, immunohistochemistry analysis of excised tumor tissues showed an upregulation of E-cadherin expression and a downregulation of β-catenin, Vimentin, Snail, Slug, and PCNA expression in Br-ORM-treated animals compared to the control (Figure 5D). All together, these results further confirm the antitumor efficacy of Br-ORM and its inhibitory action on nuclear β-catenin signaling and EMT processes.

4. DISCUSSION

Cervical cancer ranks as the fourth leading cause of cancer-related death among women worldwide.[1−3] Approximately 99.7% of cervical cancer instances involve HPV.[6−9] Accumulating studies suggest that cervical cancer is highly associated with HR-HPV, which produces oncogenic types of HPV protein.[6] Recent studies suggest a close link between the expression of E6/E7 HPV oncogenes and aberrant operation of Wnt/nuclear β-catenin signaling.[14,15] EMT plays a vital supporting role in cancer invasion, migration, growth, and drug resistance, which are all hallmarks of the metastatic phase of cancer resistance.[61−63] EMT is characterized by the disappearance of epithelial cell polarity and cell−cell junction proteins

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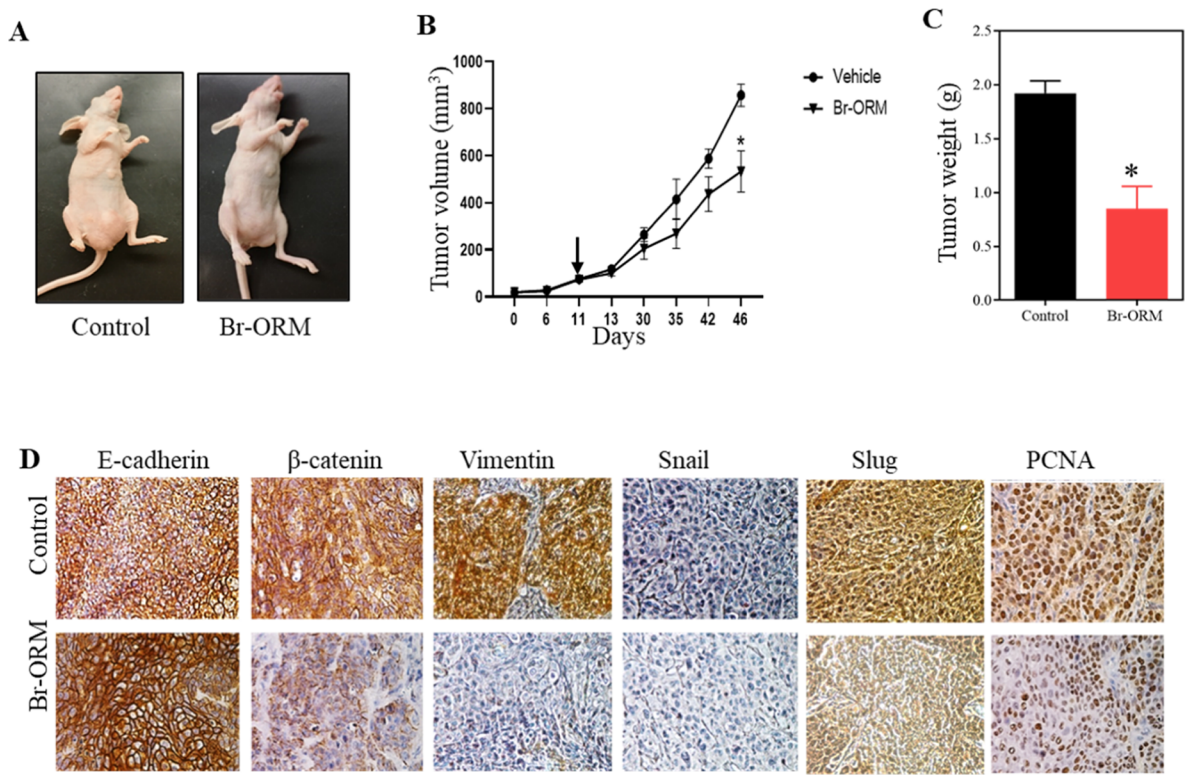

Figure 5. Br-ORM inhibits cervical tumor growth in an orthotopic xenograft mouse model. (A) Representative mouse images of control and Br-ORM-treated tumor-bearing mouse. (B) Line graph indicates regression of CaSki cell-derived xenograft tumor volume in Br-ORM-treated mice compared to the control group. (C) Bar graph representing tumor weight of control and Br-ORM-treated mice. (D) Effect of Br-ORM on the expression of E-cadherin, β-catenin, Vimentin, Snail, Slug, and PCNA as determined by immunohistochemistry in excised tumors of control and Br-ORM-treated mice.

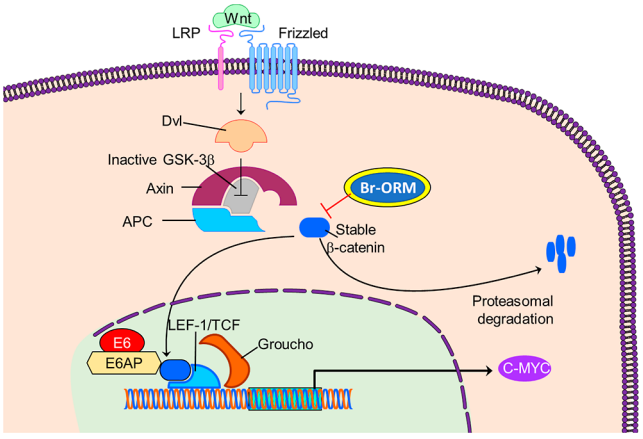

Figure 6. Hypothetical schematic model for the mechanism of action of Br-ORM in cervical cancer.

like E-cadherin and β-catenin and the appearance of mesenchymal biomarkers like N-cadherin and Vimentin.[64] Extracellular signals and transcription factors such as SNAIL members, zinc-finger E-box-binding (ZEB) families, SNAI2 (SLUG), and Twist-related protein 1 regulate EMT.[65,66] These transcription factors cause EMT reactions during tumor metastases by binding the repressed epithelial genes at their E-box sequences in the proximal promoter region.[64,67,68] Studies have shown that abnormal expression of these transcription factors is correlated with chemoresistance development in cancers. Therefore, it is essential to obtain knowledge of the mechanisms underlying EMT induction and to discover new entities/approaches to target these alterations to further improve drug sensitivity in cervical cancer patients.[69,70] We have recently revealed that ORM is a good anticancer agent and has an excellent therapeutic index, along with its safety for human use.[39] The pyrrolidine moiety in ORM is favorable and is responsible for its antagonistic activity. The receptor-binding ability and estrogen-agonistic activity of ORM are due to its benzopyran base.[39]

Herein, we synthesized a new ORM analogue, bromo-ORM, and showed that it proficiently binds to the active site of β-catenin (binding energy −7.6 kcal/mol). Functional assays have demonstrated that Br-ORM has the potential to limit the growth and proliferation of HPV-16 positive human cervical cancer (CaSki and SiHa) cells. We have shown that Br-ORM exerts its antiproliferative and pro-apoptotic effects. In addition, Br-ORM treatment was able to halt the migration and invasion of cervical cancer cells. Interestingly, Br-ORM treatment blocks the progression of the cell cycle in the G1/S phase and induces apoptosis. Furthermore, Br-ORM treatment upregulates E-cadherin expression in cervical cancer while downregulating β-catenin, Vimentin, Snail, N-cadherin, MMP-2, and MMP-9 expression. These results provide compelling evidence that Br-ORM suppresses EMT progression. Docking analysis clearly suggests that Br-ORM binds into the binding site cavity of some known inhibitors of beta-catenin, as reported previously by other groups.[71] Results from the *in vitro* efficacy of Br-ORM on cervical cancer encouraged us to set up an *in vivo* orthotopic xenograft mouse model for further study. The tumor xenograft study showed that Br-ORM inhibited cervical tumor growth in an orthotopic xenograft mouse

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model. We also observed a downregulation of β-catenin and EMT-related markers in excised xenograft tumors. Overall, our studies revealed a novel analogue of ORM (Br-ORM) that represents effective molecular docking with β-catenin and represses nuclear β-catenin-mediated EMT tumorigenesis and the cancer progression mechanism. This ORM analogue has a very high potential to develop it as a novel therapeutic modality to treat Wnt/β-catenin-derived cancers (Figure 6).

5. CONCLUSIONS

In short, using cell lines and preclinical orthotopic mouse models, we have shown prospective anticancer impacts of Br-ORM against cervical cancer. Our findings indicate that Br-ORM may represent a promising new therapeutic method for the treatment of cervical cancer and highlight an important therapeutic approach for future clinical trials.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c02277.

NMR spectra, chemical synthesis of Br-ORM, and surface view of molecular docking of Br-ORM with β-catenin (PDF)

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Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Wilailak, S.; Kengsakul, M.; Kehoe, S. Worldwide initiatives to eliminate cervical cancer. *Int. J. Gynaecol. Obstet.* **2021**, *155* (S1), 102−106.

(2) Liu, C.; Li, X.; Huang, Q.; Zhang, M.; Lei, T.; Wang, F.; Zou, W.; Huang, R.; Hu, X.; Wang, C.; et al. Single-cell RNA-sequencing reveals radiochemotherapy-induced innate immune activation and MHC-II upregulation in cervical cancer. *Signal Transduction Targeted Ther.Signal Transduct Target Ther* **2023**, *8* (1), 44.
(3) Sung, H.; Ferlay, J.; Siegel, R. L.; Laversanne, M.; Soerjomataram, I.; Jemal, A.; Bray, F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *Ca-Cancer J. Clin.CA Cancer J. Clin* **2021**, *71* (3), 209−249.
(4) American Cancer Society. *Key Statistics for Cervical Cancer*; American Cancer Society, 2023. (accessed May 4, 2023).
(5) Clarke, M. A.; Wentzensen, N.; Mirabello, L.; Ghosh, A.; Wacholder, S.; Harari, A.; Lorincz, A.; Schiffman, M.; Burk, R. D. Human papillomavirus DNA methylation as a potential biomarker for cervical cancer. *Cancer Epidemiol. Biomarkers Prev.* **2012**, *21* (12), 2125−2137.
(6) Andújar-Sánchez, M. Role of human papillomavirus genotyping in cervical cancer screening. *Enferm. Infecc. Microbiol. Clin. (Engl. Ed.)* **2023**, *41*, 259−261.
(7) Walboomers, J. M.; Jacobs, M. V.; Manos, M. M.; Bosch, F. X.; Kummer, J. A.; Shah, K. V.; Snijders, P. J.; Peto, J.; Meijer, C. J.; Muñoz, N. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J. Pathol.* **1999**, *189* (1), 12−19.
(8) Bosch, F. X.; Lorincz, A.; Munoz, N.; Meijer, C. J.; Shah, K. V. The causal relation between human papillomavirus and cervical cancer. *J. Clin. Pathol.* **2002**, *55* (4), 244−265.
(9) Carter, J. R.; Ding, Z.; Rose, B. R. HPV infection and cervical disease: a review. *Aust. N. Z. J. Obstet. Gynaecol.Aust N Z. J. Obstet Gynaecol* **2011**, *51* (2), 103−108.
(10) Reid, J. Women's knowledge of Pap smears, risk factors for cervical cancer, and cervical cancer. *J. Obstet. Gynecol. Neonatal Nurs.* **2001**, *30* (3), 299−305.
(11) Burd, E. M. Human papillomavirus and cervical cancer. *Clin. Microbiol. Rev.* **2003**, *16* (1), 1−17.
(12) Bosch, F. X.; de Sanjose, S. Chapter 1: Human papillomavirus and cervical cancer−burden and assessment of causality. *J. Natl. Cancer Inst. Monogr.* **2003**, *2003* (31), 3−13.
(13) Maglennon, G. A.; Doorbar, J. The biology of papillomavirus latency. *Open Virol. J.Open Virol J.* **2012**, *6*, 190−197.
(14) Muñoz-Bello, J.; Olmedo-Nieva, L.; Castro-Muñoz, L.; Manzo-Merino, J.; Contreras-Paredes, A.; González-Espinosa, C.; López-Saavedra, A.; Lizano, M. HPV-18 E6 Oncoprotein and Its Spliced Isoform E6*I Regulate the Wnt/β-Catenin Cell Signaling Pathway through the TCF-4 Transcriptional Factor. *Int. J. Mol. Sci.* **2018**, *19* (10), 3153.
(15) Bello, J. O.; Nieva, L. O.; Paredes, A. C.; Gonzalez, A. M.; Zavaleta, L. R.; Lizano, M. Regulation of the Wnt/β-Catenin Signaling Pathway by Human Papillomavirus E6 and E7 Oncoproteins. *Viruses* **2015**, *7* (8), 4734−4755.
(16) Xu, H.; Wang, Z.; Xu, L.; Mo, G.; Duan, G.; Wang, Y.; Sun, Z.; Chen, H. Targeting the eIF4E/β-catenin axis sensitizes cervical carcinoma squamous cells to chemotherapy. *Am. J. Transl. Res.* **2017**, *9* (3), 1203−1212.
(17) Wang, T.; Liu, Z.; Shi, F.; Wang, J. Pin1 modulates chemo-resistance by up-regulating FoxM1 and the involvements of Wnt/β-catenin signaling pathway in cervical cancer. *Mol. Cell. Biochem.* **2016**, *413* (1−2), 179−187.
(18) Kisseljov, F.; Sakharova, O.; Kondratjeva, T. Chapter 2 Cellular and Molecular Biological Aspects of Cervical Intraepithelial Neoplasia. *Int. Rev. Cell Mol. Biol.* **2008**, *271*, 35−95.
(19) Manzo-Merino, J.; Contreras-Paredes, A.; Váázquez-Ulloa, E.; Rocha-Zavaleta, L.; Fuentes-Gonzalez, A. M.; Lizano, M. The role of signaling pathways in cervical cancer and molecular therapeutic targets. *Arch. Med. Res.* **2014**, *45* (7), 525−539.
(20) Ford, C. E.; Henry, C.; Llamosas, E.; Djordjevic, A.; Hacker, N. Wnt signalling in gynaecological cancers: A future target for personalised medicine? *Gynecol. Oncol.* **2016**, *140* (2), 345−351.
(21) Bahrami, A.; Hasanzadeh, M.; ShahidSales, S.; Yousefi, Z.; Kadkhodayan, S.; Farazestanian, M.; Joudi Mashhad, M.; Gharib, M.; Mahdi Hassanian, S.; Avan, A. Clinical Significance and Prognosis Value of Wnt Signaling Pathway in Cervical Cancer. *J. Cell. Biochem.* **2017**, *118* (10), 3028−3033.
(22) Banister, C. E.; Liu, C.; Pirisi, L.; Creek, K. E.; Buckhaults, P. J. Identification and characterization of HPV-independent cervical cancers. *Oncotarget* **2017**, *8* (8), 13375−13386.
(23) Ramachandran, I.; Thavathiru, E.; Ramalingam, S.; Natarajan, G.; Mills, W. K.; Benbrook, D. M.; Zuna, R.; Lightfoot, S.; Reis, A.; Anant, S.; et al. Wnt inhibitory factor 1 induces apoptosis and inhibits cervical cancer growth, invasion and angiogenesis in vivo. *Oncogene* **2012**, *31* (22), 2725−2737.
(24) Chang, B.; Kim, J.; Jeong, D.; Jeong, Y.; Jeon, S.; Jung, S. I.; Yang, Y.; Kim, K. I.; Lim, J. S.; Kim, C.; et al. Klotho inhibits the capacity of cell migration and invasion in cervical cancer. *Oncol. Rep.* **2012**, *28* (3), 1022−1028.
(25) Tomaić, V. Functional Roles of E6 and E7 Oncoproteins in HPV-Induced Malignancies at Diverse Anatomical Sites. *Cancers* **2016**, *8* (10), 95.
(26) Jaggi, M.; Nazemi, T.; Abrahams, N. A.; Baker, J. J.; Galich, A.; Smith, L. M.; Balaji, K. C. N-cadherin switching occurs in high Gleason grade prostate cancer. *Prostate* **2006**, *66* (2), 193−199.
(27) Jaggi, M.; Johansson, S. L.; Baker, J. J.; Smith, L. M.; Galich, A.; Balaji, K. C. Aberrant expression of E-cadherin and beta-catenin in human prostate cancer. *Urol. Oncol.* **2005**, *23* (6), 402−406.
(28) De, P.; Carlson, J. H.; Wu, H.; Marcus, A.; Leyland-Jones, B.; Dey, N. Wnt-beta-catenin pathway signals metastasis-associated tumor cell phenotypes in triple negative breast cancers. *Oncotarget* **2016**, *7* (28), 43124−43149.
(29) Chesire, D. R.; Ewing, C. M.; Gage, W. R.; Isaacs, W. B. In vitro evidence for complex modes of nuclear beta-catenin signaling during prostate growth and tumorigenesis. *Oncogene* **2002**, *21* (17), 2679−2694.
(30) Hafeez, B. B.; Ganju, A.; Sikander, M.; Kashyap, V. K.; Hafeez, Z. B.; Chauhan, N.; Malik, S.; Massey, A. E.; Tripathi, M. K.; Halaweish, F. T.; et al. Ormeloxifene Suppresses Prostate Tumor Growth and Metastatic Phenotypes via Inhibition of Oncogenic β-catenin Signaling and EMT Progression. *Mol. Cancer Ther.* **2017**, *16* (10), 2267−2280.
(31) Sikander, M.; Hafeez, B. B.; Malik, S.; Alsayari, A.; Halaweish, F. T.; Yallapu, M. M.; Chauhan, S. C.; Jaggi, M. Cucurbitacin D exhibits potent anti-cancer activity in cervical cancer. *Sci. Rep.* **2016**, *6*, 36594.
(32) Zaman, M. S.; Chauhan, N.; Yallapu, M. M.; Gara, R. K.; Maher, D. M.; Kumari, S.; Sikander, M.; Khan, S.; Zafar, N.; Jaggi, M.; et al. Curcumin Nanoformulation for Cervical Cancer Treatment. *Sci. Rep.* **2016**, *6*, 20051.
(33) Heuberger, J.; Birchmeier, W. Interplay of cadherin-mediated cell adhesion and canonical Wnt signaling. *Cold Spring Harbor Perspect. Biol.* **2010**, *2* (2), a002915.
(34) Misra, N. C.; Nigam, P. K.; Gupta, R.; Agarwal, A. K.; Kamboj, V. P. Centchroman−a non-steroidal anti-cancer agent for advanced breast cancer: phase-II study. *Int. J. Cancer* **1989**, *43* (5), 781−783.
(35) Maher, D. M.; Khan, S.; Nordquist, J. L.; Ebeling, M. C.; Bauer, N. A.; Kopel, L.; Singh, M. M.; Halaweish, F.; Bell, M. C.; Jaggi, M.; Chauhan, S. C. Ormeloxifene efficiently inhibits ovarian cancer growth. *Cancer Lett.* **2015**, *356* (2), 606−612.
(36) Mishra, R.; Tiwari, A.; Bhadauria, S.; Mishra, J.; Murthy, P. K.; Murthy, P. S. Therapeutic effect of centchroman alone and in combination with glycine soya on 7,12-dimethylbenz[alpha]-anthracene-induced breast tumor in rat. *Food Chem. Toxicol.* **2010**, *48* (6), 1587−1591.
(37) Nigam, M.; Singh, N.; Ranjan, V.; Zaidi, D.; Sharma, R.; Nigam, D.; Gupta, D. K.; Sundaram, S.; Balapure, A. K. Centchroman mediated apoptosis involves cross-talk between extrinsic/intrinsic pathways and oxidative regulation. *Life Sci.* **2010**, *87* (23−26), 750−758.

https://doi.org/10.1021/acsomega.3c02277
ACS Omega 2023, 8, 38839−38848

(38) Pal, P.; Kanaujiya, J. K.; Lochab, S.; Tripathi, S. B.; Bhatt, M. L.; Singh, P. K.; Sanyal, S.; Trivedi, A. K. 2-D gel electrophoresis-based proteomic analysis reveals that ormeloxifen induces G0-G1 growth arrest and ERK-mediated apoptosis in chronic myeloid leukemia cells K562. *Proteomics* **2011**, *11* (8), 1517−1529.
(39) Singh, M. M. Centchroman, a selective estrogen receptor modulator, as a contraceptive and for the management of hormone-related clinical disorders. *Med. Res. Rev.* **2001**, *21* (4), 302−347.
(40) Lal, J. Clinical pharmacokinetics and interaction of centchroman−a mini review. *Contraception* **2010**, *81* (4), 275−280.
(41) Gara, R. K.; Sundram, V.; Chauhan, S. C.; Jaggi, M. Anti-cancer potential of a novel SERM ormeloxifene. *Curr. Med. Chem.* **2013**, *20* (33), 4177−4184.
(42) Khan, S.; Ebeling, M. C.; Chauhan, N.; Thompson, P. A.; Gara, R. K.; Ganju, A.; Yallapu, M. M.; Behrman, S. W.; Zhao, H.; Zafar, N.; et al. Ormeloxifene suppresses desmoplasia and enhances sensitivity of gemcitabine in pancreatic cancer. *Cancer Res.* **2015**, *75* (11), 2292−2304.
(43) Srivastava, V. K.; Gara, R. K.; Bhatt, M. L.; Sahu, D. P.; Mishra, D. P. Centchroman inhibits proliferation of head and neck cancer cells through the modulation of PI3K/mTOR pathway. *Biochem. Biophys. Res. Commun.* **2011**, *404* (1), 40−45.
(44) Singh, N.; Zaidi, D.; Shyam, H.; Sharma, R.; Balapure, A. K. Polyphenols sensitization potentiates susceptibility of MCF-7 and MDA MB-231 cells to Centchroman. *PLoS One* **2012**, *7* (6), No. e37736.
(45) Sikander, M.; Malik, S.; Chauhan, N.; Khan, P.; Kumari, S.; Kashyap, V. K.; Khan, S.; Ganju, A.; Halaweish, F. T.; Yallapu, M. M.; et al. Cucurbitacin D Reprograms Glucose Metabolic Network in Prostate Cancer. *Cancers* **2019**, *11* (3), 364.
(46) Sikander, M.; Malik, S.; Khan, S.; Kumari, S.; Chauhan, N.; Khan, P.; Halaweish, F. T.; Chauhan, B.; Yallapu, M. M.; Jaggi, M.; et al. Novel Mechanistic Insight into the Anticancer Activity of Cucurbitacin D against Pancreatic Cancer (Cuc D Attenuates Pancreatic Cancer). *Cells* **2019**, *9* (1), 103.
(47) Queen, A.; Khan, P.; Idrees, D.; Azam, A.; Hassan, M. I. Biological evaluation of p-toluene sulphonylhydrazone as carbonic anhydrase IX inhibitors: An approach to fight hypoxia-induced tumors. *Int. J. Biol. Macromol.* **2018**, *106*, 840−850.
(48) Morris, G. M.; Huey, R.; Lindstrom, W.; Sanner, M. F.; Belew, R. K.; Goodsell, D. S.; Olson, A. J. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *J. Comput. Chem.* **2009**, *30* (16), 2785−2791.
(49) Biovia, D. S. *Discovery Studio Modeling Environment*; San Diego: Dassault Systèmes, 2013.
(50) Ji, X. S.; Miao, Y.; Liu, Y.; Jin, T.; Song, P. Improvement for the Synthesis of Centchroman. *J. Chin. Pharmaceut. Sci.* **1998**, *7* (2), 69−71.
(51) Srivastava, A. K.; Lal, J.; Gupta, R. C.; Grover, P. K. A Facile Synthesis of a 3,4-trans-7-Hydroxy-2,2-dimethyl-3-phenyl-4-(p-pyrrolidinoethoxyphenyl)chroman. *Indian J. Chem., Sect. B: Org. Chem. Incl. Med. Chem.* **1994**, *33B*, 773.
(52) Chauhan, N.; Maher, D. M.; Hafeez, B. B.; Mandil, H.; Singh, M. M.; Yallapu, M. M.; Jaggi, M.; Chauhan, S. C. Ormeloxifene nanotherapy for cervical cancer treatment. *Int. J. Nanomed.* **2019**, *14*, 7107−7121.
(53) Chauhan, N.; Maher, D. M.; Yallapu, M. M.; B, B. H.; Singh, M. M.; Chauhan, S. C.; Jaggi, M. A triphenylethylene nonsteroidal SERM attenuates cervical cancer growth. *Sci. Rep.* **2019**, *9* (1), 10917.
(54) Shukla, S.; Shishodia, G.; Mahata, S.; Hedau, S.; Pandey, A.; Bhambhani, S.; Batra, S.; Basir, S. F.; Das, B. C.; Bharti, A. C. Aberrant expression and constitutive activation of STAT3 in cervical carcinogenesis: implications in high-risk human papillomavirus infection. *Mol. Cancer* **2010**, *9*, 282.
(55) Nakazawa, M.; Kyprianou, N. Epithelial-mesenchymal-transition regulators in prostate cancer: Androgens and beyond. *J. Steroid Biochem. Mol. Biol.* **2017**, *166*, 84−90.
(56) Chen, G.; Shukeir, N.; Potti, A.; Sircar, K.; Aprikian, A.; Goltzman, D.; Rabbani, S. A. Up-regulation of Wnt-1 and beta-catenin production in patients with advanced metastatic prostate carcinoma: potential pathogenetic and prognostic implications. *Cancer* **2004**, *101* (6), 1345−1356.
(57) Morita, N.; Uemura, H.; Tsumatani, K.; Cho, M.; Hirao, Y.; Okajima, E.; Konishi, N.; Hiasa, Y. E-cadherin and alpha-, beta- and gamma-catenin expression in prostate cancers: correlation with tumour invasion. *Br. J. Cancer* **1999**, *79* (11−12), 1879−1883.
(58) Shen, T.; Zhang, K.; Siegal, G. P.; Wei, S. Prognostic Value of E-Cadherin and β-Catenin in Triple-Negative Breast Cancer. *Am. J. Clin. Pathol.Am. J. Clin Pathol* **2016**, *146* (5), 603−610.
(59) Chen, Z.; He, X.; Jia, M.; Liu, Y.; Qu, D.; Wu, D.; Wu, P.; Ni, C.; Zhang, Z.; Ye, J.; et al. β-catenin overexpression in the nucleus predicts progress disease and unfavourable survival in colorectal cancer: a meta-analysis. *PLoS One* **2013**, *8* (5), No. e63854.
(60) Sundram, V.; Ganju, A.; Hughes, J. E.; Khan, S.; Chauhan, S. C.; Jaggi, M. Protein kinase D1 attenuates tumorigenesis in colon cancer by modulating β-catenin/T cell factor activity. *Oncotarget* **2014**, *5* (16), 6867−6884.
(61) Khan, M. I.; Adhami, V. M.; Lall, R. K.; Sechi, M.; Joshi, D. C.; Haidar, O. M.; Syed, D. N.; Siddiqui, I. A.; Chiu, S. Y.; Mukhtar, H. YB-1 expression promotes epithelial-to-mesenchymal transition in prostate cancer that is inhibited by a small molecule fisetin. *Oncotarget* **2014**, *5* (9), 2462−2474.
(62) Chaffer, C. L.; Weinberg, R. A. A perspective on cancer cell metastasis. *Science* **2011**, *331* (6024), 1559−1564.
(63) Ke, X.-P.; Liu, P.; Pei, J.-J.; Li, L.; Li, J.-W. Sildenafil Inhibits the Growth and Epithelial-to-mesenchymal Transition of Cervical Cancer via the TGF-β1/Smad2/3 Pathway. *Curr. Cancer Drug Targets* **2023**, *23* (2), 145−158.
(64) Krasnapolski, M. A.; Todaro, L. B.; Bal de Kier Joffe, E. Is the epithelial-to-mesenchymal transition clinically relevant for the cancer patient? *Curr. Pharm. Biotechnol.* **2011**, *12* (11), 1891−1899.
(65) Lamouille, S.; Xu, J.; Derynck, R. Molecular mechanisms of epithelial-mesenchymal transition. *Nat. Rev. Mol. Cell Biol.* **2014**, *15* (3), 178−196.
(66) Li, L.; Li, W. Epithelial-mesenchymal transition in human cancer: comprehensive reprogramming of metabolism, epigenetics, and differentiation. *Pharmacol. Ther. Ther* **2015**, *150*, 33−46.
(67) Nieszporek, A.; Skrzypek, K.; Adamek, G.; Majka, M. Molecular mechanisms of epithelial to mesenchymal transition in tumor metastasis. *Acta Biochim. Pol.* **2019**, *66* (4), 509−520.
(68) Imani, S.; Hosseinifard, H.; Cheng, J.; Wei, C.; Fu, J. Prognostic Value of EMT-inducing Transcription Factors (EMT-TFs) in Metastatic Breast Cancer: A Systematic Review and Meta-analysis. *Sci. Rep.* **2016**, *6*, 28587.
(69) Xiong, Y.; Sun, F.; Dong, P.; Watari, H.; Yue, J.; Yu, M. F.; Lan, C. Y.; Wang, Y.; Ma, Z. B. iASPP induces EMT and cisplatin resistance in human cervical cancer through miR-20a-FBXL5/BTG3 signaling. *J. Exp. Clin. Cancer Res.* **2017**, *36* (1), 48.
(70) Xu, F.; Zhang, J.; Hu, G.; Liu, L.; Liang, W. Hypoxia and TGF-β1 induced PLOD2 expression improve the migration and invasion of cervical cancer cells by promoting epithelial-to-mesenchymal transition (EMT) and focal adhesion formation. *Cancer Cell Int.* **2017**, *17*, 54.
(71) Graham, T. A.; Ferkey, D. M.; Mao, F.; Kimelman, D.; Xu, W. Tcf4 can specifically recognize beta-catenin using alternative conformations. *Nat. Struct. Biol.* **2001**, *8* (12), 1048−1052.

https://doi.org/10.1021/acsomega.3c02277
ACS Omega 2023, 8, 38839−38848