Bioactive Natural Products against Prostate Cancer: Mechanism of Action and Autophagic/ Apoptotic Molecular Pathways

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Abstract
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Prostate cancer is one of the leading causes of death worldwide for men. There is increasing evidence that diet and lifestyle play a crucial role in prostate cancer biology and tumorigenesis. Due to the fact that conventional chemotherapy is not adequately effective against prostate cancer and has severe side effects, numerous *in vitro* studies have been conducted in order to identify the potent cytotoxic or chemopreventive activity of naturally occurring compounds and their respective molecular mechanisms of action. In this context, many natural compounds isolated from plants have been found to inhibit cancer growth and to induce cell cycle arrest, suppress angiogenesis, and promote apoptotic or autophagic cell death. Therefore, in this article, the most promising bioactive natural products and their respective mechanisms of action for the prevention or/and treatment of prostate cancer are presented.

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Introduction
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Prostate cancer (PCa) is one of the most common malignancies in men and a leading cause of death worldwide for men. Taking into consideration that chemotherapy has severe side effects and usually a poor outcome, there is an intensive need for the development of safer and more effective agents. Since plants have been used by traditional medicine for the treatment of various diseases, in the last decades, many natural products have been isolated from plants and tested for their tumor selectivity and cytotoxic efficacy. Several of these naturally occurring compounds have been found to inhibit PCa growth and metastasis and are thus a promising approach for the treatment of this malignancy. Laboratory studies in different *in vitro* and *in vivo* systems have shown that these natural products modulate cellular processes, exhibit chemopreventive and/or chemotherapeutic effects, and induce apoptosis and autophagy. Accordingly, the antiproliferative and autophagic effects of nontoxic dietary agents could be of additional significance for the prevention, control, and management of PCa, specifically for the advanced and androgen-independent stage of the malignancy [1–3]. As there is increasing data on how natural compounds interfere with diverse molecular pathways in cancer cells, this review discusses the mechanism of action of bioactive natural products in the field of PCa and emphasizes the implicated molecular pathways of apoptosis and autophagy as important processes that control cellular homeostasis and that have been highlighted as promising targets for novel cancer therapies.

Apigenin
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Apigenin (4′,5,7-trihydroxyflavone) is a flavone found in plants of the *Asteraceae* family, such as *Anthemis* sp., and many fruits and vegetables [4]. Apigenin has been tested in various types of cancer cell lines (breast, colon, liver, lung) showing promising results [5]. In prostate cancer in particular, apigenin administered in various concentrations (1–20 μM) for 24, 48, and 72 h not only causes G1 cell cycle arrest both in androgen-dependent (LNCaP) and -independent (DU145 and PC-3) PC cell lines through the decreased expression of cyclins D1, D2, and E, but also induces apoptosis through a shift in the Bax/Bcl-2 ratio [6,7]. Further studies in PC-3 cells have also demonstrated that apigenin (5–40 μM), delivered for 24 h, suppresses cell proliferation and induces apoptosis by inhibiting IGF-IGF-IR signaling and inactivating the PI3k/Akt pathway [8]. Apigenin

treatment of PC-3M cells (25 μM for 16 h) also prevents cell motility and invasion through a disruption of the actin cytoskeleton organization and inhibition of FAK/scr signaling [9]. Apigenin is also a mediator of epigenetic events, when administered at similar concentrations (20–40 μM), as it inhibits class I HDACs both in PC-3 and 22Rv1 cells [10]. In 22Rv1 cells, induction of apoptosis is attributed to ROS generation, which subsequently triggers transcriptional, p53-dependent and -independent, pathways [11]. The antiangiogenetic potential of apigenin is also demonstrated in PC-3, LNCaP, and C4-2B cells and is attributed to a decreased production of vascular endothelial growth factor (VEGF) leading to the inhibition of cancer progression and metastasis [12]. Finally, *in vivo* studies have shown that apigenin causes growth inhibition of 22Rv1 and PC-3 tumor xenografts in athymic nude mice [13], whereas in TRAMP mice, apigenin suppresses cancer progression [14].

Artemisinin and Derivatives
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Artemisinin (3*R*,5a*S*,6*R*,8a*S*,9*R*,12*S*,12a*R*)-octahydro-3,6,9-trimethyl-3,12-epoxy-12*H*pyrano[4,3-*j*]-1,2-benzodioxepin-10 (3*H*)-one is a sesquiterpene lactone and a naturally occurring component of *Artemisia annua* (*Asteraceae*) [15]. It is a potent antimalarial compound that was shown to have antiproliferative effects on a number of human cancer cell lines. Artemisinin treatment (300 μM for 48 h) triggers G1 cell cycle arrest of LNCaP human prostate cancer cells due to the transcriptional downregulation of CDK4 expression caused by a disruption of Sp1 interactions with the CDK4 promoter [16]. Furthermore, artesunate (ART), a semisynthetic derivative of artemisinin, is found to cause G2/M cell cycle arrest in PC-3 cancer cells [17]. Other studies demonstrated that dihydroartemisinin (DHA), another derivative of artemisinin, reduces cell viability in a time- (30 μM for 24, 48, and 72 h) and dose-dependent (10–50 μM for 24 h) manner in androgen-dependent (LNCaP) and -independent (DU145 and PC-3) cells by the activation of caspases 8 and 9, suggesting that DHA is involved both in the extrinsic and intrinsic pathways of apoptosis. Finally, DHA and the artemisin dimers ON-2Py and 2Py cause a dose-dependent decrease in the proliferation of LNCaP and PC-3 cells through growth arrest [18,19].

Baicalin–Baicalein
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7-D-glucuronic acid-5,6-dihydroxyflavone is a flavone isolated from *Scutellaria baicalensis* (*Lamiaceae*) which is converted to baicalein, *in vivo* [20]. Baicalin inhibits cell proliferation of various cancer cell lines (bladder, bone, breast, colon, liver) and exerts its cytotoxic/cytostatic effect through the induction of apoptosis in DU145, PC-3, LNCaP, and CA-HPV-10 prostate cancer cell lines when administered at concentrations of 150 μM or above for 2–4 days [21]. A study in LNCaP cells revealed that baicalin increases the expression of cyclin-dependent kinase inhibitor [p27 (kip1)] and causes G1 cell cycle arrest. Similar results are found for baicalein [22]. Baicalin at doses of 50 μM and 125 μM also induces G1 arrest and apoptosis in DU145 cells through the inhibition of bcl-2, loss of Bax, and upregulation of Fas [23]. In PC-3 cells, baicalein overcomes TRAIL resistance by upregulating DR5. Finally, both baicalin and baicalein prevent angiogenesis and reduce tumor volume in xenograft models receiving different doses of baicalein (10–40 mg/kg per day) for 28 days.

Berberine
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Berberine (5,6-dihydro-9,10-dimethoxybenzo[g]-1,3-benzo-dioxolo[5,6-a]quinolizinium) is an isoquinoline alkaloid derived from the plants of the genus *Berberis* (*Berberidaceae*) [24]. Treatment of DU145, PC-3, and LNCaP cells with berberine leads to inhibition of cell proliferation combined with G1 cell cycle arrest in a dose- (10–100 μM) and time-dependent (24–72 h) manner, without affecting normal human prostate epithelial cells. The suggested molecular mechanisms refer to the inhibition of the expression of cyclins D1, D2, and E and cyclin-dependent kinase (Cdk) 2, Cdk4, and Cdk6 proteins, the increased expression of the Cdk inhibitory proteins (Cip1/p21 and Kip1/p27), and the enhanced binding of Cdk inhibitors to Cdks. Berberine induces cell death of cancer cells via modulations of the Bax/Bcl-2 ratio, disruption of the mitochondrial membrane potential, and activation of poly(ADP-ribose) polymerase and caspases [25]. Low concentrations of berberine (less than 50 μM) in RM-1 cells trigger G1 arrest associated with the activation of the p53-p21 cascade, whereas higher concentrations (over 50 μM) of berberine cause G2/M arrest. Studies in LNCaP xenografts in nude mice revealed that berberine delivered at 5 mg/kg/day inhibits tumor growth due to a reduction in AR expression [26]. Finally, berberine (at doses of 30 μM and 50 μM) enhances the radiosensitivity of human prostate cancer cells as it interferes with MAPK/caspase-3 and ROS pathways and inhibits the expression of HIF-1alpha and VEGF [27].

Betulinic Acid
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Betulinic acid [(3beta-hydroxy-20(29)-lupaene-28-oic acid] (BA) is a pentacyclic triterpene derived from the bark of *Betula papyrifera* (*Betulaceae*) [28]. BA (1–5 μM) was firstly shown to trigger apoptosis and antiangiogenic responses in LNCaP cells and then in xenograft models when delivered at doses of 10 and 20 mg/kg/day every second day for 14 days by decreasing the expression of the anti-apoptotic proteins, survivin and VEGF, caused by degradation of the transcription factors specificity proteins Sp1, Sp3, and Sp4 [29]. Treatment of PC-3 cells with BA (10 μM and 20 μM) inhibits TNFα-induced activation of NF-κB, which shifts the Bax/Bcl-2 ratio and leads to cleavage of poly(ADP)ribose polymerase and thus induces apoptosis [30]. Recently, BA was found to inhibit multiple deubiquitinases (DUBs), which results in poly-ubiquitinated protein accumulation, decreased levels of oncoproteins, and increased apoptotic cell death in LNCaP, DU145, and PC-3 cells. BA treatment (10 mg/kg for 15 days) of TRAMP mice results in inhibition of proliferation, tumor growth, and angiogenesis, and lowers the levels of the androgen receptor and cyclin D expression and the induction of apoptosis [31].

Capsaicin
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Capsaicin (8-methyl-*N*-vanillyl-6-nonenamide) is a vanilloid isolated from the plants of the genus *Capsicum* (*Solanaceae*) [32]. Capsaicin inhibits the growth of PC-3 cells, both *in vitro* (IC$_{50}$ of 20 μM) and in xenograft models (5 mg/kg), and stimulates apoptosis through reactive oxygen species generation, dissipation of the mitochondrial inner transmembrane potential, and activation of caspase 3 [33]. Further studies in PC-3 cells reveal that apoptosis is also induced by ceramide accumulation and activation

of JNK and ERK [34]. Likewise, capsaicin at varying concentrations (100–500 μM) triggers apoptosis both in androgen-dependent (LNCaP) and refractory (DU-145) prostate cancer cell lines and is associated with an increase of p53, p21, and Bax, a downregulation of both the prostate-specific antigen (PSA) and AR, and inhibition of proteasome activity [35].

Curcumin
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Curcumin (diferulolylmethane), a diphenylheptanoid isolated from *Curcuma longa* (turmeric; *Zingiberaceae*) [36], was firstly described to induce apoptosis at doses of 5–50 μM in both androgen-dependent and refractory prostate cancers by interfering with the EGF-R signaling pathway [37]. Apoptosis is also prompted by curcumin's interference with Bcl proteins, ROS generation, and the activation of mitochondrial related pathways. In PC-3 cells, the induction of apoptosis is attributed to apoptosis-inducing factor (AIF) and caspase-independent mechanisms [38]. Further studies revealed that curcumin decreases the proliferation of prostate cancer cells through the downregulation of the androgen receptor, whereas the activation of caspase-dependent apoptosis is a result of the downregulation of AP-1, NF-κB, cAMP response element-binding protein (CREB), PSA, and cyclin D [39]. In addition, prostate cancer cells are accumulated in the G1 phase by the proteasome-mediated downregulation of cyclin E and the upregulation of CDKs. In early-stage prostate cancer, curcumin acts as a chemopreventive agent affecting Wnt/β-catenin pathways, leading to autophagy [40]. Furthermore, curcumin suppresses glyoxalases, and thus modulates metabolic cellular pathways and acts as a histone acetyltransferase inhibitor [41].

Other studies have shown that curcumin prevents PC angiogenesis and metastasis by interfering with the cell cytoskeleton organization and the VEGF expression, respectively. Studies in DU145 xenografts, when curcumin is administered at doses of 5 mg/kg thrice a week for four weeks, show that invasion and metastasis suppression by curcumin can also be attributed to a reduction in metalloproteinases expressed by cancer cells [42].

Inhibition of PCa growth combined with a reduction in the metastasis rate caused by curcumin was also found in the first *in vivo* model tested, LNCaP xenograft in nude mice [43]. Interestingly, several studies have been conducted in order to enhance curcumin delivery to cancer cells through liposomal distribution, curcumin-loaded nanospheres, cellulose nanoparticles, and co-loaded lipid-based carriers with promising results [44].

Daidzein
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Daidzein (4′,7-dihydroxyisoflavone) is an isoflavone (steroid glucoside) isolated from soy beans (*Glycine max*) (*Fabaceae*) [45] and was firstly found to inhibit LNCaP and PC-3 cell growth. Even though daidzein does not influence the cell cycle of LNCaP and PC-3 cells, it decreases the expression of VEGF and AR genes in both cell lines and elevates the apoptosis percentage of LNCaP cells via the Akt pathway. Daizein has also been found to cause modulations of the cyclin-dependent kinase-related pathway genes and a downregulation of EGF and IGF in LNCaP, PC-3, and DU145 cells [46]. Recent studies show that daizein (110 μM for 48 h) can cause epigenetic modifications to DNA, such as the promoter CpG island demethylation of tumor suppressor genes, thus demonstrating a chemopreventive role [47].

Delphinidin
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Delphinidin [2-(3,4,5-trihydroxyphenyl)chromenylium-3,5,7-triol] is an anthocyanidin (coumaroyl glucoside) mostly isolated from *Viola sp.* (*Violaceae*) and *Delphinium sp.* (*Ranunculaceae*) and from many pigmented fruits and vegetables [48]. Delphinidin has been shown to induce a dose-dependent (30–180 μM) inhibition of cell growth and apoptosis in LNCaP, C4-2, 22Rv1, and PC-3 cells via the inhibition of NFκB signaling and the subsequent activation of caspases. Other studies propose that delphinidin induces cell growth inhibition and apoptosis of human PC-3 cells by inhibition of Notch-1 and/or NF-κB/PI3K pathways and Wnt/β-catenin signaling [49]. In PC-3 xenografts in athymic nude mice, delphinidin administration (2 mg/animal thrice a week) resulted in a significant inhibition of tumor growth [50].

Ellagic Acid
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Ellagic acid (2,3,7,8-tetrahydroxy-chromeno[5,4,3-cde]chromene-5,10-dione) (EA) is a polyphenolic compound [51] found in various fruits such as blackberries (*Rubus* sp., *Rosaceae*), cranberries (*Vaccinium sp., Ericaceae*), pecans, pomegranates, raspberries and strawberries. Studies in LNCaP cells show that EA can cause DNA damage while it downregulates antiapoptotic proteins, such as silent information regulator 1 (SIRT1), upregulates the tumor suppressor protein p21, and modulates the expression of AIF thus resulting in ROS-mediated and caspase-mediated apoptosis [52]. Recent studies in LNCaP cells also depict the antiangiogenetic effects of EA (at concentrations of 25 and 50 μM) as it decreases the eicosanoid biosynthesis levels and suppresses the HO system. In androgen-independent PC cells, DU145 and PC-3, EA is found to induce cell cycle arrest in the S phase and apoptosis in a dose- (15–60 μmol/L) and time-dependent (24–120 h) manner, which is associated with a decrease in cyclin B1 and cyclin D1 levels and caspase-dependent pathways. Finally, EA is shown to confine the invasive potential of PC-3 and rat PC cell lines by interfering with protease activity and decreasing the secretion of matrix metalloproteinase MMP-2 [53].

Epigallocatechin-3-Gallate
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Epigallocatechin-3-gallate [(2R,3R)-5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)chroman-3-yl)3,4,5-trihydroxybenzoate] (EGCG) is a catechin derived mainly from tea (*Camellia sinensis, Theaceae*) [54]. Studies in LNCaP and DU145 cells have shown that EGCG causes G0/G1 cell cycle arrest and induces apoptosis in a cell-type-specific manner, irrespective of the p53 status of the cells [55]. EGCG treatment upregulates the expression of WAF1/p21, KIP1/p27, INK4a/p16, and INK4c/p18 and down-modulates the expression of cyclin D1, cyclin E, cdk2, cdk4, and cdk6 proteins while it increases the binding of cyclin D1 toward WAF1/p21 and KIP1/p27 and decreases the binding of cyclin E toward cdk2 [56]. In particular, EGCG-induced apoptosis in LNCaP cells is mediated through the modulation of p53 and NF-κB expression, subsequent change in the Bax/Bcl-2 ratio, and activation of capsases 3, 8, and 9 followed by poly (ADP-ribose) polymerase cleavage when administered in 20–80 μM for 24, 48, and 72 h [57]. Other molecular mechanisms involved in the induction of apoptosis refer to the inhibition of COX-2 without affecting COX-1 expression, both in LNCaP and PC-3 cells, and ERK1/2 activation

via an MEK-independent, PI3-K-dependent signaling pathway in PC-3 cells [58, 59]. In addition, EGCG (10–60 μM) antagonizes androgen action at multiple levels, as it suppresses the activation of the agonist-dependent androgen receptor through Sp1-protein and AR-regulated gene transcription, thus resulting in the inhibition of PCa growth. Invasion and migration are also inhibited after EGCG treatment via modulations in VEGF, uPA, angiopoietin 1 and 2, MMP-2, and MMP-9 [60].

Administration of 0.06% EGCG in TRAMP mice demonstrated that EGCG leads to attenuation of AR and the IGF-1 expression and decreases the MAPK signaling, thus inducing apoptosis without toxicity. Combinational treatment with 1 μM EGCG and cisplatin (2.5 or 5 μM) promotes the expression of the proapoptotic splice isoform of caspase 9 in PC-3 cells. Furthermore, oral administration of encapsulated EGCG reduces cell viability and induces apoptosis of DU145 PC cells. Finally, chitosan nanoparticles encapsulating epigallocatechin-3-gallate cause tumor growth inhibition and a reduction of secreted PSA levels [61, 62].

Fisetin

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Fisetin (3,3′,4′,7-tetrahydroxyflavone) is a flavonol derived from many plants, such as *Acacia greggii* (*Fabaceae*) [63], which has been shown to have cytotoxic and cytostatic effects in numerous cancer cell lines (breast, blood, liver, lung, melanoma, ovary, pancreas) [5]. Studies in prostate LNCaP cells revealed that fisetin, when administered at 10–60 μM for 24 and 48 h, causes G1 cycle arrest by downregulating cyclins and cyclin-dependent kinases and triggers both caspase-dependent and -independent apoptotic pathways. Fisetin also decreases AR levels and competes with the AR ligand [64]. In highly metastatic PC-3 cells, fisetin inhibits adhesion, migration, and metastasis by interfering with the NF-κB pathway and by downregulating MMP-2 and MMP-9 [65]. The downregulation of NF-κB is accompanied with an increased TRAIL-induced apoptosis in LNCaP, DU145, and PC-3 cells [66]. It is noteworthy that fisetin also induces autophagic cell death through the inhibition of mTOR and PI3K/Akt signaling [67]. In a CWR22Rupsilon1 human xenograft model, a fisetin injection (1 mg/animal) twice weekly was found to suppress tumor growth and reduce PSA levels [64].

Formononetin

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Formononetin (7-hydroxy-4′-methoxyisoflavone) (FN) is an O-methylated isoflavone acting as a phytoestrogen, which is found in red clover plants (*Trifolium pretense*, *Fabaceae*) [45]. FN has been shown to provoke apoptosis in LNCaP and PC-3 cells through the ERK1/2 MAPK-Bax pathway [68]. Further studies in PC-3 cells show that FN-induced apoptosis is associated with the inhibition of the IGF-1/IGF-1R pathway, alterations in the Bax/Bcl-2 ratio, and modulations of the p38/Akt pathway when delivered at 25, 50, and 100 μM for 48 h [69, 70]. FN triggers apoptosis in DU145 cells as well through the activation of the mitochondrial apoptotic pathway, which follows the upregulation of RASD1 [71].

Gallic Acid

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Gallic acid (3,4,5-trihydroxybenzoic acid) (GA) is a polyphenolic constituent of grape (*Vitis vinifera*, *Vitaceae*) seed extract [72]. GA prompts a dose- (10–50 μmol/L) and time-dependent (6–24 h) growth inhibition and G2/M cell cycle arrest in DU145 cells through DNA damage and an increase of cdc25A/C-cdc2 phosphorylation. GA also results in apoptotic death of PCa cells by triggering the cleavage of caspase-9, caspase-3, and poly (ADP-ribose) polymerase (PARP) as well as by inducing ROS and mitochondria-mediated mechanisms [73]. GA has been shown to cause DNA damage and inhibit the invasion and migration of PC-3 cells in a dose- (50–200 μM) and time-dependent (12–48 h) manner by blocking the p38, JNK, PKC, and PI3K/AKT signaling pathways while it reduces the levels of the NF-κB protein, resulting in the repression of MMP-2 and −9 [74]. In addition, GA exerts a synergistic effect with doxorubicin in suppressing the growth of DU145 cells [73].

It is noteworthy that oral feeding with drinking water supplemented with 0.3% and 1% (w/v) GA until 24 weeks of age inhibits PCa growth and progression to advanced-stage adenocarcinoma in TRAMP mice by decreasing the expression levels of Cdk2, Cdk4, Cdk6, cyclin B1, and E proteins [75]. In DU145 and 22Rv1 PC xenografts in nude mice being fed water with 0.3%, 1% (w/v) of GA for 5 days/week for six weeks, GA was shown to suppress tumor cell proliferation, reduce the microvessel density of tumor xenografts, and induce apoptosis [76].

Gambogic Acid

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Gambogic acid is a xanthone isolated from *Carcinia hanburyi* (*Clusiaceae*) [77] that suppresses the viability of PC-3 cells at doses of 1–5 μmol/L, and downregulates TNF-α-induced invasion of PC-3 cells at doses of 0.125–0.5 μmol/L via inactivation of the PI3K/Akt and NF-κB signaling pathways [78]. Gambogic acid, when injected in a xenograft prostate tumor model at 3 mg/kg, was shown to suppress tumor growth and angiogenesis by inhibiting the activation of vascular endothelial growth factor receptor 2 (VEGF-2R) and its downstream protein kinases, such as c-Src and AKT [79].

Genistein

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Genistein (4′,5,7-trihydroxyisoflavone) is a flavanone isolated from *Glycine max* (*Fabaceae*) [80]. Genistein acts as a tyrosine protein kinase inhibitor, thus causing a dose-dependent growth inhibition of DU145, PC-3, and LNCaP PCa cell lines via the suppression of protein phosphorylation [81]. Another study conducted in LNCaP and PC-3 cells concluded that genistein-mediated growth inhibition is caused by the downregulation of survivin, DNA topoisomerase II, cell division cycle 6 (CDC6), and mitogen-activated protein kinase 6, and the augmented regulation of glutathione peroxidase. In PC-3 cells, suppression of cell growth is also attributed to the downregulation of the IGF-1/IGF-1R signaling pathway [82, 83].

Recent studies have shown that genistein exerts its apoptotic and antiproliferative effects by regulating microRNAs. Thus, genistein is found to cause apoptosis through the downregulation of miR-1260b and its target genes sRRP1 and Smad4 [84]. Furthermore,

studies in PC-3 and DU145 cells show that genistein inhibits cell growth by modulating miR-34a and HOTAIR expression [85]. Apoptosis is also attributed to various mechanisms such as inhibition of proteasomal chymotrypsin-like activity, inactivation of NF-κB, and inhibition of Akt [86]. Genistein in high doses has inhibitory effects due to modulating the expression of the AR function, but its growth inhibitory effect is independent of PSA expression [87]. However, genistein at physiological concentrations (0.5–5 μM) activates mutant types of AR present in advanced PC [88]. Moreover, numerous genes involved in cell adhesion and migration (MMP-9, protease M, uPAR, VEGF) are downregulated in PC-3 cells after genistein treatment [89]. A recent study showed that genistein also targets cancer stem cells (CSC) and can contribute to an anti-CSC effect, which is important for inhibiting PC relapse and metastasis [90]. Epigenetics effects of genistein administrated at 40 μM are also found in DU-145 and PC-3 cells, as it reverses DNA hypermethylation of tumor suppression genes leading to their activation and subsequent inhibition of cancer progression [91].

In vivo studies reveal a chemopreventive activity of genistein. Lobund-Wistar (L–W) rats that are susceptible to spontaneous and induced metastasizing adenocarcinomas in the prostate-seminal vesicle complex were found to exert a reduced incidence of induced prostate-related cancer after genistein feeding [92]. TRAMP mice fed with a phytoestrogen-rich diet containing 100, 250, or 500 mg of genistein per kg showed a low percentage of PD (poorly differentiating) developed cancer [93].

Oral administration of genistein in PCa patients does not affect PSA levels, yet a more recent study showed that 30 mg of synthetic genistein, given daily for three to six weeks, reduces serum PSA levels [94]. In addition, combinational treatment of metastatic-castration-resistant PCa with Cabazitaxel and genistein was found to have an enhanced apoptotic effect [95]. Clinical use of genistein against cancer is limited by its extremely low aqueous solubility, poor bioavailability, and pharmacokinetics. Based on structural analogy with steroidal compounds, liposomal vehicle compositions are designed and optimized for maximum incorporation of genistein's flavonoid structure. The pharmaceutical design of genistein-loaded liposomes seems to improve cellular delivery and specific proapoptotic effectiveness of the incorporated drug against various cancers [96]. Finally, a meta-analysis of studies that investigated soy food consumption and risk of PCa was reported. The results of this meta-analysis suggested that high consumption of non-fermented soy foods (e.g., tofu and soybean milk) might significantly decrease the risk of PC [97].

Ginsenosides
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Ginsenosides are compounds isolated exclusively from the plants of the genus *Panax* (*Araliaceae*). Ginsenoside Rg3 is one of the bioactive components found in ginseng root extract [98]. Rg3 has been found to arrest LNCaP cells at the G1 phase and subsequently induce a caspase3-mediated apoptosis mechanism by activating the expression of cyclin-kinase inhibitors, p21 and p27. Rg3 with an IC_{50} of 8.4 μM was also shown to modulate the expression of MAP kinases, inducing cell detachment of LNCaP and PC-3 cells [99]. Further studies suggest that Rg3 interferes with the p38 MAPK pathway, causing a downregulation of AQP1 expression (water channel protein, involved in cell migration), which leads to the inhibition of cell migration and metastasis of PC-3M cells [100]. Combinational treatment of Rg3 with various

chemotherapeutics (docetaxel, cisplatin, and doxorubicin) exhibits a more effective inhibition of PC cell growth in LNCaP, DU145, and PC-3 cells through the suppressed activation of NF-κB [101]. Rh2 ginsenoside (β-D-glucopyranoside) is a glycoside isolated from the root of *Panax ginseng* [102], which is also found to have antiproliferative effects and cause cell detachment, with an IC_{50} of 5.5 μM in LNCaP and PC-3 cells, through modulations in MAP kinase expression [99]. Rh2 (0.5–40 μM) and paclitaxel act synergistically and cause a significant decrease of LNCaP cell proliferation and LNCaP tumor growth [103]. Oral administration of Rh2 at a dose of 120 mg/kg in a PC-3 human xenograft model in nude mice was found to decrease tumor cell proliferation, significantly delay the tumor growth, and eventually increase the rate of apoptosis [104].

Hydroxyprotopanaxadiol (25-OH-PPD) and 20(S)-25-methoxyl-dammarane-3β,12β,20-triol (25-OCH3-PPD) were isolated from *Panax ginseng* as well [105]. Both were found to decrease the expression levels of proteins associated with cell proliferation (MDM2, E2F1, cyclin D1, and Cdk 2 and Cdk 4), thus causing cell cycle arrest in the G1 phase and in LNCaP and PC-3 cells. Furthermore, both compounds engender the apoptosis of LNCaP and PC-3 cells through the activation of proapoptotic proteins (p21, p27, Bax, cleaved PARP, cleaved caspases). Finally, 25-OH-PPD inhibits tumor growth in PC-3 xenograft tumors in a dose-dependent manner, while 25-OCH3-PPD represses tumor growth in PC-3 and LNCaP xenograft tumors [106].

Glycyrrhiza Compounds
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The hexane/ethanol extract of *Glycyrrhiza uralensis* (*Fabaceae*) (HEGU), comprising the two active compounds isoangustone A and licoricidin, has been shown to exert anticarcinogenic effects [107].

HEGU and its active flavonoid compound isoangustone A (5,7,3′,4′-tetrahydroxy-6,5′-diprenylisoflavone) were found to induce apoptosis of androgen-insensitive DU145 cells by augmenting the levels of cleaved caspase-9, caspase-7, caspase-3, and poly (ADP-ribose) polymerase (PARP) in combination with mitochondrial membrane depolarization and cytochrome C release to the cytosol [107]. Additionally, HEGU and its active component, isoangustone A, diminish DNA synthesis in a dose-dependent manner, reduce the levels of CDK2, CDK4, cyclin A, and cyclin D1 proteins and decrease the CDK2 activity causing G1 phase arrest in DU145 cells [108]. HEGU also contains licoricidin, which has been shown to act as a potent antimetastatic agent. Licoricidin inhibits the metastatic and invasive capacity of malignant PCa cells by suppressing the expression of adhesion molecules and restricting the secretion and activation of the matrix metalloproteinases (MMP-2, MMP-9), TIMP-1, urokinase-type plasminogen activator, and VEGF [109].

Licochalcone (LA) (3-dimethylallyl-4,4′-dihydroxy-6-methoxy-chalcone) is an estrogenic flavonoid isolated from licorice root (*Glycyrrhiza glabra*) [110]. LA is found to cause G2/M cell cycle arrest of PC-3 prostate cells, accompanied with the suppression of cyclin B1 and cdc2 [111]. LA can also induce caspase-dependent and autophagy-related cell death in LNCaP cells [112].

Glycyrrhetinic acid (18β-glycyrrhetinic acid) is an active triterpenoid metabolite abundantly present in licorice roots, which inhibits proliferation and growth of DU-145 cells (10–500 μM) by the induction of apoptosis. It also reduces HUVEC tube formation and prevents the invasion of DU-145 PC cells on matrigel-coated

wells via the downregulation of NF–κB (p65), VEGF, and MMP-9 expression [113]. In LNCaP androgen-dependent PC cells, glycyrrhetinic acid was shown to reduce the proliferation rate as well as the production of prostate-specific antigen [114].

Gossypol

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Gossypol [2,2-bis-(formyl-1,6,7-trihydroxy-5-isopropyl-3-methylnaphthalene] is a polyphenolic aldehyde present in cottonseed (*Gossipium hirsutum*, *Malvaceae*) [115], which has been shown to exert antiproliferative and cytotoxic effects in PC cell lines and implanted MAT-LyLu cells in Copenhagen rats. In MAT-LyLu cells, gossypol modulates TGFβ1 and Akt signaling, altering the expression of regulatory proteins such as cyclin D1, Cdk4, and phospho-Rb and finally causing G0/G1 cell cycle arrest when delivered at 0.5–4.0 μM for 24, 48, and 72 h [116]. Gossypol has also been found to induce G0/G1 cell cycle arrest in PC-3 cells and prostatic cells from human benign prostatic hyperplasia (BPH) patients as it evokes alterations in TGF-beta 1 expression levels. In addition, gossypol at doses of 5–20 μM downregulates Bcl-xL resulting in the inhibition of the heterodimerization of Bcl-xL/Bcl-2 with proapoptosis molecules, which is followed by caspase-dependent and -independent apoptotic processes [117].

Recently, gossypol was shown to induce autophagy in androgen-independent PCa cells that have high levels of Bcl-2 and are resistant to apoptosis, both *in vitro* and *in vivo* (PC xenografts), by interrupting the interactions between Beclin1 and Bcl-2/Bcl-xL at the endoplasmic reticulum, thus releasing the BH3-only pro-autophagic protein Beclin1, which in turn triggers the autophagic cascade [118].

Gossypol also inhibits metastatic behaviors (adhesion, migration, and invasion) and angiogenesis. In PC-3 cells, GP suppresses AP-1 and NF-κB activity, resulting in the inhibited secretion of the urokinase plasminogen activator and VEGF in combination with the downregulation of chemokine receptor 4 [119]. In human prostate tumor PC-3 xenografts in mice, gossypol at a dosage of 15 mg/kg/day prompts the suppression of angiogenesis in the solid tumors as it blocks the activation of VEGF receptor 2 kinase causing the subsequent suppression of phosphorylation of focal adhesion kinase, extracellular signal-related kinase, AKT kinase, and key intracellular proangiogenic kinases such as Src family kinase [120].

Combinational treatment of docetaxel and gossypol was found to be cytotoxic and apoptotic in PC-3 cells in a dose- and time-dependent manner [121]. Gossypol (0.5–10 μM) and sorafenib (2–20 μM) were found to induce cell death via apoptotic pathways in DU-145 cells and via autophagic pathways in PC-3 cells, respectively [122]. Finally, administration of AT-101 (gossypol), at 20 mg/day for 21 days, was found to decline PSA levels in some men with chemotherapy-naïve, castrate-resistant PCa [123].

Luteolin

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Luteolin (3′,4′,5,7-tetrahydroxyflavone) is a flavone isolated from *Terminalia chebula* (Combretaceae) [124]. Luteolin induces apoptosis in various cancer cell lines (bladder, blood, bone, breast, colon, liver, lung) [5]. Induction of apoptosis in prostate DU145 cells is attributed to the up-regulation of death receptor 5 when administered at concentrations of 5–40 μM for 24 h [125]. It also suppresses cell growth and proliferation of DU145, PC-3 cells, and

PC-3 *in vivo* models by inhibiting insulin like growth factor 1 (IGF-1) and the subsequent activation of IGF-1R, AKT, EGFR, and MAPK/ERK signaling [126]. In PC-3 cells, luteolin at doses of 1–100 μM acts as a ligand for the nuclear type II [(3)H] estradiol binding site resulting in epigenetic changes in various genes (CCNA2 CCNE2, CDC25A, etc.) involved in the cell cycle [127]. Luteolin (10–40 μM) also suppresses angiogenesis and invasion through the downregulation of VEGF-2R in PC-3 cells *in vitro* and *in vivo* and through the downregulation of AR and PSA expression in LNCaP cells, respectively [128].

Lycopene

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Lycopene (ψ,ψ-carotene) is a carotenoid mostly isolated from *Solanum lycopersicum* (tomato; *Solanaceae*) [129]. Extensive research has been conducted both *in vitro* and in clinical trials in order to identify the mechanisms of lycopene's cytotoxic and chemopreventive effects against PCa. More specifically, lycopene is found to induce cell cycle arrest and apoptosis in PC-3, LNCaP, DU145 cells, and DU145 xenografts. In LNCaP cells, lycopene induces mitochondrial-related apoptosis when delivered in physiologic concentrations (0.3–3.0 μM), whereas in high concentrations (> 5 μM), it leads to DNA damage [130]. A lycopene-mediated reduction in cholesterol synthesis was also shown through the activation of the PPARγ-LXRα-ABCA1 pathway both in LNCaP and DU145 cells [131]. In PC-3 cells and xenograft models, high concentrations of lycopene (16 mg/kg twice a week for seven weeks) induced apoptosis through alterations in IGF-I, IGF-IR, and IGFBP-3 expression levels [132]. Both in LNCaP and PC-3 cells, G0/G1 cell cycle arrest is caused by lycopene via its interference with phosphatidylinositol 3-kinase signaling, which leads to a decrease in Cdk4, cyclins D1 and E, and Rb phosphorylation. Cell cycle arrest and apoptosis are also attributed to a reduced activation of NF-κB in combination with an increased expression of p21, p27, and p53, shifting the Bax : Bcl-2 ratio. Migration and invasion of LNCaP and PC-3 cells are also suppressed by lycopene via a reduction in the expression levels of integrins [133, 134].

Moroever, lycopene acts also as a chemopreventive agent, delaying or preventing the establishment of PCa. In LNCaP cells, lycopene exerts its chemopreventive effect through an increase in detoxification proteins and subsequent prevention of DNA damage, and suppresses ROS generation and oxidative stress as well [135]. Chemopreventive activity of lycopene was also found in TRAMP mice fed 28 mg lycopene per kg for 20 weeks [136].

In clinical trials, lycopene was found to be more of a chemopreventive agent than a cytostatic agent of established tumors. Lycopene given to patients at a dose of 4 mg twice a day for one year was shown to delay or prevent high-grade prostate intraepithelial neoplasia from developing into PC [137], whereas whole tomato lycopene administration in men with established PCa at a dose of 10 mg per day for one year resulted in a reduced PSA velocity [138]. Finally, recent epidemiologic studies have suggested a potential benefit of lycopene against the risk of PCa. Five studies support a 30 to 40% reduction in risk associated with high tomato or lycopene consumption, three are consistent with a 30% reduction in risk, but the results were not statistically significant, and seven were not supportive of an association [139].

Compounds Derived from *Magnolia* sp.
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Honokiol (2-(4-hydroxy-3-prop-2-enyl-phenyl)-4-prop-2-enyl-phenol), a lignan isolated from *Magnolia officinalis* (*Magnoliaceae*) [140], has been found to decrease the viability of PC-3 and LNCaP human PCa cells in a dose- and time-dependent manner through G0–G1 phase cell cycle arrest. Honokiol also triggers apoptotic DNA fragmentation in a dose- (20–60 µM) and time-dependent (24–72 h) manner both in androgen-dependent and -independent prostate cell lines (PC-3, LNCaP, and C4-2), which is correlated with the induction of Bax, Bak, and Bad in addition to a decrease in Bcl-xL and Mcl-1 protein levels [141, 142]. Likewise, honokiol treatment exhibits growth inhibitory, apoptotic, and antiangiogenic effects on PC xenografts fed with 1–3 mg honokiol thrice a week [141].

Magnolol (4-allyl-2-(5-allyl-2-hydroxy-phenyl)phenol) is a hydroxylated biphenyl (lignan) isolated from the root and stem bark of *Magnolia officinalis* [140]. Magnolol was shown to induce apoptotic cell death in a dose-dependent (10–60 µM) manner in PC-3 cancer cells through epidermal growth factor receptor (EGFR)-mediated signaling transduction pathways and also inhibits the adhesion, invasion, and migration of PC-3 human prostate [143].

Obovatol (5-prop-2-enyl-3-(4-prop-2-enylphenoxy)benzene-1,2-diol), a biphenyl ether lignan isolated from *Magnolia obovata* [144], engages LNCaP and PC-3 cells to apoptotic cell death through the inhibition of NF-κ B activity and also enhances the cell growth inhibition of chemotherapeutics (docetaxel, paclitaxel, cisplatin, and doxorubicin) [145].

Oridonin
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Oridonin (7a,20-epoxy-1a,6b,7,14-tetrahydroxy-Kaur-16-en-15-one) is an isoprenoid (kaur-type diterpenoid) isolated from *Rabdosia rubescens* (*Labiatae*) [146]. Oridonin has been found to elicit G0/G1 cell cycle arrest and apoptosis of LNCaP cells through the upregulation of p53 and Bax and the downregulation of Bcl-2 expression in a dose-dependent manner [147]. Oridonin has also been shown to trigger G2/M cell cycle arrest, autophagy, and apoptosis in LNCaP and PC-3 cells by upregulating the expression of p21 in a time- (12–72 h) and dose-dependent (10, 25–100 µM) manner [148].

Phenethyl-Isothiocyanat
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Phenethyl-isothiocyanat [(2-isothiocyanato-ethyl)benzene] (PEITC) is one of the most extensively studied isothiocyanates (ITCs) that is found in cruciferous vegetables such as broccoli (*Brassica oleracea*) and watercress (*Nasturtium officinale*) of the *Brassicaceae* family, brussels sprouts, cabbage, Japanese radish, and cauliflower [149].

In DU145 cells, PEITC (1–20 µM) suppresses cell proliferation and causes cell cycle arrest in the G2/M phase and apoptosis in a dose-dependent manner. The mechanism of PEITC action suggests that it increases p53 expression while it reduces CDC25C, inhibits the JAK-STAT3 signal cascade, and modulates the activation of the caspases pathway [150].

PEITC (5–20 µM) has also been described to induce G2-M cell phase arrest and inhibit the expression of α- and β-tubulin proteins in LNCaP, DU145, PC-3, and C4-2B cells through reactive oxygen species generation and protein degradation [151]. Other studies show that PEITC represses the androgen receptor's expression through the inhibition of Sp1 transcription, thus mediating growth arrest both in androgen-dependent and -independent PC cells [152].

In LNCaP and PC-3 cells, PEITC (2.5 and 5 µM) triggers apoptotic mechanisms via the activation of Bax and ROS production, whereas it downregulates survivin and X-linked inhibitors of apoptosis [153]. It is noteworthy that PEITC induces both apoptotic and autophagic cell death in PC-3 and LNCaP cells regulated by Atg5 protein [154].

Furthermore, PEITC (2.5 and 5 µM) was shown to restrain migration of PC-3 and LNCaP cells. The suggested mechanisms propose that PEITC treatment leads to inactivation of Akt with a subsequent suppression of VEGF and interference with the Notch pathway [155].

PEITC was found to cease angiogenesis both in human umbilical vein endothelial cells (HUVEC) and in *ex vivo* experiments (chicken egg chorioallantoic membrane assay) [156]. In xenograft models, similar results were observed. When administered orally at a dose of 12 µM/day for five days per week, PEITC delays growth of PC-3 xenografts in athymic mice [157]. In an LNCaP xenograft model, PEITC regulates tumor growth by suspending the expression of the platelet/endothelial cell adhesion molecule (PECAM1-CD31) and by the suppression of angiogenesis [158]. Studies in transgenic adenocarcinoma of the mouse prostate, in mice fed with 3 µmol PEITC/g for 19 weeks, also revealed inhibition of prostate carcinogenesis induced by the overexpression of E-cadherin and autophagy-regulated pathways [159].

Finally, the combination treatment of PEITC (2 µM) with docetaxel (1 nM) increased the rate of apoptosis in PC-3 and DU145 cells by the suppression of Bcl2 and the induction of Bax and Bak proteins, while combinational treatment of PEITC with adriamycin and etoposide led to PC-3 cell death through the downregulation of protein kinase C and inhibition of telomerase [160].

Quercetin
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Quercetin (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-chromen-4-one) is a polyphenol (flavonol) isolated from grapes (*V. vinifera*, *Vitaceae*) [63]. Quercetin was found to reduce cell growth and cause apoptosis in various cell lines (bladder, blood, bone, breast, colon, liver, lung, mouth, esophagus) [5]. Quercetin reduces cell growth of PC-3, LNCaP, and DU145 PC cells in a dose-dependent manner by interfering with the expression levels of numerous oncogenes and tumor suppressor genes. In LNCaP, quercetin causes G2/M cycle arrest due to p21 upregulation and cyclin B suppression [161]. Studies in PC-3 cells suggest that growth inhibition is caused by a decreased phosphorylation of ErbB-2, ErbB-3, c-Raf, MAPK kinase 1/2 (MEK1/2), and MAPK, Akt-1 and is combined with a reduced metastatic rate and drug resistance. In addition, quercetin has been described as interfering with c-Jun and SP1, causing AR reduction [162].

Quercetin at doses of 5–100 µM was also shown to provoke apoptosis in PCa cell lines through inhibition of fatty acid synthase and downregulation of heat shock protein 90 [163]. More studies showed that quercetin induces G2/M cycle arrest and apoptosis of PC-3 cells via a decrease in Cdc2/Cdk-1, cyclin B1, phosphorylated pRb, IGF-I, and IGF-II and an increase in p21, Bax, and caspase-3, and modulations of the Bcl-2/Bax ratio [164]. In addition, quercetin augments TRAIL-induced cytotoxicity through caspase

activation, inhibition of surviving, and Akt phosphorylation [165]. Moreover, *in vitro* and *in vivo* studies in prostate xenograft mouse models depict quercetin's antiangiogenetic effects as it interacts with the VEGF-R2-regulated autophagic (AKT/mTOR/P70S6K) pathway when administered at a dose of 20 mg/kg/day [166].

Sanguinarine
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Sanguinarine (13-methyl-[1,3]benzodioxolo[5,6-c]-1,3-dioxolo [4,5-i]phenanthridinium) is a benzophenanthridine alkaloid derived from *Sanguinaria canadensis* (*Papaveraceae*) (the bloodroot plant) [167]. In LNCaP and DU145, sanguinarine causes G0/G1 cell cycle arrest in a dose-dependent manner (0.1–2 µM) by interfering with the expression of cyclin kinase inhibitors p21/WAF1 and p27/KIP1, cyclin E, D1, and D2 and cyclin-dependent kinases 2, 4, and 6 [168].

Sanguinarine has also been shown to confine PCa cells growth and induce apoptosis at concentrations of 0.1–8 µM. This has been attributed to the suppressed expression of survivin and protein degradation via the ubiquitin-proteasome system [169]. Treatment of DU145, C4-2B, and LNCaP cells with sanguinarine (2 µM and 4 µM, for 1–12 h) revealed that it restricts PCa growth, migration, and invasion through Stat3 inactivation [170]. In DU145 cell xenografts, the administration of sanguinarine (0.25 mg/kg and 0.5 mg/kg) reduced tumor weight and volume after 31 days [169].

Silibinin
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Silibinin or silybin (3,5,7-trihydroxy-2-(3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-1,4-benzodioxan-6-yl)-4-chromanone) is a flavolignan isolated from the fruits of *Silybum marianum* (*Asteraceae*) [171]. Silibinin has been described as causing G1 cell cycle arrest and decreasing both intracellular and secreted forms of PSA in LNCaP cells in a dose- (50–200 µM) and time-dependent (12–48 h) manner, which has been attributed to modulations of retinoblastoma (Rb) levels and its phosphorylation status combined with a decreased activity of cyclin-dependent kinases (CDKs) [172]. Further studies in LNCaP cells revealed that the decrease of PSA was caused by the downregulation of the androgen receptor's coactivator and the epithelium-derived Ets transcription factor (PDEF) [173]. Silibinin was not only shown to suppress global protein translation, thus inhibiting HIF-1 alpha expression and telomerase activity [174], but also, as a lipophilic compound, was found to compete in the EGF-erbB1 interaction and to interfere with the mitogenic signaling and DNA synthesis in LNCaP and DU145 cells [175].

In DU145 cells, silibinin treatment (50–200 µM, for 24 and 48 h) caused G1 cell cycle arrest mediated by a decrease in p21 and p27 expression [176]. Silibinin also restrains Wnt/LRp6 signaling and induces apoptosis through the inhibition of active Stat3 while it sensitizes cells to TNFα-induced apoptosis through constitutive NF-κB inactivation [177].

Silibinin at pharmacologically achievable concentrations (0.02–20 µM) causes G1 and G2/M cycle arrest in PC-3 cells by interfering with the expression levels of cyclins and CDKs [178] and the insulin-like growth factor I receptor-mediated signaling pathway [179].

Silibinin has also been found to prevent migratory and invasive potential of PC-3, PC-3MM2, C4-2B LNCaP, and DU145 cells [180]. In general, silibinin inhibits the epithelial to mesenchymal transition of PC cells through interference with the NF-κB pathway and subsequent downregulation of ZEB1 and SLUG transcription factors and by downregulating vimentin and MMP2 [181]. Silibinin also exerts inhibitory effects in high bone metastatic prostate models and prevents PC cells-induced osteoclastogenesis [182].

In xenograft models, silibinin is described as having antiproliferative, proapoptotic, and antiangiogenic effects. Studies in PC-3 tumor xenografts in athymic mice revealed that silibinin effects are attributed to an increase in IGFBP-3, Cip1/p21, and Kip1/p27 levels, activation of ERK1/2, and a decrease in Bcl-2 and VEGF levels. In TRAMP mice fed 0.5% and 1% w/w silybin-phytosome diets for 11 weeks, silibinin blocked PCa growth and progression through IGF-IGFBP-3 axis modulation, whereas it suppressed tumor microvessel density via a decrease in VEGF, VEGFR-2, MMPs, and vimentin [183]. Finally, in patients receiving a silybin-phytosome (13 g/day) for 14–31 days, high blood concentrations were found transiently, but low levels of silibinin were detected in prostate tissue. Silibinin's lack of tissue penetration may be explained by its short half-life, the brief duration of therapy in this study, or an active process of removing silibinin from the prostate [184].

Sulforaphane
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Sulforaphane (1-isothiocyanato-4-methylsulfinylbutane) (SFN) is a natural isothiocyanate found in many cruciferous vegetables, firstly isolated from *Brassica oleracea* (broccoli; *Brassicaceae*) [185]. Many studies have shown that SFN can provoke cell cycle arrest and apoptosis in androgen-dependent and androgen-refractory PC cell lines. SFN (IC$_{50}$ of 10 µM) causes G2/M phase arrest in DU145 cells [186] and G1 cell cycle arrest in LNCaP and PC-3 cells. The antiproliferative effects of SFN at doses of 10–40 µM involve mechanisms such as the modulation of methyltransferases expression, which leads to an increase in cyclin D2 in LNCaP cells, and protein synthesis inhibition through decreased phosphorylation of mTOR substrates in PC-3 cells [187]. Apoptosis is induced through caspase activation in LNCaP cells, ROS generation that triggers intrinsic and extrinsic caspase cascades in PC-3 and DU145 cells [186], and inhibition of histone deacetylase 6 in BPH-1, LNCaP, and PC-3 cells [188, 189]. SFN has also been reported to inhibit HIF-1α with a subsequent decrease in VEGF expression, thus preventing prostate cell angiogenesis [190]. Cell migration of PC-3 and LNCaP cells is also restricted by SFN when delivered at 20 µM for 8 and/or 24 h due to modulations of the Notch pathway [191].

In vivo studies have deduced that oral administration of a daily dose of 7.5 mmol per animal for 21 days in PC-3 xenografts in nude mice causes a > 50% reduction in tumor volume due to a decrease in HDAC activity. Finally, TRAMP mice that were fed broccoli sprouts exhibited a decrease in prostate tumor growth [192].

Thymoquinone
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Thymoquinone (2-isopropyl-5-methylbenzo-1,4-quinone) (TQ) is a phytochemical isolated from the plant *Nigella sativa* (*Ranunculaceae*) [193]. TQ (40–100 µM) has been found to reduce cell
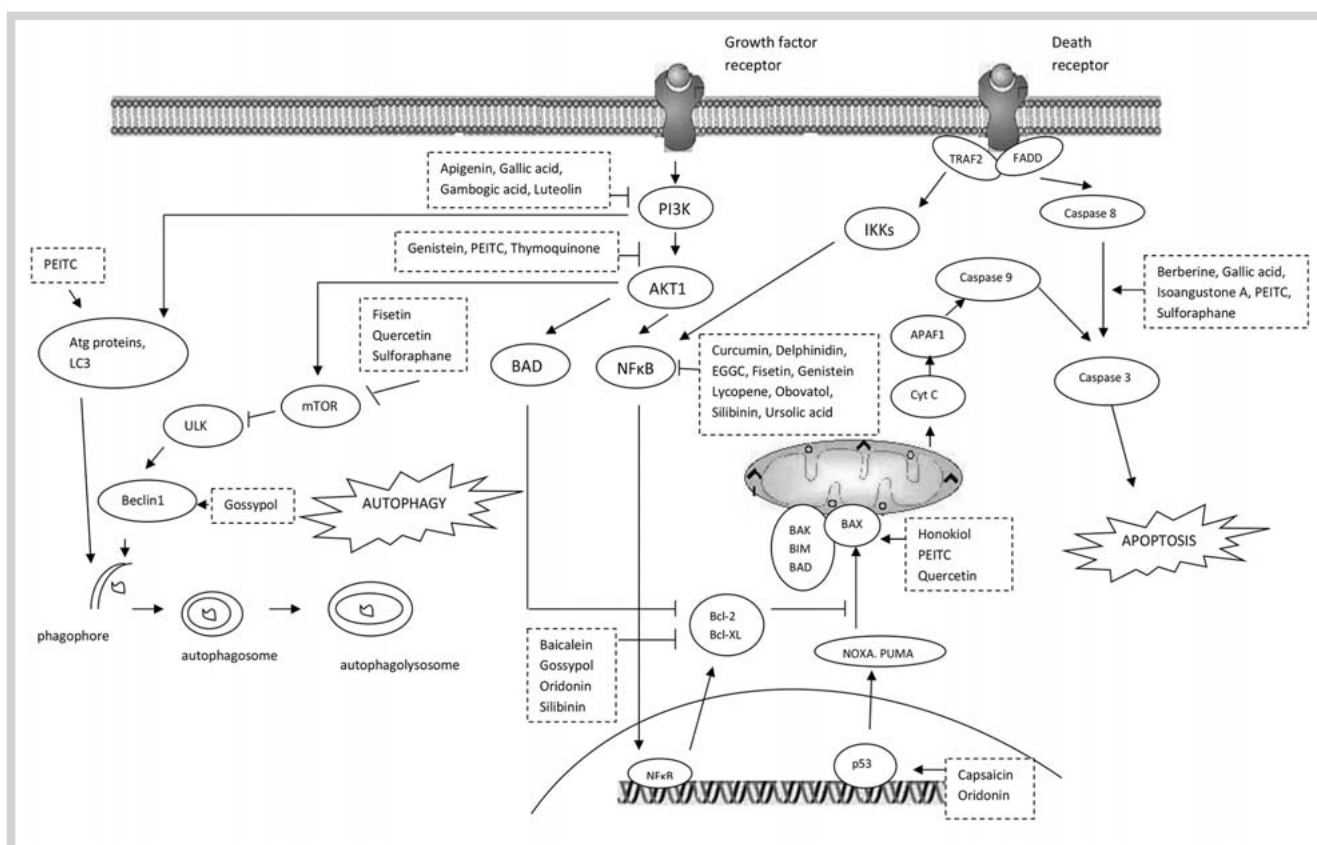
Fig. 1 Mechanism of action of bioactive natural products in prostate cancer cells. Natural compounds provoke apoptosis of PCa cells by triggering caspases (berberine, gallic acid) and mitochondrial-dependent cascades (honokiol) or by inhibiting oncogenes (baicalein), and by suppressing the NFκB signaling pathway (curcumin). Other compounds trigger autophagy via inhibition of the PI3K signaling pathway (apigenin), suppression of the mTOR complex (fisetin), or activation of the phagophore formation-related proteins (PEITC, gossypol).

growth both in androgen-dependent (LNCaP, C4-B) and androgen-refractory (DU145, PC-3) PC cell lines. Cell growth reduction is attributed to a decrease in AR and E2F-1 as well as the E2F-1 regulated proteins [194]. In PC-3 and C4-B cells, TQ (IC$_{50}$ values of approximately 50 and 80 mM) was shown to induce apoptosis through increased ROS generation and decreased GSH levels [195]. Other studies in PC-3 cells also demonstrated that TQ inhibits cell proliferation through suppression of AKT and prevents tumor angiogenesis via the repressed activation of induced extracellular signal-regulated kinase by VEGF [196].

Ursolic Acid
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Ursolic acid ((3*β*)-3-hydroxyurs-12-en-28-oic acid) (UA) is a pentacyclic triterpenoid compound derived from *Cornus officinalis* (*Cornaceae*) [197].

In PC-3 cells, UA evokes apoptosis via extrinsic and intrinsic apoptotic pathways while it confines cell invasion by inhibiting Akt and downregulating matrix metalloproteinase-9 [198]. UA was also shown to induce apoptosis through JNK activation, which results in Bcl-2 phosphorylation and degradation causing the activation of caspase 9, both in androgen-dependent (LNCaP) and androgen-independent PCa cell lines (LNCaP-AI and DU145 cells) [199]. In addition, UA displays a role in the suppressed activation of NF-κ B and STAT3 by downregulating the expression of various NF-κB and STAT3 gene products involved in proliferation, survival, and angiogenesis, and thus induces apoptosis in PCa cell lines

(LNCaP, DU145) and TRAMP mice [200]. UA was also found to restrict metastasis through the suppression of CXCR4 expression in PC both *in vitro* (PC-3, LNCaP, DU145 cells) and *in vivo* (TRAMP mice fed 1% w/w UA for 6 to 8 weeks) [201]. Finally, in DU145 cells, UA and its *cis*- and *trans*-3-O-p-hydroxycinnamoyl esters derived from American cranberries, such as *Vaccinium macrocarpon*, were shown to limit tumor cell growth at micromolar concentrations through matrix metalloproteinase (MMP-2 and MMP-9) inhibition [202].

Conclusion
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In this review article, the most promising bioactive natural products and their respective mechanisms of action for the treatment of PCa are presented, as they affect the processes of cell proliferation, cell cycle control, apoptosis, autophagy, tumor angiogenesis, invasion, and metastasis (○ **Fig. 1**, **Table 1**). Indeed, a variety of natural products have gained widespread use in the clinical treatment of a number of malignancies, such as carcinomas of the colon, breast, ovary, lung, and prostate. Unlike conventional chemotherapy, targeted agents have a relatively wide therapeutic window and have nonoverlapping toxicity profiles. Natural compounds that interfere with essential carcinogenic pathways, without demonstrating severe side effects, could exert a significant role as chemotherapeutic or chemopreventive agents, thus offering an alternative or complementary approach to the treatment of cancer. However, further *in vivo* studies should be con-

Table 1 Bioactive natural products against prostate cancer.

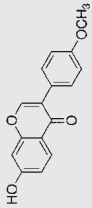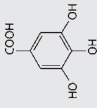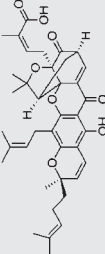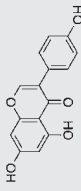| Compound	In vitro	In vivo	Mechanism	Molecular pathway	Plant	References
Apigenin (flavonoid)	DU145, LNCaP, PC-3, 22Rv1		G1 cell cycle arrest, Apoptosis	Cyclin D1,D2,E, Bax/Bcl-2 ratio, NF-κB, ROS, IGF-IGF-IR, PI3k/Akt	Anthemis sp. (Asteraceae)	[4–14]
			Epigenetic effects	HDACs		
			No invasion	Actin, FAK/scr		
	LNCaP, PC-3, C4-2B		Antiangiogenesis	TGF-β, ↓VEGF		
		22Rv1, PC3 xenografts, TRAMP mice	Growth inhibition	IGF-I/IGFBP-3, PI3K/Akt/FoxO		
Artemisin artesunate dihydroartemisin	LNCaP		G1 cycle arrest	CDK4	Artemisia annua (Asteraceae)	[15–19]
	PC-3		G2/M cycle arrest			
	DU145, PC-3		Apoptosis	Caspases 8, 9		
	C4-2, LNCaP		Cell cycle arrest			
Baicalin-baicalein (flavonoid)	DU145, PC-3, LNCaP, CA-HPV-10	Xenografts	G1 cycle arrest, Apoptosis	P27(kip1), Bcl-2, Bax, Fas	Scutellaria baicalensis (Lamiaceae)	[20–23]
			Antiangiogenesis			
Berberine (alkaloid)	DU145, PC-3, LNCaP		G1 cycle arrest, Apoptosis	Cyclins, CDKs, CDKs inhibitors Bax/Bcl2 ratio, caspases, cytochrome c	Berberis sp. (Berberidaceae)	[24–27]
		LNCaP xenografts	↓Tumor growth	↓AR expression		
Betulinic acid (triterpene)	LNCaP, PC-3, DU145		Apoptosis, antiangiogenesis	NF-κB, survivin, VEGF	Betula papyrifera (Betulaceae)	[28–31]
		TRAMP mice	↓Tumor growth, antiangiogenesis	Cyclin D, ↓AR		
Capsaicin (vanilloid)	PC-3, DU145, LNCaP	PC-3 xenografts	Apoptosis	ROS, JNK, ERK, ↑p53, p21, Bax, ↓PSA, AR	Capsicum sp. (Solanaceae)	[32–35]
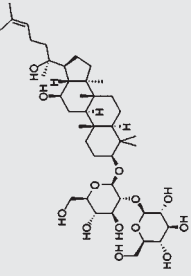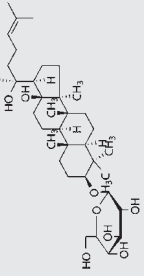
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Table 1 Continued

Compound	In vitro	In vivo	Mechanism	Molecular pathway	Plant	References
Curcumin (diphenylheptanoid)	PC-3, LNCaP	LNCaP xenografts, Curcumin-loaded PLGA nanospheres, Cellulose, Nanoparticles, Lipid-based carriers	Cycle arrest; Apoptosis; Metabolism; ↓ Angiogenesis; ↓ Growth	Cyclins, Cdks, Akt; EGF-R, Bcl, ROS, AIF; ↓ AR, NF-κB, AP-1, CREB, PSA; Glyoxylase; VEGF, MMPs; β-catenin, NF-κB	*Curcuma longa* (*Zingiberaceae*)	[36–44]
Daidzein (isoflavone)	PC-3, DU145, LNCaP		↓ Cell growth; Apoptosis	CDKs, IGF, EGF, VEGF; Akt; Epigenetic modifications	*Glycine max* (*Fabaceae*)	[45–47]
Delphinidin (anthocyanidin)	LNCaP, C4-2, 22Rnu1, PC3	PC3 xenografts	↓ Cell growth, Apoptosis; ↓ Tumor growth	Caspases, NF-κB/PI3K, Notch-1, Wnt/β-catenin	*Delphinium sp.* (*Ranunculaceae*)	[48–50]
Ellagic acid (polyphenol)	LNCaP; DU145, PC-3		Apoptosis; Antiangiogenesis; S cycle arrest, apoptosis; No invasion	SIRT1, p21, AIF, ROS, caspases; ↓ Eicosanoid, HO system; Cyclin D1, B1, Bcl2/Bax, caspases; MMP-2	*Rubus sp.* (*Rosaceae*)	[51–53]
Epigallocatechin-3-gallate (catechin)	LNCaP, DU145, PC-3	TRAMP mice	G0/G1 cycle arrest; Apoptosis; ↓ Invasion-metastasis; Apoptosis	↑ WAF1/p21, KIP1/p27, INK4, ↓ cyclins, CDKs; P53, NF-κB, Bax/Bcl-2 ratio, caspases, COX-2, PI3K, AR antagonist; VEGF, MMPs, uPA, angiopoietins; ↓ IGF-1, MAPK	*Camellia sinensis* (*Theaceae*)	[54–62]
Fisetin (flavonoid)	PC-3, LNCaP	CWR22 Rupsilon1 human xenograft	Cell cycle arrest; No adhesion, migration, autophagy; ↓ Tumor growth	Cyclins, Cdks, ↓ AR; NF-κB, MMP2, MMP9, mTOR, PI3K/Akt; ↓ PSA	*Acacia greggii* (*Fabaceae*)	[63–67]

cont.

Table 1 Continued

Compound	In vitro	In vivo	Mechanism	Molecular pathway	Plant	References
Formononetin (isoflavone)	PC-3, LNCaP		Apoptosis	ERK1/2 MAPK-Bax, IGF, Bax/Bcl-2 ratio, p38/Akt; ↑RASD1	*Trifolium pretense* (*Fabaceae*)	[68–71]
	DU145					
Gallic acid (polyphenol)	DU145		G2/M cycle arrest	ATM-Chk2	*Vitis vinifera* (*Vitaceae*)	[72–76]
	LNCaP		Apoptosis	ROS, caspases 3,9 PI3K/AKT, NF-κB, MMP-2		
	PC-3		No migration			
		TRAMP mice DU145, 22Rv1 xenografts	↓Tumor growth apoptosis	Cdks, cyclin B1, E		
Gambogic acid (xanthone)	PC-3		Apoptosis, no invasion	NF-κB, PI3K/Akt	*Carcinia hanburyi* (*Clusiaceae*)	[77–79]
		Xenografts	Antiangiogenesis	VEGFR-2, c-Scr, Akt		
Genistein (flavanone)	DU145, PC-3, ND1, LNCaP, ALVA31, JCA1		Cycle arrest, growth inhibition	↑p21WAF1, ↓Cdks, cyclins, survivin, DNA topoisomerase II, ↓IGF-1/IGF-1R	*Glycine max* (*Fabaceae*)	[80–97]
			Apoptosis	NF-κB, Akt, caspases		
			↓Angiogenesis	IL-10		
			No metastasis	MMP-9, protease M, uPAR, VEGF		
		PC-3, DU145 xenografts	Growth inhibition	Epigenetic modifications		
		Lobund-Wistar rats, TRAMP mice	Chemoprevention			
		Cabazitaxel + genistein Genistein-loaded liposomes	Apoptosis			
Ginsenoside Rg3	PC-3, LNCaP		G1 cycle arrest	P21, p27	*Panax sp.* (*Araliaceae*)	[98–101]
			Apoptosis	Caspase-3		
			Cell detachment	MAP kinases		
			No metastasis	P38 MAPK		
Ginsenoside Rh2	PC-3, LNCaP		↓Cell proliferation, detachment	MAP kinases	*Panax sp.* (*Araliaceae*)	[102–104]
		PC-3 xenograft	Apoptosis			

cont.

Table 1 *Continued*
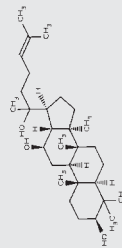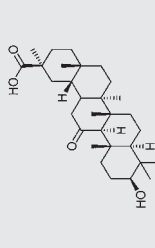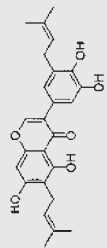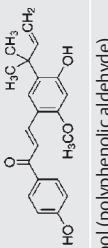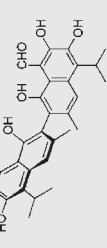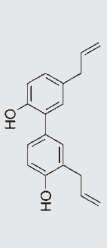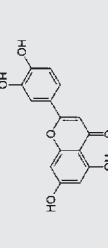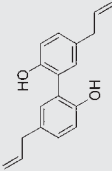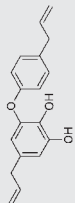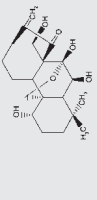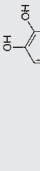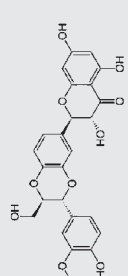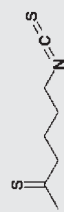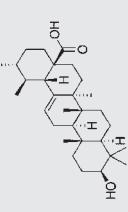
Compound	In vitro	In vivo	Mechanism	Molecular pathway	Plant	References
25-OH-PPD 25-OCH3-PPD	PC-3, LNCaP	PC-3, LNCaP xenografts	G1 cycle arrest Apoptosis Tumor growth	p21, p27, Bax	*Panax sp.* *(Araliaceae)*	[105, 106]
Glycerrhitinic acid (triterpenoid)	DU145, LNCaP		Apoptosis, no invasion Cell proliferation	NF-κB, VEGF, MMP-9 PSA	*Glycyrrhiza glabra* *(Fabaceae)*	[113, 114]
Isoangustone A (flavonoid)	DU145		G1 cycle arrest Apoptosis	Cdks, cyclin A, D1 Caspases	*Glycyrrhiza uralensis* *(Fabaceae)*	[107–109]
Licochalcone (flavonoid)	PC-3, LNCaP		G2/M cycle arrest Apoptosis, autophagy	Cyclin B1, cdc2	*Glycyrrhiza glabra* *(Fabaceae)*	[110–112]
Gossypol (polyphenolic aldehyde)	PC-3, BPH DU145 Docetaxel + gossypol Sorafenib + gossypol	PC-3 xenograft	G0/G1 cycle arrest Apoptosis, no invasion Apoptosis Autophagy Antiangiogenesis Apoptosis Apoptosis, autophagy	TGF-1 Bcl-X/Bcl-2,NF-κB, AP1 P53, Bcl-2, Bcl-xL, caspases Beclin1, Bcl-2/Bcl-xL VEGFR-2, Akt, Scr Noxa, Puma	*Gossypium hirsutum* *(Malvaceae)*	[115–123]
Honokiol (lignan)	PC-3, LNCaP C4-2		G0/G1 cycle arrest Apoptosis	Bax, Bak, Bcl-xL	*Magnolia officinalis* *(Magnoliaceae)*	[140–142]
Luteolin (flavonoid)	PC-3, DU145 LNCaP, PC-3	PC-3 xenografts	Cell growth Apoptosis Antiangiogenesis, no invasion	IGF-1, Akt, EGFR, MAPK/ERK DR5 VEGF-2R, AR, PSA	*Terminalia chebula* *(Combretaceae)*	[124–128]

cont.

Table 1 Continued

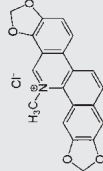| Compound	In vitro	In vivo	Mechanism	Molecular pathway	Plant	References
Lycopene (carotenoid)	LNCaP, PC-3		Cell cycle arrest	Cdk4, cyclins D1, E and Rb	Solanum lycopersicum (Solanaceae)	[129–139]
	LNCaP, DU145		↓Cholesterol synthesis	PPARγ-LXRα-ABCA1		
	LNCaP, PC-3		Apoptosis	p21, p27, p53,Bax/Bcl-2, IGF		
			↓Invasion	Integrins		
		PC-3	Apoptosis	↓ROS, ↑detoxification proteins		
		DU145 xenografts, TRAMP mice	Chemoprevention			
		Clinical trials	Chemoprevention	PSA, androgen-estrogen metabolism		
Magnolol (biphenyl)	PC-3		Apoptosis, no invasion	EGFR	Magnolia officinalis (Magnoliaceae)	[143]
Obovatol (biphenyl)	LNCaP, PC-3		Apoptosis	NF-κB	Magnolia obovata (Magnoliaceae)	[144, 145]
Oridonin (isoprenoid)	LNCaP, PC-3		G2/M cycle arrest, Apoptosis, autophagy	p21	Rabdosia rubescens (Labiatae)	[146–148]
PEITC (isothiocyanate)	DU145		G2/M cycle arrest	JAK/STAT-3, p53	Brassica oleracea (Brassicaceae)	[149–160]
	C4-2B		Apoptosis	ROS, survivin, caspases		
	LNCaP, PC-3		Autophagy	ATg5		
			No migration	Akt, VEGF, Notch		
		PC3, LNCaP xenografts	↓Tumor growth	PECAM1-CD31		
			Autophagy	E-cadherin		
	PEITC + docetaxel	TRAMP mice	Apoptosis			
	PEITC + etoposide + adriamycin		Apoptosis	↓Protein kinase C, telomerase		
Quercetin (polyphenol)	LNCaP, DU145	LNCaP, DU145	Cell cycle arrest	Cyclins, Cdks, Rb, ErbB	Vitis sp. (Vitaceae)	[161–166]
	PC-3	PC-3	Apoptosis	Bax, Bcl, caspases, IGF, ↓fatty acid synthase, ↓HS90, ↓AR		
			↓Adhesion, metastasis	MMPs, uPA, uPAR		
			↓Angiogenesis	EGF, EGF-R		
	LAPC-4 xenografts		↓Tumor growth	AKT/mTOR/P70S6K		
Sanguinarine (alkaloid)	LNCaP, DU145		G0–G1 cycle arrest	p21/WAF1, p27/KIP1, Cdks	Sanguinaria canadensis (Papaveraceae)	[167–170]
	PC-3		Apoptosis	Survivin		
	C4-2B	DU145 xenografts	No invasion, metastasis	Stat3		
			↓Tumor growth			

cont.

Table 1 *Continued*

Compound	In vitro	In vivo	Mechanism	Molecular pathway	Plant	References
Silibinin (flavolignan)	LNCaP		G1 cycle arrest	Rb, Cdks, PDEF, ↓ PSA	*Silybum marianum* (*Asteraceae*)	[171–184]
	DU145, 22Rv1			P21,p27, Wnt/LRp6		
	PC-3		G1, G2/M cycle arrest	HIF1,Wnt/LRp6,cyclins,Cdks		
	PC-3MM2, C4-2B		No invasion, metastasis	NF-κB, vimentin, MMP2		
		PC3 xenografts	↓ Tumor growth, apoptosis, angiogenesis	↑ IGFBP-3, Cip1/p21, Kip1/p27, ↓ Bcl-2		
		TRAMP	↓ Angiogenesis	VEGF, vimentin, MMPs		
Sulforaphane (isothiocyanate)	LNCaP, PC-3		G1 cycle arrest	Methyltransferase, cyclins	*Brassica oleracea* (*Brassicaceae*)	[185–192]
	DU145		G2/M cycle arrest			
			Apoptosis	ROS, caspases, HDAC6, ↓ AR		
			↓ Angiogenesis	HIF-1, VEGF		
			No migration	Notch		
		PC3 xenografts, TRAMP	↓ Tumor growth	HDAC		
Thymoquinone	LNCaP, C4–2B	LNCaP, C4–2B	↓ Growth	E2F-1, AR, AKT	*Nigella sativa* (*Ranunculaceae*)	[193–196]
	PC-3, DU145	PC-3, DU145 Xenografts	Apoptosis	ROS		
			↓ Angiogenesis	VEGF		
Ursolic acid (triterpenoid)	PC-3, LNCaP	TRAMP mice	Apoptosis	JNK, caspases	*Corni fructus* (*Cornaceae*)	[197–202]
	DU145		↓ Angiogenesis	NF-κB, STAT3		
			No metastasis	CXCR4		

ducted in order to clarify whether these compounds can exert their effects in physiologic concentrations or have a combinational effect when administered with the traditional chemotherapeutic agents in order to determine if they are possible candidates for clinical trials.

Conflict of Interest
▼

The authors declare that no conflict of interest or any financial disclosure exists.

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