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REVIEW ARTICLE

Anticancer mechanisms of phytochemical compounds: focusing on epigenetic targets

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Abstract

It has recently been proven that epigenetic dysregulation is importantly involved in cell transformation and therefore induces cancerous diseases. The development of molecules called epidrugs, which target specifically different epigenetic modifications to restore cellular memory and therefore the treatment, became a real challenge currently. Currently, bioactive compounds of medicinal plants as epidrugs have been can identified and explored in cancer therapy. Indeed, these molecules can target specifically different epigenetic modulators including DNMT, HDAC, HAT, and HMT. Moreover, some compounds exhibit stochastic epigenetic actions on different pathways regulating cell memory. In this work, pharmacodynamic actions of natural epidrugs belonging to cannabinoids, carotenoids, chalcones, fatty acids, lignans, polysaccharides, saponins, secoiridoids, steroids, tannins, tanshinones, and other chemical classes we reported and highlighted. In this review, the effects of several natural bioactive compounds of epigenetic medications on cancerous diseases were highlighted. Numerous active molecules belonging to different chemical classes such as cannabinoids, carotenoids, fatty acids, lignans, polysaccharides, saponins, secoiridoids, steroids, tannins, and tanshinones are discussed in this review.

Keywords Epigenetic, Cancer · Epidrugs · Cannabinoids · Carotenoids · Chalcones

Introduction

Cancer represents a complex pathology induced by several risk factors such as microbial infections, genetic mutations, environmental changes, and epigenetic variability (Yahya and Alqadhi 2021). These risk factors contribute directly or indirectly to the mechanisms of tumor transformation as well as the spread of cancer. Today with the mechanistic understanding of cellular signaling pathways, molecular evidence has shown that cancer may be the result of genetic instability leading to disruption of epigenetic pathways which lead to the transformation of cells into tumor cells (Feng and De Carvalho 2021).

Indeed, epigenetics signify all changes in gene expression that are the physical modification of the sequence of genetic material (Mancarella and Plass 2021). These modifications control transcription via the spatiotemporal regulation of transcription

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factors. The modifications of epigenetic include DNA methylation on CpG islands, histone acetylation and deacetylation, and chromatin remodeling (Mancarella and Plass 2021).

The disturbances of these epigenetic modifiers (loss or gain of function) often lead to major phenotypes which can predispose to a loss of cellular memory and subsequently to the mechanism of cancerization (Khan et al. 2021). Recently, therapeutic strategies that specifically target the epigenome have started to implement molecules (epidrugs) that operate in a targeted manner for one or another epigenetic modifier. Indeed, certain epidrugs have already been validated and used clinically in chemotherapy for cancer treatment (Khan et al. 2021).

Among the sources of major epidrugs, nature constitutes an inexhaustible reservoir of bioactive natural molecules acting specifically against epigenetic pathways and thus demonstrating remarkable anticancer effects (Manso et al. 2021). Indeed, natural substances such as those identified in medicinal plants showed their capacity to have epidrug activity. In this review, a systemic research was carried out on anticancer actions of bioactive compounds belonging to carotenoids, fatty acids, lignans, polysaccharides, saponins, secoiridoids, steroids, tannins, and tanshinones chemical classes and their action on different epigenetic ways inducing and/or involving human cancer. Therefore, we highlighted the role of these phytochemical compounds in cancer chemoprevention.

Methodology

The literature on anticancer effects with epigenetic mechanisms of carotenoids, fatty acids, lignans, polysaccharides, saponins, secoiridoids, steroids, tannins, and tanshinones was carried out and highlighted. The collection of data was performed by using different databases, including Google Scholar, ScienceDirect, PubMed, SpringerLink, Web of Science, Scopus, Wiley Online, and SciFinder. The used keywords are cannabinoids, carotenoids, fatty acids, lignans, polysaccharides, saponins, secoiridoids, steroids, tannins, and tanshinones with the screening of all published papers to select those evaluated their anticancer actions on epigenetic targets. The collected data were classified and organized in tables to facilitate and then discussed and highlighted. The structures of all highlighted natural epidrugs in this study were drawn by the ChemDraw Pro 8.0 software. Moreover, for checking the IUPAC names of these molecules, PubChem database was utilized.

Results and discussion

Cannabinoids as epidrug agents against cancer

Tetrahydrocannabinol (THC) is an exogenous cannabinoid isolated from the *Cannabis sativa* plant (Fig. 1). This compound has long been known for its anti-inflammatory efficacy and antitumor immune response suppression. In 2015, Sido et al. (2015) studied the effect of THC on the methylation of promoters of different genes involved in the activation and therefore the differentiation of MDSCs using an in vivo model (Female BL6 (wild-type) mice methylated DNA) and also using many in vitro assays such as Western blot analysis and methylation-specific PCR (MSP) analysis. Results of this study revealed that THC increases the T cell suppression in THC-induced MDSCs (myeloid-derived suppressor cells) and upregulates STAT3-associated cytokines in THC-induced MDSCs. Moreover, THC elevated the S100A8 expression, which promoted the suppressive function of MDSCs and induced hypomethylation of major key MDSC functional genes (Table 1). Very recently, a review by Griffiths and colleagues was carried out to investigate the impact of cannabidiol on epigenetic treatments and on the effectiveness of chemotherapy in oncology (Griffiths et al. 2021). Indeed, the authors have observed from the results of several research works that this compound can be very promising when it is used alone or in combination, as in the case of immunotherapy and epigenetics (Griffiths et al. 2021).

Carotenoids as epidrug agents against cancer

The carotenoids are the natural substances, known also as tetraterpenoids, and present in different vegetables and algae. Some carotenoid compounds such as astaxanthin, fucoxanthin, lycopene, and β-carotene (Fig. 2) showed remarkable effects against cancer cell lines, particularly by their ability to modulate epigenetic modifications (Table 2).

Astaxanthin and fucoxanthin

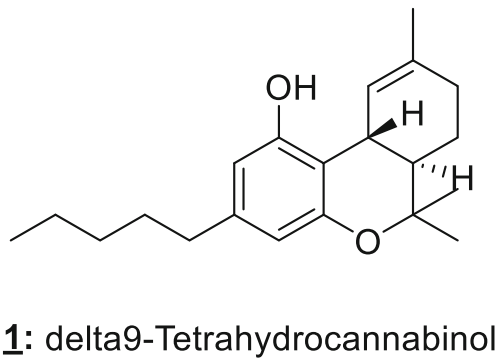
Yang et al. (2017) performed in vitro testes to evaluate the capacity of astaxanthin (AST) to reactivate Nrf2 and GSTP1 expression (in human prostate LNCaP cells) via some epigenetic modifications. During this study, several assays were conducted to demonstrate this effect such as genomic sequencing, Western blot analysis, DNMT and HDAC activity assays, and others. The findings of this investigation revealed that this compound was able to reduce the methylation of 21 CpG sites of the GSTP1 CpG island and decrease the transcription of DNMT3b, as well as significantly inhibit the enzymatic activities of DNMT and HDAC. Moreover, AST increased the expression of NQO1 mRNA in sh-mock LNCaP and also induced the expression of mRNA and certain proteins such as Nrf2 and GSTP1. However, AST did not induce effects on the methylation of Nrf2 gene promoter region.

The same authors carried out a study in 2018 on mouse skin epidermal JB6 P+ cells to investigate the epigenetic regulation of Nrf2 by AST and fucoxanthin (FX) (Yang et al. 2018). The same assays, as those performed in the previous

Table 1 Effects of cannabinoid on epigenetic pathways in cancer

Bioactive molecules	Origin	Experimental methods	Key results	References
Δ9-Tetrahydrocannabinol (THC)	Commercial sample	Female BL6 (wild-type) mice Methylated DNA immunoprecipitation sequencing service Methylation-specific PCR analysis Western blot analysis Quantitative PCR approach	Increased the T cell suppression in THC-induced MDSCs (myeloid-derived suppressor cells) Upregulated the STAT3-associated cytokines in THC-induced MDSCs Elevated the S100A8 expression, which promoted the suppressive function of MDSCs Altered the methylation profile in MDSCs as a result of decreased DNMT3a and DNMT3b Decreased the methylation of key MDSC functional genes	Sido et al. 2015

study, were conducted in this work and it was found that AST is able to decrease colony formation in TPA-induced transformation of JB6 P+ cells and reduce DNMT activity, while it has not showed inhibitory effects against HDAC activity in JB6 P+ cells. On the other hand, the results related to the effect of FX show that this compound has the same effect as AST to decrease colony formation in TPA-induced transformation of JB6 P+ cells and to reduce DNMT activity, while it did not show inhibitory effects against HDAC activity in JB6 P+ cells. In addition, in the same cell lines, FX suppressed the methylation of Nrf2 promoter region. Moreover, in HepG2-C8 cell lines, this epidrug induced the epigenetic demethylation of CpG sites in Nrf2, increased the antioxidant response element (ARE)-luciferase, and upregulated protein and mRNA levels of Nrf2 downstream genes.



1: delta9-Tetrahydrocannabinol

Fig. 1 Chemical structure of the cannabinoid

Lycopene

Lycopene is another naturally occurring compound that is abundant in tomatoes and belonging to the carotenoid family. This compound is known for its antioxidant properties and nutrient protection. In 2008, King-Batoon et al. (2008) studied the effect of lycopene on the methylation of the GSTP1 promoter and important methylation in prostatic cancer cell lines (PC3 and LNCaP cells). The results showed that this compound had no effect on the *RARβ2* gene in any of the breast cancer cell lines. However, it induced demethylation of GSTP1 gene (the tumor suppressor) in MDA-MB-468 cells and restored the GSTP1 expression. Moreover, lycopene caused demethylation of *RARβ2* and *HIN-1* genes in non-cancer MCF10A fibrocystic breast cells. The same cells were used by Fu et al. (2014) to study the effect of lycopene on the epigenetic regulation by methylation of the main gene in these cells. A decrease in methylation levels of the GSTP1 promoter was observed, as well as an increase in protein and mRNA levels of GSTP1 in an androgen-independent PC-3 cell line. Moreover, the methylation levels of the long interspersed element (LINE-1) were reduced, as well as the short interspersed element ALU. It has also been observed that lycopene

Table 2 Effects of carotenoids on epigenetic pathways in cancer

Bioactive molecules	Origin	Experimental methods	Key results	References
Astaxanthin	Commercial sample	Human prostate LNCaP cells Cell viability test: MTS assay DNA extraction and bisulfite genomic sequencing RNA isolation and reverse transcription PCR Western blot analysis DNMT and HDAC activity assays	Reduced the methylation of 21 CpG sites of the GSTP1 CpG island Induced the mRNA expression and protein expression of both Nrf2 and GSTP1genes No effect on the methylation status of the Nrf2 gene promoter region Increased the mRNA expression of NQO1 in sh-mock LNCaP cells but not in sh-SETD7 LNCaP cells Reduced the protein expression of DNMT3b and significantly inhibited DNMT and HDAC activities in vitro	Yang et al. 2017
	Commercial sample	Mouse epidermal JB6 P+ cells Cell viability test: MTS assay RNA Extraction and qRT-PCR DNA Extraction and pyrosequencing Western blot analysis DNMT and HDAC activity assays	Decreased colony formation in TPA-induced transformation of JB6 P+ cells Reduced DNMT activity but did not affect HDAC activity in JB6 P+ cells	Yang et al. 2018
Fucoxanthin	Commercial sample	Human hepatocellular HepG2-C8 cell line Mouse epidermal JB6 P+ cells Cell viability test: MTS assay RNA extraction and qRT-PCR DNA extraction and pyrosequencing Western blot analysis DNMT and HDAC activity assays	Induced the antioxidant response element (ARE)-luciferase and upregulated the mRNA and protein levels of Nrf2 and Nrf2 downstream genes in HepG2-C8 cells Induced the epigenetic demethylation of CpG sites in Nrf2 Decreased colony formation in TPA-induced transformation of JB6 P+ cells Decreased the methylation of the Nrf2 promoter region in the JB6 P+ cells by the bisulfite conversion and pyrosequencing Reduced DNMT activity but did not affect HDAC activity in JB6 P+ cells	Yang et al. 2018
Lycopene	Commercial sample	Breast cancer cell lines MCF-7 and MDA-MB-468 Non-cancer fibrocystic MCF10A breast cells Bisulfite modification of DNA MSP RT-PCR	Demethylated the promoter of the *GSTP1* tumor suppressor gene in MDA-MB-468 cells Restored the *GSTP1* expression No effect on the *RARβ2* gene in any of the breast cancer cell lines Induced the demethylation of *RARβ2* and the *HIN-1* genes in the non-cancer MCF10A fibrocystic breast cells	King-Batoon et al. 2008
	Commercial sample	LNCaP and PC-3 cells Genomic DNA extraction Bisulfite modification Bisulfite sequencing PCR RNA isolation and qRT-PCR Western blot analysis	Decreased the methylation levels of the GSTP1 promoter and increased the mRNA and protein levels of GSTP1 in an androgen-independent PC-3 cell line Downregulated the DNMT3A protein levels in PC-3 cells No effect on any detected DNMT protein expression in LNCaP cells Decreased the methylation levels of the long interspersed element (LINE-1) and short interspersed element ALU	Fu et al. 2014
	Not reported	Molecular docking studies Biological activity analysis	The lycopene-DNMT binding has been compared with other ligands known to bind the DNMT in its active sites (SFG and SAH) The binding affinity of lycopene and DNMT was lower than other ligand	Sukirman 2018
β-Carotene	Commercial sample	$CD44^{+}CD133^{+}$ colon cancer stem cells (CSCs) Cell proliferation (MTT assay) miRNA sequencing array Enzyme-linked immunosorbent assay Western blot analysis qRT-PCR	Reduced the cell proliferation and sphere formation Regulated the expression of miRNAs associated with histone acetylation Elevated the histone H3 and H4 acetylation levels Downregulated the DNMT3A mRNA expression and global DNA methylation in colon CSCs	Kim et al. 2019

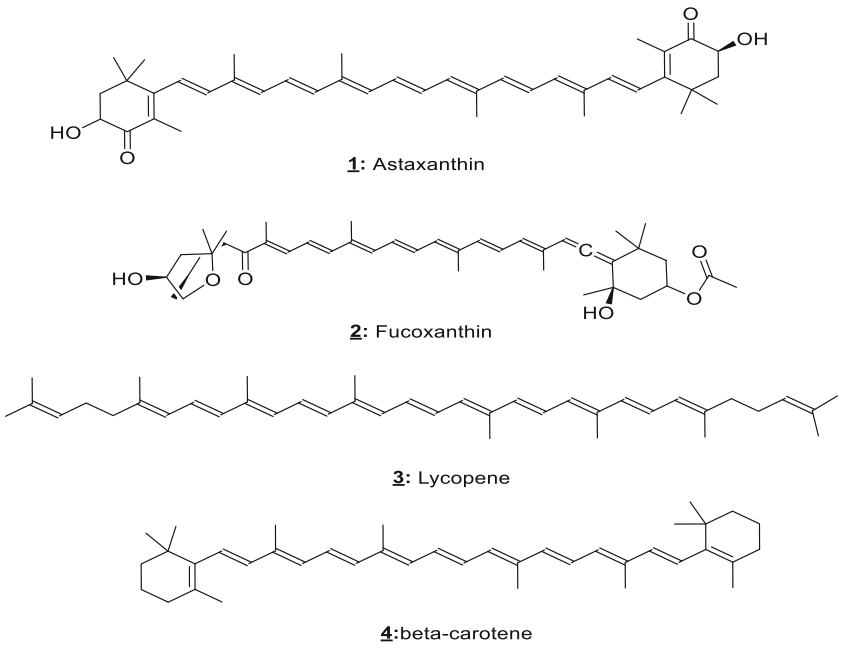


Fig. 2 Chemical structures of carotenoids

is able to downregulate the DNMT3A protein levels in PC-3 cells. However, this compound had no effect on DNMT protein expression in LNCaP cells. The last study was conducted in 2018 to investigate the mechanism of lycopene prostate cancer by targeting DNA methyltransferase (Sukirman 2018). In this study, the binding of lycopene and other ligands to the active sites (SFG and SAH) of the DNA methyltransferase was compared. Therefore, this compound has a lower binding affinity than other ligand to bind to DNMT active sites.

β-Carotene

In 2019, colon cancer stem cells were used by Kim et al. (2019) to study the mechanisms of the effects of β-carotene on microRNA expression, DNA methylation, and histone acetylation. Indeed, β-carotene is known to have anticancer effects in different cancers via numerous mechanisms, including cell cycle arrest, apoptosis, cell growth regulation, immune system modulation, inhibition of cell proliferation, and antioxidant activity. The results obtained in this study were more in-depth because they showed that this constituent reduced cell proliferation and sphere formation in $CD44^{+}CD133^{+}$ colon CSCs. However, treatment with β-carotene elevated acetylation levels of histones H3 and H4. Further, after miRNA sequencing array analysis, it was found that β-carotene is able to regulate the expression of miRNAs associated with histone acetylation. In addition, this compound downregulated the expression of DNMT3A mRNA and the methylation of DNA in colon CSCs.

Chalcones as epidrugs against cancer

Some natural chalcones may also play a role in epigenetic targeting of cancerous diseases, such as isoliquiritigenin (ISL) and phloretin (Fig. 3), belonging to the chalcone family. Indeed, Lee and colleagues (Lee et al. 2015) carried out an investigation to assess the antitumor and antiviral effects of ISLa against EBVaGC. The results revealed that this molecule induces apoptosis in SNU719 cells, affects cell cycle progression of SNU719, induces the transduction of signal, stimulates apoptosis, and induces EBV (Epstein–Barr virus) gene transcription. In addition, ISL eliminated DNMT1 and DNMT3A expressions. Moreover, it decreased the use of F promoter and increased frequency of use of Q promoter, which supports the functional role of this molecule in EBV latency establishment. In the same year, a mouse mammary tumor model was used by Wang et al. (2015) to demonstrate the effects of ISL treatment on breast cancer. Analysis in this study showed that ISL suppressed the mammary hyperplasia and breast cancer initiation in mice and inhibited lung metastasis and breast cancer growth in these animals. Furthermore, molecular docking analysis identified the Wnt inhibitory factor-1 (WIF-1) as the primary target of ISL in limiting breast CSC. Wherefore, the ISL increased the WIF1 expression by demethylation of the promoter through inhibiting DNMT1 methyltransferase and limited the self-renewal ability of human breast CSCs in mice (Table 3).

Regarding phloretin, Paluszczak et al. (2010) evaluated the effect of this molecule on the epigenetic modifications in MCF7 breast cancer cells. The results show that phloretin inhibited DNMT activity and increased the level of DNMT1

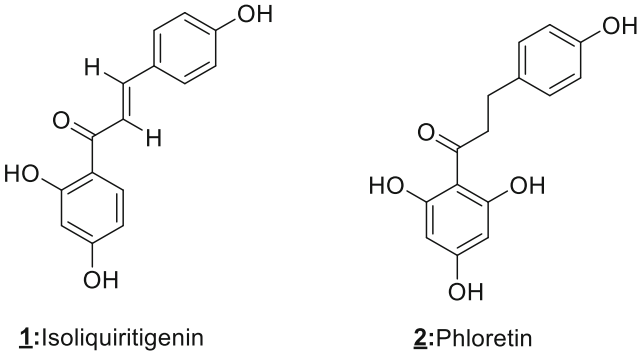


Fig. 3 Chemical structures of chalcones

transcription. Phloretin did not cause any effect on the methylation pattern or expression of *RASSF1A*, *GSTP1*, or *HIN-1* in MCF7 cells. In addition, it did not affect histone H3 methylation.

Fatty acids as epidrugs against cancer

Fatty acids are essential metabolites in vegetables and contain molecules with important biological effects. Recent data showed that several fatty acids isolated from vegetables such as linoleic acid, docosahexaenoic acid, eicosatetraenoic acid, butyrate, and butyric acid (Fig. 4) exhibit anticancer properties with different mechanisms, including their action on epigenetic pathways involved in cancer inducing and promotion (Table 4).

Butyrate

It is a fatty acid presents in several natural sources, including medicinal plants. Several studies have shown the importance of honey, alone and in combination with other compounds, in

Table 3 Effects of chalcones on epigenetic pathways in cancer

Bioactive molecules	Origin	Experimental methods	Key results	References
Isoliquiritigenin (ISL)	Commercial sample	Gastric carcinoma cell line (SNU719) RT-qPCR Intracellular and extracellular EbV genomic DNA copy number quantification Cell cycle analysis Cytotoxicity assay Western blot analysis	Induced the apoptosis of SNU719 cells Eliminated DNMT1 and DNMT3A expressions Affected the cell cycle progression of SNU719 Induced the signal transductions to stimulate apoptosis and induced EBV (Epstein–Barr virus) gene transcription Enhanced the frequency of Q promoter use	Lee et al. 2015
	Commercial sample	Human breast cancer cell lines MDA-MB-231 and MCF-7 Microarray analysis RT-PCR analysis Western blot analysis DNMT1 siRNA construction and transfection Molecular docking	Suppressed the mammary hyperplasia and breast cancer initiation in vivo Inhibited the breast cancer growth and lung metastasis in vivo Identified the Wnt inhibitory factor-1 (WIF-1) as the primary target of ISL in limiting breast CSC Limited the self-renewal ability of human breast CSCs in vitro Increased the WIF1 expression by promoter demethylation through inhibiting DNMT1	Wang et al. 2015
Phloretin	Commercial sample	Epithelial breast cancer MCF7 cell line Determination of the activity of DNMTs DNA methylation analysis Real-time PCR Western blot analysis	Inhibited the DNMT activity No effect on the methylation pattern or the expression of *RASSF1A*, *GSTP1*, or *HIN-1* in MCF7 cells No effect on the global methylation of histone H3 Increased the DNMT1 transcript level	Paluszczak et al. 2010

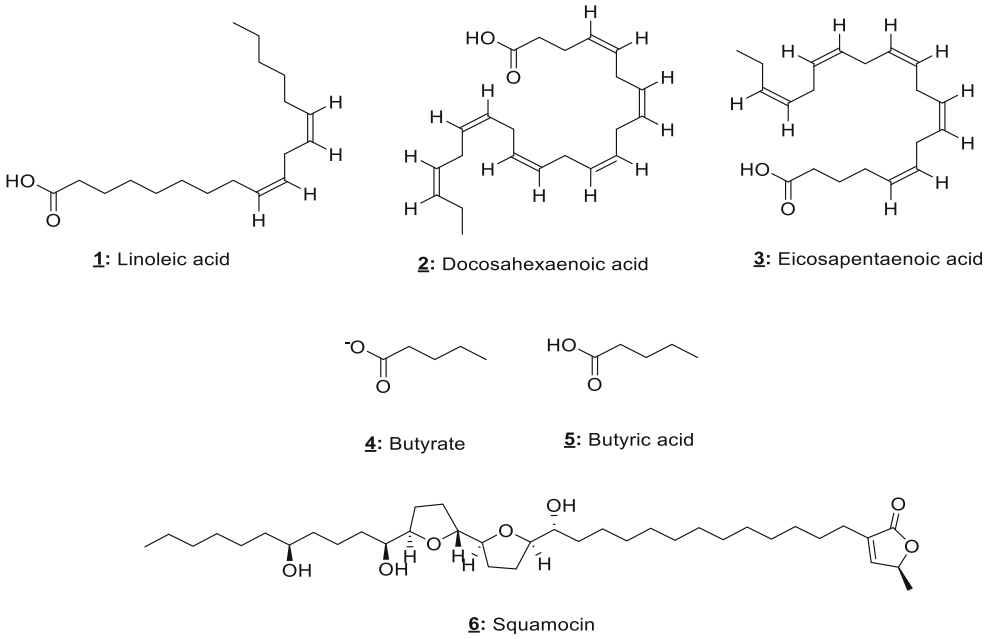


Fig. 4 Chemical structures of fatty acids

the treatment of various types of cancer. Cho et al. (2014) used HCT-116 cells to demonstrate the effect of butyrate on the epigenetic regulation of apoptosis-related genes in these cells. Moreover, this molecule can also affect DNA methylation of apoptosis-related genes; the transcription of *Cideb*, *Dapk1*, and *Tnfrsf25*; and the modification of histone acetylation.

The authors found also that butyrate increases the colonocyte apoptosis. In addition, the treatment of HCT-116 cells with butyrate combined with docosahexaenoic acid importantly decreased the methylation of the proapoptotic genes (*Cideb*, *Dapk1*, *Bcl2l11*, *Ltbr*, and *Tnfrsf25*) compared to untreated control cells. In the same year, Zheng et al. (2014) studied the effect of butyrate on aberrant epigenetic alteration in Eca9706 cells. Consequently, in a dose-dependent manner, butyrate suppressed the human esophageal 9706 cancer cell growth. Moreover, treatment of Eca9706 cells with butyrate in combination with quercetin inhibited Eca9706 cell proliferation more than that induced by the compound alone. Also, using the immunoblotting assay, it was shown that butyrate in combination with quercetin could downregulate the reverse expressions of global DNMT1, NF-κB p65, HDAC1, and cyclin D1, whereas it upregulated the expressions of caspase-3 and $p16^{INK4\alpha}$. In addition, the treatment of Eca9706 cells with butyrate in combination with quercetin displayed an inverse effect targeting both altered DNA methylation and histone acetylation, acting as an HDAC inhibitor mediated by the epigenetic-NF-κB cascade signaling. Saldanha et al. (2014) demonstrated that the treatment of colon cancer cells with sodium butyrate (NaB) in combination with epigallocatechin gallate (EGCG) induced apoptosis and cell cycle arrest in RKO, HCT-116, and HT-29 colorectal cancer cells; this arrested mainly in the G_2/M phase and for HT-29 CRC cells in the G1 phase. Additionally, real-time PCR analysis showed an increase in p21, NF-κB p65, and HDAC1 and a decrease in DNMT1 and survivin when RKO CRC cells were treated with the two molecules combined. While the combined treatment inhibited HDAC1, DNMT1, and survivin in the three CRC cells tested. Further assays from this study, conducted to clarify the combination treatment mechanism, showed that this combination inhibits DNMT3A and DNMT3B and induces p21 through a p53-dependent mechanism in RKO CRC cells. In addition, the Western blot assay showed that the combined treatment significantly decreased the percentage of CpG methylation.

Two years later, a study was carried out by Fialova et al. (2016) to evaluate the role of NaB on epigenetic modulation of androgen receptors (AR) gene expression in DU145 prostate cancer cells. The results of this study showed that NaB combined with 5′-aza-2′-deoxycytidine (Aza-dC) induced both AR gene expression at the mRNA level and the increase in histone H4 acetylation in AR gene promoter. In addition, the combination of the two molecules activated and maintained the G_2/M cell cycle arrest with better survival in normal cells compared to prostate cancer DU145 cells. One of the reasons for combining two compounds is that one of the compounds reduces the toxicity of the other. In this study, it was found that the combination of NaB with Aza-dC reduces the toxicity of NaB.

In 2017, CHO DP-12 cells were used by Wippermann et al. (2017) to analyze genome-wide butyrate-induced several changes in the methylation of numerous gene expression and affected therefore their expression. The results reported

Table 4 Effects of fatty acids on epigenetic pathways in cancer

Bioactive molecules	Origin	Experimental methods	Key results	References
Butyrate	Commercial sample	Cell culture (HCT-116 cells) Global DNA methylation assay Gene-specific DNA methylation Gene expression using qRT-PCR Histone H3 and H4 acetylation	Enhanced the colonocyte apoptosis The combination of butyrate and docosahexaenoic acid significantly reduced methylation of the proapoptotic *Bcl2l11*, *Cideb*, *Dapk1*, *Ltbr*, and *Tnfrsf25* genes compared to untreated control cells	Cho et al. 2014
	Commercial sample	MTT assay (Eca9706 cells) Immunoblotting of Eca9706 cells Methylation-specific PCR (MSP) of p16INK4a gene promoter	Suppressed the human esophageal 9706 cancer cell growth in a dose-dependent manner Butyrate + quercetin inhibited the Eca9706 cell proliferation than that induced by the compound alone Downregulated the reverse expressions of global DNMT1, NF-κB-p65, HDAC1, and cyclin D1 Upregulated the expressions of caspase-3 and p16INK4α Butyrate + quercetin displayed a reverse effect targeting both altered DNA methylation and histone acetylation, acting as HDAC inhibitor mediated via epigenetic-NF-κB cascade signaling	Zheng et al. 2014
	Commercial sample	Prostate cancer cell line DU145 Cell culture and viability assay mRNA analysis using RT-qPCR Chromatin immunoprecipitation coupled with quantitative PCR (ChIP-qPCR)	Sodium butyrate (NaB) combined with 5′-aza-2′-deoxycytidine (Aza-dC) induced both androgen receptor (AR) gene expression on the mRNA level and increased histone H4 acetylation in AR gene promoter NaB + Aza-dC activated and maintained the G_2/M cell cycle arrest with better survival in normal cells compared to cancer DU145 cells	Fialova et al. 2016
	Commercial sample	RKO (CRL-2577), HCT-116 (CCL-247), and HT-29 (HTB-38) CRC cells Cell viability assessment Apoptosis analysis Real-time quantitative PCR HDAC activity and CpG percent methylation ChIP assay	Combinatorial effects of sodium butyrate and EGCG: Induced apoptosis and cell cycle arrest in RKO, HCT-116, and HT-29 colorectal cancer cells Suppressed RKO CRC cell proliferation Arrested RKO, HCT-116 CRC cells predominantly in the G_2/M phase, and HT-29 CRC cells in the G_1 phase Increased p21, NF-κB-p65, HDAC1 and decreased DNMT1 and survivin in RKO CRC cells Inhibited the HDAC1, DNMT1, and survivin in all the three CRC cells tested Inhibited the DNMT3A and DNMT3B in total protein assessed in RKO CRC cells Induced the p21 through a p53-dependent mechanism in RKO CRC cells Affected the global DNA methylation and chromatin structure	Saldanha et al. 2014
	Commercial sample	Cell culture (CHO DP-12 cells) Gene expression profiling by microarrays Functional analysis of gene sets	Affected the cell cycle, apoptosis, central energy metabolism, and protein biosynthesis Differentially methylated regions were found to contain binding-site motifs of specific transcription factors and were hypothesized to represent regulatory regions closely connected to the cellular response to butyrate	Wippermann et al. 2017
Butyric acid	Commercial sample	Excised skin from the painted area (female mice) Quantitative real-time RT-PCR Bisulfite modification of DNA and MSP assay RT-PCR Western blot analysis	Prevented tumor development but protection was greatly improved when combined with nicotinamide (NA) and calcium glucarate (CAG) Downregulated the miR-203 levels at 16 weeks Upregulated the HDAC, DNMT, promoter methylation of miR-03 at 4 or 16 weeks Prevented altered gene expression (after 16 weeks), while co-administration with NA and CAG had a more pronounced effect than that of the individual compound, by regulating miR-203 status through epigenetic or biogenetic modulations	Tiwari and Gupta 2014
Docosahexaenoic acid (DHA)	Commercial sample	Cell culture (HCT-116 cells) Global DNA methylation assay Gene specific DNA methylation Gene expression using qRT-PCR	Enhanced the colonocyte apoptosis The combination of DHA and butyrate significantly reduced methylation of the proapoptotic *Bcl2l11*,	Cho et al. 2014

Table 4 (continued)

Bioactive molecules	Origin	Experimental methods	Key results	References
		Histone H3 and H4 acetylation	*Cideb*, *Dapk1*, *Ltbr*, and *Tnfrsf25* genes compared to untreated control cells Reduced the methylation of Cideb, Dapk1, and Tnfrsf25	
	Commercial sample	Cell culture CRC cell lines (HCT116, HT29/219, and SW742) DNA methylation analysis RNA extraction and RT-PCR	Cell-type specific differences in expression of DNMT1, DNMT3a, and 3b genes Induced global hypermethylation in HT29/219 and HCT116 cells, but reduced methylation in Caco2 cells Induced the promoter demethylation of Cox2 in HT29/219, p14 and PPARγ in HCT116, and ECAD in SW742 cells	Sarabi and Naghibalhossaini 2018
Eicosapentaenoic acid (EPA)	Commercial sample	Cell culture (McRH-7777 cells) Cell cycle and viability qRT-PCR Immunoblot analysis DNA isolation and p21 CpG island quantitative DNA methylation Bisulfite modification of genomic DNA and sequencing Hydroxymethylated DNA immunoprecipitation and methylated DNA immunoprecipitation assays ChIP	Demethylated the DNA in hepatocarcinoma cells Increased the 5-hydroxymethylcytosine (5hmC) on DNA, inducing $p21^{Waf1/Cip1}$ gene expression, which slows cancer cell cycle progression Simultaneous binding to peroxisome proliferator-activated receptor γ (PPARγ) and retinoid X receptor α (RXRα), thus promoting their heterodimer and inducing a PPARγ-ten-eleven translocation (TET1) interaction Regulated the DNA methylation levels, allows TET1 to exercise its antitumor function	Ceccarelli et al. 2018
	Commercial sample	Cell culture CRC cell lines (HCT116, HT29/219, and SW742) DNA methylation analysis RNA extraction and RT-PCR	Cell-type-specific differences in expression of DNMT1, DNMT3a, and 3b genes Induced global hypermethylation in HT29/219 and HCT116 cells, but reduced methylation in Caco2 cells Induced the promoter demethylation of Cox2 in HT29/219, p14 and PPARγ in HCT116, and ECAD in SW742 cells	Sarabi and Naghibalhossaini 2018
	Commercial sample	Cell culture (McRH-7777 cells) Flow cytometry analysis of apoptosis qRT-PCR Immunoblot analysis DNA isolation and HIC-1 gene analyses for CpG islands ChIP	EPA inhibited HDAC1 and DNMT expression and activity, thus promoting tumor suppressor gene expression EPA bound and activated PPARγ thus downregulating HDAC1 which sequentially reduced expression of DNMT1, 3A and 3B, in hepatocarcinoma cells (HCC) EPA-bound PPARγ induced re-expression of the tumor suppressor gene Hic-1	Ceccarelli et al. 2020
Omega-3 polyunsaturated fatty acids	Not reported	LC-MS/MS qRT-PCR	Lowered the incidence and size of the tumor compared with colorectal cancer (CRC) model rats Increased the 5hmC percentage Positive correlation of the anticancer effect of the molecule with global 5hmC accumulation and TET1 expression	Sarabi and Naghibalhossaini 2018
Linoleic acid	Commercial sample	Cell culture CRC cell lines (HCT116, HT29/219, and SW742) DNA methylation analysis RNA extraction and RT-PCR	Cell-type specific differences in expression of DNMT1, DNMT3a, and 3b genes	Sarabi and Naghibalhossaini 2018
Squamocin	Commercial sample	Cell culture (GBM8401, Huh-7, and SW620 cell lines) Cell growth inhibition assay Western blot analysis qRT-PCR	Inhibited the proliferation of GBM8401, Huh-7, and SW620 cells Arrested the cell cycle at the G_1 phase Activated both intrinsic and extrinsic pathways to apoptosis Modulated the phosphorylation levels of H3S10 (H3S10p) and H3S28 (H3S28p) in association with the downregulation of aurora B and pMSK1 expressions Affected the epigenetic alterations by modulating histone H3 phosphorylation at S10 and S28	Lee et al. 2011

Table 4 (continued)

Bioactive molecules	Origin	Experimental methods	Key results	References
Inositol hexaphosphate (IP6)	Commercial sample	ENU-induced lung tumors DNA isolation Bisulfite modification of DNA and MSP Global DNA methylation analysis using the Sss1 methylase assay RT-PCR Western blot analysis	Reduced the expressions of DNMT1, MeCP2, MBD1, and HDAC1 Reduced the expression of COX-2 (14%) and MLH1 (21%) Reduced the downregulation of the tumor suppressor gene p16 (6%) Reduced the upregulation of DNMT1 (13%), MeCP2 (18%), MBD1 (30%), MBD2 (5.2%), and HDAC1 (12%)	Pandey and Gupta 2010
	Commercial sample	Colorectal cancer cell (CRC) DMH-induced rat CRC model Real-time PCR analysis of mRNA expression Western blot analysis Immunohistochemical staining	Inhibited the tumors, in terms of tumor incidence, number, weight, and volume in DMH-induced rats Decreased the Akt and c-Myc mRNA levels Downregulated the Akt, pAkt, pGSK-3β, and c-Myc protein expression Upregulated the pβ-catenin protein expression	Yu et al. 2017

that butyrate affects the cell cycle, apoptosis, central energy metabolism, and protein biosynthesis in the CHO DP-12 cell line. On the other hand, the authors found that differential methylated regions contain binding-site motifs of transcription factors.

Butyric acid

This acid is a short-chain fatty acid (CH_3 CH_2 CH_2CO_2 H) formed when the good bacteria in our gut breakdown dietary fiber. Tiwari and Gupta (2014) studied in vivo the chemopreventive effect of butyric acid alone or in combination with nicotinamide and calcium glucarate. After 16 weeks of treatment, butyric acid was shown to prevent tumor development, but protection was greatly improved when combined with nicotinamide and calcium glucarate. Molecular analyses revealed that butyric acid had an antitumor effect against 7,12-dimethylbenz[a]anthracene (DMBA)-induced tumor by downregulating miR-203 levels at 16 weeks and upregulating HDAC, DNMT, and DNA methylation promoter of miR-203 at 4 or 16 weeks. In addition, the co-administration of butyric acid combined with nicotinamide and calcium glucarate prevented the effect of altered gene expression (after 16 weeks) more than that of the single compound through regulation of miR-203 status via epigenetic modifications.

Docosahexaenoic acid (DHA)

It is an omega-3 fatty acid present in different vegetables. Some studies demonstrated the activity of this acid against various cancer cells through their epigenetic regulation. Cho et al. (2014) conducted a study to illustrate the apoptosis effect of DHA combined with butyrate. The results of this study were previously reported in the butyrate section. In addition to this, the acid decreased *Cideb*, *Dapk1*, and *Tnfrsf25* methylation promoters. In 2018, Sarabi and Naghibalhossaini (2018) studied the effect of some polyunsaturated fatty acids, including DHA, on DNA methylation and DNMT expression in HCT116, HT29/219, SW742, LS180, and Caco2 cell lines (human colorectal cancer cells). After 6 days of treatment with DHA, cell-type-specific differences in the expression of the DNMT1, DNMT3a, and 3b genes were observed. Moreover, this acid induced global hypermethylation in HT29/219 and HCT116 cells, while reducing methylation in Caco2 cells were observed. In addition, the demethylation of the Cox2 promoter in the HT29/219, p14, and PPARγ promoters in HCT116 cells and the ECAD promoter in SW742 cells was induced when the cells were treated with DHA.

Eicosapentaenoic acid

Eicosapentaenoic acid (EPA) is also an omega-3 fatty acid. The mechanism of action of DNA demethylation induced by EPA in carcinoma cells revealed that this molecule increases 5-hydroxymethylcytosine (5hmC) on DNA, inducing $p21^{WAF1/CIP1}$ gene expression, which slows the progression cycle of cancer cells, as well as the simultaneous binding to the peroxisome proliferator-activated receptor γ (PPARγ) and retinoid X receptor α (RXRα), thereby promoting their heterodimer and inducing a PPARγ-ten-eleven translocation (TET1) interaction (Ceccarelli et al. 2018). In addition, these results indicate that eicosapentaenoic acid exerts its anticancer activity by regulating DNA methylation levels allowing TET1 to perform its function. In the same year, a study carried by Sarabi and Naghibalhossaini (2018), for the evaluation of the effect of EPA on DNA methylation and DNMT expression in

human colorectal cancer cells, showed results identical to those previously obtained by docosahexaenoic acid.

A recent study aimed to demonstrate the molecular mechanism of EPA in inhibiting the expression of HDAC1 and DNMT in carcinoma cells (Ceccarelli et al. 2020). The results of this work reported that this acid inhibits HDAC1 and DNMT expression and thus promotes the expression of the tumor suppressor gene. This mechanism is produced by the binding of EPA to PPARγ and activates it in hepatocellular carcinoma (HCC) cells, thereby downregulating HDAC1, which sequentially reduces the expression of DNMT1, 3A, and 3B. Moreover, EPA bound to PPARγ induced re-expression of the tumor suppressor gene Hic-1.

Omega-3 polyunsaturated fatty acids (omega-3 PUFAs)

In 2019, colorectal cancer (CRC) model rats used by Huang et al. (2019) to investigate the antitumor effect of omega-3 PUFAs on DNA demethylation pathways. From the results, it was found that the group treated with omega-3 PUFAs had a lower incidence and tumor size than the control group. In addition, a significant increase in the 5hmC percentage was observed in the omega-3 PUFA-treated group compared to the control group. In summary of this study, a positive correlation was noted between the anticancer effect of the omega-3 PUFAs and global 5hmC accumulation and TET1 expression.

Linoleic acid

A study by Sarabi and Naghibalhossaini (2018) to test the antitumor activity of polyunsaturated fatty acids, including linoleic acid, on DNMTs in HCT116, HT29/219, SW742, LS180, and Caco2 cell lines. After 6 days of treatment with linoleic acid, the authors recorded cell-type-specific differences in the expression of the DNMT1, DNMT3a, and 3b genes.

Squamocin

In 2011, Lee et al. (2011) studied the possible antitumor mechanism of squamocin on different cell lines (GBM8401, Huh-7, and SW620). It was found that squamocin inhibited the proliferation of cells tested by inducing changes in apoptosis and cell cycle. Moreover, squamocin also affects histone H3 phosphorylation, which is associated with the expressions of these histone-modifying enzymes. Moreover, squamocin caused an arrest in the cell cycle at the G_1 phase and induced apoptosis via extrinsic and intrinsic pathways. In addition, the results showed that squamocin affects the epigenetic pathways in S10 and S28 cells by modulating histone H3 phosphorylation with downregulation of pMSK1 and aurora B expressions.

Inositol hexaphosphate

Pandey and Gupta (2010) evaluated in vivo the antitumor effect of inositol hexaphosphate (IP_6) in mouse lungs before tumor development, using female albino Swiss mice made diabetic by intravenous injection of ethylnitrosourea (120 mg/kg). After 3 months of treatment, IP_6 reduced the expressions of DNMT1, MeCP2, MBD1, and HDAC1 through the reduction of the upregulation of these genes; it thus reduced the expression of COX-2 (14%) and MLH1 (21%). IP_6 also reduced the downregulation of the tumor suppressor gene p16 (6%).

Furthermore, the anticancer and antiproliferative effects of this molecule have been studied by Yu et al. (2017) in rats with CRC. Consequently, it inhibited tumors, in terms of incidence, number, weight, and volume. In terms of antitumor mechanism, IP_6 decreased the Akt and c-Myc mRNA levels through downregulation of Akt, pAkt, pGSK-3β, and c-Myc protein expression, as well as upregulation of the expression of the pβ-catenin protein. These results revealed that IP_6 may have an antiproliferative effect against colorectal cancer by crosstalk between the PI3K/Akt and Wnt pathways.

Lignans as epidrugs against cancer

Lignans are phenolic compounds belonging to phytoestrogen metabolism. Recent findings revealed the anticancer effects of some lignan's compounds such as honokiol, peperomin E, and nordihydroguaiaretic acid (Table 5 and Fig. 5).

Honokiol

Honokiol is a poly-phenolic compound found in species of the genus *Magnolia.* Prasad et al. (2017) examined the effects of this compound on UVB-induced immunosuppression by utilizing contact hypersensitivity (CHS) in C3H/HeN mice. Topical application of this molecule to the skin at a dose of 0.5 and 1.0 mg/cm^2 inhibited the UVB-induced suppression of the CHS response in mice and inhibited the COX-2 expression and the PGE2 production in the UVB-exposed skin. In addition, the global DNA methylation analysis shows that the honokiol prevented the UVB-induced DNA hypermethylation in mouse skin by increasing the levels of TET enzyme, which is responsible for this effect. A year later, Prasad and Katiyar (2018) studied the effect of honokiol in vitro on DNA methylation using two cell lines, PANC-1 and AsPC-1. The results show that treatment of these cells with honokiol for 5 days decreased the global DNA methylation levels (60–80%) compared to control cells. This molecule also increased the 5-hmC levels in a dose-dependent manner as well as the levels of TET activity and protein expression in pancreatic cancer cell lines. Moreover, cells treated with honokiol reactivated the tumor

Table 5 Effects of lignans on epigenetic pathways in cancer

Bioactive molecules	Origin	Experimental methods	Key results	References
Honokiol	Commercial sample	Female C3H/HeN mice Mouse skin Global DNA methylation analysis DNMT and TET activity assays Western blot analysis	Inhibited the UVB-induced suppression of the CHS (contact hypersensitivity) response in mice Inhibited the COX-2 expression and the PGE2 production in the UVB-exposed skin Prevented the UVB-induced DNA hypermethylation in mouse skin	Prasad et al. 2017
	Commercial sample	Pancreatic cancer cell lines (PANC-1 and AsPC-1) Global DNA methylation analysis Flow cytometry analysis	Decreased the global DNA methylation levels (60–80%) Increased the 5-hmC levels in pancreatic cancer cells Increased the levels of TET activity and protein expression in cancer cell lines Reactivated the tumor suppressor genes/proteins levels, such as $p16^{INK4a}$ and RASSF1A in cancer cells	Prasad and Katiyar 2018
Nordihydroguaiaretic acid	Not reported	Human breast cancer cell line T47D Growth inhibition assay Bisulfite modification and MSP DNA sequencing RT-PCR Western blot analysis Cell cycle analysis by flow cytometry	Inhibited the growth of cancer cells Induced a partial demethylation of the 5' region of the $p16^{INK4a}$ gene in both cell lines Restored the expression of $p16^{INK4a}$ mRNA Induced a cell cycle arrest in the G_1 phase	Cui et al. 2008a
	Commercial sample	Human breast cancer cell lines SKBR3 and MDA-MB-435 DNA and RNA extraction MTT cell proliferation assay RT-PCR Western blot analysis Tumorigenesis assay in nude mice	Inhibited the growth of human breast cancer cell lines SKBR3 (IC_{50} = 31.09 ± 1.6 μmol/L) and MDA-MB-435 (IC_{50} = 38.8 ± 2.1 μmol/L) Inhibited the growth of human breast carcinoma cells in both animal and cell-based models Reactivated the methylation-silenced E-cadherin gene in vitro and in vivo	Cui et al. 2008b
Peperomin E	*Peperomia dindygulensis*	BEAS-2B, A549, H1229, and NCI-H460 cells In vitro cytotoxicity assay In vivo tumorigenicity assay Apoptosis and cell cycle analysis Determination of genomic DNA methylation levels In vitro DNMT inhibition assay Bisulfite modification and MSP Quantitative real-time PCR	Inhibited cell proliferation Induced the apoptosis and cell cycle arrest in non-small-cell lung cancer (NSCLC) cell lines in a dose-dependent manner Inhibited the tumor growth in A549 xenograft BALB/c nude mice Interacted with the active domain of DNMT1, potentially affecting its genome methylation activity Decreased DNMT1 activity and expression Decreased global methylation Reactivated the epigenetically silenced tumor suppressor genes including *RASSF1A*, *APC*, *RUNX3*, and $p16^{INK4}$	Wang et al. 2016a
	Peperomia dindygulensis	Gastric cancer cells Proliferation assay Orthotopic xenograft NOD-SCID mouse model DNMT activity assay Molecular modeling Quantitative real-time RT-PCR assay MSP assay Western blot analysis Global DNA methylation analysis	Suppressed invasion and migration of poorly differentiated gastric cancer cells (in vitro and in vivo) in a dose-dependent manner Exhibited a covalent bond with the catalytic domain of DNMT1 Inhibited the DNMT1 activity (IC_{50} = 3.61 μM) Downregulated the DNMT1, 3a, and 3b mRNA and protein expression in gastric cancer cells Induced a relative global DNA hypomethylation	Wanga et al. 2018

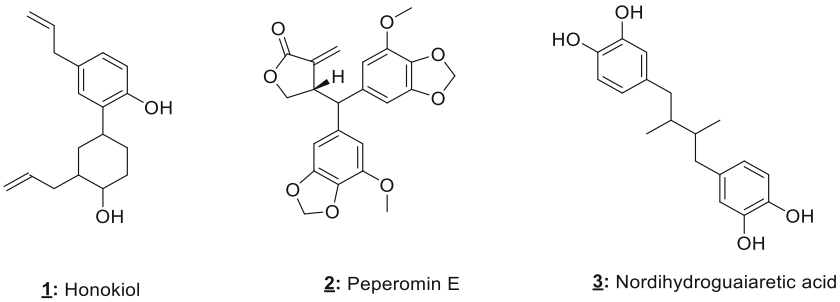


Fig. 5 Chemical structures of lignans

suppressor genes/proteins levels, such as $p16^{INK4a}$ and RASSF1A in cancer cells.

Nordihydroguaiaretic acid

Nordihydroguaiaretic acid (NDGA) is a lignan present in large amounts in *Larrea tridentate*. Cui et al. (2008a) carried out two studies to demonstrate the prevention mechanism that NDGA plays in the epigenetic modifications in human breast cancer cells, using two cancer cell lines, T47D and RKO. NDGA was able to inhibit the growth of cancer cells by arresting the cell cycle in the G_1 phase. Moreover, NDGA induced a partial demethylation of the 5′ region of the $p16^{INK4a}$ gene in both cell lines, which restored the expression of $p16^{INK4a}$ mRNA. The other study was performed in vitro on SKBR3 and MDA-MB-435 cancer cell lines, also in vivo on T cell-deficient nude (nu/nu) mice. The in vitro results show that NDGA limited the growth of human breast cancer cell lines SKBR3 (IC_{50} = 31.09 ± 1.6 μmol/L) and MDA-MB-435 (IC_{50} = 38.8 ± 2.1 μmol/L). In addition, in both models, it was shown that NDGA inhibits the growth of human breast carcinoma, as well as reactivates the methylation-silenced E-cadherin gene (Cui et al. 2008b).

Peperomin E

The antitumor effects of peperomin E and its possible mechanisms have been evaluated (in vitro and in vivo) by Wang et al. (2016a). This molecule is a natural secolignan isolated from *Peperomia dindygulensis*. The assays applied have shown that this molecule inhibits the proliferation of BEAS-2B, A549, H1229, and NCI-H460 cells in a dose-dependent manner and induces apoptosis and cell cycle arrest in non-small-cell lung cancer (NSCLC) cell lines of the same way. In vivo treatment of A549 xenograft in BALB/c nude mice model with peperomin E inhibited the tumor growth without side effects. Furthermore, using an in silico target fishing method, it was found that peperomin E inhibits DNMT1 by the interaction with this active domain of DNMT1, which importantly affect genome methylation status. In addition, other results showed that this molecule decreased global methylation and reactivated silenced tumor suppressor genes via epigenetic modulators such as RASSF1A, APC, RUNX3, and $p16^{INK4}$DNMT1 activity and expression, through decreased DNMT1 activity and its expression. Two years later, the same authors (Wanga et al. 2018) studied the same in vitro activity of peperomin E on GC cancer cell and on an orthotopic xenograft mouse model in vivo. Peperomin E, in a dose-dependent manner, suppressed the invasion and migration of poorly differentiated gastric cancer cells (in vitro and in vivo). This molecule also exhibited a covalent bond with the catalytic domain of DNMT1 and inhibited the DNMT1 activity with an IC_{50} value of 3.61 μM by downregulating the DNMT1, 3a, and 3b mRNA and protein expression in gastric cancer cells. The inhibition mechanism that peperomin elicits on DNMT1activity was caused by the induction of relative global DNA hypomethylation.

Polysaccharides as epidrugs against cancer

Fucoidan is a natural sulfated polysaccharide substance (Fig. 6) of marine origin located in brown algae cell membrane. Yan et al. (2015) revealed that fucoidan may have an antitumor effect against hepatocellular carcinoma (HCC) by upregulating miR-29b of human HCC cells and suppressing DNMT3B, which led to the MTSS1 upregulation. On the other hand, the signaling pathway of HCC cells for TGF-β receptors and Smad signaling of HCC cells were also

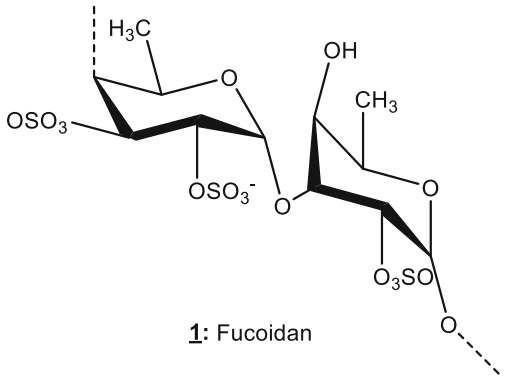


Fig. 6 Chemical structures of fucoidan

"downregulated." These findings led to the reduction of EMT and the prevention of degradation of the extracellular matrix, which reduced HCC cell invasion activity (Table 6).

Another study by Liao et al. (2019) proved the inhibitory effect of oligo-fucoidan (OF) which clearly inhibited the proliferation of malignant glioma cells but only partially influenced that of SVGp12 cells, the results showed. Supplemented by strong induction of mRNA differentiation marker (MBP, OLIG2, S100β, GFAP, NeuN, and MAP2) in U87MG and GBM8401 cells, OFs inhibited the expression of DNMT1, 3A, and 3B protein expression, as a consequence, OF reduced the methylation of p21, a DNMT3B target gene. OF could also establish synergies between growth inhibition and MBP induction in U87MG cells in combination with the clinical inhibitor of DNMT, decitabine.

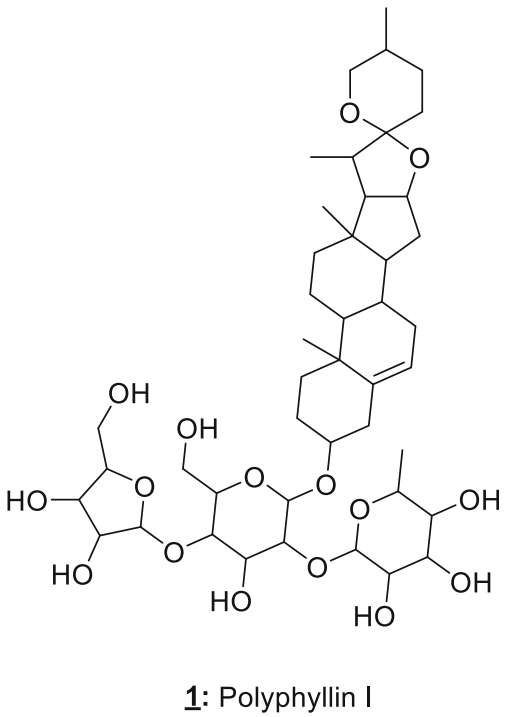
Table 6 Effects of polysaccharides on epigenetic pathways in cancer

Bioactive molecules	Origin	Experimental methods	Key results	References
Fucoidan	Not reported	Human hepatocellular carcinoma cell lines (Huh6, Huh7, SK-Hep1, and HepG2) Cell viability (MTS assay) Colony formation assay RNA isolation and RT-PCR MicroRNA profiling Quantitative RT-PCR Western blot analysis	Upregulated the miR-29b of human HCC cells Suppressed the downstream target DNMT3B of miR-29b in a dose-dependent manner Increased the mRNA and protein levels of MTSS1 (metastasis suppressor 1), a target silenced by DNMT3B Downregulated the TGF-β receptor and Smad signaling of HCC cells	Yan et al. 2015
	Not reported	Human glioblastoma multiforme cell line GBM8401 Cell viability Quantitative RT-PCR Western blot analysis DNA isolation MSP	Suppressed the proliferation of malignant glioma cells Inhibited the protein expressions of DNMT1, 3A, and 3B accompanied with obvious mRNA induction of differentiation markers (*MBP*, *OLIG2*, *S100β*, *GFAP*, *NeuN*, and *MAP2*) both in U87MG and GBM8401 cells Decreased the methylation of p21, a DNMT3B target gene	Liao et al. 2019

Saponins as epidrugs against cancer

Polyphyllin I

Polyphyllin I (PPI), an active compound (Fig. 7) belongs to saponin subclass, isolated from *Parispolyphylla*. Several studies were undertaken to discuss its inhibitory effect on tumor growth. Li et al. (2016) tested this compound on NSCLC cells. The results showed growth inhibition and cell cycle arrest in a dose-dependent manner, with increased phosphorylation of SAPK/JNK signaling cascades, decreased the expression of p65 and DNMT1 proteins, and decreased the activity of EZH2 protein, mRNA, and promoter. In addition, PPI was shown to have a decreasing effect on tumor growth, protein expression levels of p65, DNMT1 and EZH2, with increased phosphorylation of SAPK/JNK in vivo. A study by Xiang et al. (2018) aimed to assess the effect of this compound on castration-resistant prostate cancer (CRPC) cells. The findings revealed that PPI exerts an inhibitory effect on growth and



1: Polyphyllin I

Fig. 7 Chemical structures of polyphyllin

induces cell cycle arrest, limiting migration and invasion in this type of cell, with down-expressing proteins EZH2 and DNMT1 and lowering levels of HOTAIR mRNA (Table 7).

Compound K

In 2013, a study by Kang et al. (2013) was performed to determine whether this compound has the capacity to reactivate tumor suppressor genes in human colorectal cancer HT-29 cells by reducing DNMT activity. The results revealed that this compound inhibits growth of the HT-29 cell at a dose- and time-dependent manner. In addition, compound K inhibited the expression and activity of DNMT1, which led to reverse the methylation and reactivated the tumor suppressor gene RUNX3. It was revealed that this compound induces also the expression of Smad4 and Bim as well as the upregulation of extracellular signal-regulated kinase (ERK) inhibition.

Secoiridoids as epidrugs against cancer

Oleacein

Oleacein is a polyphenol (Fig. 8) of the secoiridoid group found in *Olea europaea* L. Juli et al. (2019) studied in vitro

Table 7 Effects of saponins on epigenetic pathways in cancer

Bioactive molecules	Origin	Experimental methods	Key results	References
Polyphyllin I (PPI)	Commercial sample	Non-small cell lung cancer (NSCLC) cells Cell viability and cell cycle distribution Flow cytometry assays qRT-PCR) Western blot analysis Transient transfection assays A xenografted tumor model in nude mice	Inhibited growth and induced cell cycle arrest of NSCLC cells in a dose-dependent manner Increased the phosphorylation of SAPK/JNK Reduced the protein expression of p65 and DNMT1 Reduced EZH2 protein, mRNA, and promoter activity Inhibited the tumor growth, protein expression levels of p65, DNMT1, and EZH2 Increased phosphorylation of SAPK/JNK in vivo	Li et al. 2016
	Commercial sample	Castration-resistant prostate cancer (CRPC) cells Cell viability assay Cell cycle analysis Transwell Matrigel invasion assay qRT-PCR Western blot analysis Xenografted tumor model	Inhibited the growth and induced cell cycle arrest of CRPC cells Reduced the migration and invasion in CRPC cells Decreased the protein expressions of EZH2, DNMT1, and levels of HOTAIR (HOX transcript antisense RNA)	Xiang et al. 2018
Compound K		Human colorectal cancer HT-29 cells Cell proliferation assay RT-PCR MSP assay Immunocytochemistry DNMT activity assay ChIP assay Transient transfection of small RNA interference (siRNA)	Inhibited the HT-29 cell growth in a dose- and time-dependent manner Reversed the methylation and reactivated the tumor suppressor gene RUNX3 Inhibited the expression and activity of DNMT1 Induced the RUNX3-mediated expression of Smad4 and Bim Suppressed the DNMT1 activity by inhibiting the ERK pathway	Kang et al. 2013

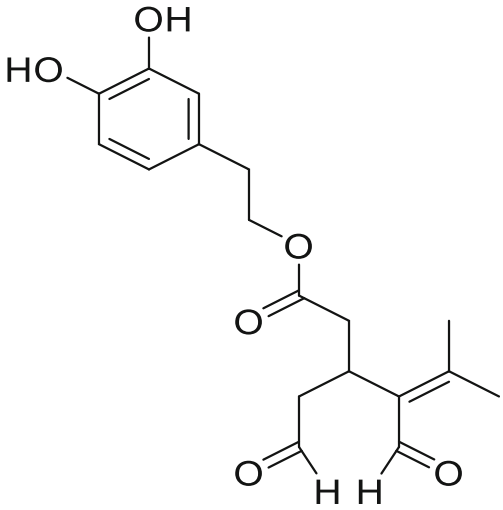


Fig. 8 Chemical structure of oleacein

the antitumor potential of oleacein against human multiple myeloma (MM) cell and its underlying bio-molecular sequelae. The results showed that oleacein was able to reduce the viability of primary human MM samples and cell lines, inhibit the clonogenicity of human MM, and induce cell cycle blockade and trigger apoptosis (Table 8). Oleacein has shown an effect in the epigenetic regulation by induced accumulation of acetylated histones and α-tubulin in a dose-dependent manner, as well as the downregulation of several class I/II HDACs both at the mRNA and protein level. However, this molecule had no effect on global DNA methylation. In terms of the mechanism of action, it was found that oleacein affected the downregulation of Sp1, the major transactivator of the HDAC promoter, via the activation of caspase-8, which inhibited HDAC. These results indicate that the oleacein inhibited the in vitro anti-MM effect of the proteasome inhibitor carfilzomib.

Oleosidedecarboxymethyl oleuropein aglycone (DOA)

In 2018, cancer stem cells (CSC) and female athymic nude mice were used by Corominas-Faja et al. (2018) to clarify the ability of DOA to suppress functional traits of CSC in breast cancer (BC). The results showed that DOA targets subpopulations of epithelial-like, mesenchymal-like, aldehyde dehydrogenase (ALDH)-positive, and $CD44^{+}/CD24^{-/low}$ CSC, as well as blocking the formation of multicellular tumor spheres generated from single founder stem-like cells in a panel of genetically diverse BC models. An in vivo study also showed that mice treated with DOA reduced subsequent tumor-forming capacity and inhibited the tumor growth for several months in mice orthotopically injected with CSC-enriched BC cell populations. In order to determine the mechanism by which DOA acts, an in silico assay was used to subsequently find that DOA inhibits the ATP-binding kinase domain site of mTOR. The following year, Verdura et al. (2019) showed that DOA is a phenol-conjugated oleoside present in extra-virgin olive oil (EVOO). The aim of their study was to assess the ability of DOA in the inhibition of neomorphic activity of mutant IDH1 (R132H) and reducing of 2HG production, reversing 2HG-driven rewiring of epigenetic and immunological landscape, and restricting 2HG-enhanced tumor-initiating capacity. The results showed that DOA inhibited the production of 2HG (2-hydroxyglutaratye) by neomorphic IDH1 (isocitrate dehydrogenase 1) mutations. In silico studies and molecular dynamic analyses showed that DOA preferentially occupied the allosteric pocket of the IDH1 mutant and inhibited the enzymatic activity of the recombinant IDH1 mutant protein (R132H) in the low micromolar range. After enzymatic analyses of IDH1 activity/inhibition, it was found that DOA suppresses 2HG overproduction in engineered human cells expressing heterozygous IDH1-R132H mutation and restores the 2HG-suppressed activity of histone demethylases. Further tests conducted during this study showed that DOA reactivated the expression of PD-L1 (via epigenetic control through an immunosuppressive gene silenced in IDH1 mutant cells). In addition, DOA inhibited the formation of IDH1 mutant colony cells.

Steroids as epidrugs against cancer

Natural steroids isolated from some medicinal plants exhibited anticancer effects on different cancer cell lines. Indeed, guggulsterone, prostaglandin E2, and withaferin A (Fig. 9) presented remarkable anticancer activity by targeting epigenetic pathways involved in cell transformation and cancerization (Table 9).

Withaferin A and guggulsterone

The steroid compounds, withaferin A (WA) and guggulsterone, exhibit remarkable cytotoxic activity as they induce beneficial changes in gene expression through epigenetic mechanisms (Mirza et al. 2013; Szarc vel Szic et al. 2014). Therefore, at low concentrations, these two compounds showed a significant effect on the regulation of epigenetic marks involved in the activation of tumor suppressor genes, where WA compound was the most investigated. Mirza et al. (2013) detected the potential of these molecules to reverse the epigenetic changes caused by DNA hypermethylation, with a decrease in the transcript levels of the DNA methyltransferases (DNMT1, DNMT3a, and DNMT3b) and their associated proteins (DNMT1, HDAC1, and MeCP2) in two human breast cancer cell lines, MCF7 and MDA-MB-231. Moreover, WA used alone or in combination with sulforaphane (SFN) (1 and 5 μM, respectively) has also been shown to

Table 8 Effects of secoiridoids on epigenetic pathways in cancer

Bioactive molecules	Origin	Experimental methods	Key results	References
Oleacein	*Olea Europaea* L.	Multiple myeloma (MM) cell lines NCI-H929, RPMI-8226, U266, MM1s, and JJN3 Cell viability, apoptosis, and cell cycle assay Western blot analysis qRT-PCR HDAC activity assay Quantification of global 5-methylcytosine levels	Reduced the viability of MM primary samples and cell lines Inhibited the MM cell clonogenicity Prompted cell cycle blockade Triggered apoptosis Induced a dose-dependent accumulation of both acetylated histones and α-tubulin, along with downregulation of several class I/II HDACs both at the mRNA and protein level No effect on global DNA methylation HDAC inhibition was associated with downregulation of Sp1, the major transactivator of HDACs promoter, via caspase-8 activation Enhanced the in vitro anti-MM activity of the proteasome inhibitor carfilzomib	Juli et al. 2019
Oleoside decarboxymethyl oleuropein aglycone (DOA)	Not reported	Cancer stem cells (CSC) in breast cancer (BC) Cell viability assay Docking calculations DNMT activity/inhibition assays Tumor xenograft studies	DOA could selectively target subpopulations of epithelial-like, aldehyde dehydrogenase (ALDH)-positive and mesenchymal-like, $CD44^{+}CD24^{-/low}$ CSC Blocked the formation of multicellular tumor spheres generated from single founder stem-like cells in a panel of genetically diverse BC models Reduced subsequent tumor-forming capacity in vivo Inhibited the tumor growth for several months in mice orthotopically injected with CSC-enriched BC cell populations Bound and inhibited the ATP-binding kinase domain site of mTOR and the S-adenosyl-l-methionine (SAM) cofactor- binding pocket of DNMTs	Corominas-Faja et al. 2018
	Extra-virgin olive oil	Computational modeling of IDH1 Docking calculations Binding free energy analysis IDH1 activity/inhibition enzymatic assays X-MAN™ isogenic cell lines Quantification of 2-hydroxyglutarate H3K9me3 western blotting RNA isolation and reverse transcription PD-L1 gene expression Flow cytometry Colony formation assays	Inhibited the production of 2HG (2-hydroxyglutaratye) by neomorphic IDH1 (isocitrate dehydrogenase 1) mutations Occupied, preferentially, the allosteric pocket of mutant IDH1 Inhibited the enzymatic activity of recombinant mutant IDH1 (R132H) protein in the low micromolar range Suppressed 2HG overproduction in engineered human cells expressing a heterozygous IDH1-R132H mutation Restored the 2HG-suppressed activity of histone demethylases Restored, epigenetically, the expression of PD-L1, an immunosuppressive gene silenced in IDH1 mutant cells via 2HG-driven DNA hypermethylation Blocked colony formation of IDH1 mutant cells while sparing wild-type IDH1 isogenic counterparts	Verdura et al. 2019

be able to regulate the epigenetic marks (DNMT, HDAC, HMT, and HAT) (Rodríguez et al. 2017; Royston et al. 2018). In fact, WA induced a decrease of DNMT1, DNMT3A, and DNMT3B mRNA expression in MCF-7 cells, while only a significant decrease in DNMT3B mRNA expression was observed in MDA-MB-231 cells. However, the

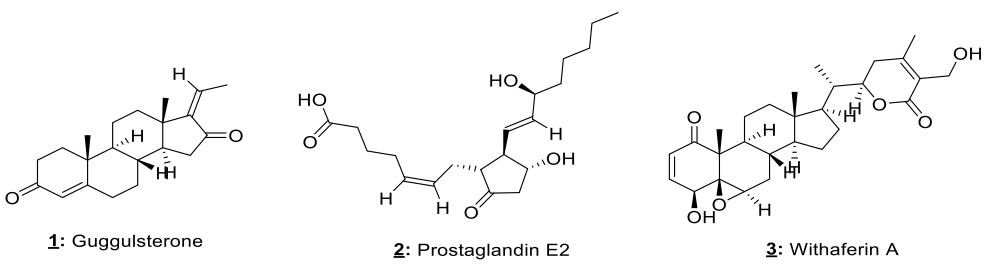


Fig. 9 Chemical structures of steroids

Table 9 Effects of steroids on epigenetic pathways in cancer

Bioactive molecules	Origin	Experimental methods	Key results	References
Guggulsterone	Commercial sample	Human breast carcinoma cell lines MCF7 and MDA-MB 231 Bisulfite conversion and MSP Real-time RT-PCR analysis Western blot analysis	Decreased the transcript levels of all the DNMTs investigated (DNMT1, DNMT3a, and DNMT3b) Decreased the protein levels of DNMT1, HDAC1, and MeCP2	Mirza et al. 2013
Prostaglandin E_2 (PGE_2)	Not reported	Colorectal cancer cells ApcMin/+ mice	Upregulated the expression of DNMT1 and DNMT3B, which resulted to increased DNA methylation in the promoters of target genes O6-methylguanine-DNA methyltransferase (MGMT) and cannabinoid receptor 1 (CB1) Decreased the expression of MGMT and CB1 Increased the expression of DNMT1 and DNMT3B and the DNA methylation of MGMT and CB1 promoters, and reduced the expression of MGMT and CB1 in vivo	Xia et al. 2011
	Commercial sample	Female C3H/HeN mice Dot-blot analysis of 5-mC for DNA methylation Analysis of global DNA methylation Assay of DNMT activity Western blot analysis	Decreased the levels of global DNA methylation in the mouse skin Decreased the levels of DNMT activity in the AH6809-treated UVB-exposed mouse skin Decreased the levels of DNMT1, DNMT3a, and DNMT3b proteins in the AH6809-treated UVB-exposed mouse skin Reduced the expression of global DNA methylation and DNMT activity and lowered the levels of DNMT proteins Enhanced the levels of global DNA methylation Increased the DNMT3a expression through increased expression of Sp1 and Sp3	Prasad and Katiyar 2013
	Not reported	Myeloid-derived suppressor cells (MDSCs) DNA methylation profiling using universal bead arrays Detection of differentially methylated CpGs Bisulfite pyrosequencing Expression array	Upregulation of MDSC-specific DNMT3A, which is PGE_2 dependent, that is necessary for the acquisition of their immunosuppressive capacity, providing a novel target for therapeutic intervention	Rodríguez et al. 2017
Withaferin A (WA)	Commercial sample	Human breast carcinoma cell lines MCF7 and MDA-MB 231 Bisulfite conversion and MSP Real-time RT-PCR analysis Western blot analysis	Decreased the transcript levels of all the DNMTs investigated (DNMT1, DNMT3a, and DNMT3b) Decreased the protein levels of DNMT1, HDAC1, and MeCP2	Mirza et al. 2013
	Commercial sample	MDA-MB-231 and MCF-7 cell lines Cell viability Cell cycle analysis Invasion assay RNA extraction and microarray processing RT-PCR and real-time quantitative PCR Western blot analysis Human epigenetic chromatin modification enzyme qPCR array	Demonstrated the attenuation of multiple cancer hallmarks Targeted the specific cancer processes related to cell death, cell cycle, and proliferation Decreased the MDA-MB-231 invasion Decreased gene expression of extracellular matrix-degrading proteases (uPA, PLAT, ADAM8) and cell adhesion molecules (integrins, laminins) Increased the expression of the validated breast cancer metastasis suppressor gene (BRMS1) Decreased protein levels and corresponding activity of uPA in MDA-MB-231 cell supernatant Reprogrammed the transcription levels of invasive mesenchymal MDA-MB-231 cells	Szarc vel Szic et al. 2014
	Commercial sample	Breast cancer cells (MCF-7 and MDA-MB-231) Cell density assay MTT assay RNA isolation qRT-PCR Western blot analysis DNMTs and HDAC activity assay	WA + SFN promoted cell death WA + SFN decreased the HDAC expression and promoted varying changes in DNMT expression WA + SFN induced the changes in BAX and BCL-2	Royston et al. 2018

Table 9 (continued)

Bioactive molecules	Origin	Experimental methods	Key results	References
	Commercial sample	MDA-MB-231 and MCF-7 cell lines DNA extraction and bisulfite conversion RT-PCR and real-time quantitative PCR Western blot analysis ChIP assay	No effect on global DNA methylation changes in aggressive metastatic MDA-MB-231 human breast cancer cells Induced the DNA hypermethylation and gene expression alterations reveals epigenetic reprogramming of ADAM8, PLAU, TNFSF12, ME3, and GSTM1 target genes, associated with HER2/PR/ESR status in TNBC (triple negative breast cancer) Induced the chromatin silencing at the *PLAU* gene promoter	Szarc vel Szic et al. 2017
	Commercial sample	Breast cancer cell lines (MCF-7 and MDA-MB-231) Flow cytometry cell cycle analysis DNA extraction Quantitative RT-PCR Western blot analysis Global methylation activity assay Histone acetyltransferase activity/Inhibition assay Histone methyltransferase activity/Inhibition assay ChIP assay	WA + SFN regulated cell cycle progression from S to G_2 phase through inhibition of cell cycle genes in breast cancer cells WA + SFN promoted changes in epigenetic regulators in MCF-7 and MDA-MB-231 cells WA + SFN promoted changes in p53 and p21 in breast cancer cells	Royston et al. 2018

combinatorial treatment (SFN + WA) showed a high significant decrease in DNMT1, DNMT3A, and DNMT3B mRNA and their protein expression in both breast cancer cell lines. Regarding the effects of WA and WA + SFN on HDAC, WA induced considerable downregulation of mRNA and protein expression of HDAC1 in both cell lines, with a potent effect in combination with SFN. Additionally, WA and combination therapy decreased histone methyltransferase (HMT) and increased histone acetyltransferase (HAT) activities in both cell lines, with significant impact on MDA-MB-231 cells (Royston et al. 2018). In contrast, Szarc vel Szic et al. (2014) revealed that WA induced a small change in expression of DNMT manifested by only decreased expression of DNMT3B in MCF-7 cells. These activities of WA and the combinatorial treatment (SFN + WA) have been associated with decreasing anti-apoptotic BCL-2 and an increasing proapoptotic BAX (Rodríguez et al. 2017). Furthermore, a significant downregulation of cyclin D1, CDK4, and p-RB, known by their various roles in the cell cycle, and an increase in the levels of E2F mRNA and tumor suppressor p21 were shown under the WA and SFN + WA treatments (Royston et al. 2018). Furthermore, both transcription activators, FOXM1 and E2F1, were inhibited and a cyclin-dependent kinase inhibitor 2A (CDKNA2A) was activated after WA treatment. While WA administration is predicted to repress histone demethylase JARID1B and activate histone acetylase p300 activities, in MDA-MB-231 and MCF-7 cell lines. On the other hand, WA could dramatically reduce cell motility, invasion, and epithelial-mesenchymal transition, all of which are key processes in breast cancer metastasis in MDA-MB-231 cells (Szarc vel Szic et al. 2017). Cell motility and invasion were inhibited both by the activation of the transcription factor SPDEF known by its inhibitory role in the migration of several types of cancer and by an increase in the expression of a well-established breast cancer metastasis suppressor 1 (BRMS1) following WA treatment (Szarc vel Szic et al. 2014). Moreover, the impact of WA treatment on MDA-MB-231 cells and their invasion is also mediated by the downregulation of tumor-promoting genes, such as urokinase-type plasminogen activator (PLAU), tumor necrosis factor (ligand) superfamily, ADAM metallopeptidase domain 8 (ADAM8), member 12 (TNFSF12), genes implicated in the detoxification (glutathione-S-transferase mu 1, GSTM1), and/or mitochondrial metabolism (malic enzyme 3, ME3) (Szarc vel Szic et al. 2014; Szarc vel Szic et al. 2017).

Tannins as epidrugs against cancer

Ellagic acid

Ellagic acid (EA) (Fig. 10), a ubiquitous polyphenol biosynthesized by many fruits and vegetables, showed potent cytotoxicity activity against MCF7 cells with a noticeable decrease in the activity of PARG (enzyme which might play a role in the anticytotoxic effect). In addition, EA significantly inhibited DNMT activity in MCF7 cells, without affecting the DNMT1 transcription or DNMT1 protein levels (Table 10). This compound, however, had no effect on the global

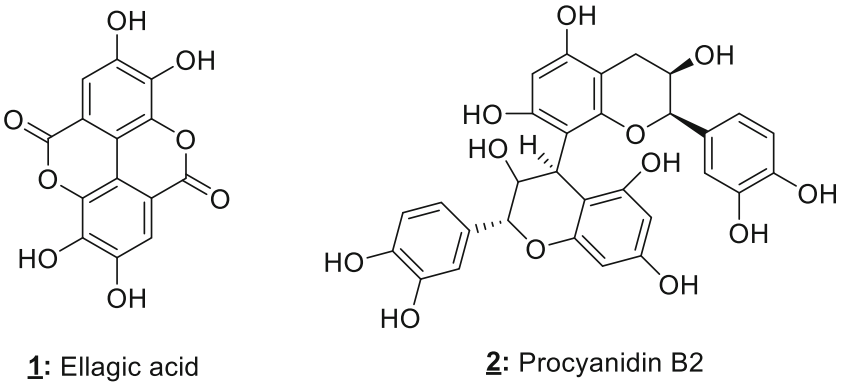


Fig. 10 Chemical structures of tannins

Table 10 Effects of tannins on epigenetic pathways in cancer

Bioactive molecules	Origin	Experimental methods	Key results	References
Ellagic acid (EA)	Commercial sample	Epithelial breast cancer MCF7 cell line	Inhibited the DNMT activity	Paluszczak et al. 2010
		Determination of the activity of DNMTs	No effect on the methylation pattern or the expression of *RASSF1A*, *GSTP1*, or *HIN-1* in MCF7 cells	
		DNA methylation analysis	No effect on the global methylation of histone H3	
		Real-time PCR	Increased *GSTP1* transcript level	
		Western blot analysis	No effect on DNMT1 transcription or on DNMT1 protein level	
	Not reported	Human prostatic cancer cell lines, LnCap, and DU145	Reduced the levels of chromogranin A (CgA)	Vanella et al. 2013
		Immunocytochemistry	Increased the expression of p75-NGFR	
		Western blot analysis	Decreased the phospho-Rb (p-Rb), DNMT1 levels, and Akt activation/phosphorylation	
		ELISA		
		COMET assay	Increased the DNA damage, in a dose-dependent manner, both in LnCap and DU145 cell lines	
	Commercial sample	Human adipogenic stem cells (hASCs)	Inhibited the HDAC9 downregulation	Kang et al. 2014
		qPCR and microarray analysis	Reduced the histone acetylation levels	
		Western blot analysis	Reduced the histone 3 arginine 17 methylation levels (H3R17me2), implying the inhibitory role of EA in coactivator-associated arginine methyltransferase 1 (CARM) 1 activity during adipogenesis	
		HDAC enzyme activity assays		
		Depletion of HDAC9 using siRNA		
		Immunocytochemistry of H3R17me2 and HDAC9		
Procyanidin B2	Commercial sample	MDA-MB-231 and human keratinocyte cells HaCaT	Interacted as a potent inhibitor having medicinally relevant chemical space	Shilpi et al. 2015
		In silico investigations	Attenuated the DNMT activity (IC_{50} = 6.88 ± 0.647 μM)	
		Docking protocols	Enhanced the expression of DNMT target genes, E-cadherin, Maspin and *BRCA1*	
		Molecular dynamic simulation analysis		
		DNMT inhibition assay		
		qRT-PCR		

methylation of histone H3 in MCF7 cells, as well as on the demethylation of RASSF1A, GSTP1, and HIN-1 genes, with only a slight increase in GSTP1 expression at the dose of 40 μM (Paluszczak et al. 2010). EA could also reduce the differentiation of adipocytes through the methylation of histone arginine and subsequent histone acetylation levels via epigenetic modification mediated by CARM1 inhibition (Kang et al. 2014). In fact, HDAC9 is characterized by a negative regulator role on adipogenesis, where in human adipogenic stem cells (*h*ASCs), high levels of HDAC9 repress the transcriptional activation of adipogenic genes (Chatterjee et al. 2011). During an adipogenic stimulus, CARM1 enzyme facilitates the transfer of two methyl moieties to H3R17, which is accompanied by H3K9 acetylation and HDAC9 dissociation. In contrast, the EA repression activity against adipogenesis is mediated by the inhibition of CARM1 enzyme activity leading to the suppression of H3R17 methylation and consequently reduces H3K9 acetylation and HDAC9 dissociation (Kang et al. 2014). Furthermore, EA has a significant activity in the treatment of prostate cancer with antiproliferative and pro-differentiation properties. Indeed, treatment of two prostate cancer cell lines, LnCap and DU145, with EA showed an important decline of glycoprotein chromogranin A (CgA), where high levels of CgA are used as a marker of neuroendocrine tumors (NET). Besides, EA induced an increase in p75NGFR (NGF receptor) expression, which is also considered to be a marker of prostate cancer (p75NGFR levels decrease during tumorigenesis). Moreover, a decrease in p-Rb, Akt activation/phosphorylation and DNMT1 had been demonstrated under EA treatment (Vanella et al. 2013; Ebert et al. 2020).

Procyanidin B2

Procyanidin B2 (Fig. 10) was shown to be cytotoxic against MDA-MB231 cells. At low doses, it is able to inhibit DNMT activity and decrease DNMT expression in correlation with the upregulation of the expression of the E-cadherin, Maspin, and BRCA1 genes in MDA-MB-231 cells (Shilpi et al. 2015).

Tanshinones as epidrugs against cancer

Tanshinone IIA

Tanshinone IIA (TIIA) (Fig. 11), a compound detected in *Salvia miltiorrhiza* species, showed a considerable anticancer effect against mouse skin epidermal JB6 cells through an epigenetic mechanism (Table 11). This compound has been shown to be effective in activating the Nrf2 signaling pathway (regulator of the antioxidative stress response) by the demethylation of the CpGs of Nrf2. This activity was accompanied by a decrease in DNMT1, DNMT3a, DNMT3b, and HDAC3 protein levels and an inhibition of HDAC enzyme activity.

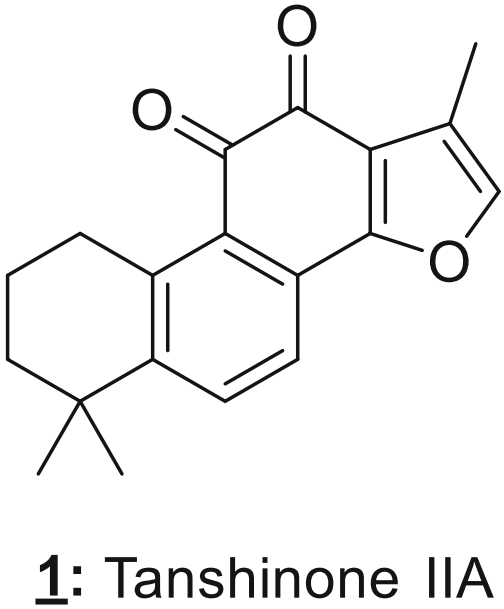


Fig. 11 Chemical structure of tanshinones

These findings also confirmed the important role of oxidative stress in carcinogenesis and cancer progression and, therefore, the importance of antioxidant compounds in cancer prevention and therapy (Slattery et al. 2014; Wang et al. 2014).

Other bioactive compounds as epidrugs against cancer

Other natural bioactive compounds isolated from medicinal plants have also shown anticancer properties against various human cancers. These substances include curcumol, chlorogenic acid, ascorbic acid, folic acid, vitamins, and others (Fig. 12). Currently, several pharmacological investigations revealed that these molecules exhibit their anticancer effects via epigenetic mechanisms (Table 12).

Amarogentin, eugenol, and EGCG

Amarogentin (detected in chirata), eugenol (produced by clove), and epigallocatechin gallate (EGCG, found in green tea), used alone or in combination (EGCG with eugenol and EGCG with amarogentin) have shown their ability to block cell proliferation and colony formation, as well as induce apoptosis and epigenetic modification in the HeLa cell line (Pal et al. 2019). The combinatorial treatments were more effective than each of the compounds tested alone. In fact, these compounds produce a downregulation of DNMT1, with an increase in cell cycle inhibitors (LIMD1, RBSP3, and p16) and a decrease in cyclin D1 that plays a role in the regulation of cell cycle progression (Pal et al. 2019). The upregulation of

Table 11 Effects of tanshinone on epigenetic pathways in cancer

Bioactive molecules	Origin	Experimental methods	Key results	References
Tanshinone IIA	Commercial sample	JB6 P+, JB6-shNrf2, and HepG2-C8 cells Cell viability tests Luciferase reporter activity assay RNA extraction and qRT-PCR Western blot analysis HDAC and DNMT activity assay DNA isolation and bisulfite genomic sequencing ChIP assay	Inhibited the TPA-induced JB6 P+ cell transformation The IC_{50} values of the viability of JB6 P+, JB6-shNrf2, and HepG2-C8 cells were 28.9, 20.1, and 26.3 μM, respectively Induced a luciferase activity in a dose-dependent manner at concentrations ranging from 5 to 25 μM Upregulated the mRNA and protein levels of Nrf2 target enzymes in JB6 P+ cells Inhibited the mRNA and protein expression of epigenetic modification enzymes in JB6 P+ cells Inhibited the HDAC activity by 50% with a treatment of 5.0 or 10.0 μM No inhibition of DNMT activity despite the inhibition of DNMT1, DNMT3a, and DNMT3b expression Decreased the proportion of methylated CpG in the Nrf2 gene promoter region Increased the recruitment of RNA polymerase complex II at Nrf2 transcription start site	Wang et al. 2014

cyclin D1 in cancer cells correlates with tumor differentiation and increased metastasis (Shan et al. 2017).

Chlorogenic acid

Chlorogenic acid was found to be a potent inhibitor of HDAC, M.SssIDNMT, and human DNMT1 (Lee and Zhu 2006; Bora-Tatar et al. 2009). The inhibition of DNMT1 is mainly due to the upregulation of S-adenosyl-L-homocysteine (SAH, a potent inhibitor of DNA methylation) (Lee and Zhu 2006). As well, chlorogenic acid could partially induce a hypomethylation of retinoic acid receptor beta (RARβ) gene promoter, a tumor suppressor gene encoding the RARβ receptor (Lee and Zhu 2006).

Butyric acid, nicotinamide, and calcium glucarate

Butyric acid (BA), calcium glucarate (CAG), and nicotinamide (NA), used alone or in combination, possess chemopreventive effects on mouse skin tumorigenesis by modification of genetic marks, where the combinatorial treatment was more effective. These compounds used alone or in combination have been shown to inhibit the epigenetic silencing of miR-203 and p16 tumor suppressor genes. This later is correlated with the reduction in DNMT1 and HDAC1 expression (Tiwari and Gupta 2014).

Proanthocyanidins and resveratrol

Both dietary components, grape seed proanthocyanidins (GSP) and resveratrol (Res) produced by many plants, were reported to be effective against MDA-MB-231 and MCF-7 human breast cancer cells, when used in combination. The combined treatment of GSP and Res induced decreased cell viability and proliferation as well as downregulation of DNMT and HDAC activity, with synergistic effects in both cell lines. In addition, the combinatorial treatment induced apoptosis in MDA-MB-231 cells correlating with an increase in proapoptotic Bax and a decrease in anti-apoptotic Bcl-2 expression (Gao and Tollefsbol 2018).

Curcumol

Curcumol showed a potent antitumor activity, in vitro and in vivo, on cancer stem-like cells (CSLCs) of choriocarcinoma, through epigenetic modifications. Curcumol is one of the major compounds of *Curcuma zedoaria* plant, mainly used as a spice and medicinal plant. This compound reduced the expression of DNMT1, DNMT3b, HDAC1, and HDAC3 that

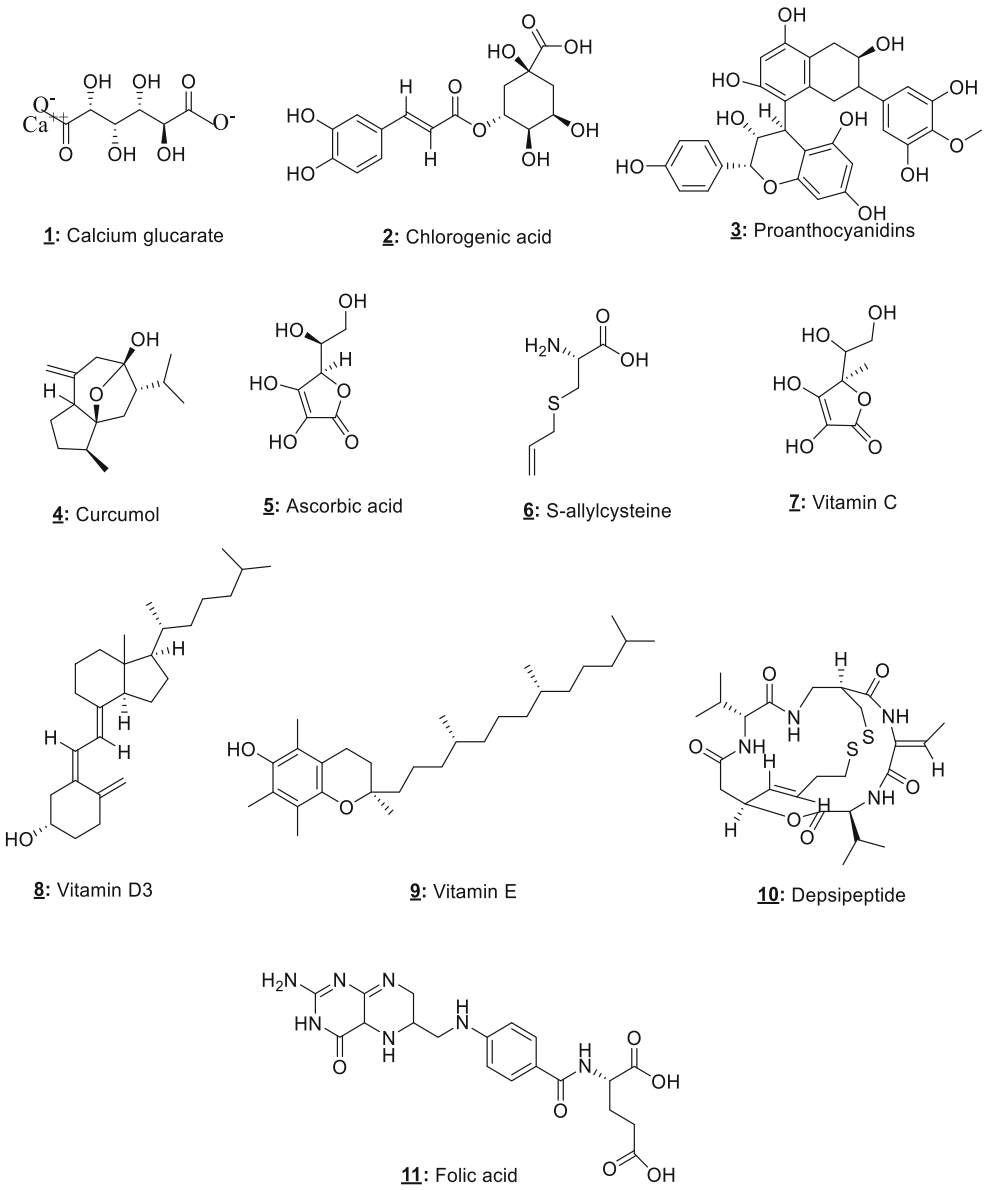


Fig. 12 Chemical structures of other bioactive compounds

have been shown to be significantly upregulated in CSLCs (Peng et al. 2018). Further studies revealed that curcumol possesses the ability to upregulate cell cycle regulator p27 and p16 tumor suppressor (Cao et al. 2017).

Depispeptide

Depispeptide has been shown to be an effective compound capable of changing DNA methylation and histone modification. In prostate cell lines (PC-3, LNCaP, and BPH-1), this compound could reduce H3K9me2/3 and H3K27me2/3 (transcriptional repressor) expression and enhance that of H3K18Ac (transcriptional activator). These modifications were related to an upregulation of GSTP1 mRNA (Hauptstock et al. 2011). In fact, GSTP1, downregulated in prostate cancer, plays a key role in detoxification and reduction of oxidative damage in cells (Cui et al. 2006). In addition, depsipeptide has been shown to inhibit HDAC by suppressing histone methyltransferases (G9A and SUV39H1) and reducing DNMT1 activity and heterochromatin-associated proteins (HP1 and HP1β) in several genes. Consequently, these significant demethylation activities lead to increased expression of p16, GATA4, and SALL3 in human lung cancer cell lines (H719 and H23), pancreatic cancer cell line (PANC1), and colon cancer cell line (HT-29) (Wu et al. 2008). Depsipeptide (2 μM for 4 h) also induced a noticeable upregulation of HAT3 in three cell lines (HepG2, CL-48, and Hep3B) and a downregulation of HDAC3 expression in two

Table 12 Effects of other molecules on epigenetic pathways in cancer

Bioactive molecules	Origin	Experimental methods	Key results	References
Arsenic trioxide (AS_2O_3)	Commercial sample	HepG2 and Huh-7 cells Bisulfite modification and MSP Quantitative real-time RT-PCR Western blot analysis Immunohistochemistry DNMT enzyme activity assay	Restored the mRNA expression of 4 genes ($p16^{INK4a}$, RASSF1A, E-cadherin, and GSTP1) by low concentrations (2-6 μmol/L) through demethylation Decreased the mRNA expression of DNMT 1 and dose dependently inhibited DNMT activity	Cui et al. 2006
	Commercial sample	Human leukemia K562, U937, and HL-60 cell lines Analysis of genomic deoxyribonucleic acid methylation levels Analysis of multiple drug effects	Downregulated the global DNA methylation level in HL-60 cells AS_2O_3 + TSA caused an antagonistic interaction in U937 and K562 cells	Peng et al. 2010
	Commercial sample	MDA-MB-435s and MCF-7 cells Breast cancer xenografts in nude mice MTT assay MSP assay RNA isolation and semi-qRT-PCR Immunohistochemistry Western blot analysis	AS_2O_3 + tamoxifen suppressed cell proliferation in MDA-MB-435s cells Decreased the DNMT1-dependent methylation of the ERα gene and restored the mRNA expression Enhanced the ERα expression in MDA-MB-435s cells Increased the ERα and decreased the DNMT1 protein expression levels in MDA-MB-435 s cells AS_2O_3 + tamoxifen inhibited the tumor growth in human breast cancer xenografts in nude mice	Zhang et al. 2011
	Commercial sample	MDA-MB-231, Hs578T, and MCF-7 Cell growth assay Nude mice xenograft model RNA isolation and RT-PCR Western blot analysis Transfection and luciferase reporter gene assays MSP analysis ChIP assay Immunohistochemistry	Induced the re-expression of ERα mRNA and protein in ER-negative human breast cancer cells Induced the restoration of ERα function in ER-negative human breast cancer cells Induced the functional re-expression of ERα in in vivo Induced the demethylation of ER promoter CpG island Altered the expression of DNMTs and MBD2 proteins in MDA-MB-231 cells Altered the association of DNMT1 with ERα promoter in MDA-MB-231 cells	Du et al. 2012
	Commercial sample	Human prostate cancer PC-3 and LNCaP cells Tube formation assay Western blot analysis Quantitative RT-PCR Cell transfection Immunohistochemistry DNA methylation analysis	Attenuated the angiogenic ability through microRNA-155 (miR-155)-mediated inhibition of transforming growth factor beta (TGF-β)/SMAD signal pathway in prostate cancer cells in vitro and in vivo Inhibited the activations of both TGF-β-induced and endogenous SMAD2/3 Improved the expression of miR-155 via DNA demethylation	Ji et al. 2014
	Not reported	Human acute myeloid leukemia cell line (HL-60) Cell proliferation assay Cell apoptosis Real-time PCR	Inhibited the proliferation and induced the apoptosis of HL-60 cells in a dose- and time-dependent manner AS_2O_3 + DAC increased the proliferation inhibition rate, apoptosis rate, and DAPK (death-associated protein kinase)	Ren et al. 2015
	Commercial sample	K562 cell line RNA extraction and RT-PCR DNA extraction and bisulfite modification DNA MSP assay Apoptosis assay Western blot analysis HeLa or HEK293T cells	Restored the expression of TMS1 by inhibiting DNMT to reverse the hypermethylation and induced apoptosis of K562 cells by downregulation of Bcl-2/Bax expression	Li et al. 2015

Table 12 (continued)

Bioactive molecules	Origin	Experimental methods	Key results	References
	Commercial sample	Luciferase reporter assay RT-PCR HAT assay Transient transfection or RNAi treatment ChIP assay Flow cytometry	Reduced the global histone H4 acetylation at lysine 16 (H4K16ac) through direct binding to histone acetyl transferase hMOF in human cells Increased HDAC4 expression in HeLa or HEK293T cells Inhibited the hMOF activity	Liu et al. 2015
	Commercial sample	MDA-MB-231 and BT-549 cells Cell vitality assay Migration assay Invasion assay Western blot analysis RT-PCR and qRT-PCR Cell transfection MeDIP analysis	Attenuated the migratory and invasive capacities of breast cancer cells Induced an MET (mesenchymal to epithelial transition) in vitro and in vivo, as determined by the increased expression of the epithelial marker, E-cadherin, and decreased expressions of mesenchymal markers, N-cadherin, and vimentin Upregulated the expression of miR-200c through demethylation	Si et al. 2015
	Not reported	Human colorectal cancer cell lines HCT-116, HT-29, and SW-480 MTT assay Quantitative real-time PCR Methylation analysis Flow cytometry analysis	Decreased the DNA methylation in promoter regions of these genes and restored their expression Reduced the expression of DNMT1 and increased arsenic methyltransferase (AS3MT) Altered the transcriptional activity of several unmethylated cell cycle regulatory genes including cyclin B1, E1, D1, GADD45A, and p21 Induced the G_2/M arrest in all three cell lines	Eyvani et al. 2016
	Not reported	Cervical cancer cell lines (HeLa and C33a) and human keratinocytes (HK) Inductively coupled plasma optical emission spectrometer (ICP-OES) Flow cytometry analysis Fluorescent double immunocytochemical staining	Reduced the protein levels of HPV-E6 and induced the cell apoptosis in HeLa cells Liposome-delivered AS_2O_3 protected cells from the direct toxic effects induced by higher concentrations of intracellular AS_2O_3	Wang et al. 2016b
	Commercial sample	Acute promyelocytic leukemia (APL) cell line Cell cycle distribution analysis Flow cytometry analysis Preparation of DNA and RNA EpiTect methyl QPCR array Quantitative real-time PCR Western blot analysis	Reduced the expression of DNMT1, 3A, and 3B Induced the expression of CCND1, CCNE1, and GADD45α genes Suppressed the expression of CCNF and CDKN1A genes, which were consistent with decreased number of cells in G1 and S phases and increased number of cells in G_2/M phase	Hassani et al. 2018
	Commercial sample	Cancer stem-like cells (CSCs) Cell viability assay qRT-PCR Western blot analysis DNA methylation analysis	Resensitized the MDR Bel 7402 cells (Bel^{MDR}) cells to chemotherapeutic drugs, an effect mediated by the inhibition of NF-κB pathway and CSCs properties Activated the miR-148a, leading to the repression of NF-κB pathway by targeting the 3′-UTR of p65	Wang et al. 2020
Calcium glucarate (CAG)	Commercial sample	Excised skin from the painted area (female mice) Quantitative real-time RT-PCR Bisulfite modification of DNA and MSP assay RT-PCR Western blot analysis	Prevented tumor development but protection was greatly improved when combined with nicotinamide (NA) and butyric acid (BA) Downregulated the miR-203 levels at 16 weeks Upregulated the HDAC, DNMT, promoter methylation of miR-203 at 4 or 16 weeks Prevented altered gene expression, while co-administration with NA and BA had a more pronounced effect than that of the individual compound, by regulating miR-203 status through epigenetic or biogenetic modulations	Tiwari and Gupta 2014

Table 12 (continued)

Bioactive molecules	Origin	Experimental methods	Key results	References
Chlorogenic acid	Commercial sample	Human breast cancer cells (MCF-7 and MAD-MB-231) Assay of enzymatic DNA methylation in vitro Determination of the methylation status of the *RAR*β gene in cultured cancer cells	Inhibited the M.SssI DNMT-mediated DNA methylation (IC_{50} = 0.75 μM) Inhibited the human DNMT1-mediated DNA methylation (IC_{50} = 0.9 μM) Inhibited the DNA methylation predominantly through a non-competitive mechanism, largely due to the increased formation of S-adenosyl-$_L$-homocysteine Inhibited the methylation of the promoter region of the *RAR*β gene	Lee and Zhu 2006
	Commercial sample	In vitro HDAC inhibition activity screening	Inhibited the HDAC activity (IC_{50} = 375 μM)	Bora-Tatar et al. 2009
Proanthocyanidins (GSPs)	Commercial sample	MDA-MB-231 and MCF-7 cells MTT assay Clonogenic assay Apoptosis assay Western blot analysis DNMT activity assay HDAC activity assay	GSPs + RSV synergistically decreased cell viability and cell proliferation in both cell lines GSPs + RSV synergistically induced apoptosis in MDA-MB-231 cells by upregulating Bax expression and downregulating Bcl-2 expression GSPs + RSV synergistically reduced DNMT and HDAC activities in MDA-MB-231 and MCF-7 cells	Gao and Tollefsbol 2018
Curcumol	Not reported	Choriocarcinoma cancer stem-like cells (CSLCs) Flow cytometry analysis qRT-PCR Western blot analysis Global DNA methylation quantification HDAC activity analysis Xenograft tumorigenicity assay Immunohistochemistry	Suppressed the CSLCs self-renewal and DNMT/HDAC activity in vitro and in vivo Affected the CSLCs through regulation of DNMTs and HDACs	Peng et al. 2018
Folic acid (FA)	Not reported	Glioma cells Flow cytometry Measure of ROS production qPCR and RT-PCR ChIP assay Clonogenicity assay MSP assay	Enhanced the DNA remethylation through the Sp1/Sp3-mediated transcriptional upregulation of genes coding for DNMT3a and DNMT3b proteins, two de novo methyltranferases	Hervouet et al. 2009
	Commercial sample	MCF-7 and MDA-MB- 231 cells Cell viability assay Methylation gene analysis cDNA synthesis and real-time PCR	Induced a dose-dependent downregulation of tumor suppressor genes which may be linked to the increased DNA methylation detected within their promoter regions Upregulated the DNMT1 expression at the highest FA concentration	Lubecka-Pietruszewska et al. 2013
	Not reported	Streptozocin-induced diabetic rats Genomic DNA extraction and MSP analysis RNA extraction and quantitative real-time RT-PCR	FA + vit E improved the negative effects of diabetes by decreasing the expression of TGF-β-1 and ESR-1 and increasing that of CDH-1 in diabetic rats	Tabebordbar et al. 2020
Vitamin C (vit C)	Commercial sample	Human skin cancer A431 cells Apoptosis analysis RNA extraction and quantitative real-time PCR Global DNA methylation level determination DNA immunoprecipitation-qPCR Western blot analysis	Decreased the increasing apoptosis of A431 cells under prolonged UV irradiation Reduced the global DNA methylation in a time- and dose- dependent manner in A431 cells Reactivated the expression of p16 and p21 at mRNA and protein levels Increased the 5hmC enrichment at p16 and p21 promoter regions Decreased the expression of p16 and p21 in Tet1/2 double-knockdown cells	Lin et al. 2014
	Not reported	Human colorectal carcinoma cell line (HCT116)	Enhanced the global levels of 5-hmdC, without altering 5-mdC	Gerecke et al. 2018

Table 12 (continued)

Bioactive molecules	Origin	Experimental methods	Key results	References
		Cell viability assay Determination of genome-wide DNA methylation Apoptosis assay RNA extraction and quantitative real-time PCR Western blot analysis	Vit C + DAC or AZA (azacytidine) resulted in a high increase of global 5-hmdC levels Vit C + DAC or AZA increased expression of the tumor suppressor *p21* (*CDKN1A*), and a significant increase in apoptotic cell induction	
	Commercial sample	Human colorectal carcinoma cell line HCT116 MTT assay Determination of genome-wide DNA methylation Flow cytometric analysis RNA extraction and quantitative real-time PCR	Produced only minimally changes of the oncometabolite Vit C + ML309, in mutated cells, induced pronounced reduction of 2-HG leading to levels comparable to those in wild type cells Vit C + ML309 enhanced the global DNA hydroxymethylation and increased the gene expression of certain tumor suppressors Vit C + ML309 increased the percentage of apoptotic cells in mutated cells	Gerecke et al. 2020
Vitamin D_3	Commercial sample	MCF-7 and MDA-MB-231 cells Cell culture, proliferation and viability assay RNA and DNA extraction Methylation analysis of *RARbeta2* promoter Quantitative analysis of *RARbeta2* expression on mRNA level	Methylated, partially, the *RARbeta2* promoter in the tested fragment in MCF-7 cells Inhibited the promoter methylation and increased the expression of *RARbeta2* in MCF-7 cells Improved the action of 2CdA (2-chloro-2′-deoxyadenosine) and F-ara-A (9-beta-$_D$-arabinosyl-2-fluoroadenine) on *RARbeta2* methylation and/or expression	Stefanska et al. 2010
	Commercial sample	MCF-7 and MDA-MB-231 cells Cytotoxicity assay RNA extraction and complementary DNA synthesis Real-time PCR Methylation-sensitive restriction analysis	Reduced the *PTEN* promoter methylation in MCF-7 cells Downregulated the DNMT and upregulated the p21 Improved the inhibitory effects of 2CdA and F-ara-A on *PTEN* methylation in MCF-7 cells	Stefanska et al. 2012
Vitamin E (vit E)	Not reported	Human colorectal adenocarcinoma cell line Caco-2 Total ROS and superoxide level RNA/gDNA extraction and bisulfite conversion Gene expression analysis Real-time PCR DNA methylation analysis	Induced a dose-dependent counteracting effect on the oxidative stress induced by H_2O_2 Reduced the malondialdehyde at 10 μM vit E Induced the *MLH1* and *DNMT1* gene expression Increased the global methylation	Zappe et al. 2018
	Not reported	Streptozocin-induced diabetic rats Genomic DNA extraction and MSP analysis RNA extraction and quantitative real-time RT-PCR	Vit E + FA improved the negative effects of diabetes by decreasing the expression of TGF-β-1 and ESR-1 and increasing that of CDH-1 in diabetic rats	Tabebordbar et al. 2020
Amarogentin	Commercial sample	Human cervical cancer cell line HeLa Cytotoxicity analysis Apoptosis analysis mRNA expression analysis Protein extraction Western blot analysis Methylation analysis	Amrogentin in combination with eugenol + EGCG could highly: Inhibited cellular proliferation and colony formation Induced the apoptosis Downregulated the cyclin D1 and upregulated the cell cycle inhibitors LIMD1, RBSP3, and p16 at G_1/S phase of cell cycle Induced promoter hypomethylation of LimD1 and P16 genes as a result of reduced expression of DNMT1	Pal et al. 2019
Ascorbate	Not reported	Human metastatic MeWo and BLM melanoma cells Cell cycle analysis In silico analysis of HDAC inhibition HDAC inhibitor screening assay	Induced the apoptosis in human metastatic BLM melanoma cells in a time-dependent manner Inhibited the DNMT activity in nuclear extracts of MeWo and BLM melanoma cells, but did	Venturelli et al. 2014

Table 12 (continued)

Bioactive molecules	Origin	Experimental methods	Key results	References
		HDAC inhibitor profiling assay Measurement of DNMT activity miRNA expression analysis	not inhibit human HDAC enzymes of classes I, II, and IV Altered the expression of 151 miRNAs of BLM cells	
Ascorbic acid	Not reported	S180 mouse sarcoma cell line Tumor inoculation Necropsy and gene expression analysis Real-time PCR	Increased the expression of NRF2 Reduced the expression of the tumor-promoting gene HIF Increased the expression of tumor suppression gene p53 Increased the TERT Reduced the DNMT1	Mikirova and C. Scimeca 2016
	Commercial sample	Mouse embryonic fibroblasts (MEFs) RNA extraction and cDNA synthesis mRNA quantitation by real-time PCR	Enhanced the expression of DNMT1 Decreased the expression of DNMT3a	Amirabad et al. 2017
	Commercial sample	Diffuse large B cell (DLBCL) and peripheral T cell (PTCL) lymphomas	Increased the TET activity, in DLBCL and PTCL cells, leading to DNA demethylation Increased the expression of SMAD1, a tumor suppressor gene known to be suppressed by methylation Increased the chemosensitivity of lymphoma cells	Shenoy et al. 2017
Depsipeptide	Not reported	MDA-MB-231 and MDA-MB-435 cells Inhibition of DNA synthesis assay Clonogenic assay Isolation of RNA and RT-PCR analysis	Depsipeptide + DAC produced a synergistic antineoplastic effect against the tumor cells Depsipeptide + DAC produced a great reactivation of *maspin* and *gelsolin* (2 genes tumor metastasis suppressor)	Primeau et al. 2003
	Not reported	Human liver HepG2 and Hep3B cell lines Cell viability assay RT-PCR ChIP assay Western blot analysis	Reactivated the growth-arrest DNA damage-inducible gene 45, (Gadd45β) gene expression considerably within 4 h in HepG2 cells, but not in Hep3B cells Depsipeptide + 5-azacytidine exhibited a synergistic effect on Gadd45β gene reactivation in the HepG2 cells	Jiang et al. 2007
	Not reported	Human lung cancer cell lines H719 and H23, colon cancer cell line HT-29, and pancreatic cancer cell line PANC1 Cell viability and cell growth assays RNAi MSP assay Bisulfite sequencing Measurement of DNA de novo and maintenance methyltransferase activity DNMT1 activity	Exhibited significant demethylating activity on the promoters of several genes, including *p16*, *SALL3*, and *GATA4* in H719, H23, HT-29, and PANC1 cells No effect on the DNMT1expression Suppressed the expression of histone methyltransferases G9A and SUV39H1 Reduced both loading of heterochromatin-associated protein 1 (HP1α and HP1β) to methylated H3K9 and binding of DNMT1 to these genes' promoter	Wu et al. 2008
	Commercial sample	Prostate cell lines (PC-3, LNCaP, and BPH-1) Cell cycle analysis RNA isolation and mRNA quantification DNA isolation, bisulfite treatment, and MSP assay ChIP assay	Induced the apoptosis in PCA cells, but not a cell cycle arrest Reversed the DNA hypermethylation and repressive histone modifications (reduction of H3K9me2/3 and H3K27me2/3; increase of H3K18Ac), thereby inducing *GSTP1* (Glutathione-S-transferase pi 1) mRNA reexpression	Hauptstock et al. 2011
S-Allylcysteine (SAC)	Commercial sample	Epithelial ovarian cancer cell line A2780 Cell cycle analysis Global DNA methylation assay DNMT activity assay	Inhibited the proliferation of A2780 cells in a dose- and time-dependent manner Resulted in G_0/G_1 phase cell cycle arrest Reduced the global DNA methylation Induced the DNA 5-mC demethylation SAC + 5-aza-dc decreased the DNMT activity, mRNA, and protein expression of DNMTs	Xu et al. 2018

Table 12 (continued)

Bioactive molecules	Origin	Experimental methods	Key results	References
		Quantitative mRNA analysis of DNMTs and tumor suppressor gene *CDKN1A* using RT-PCR Western blot analysis	SAC + 5-aza-dc reactivated the *CDKN1A*	
Selenium (Se)	Not reported	Human LNCaP prostate cancer cells DNMT activity assay HDAC activity assay	Induced partial demethylation of the GSTP1 promoter and re-expression of GSTP1 in a dose- and time-dependent manner Decreased the mRNA levels of DNMT1, 3A, and 3B, but not DNMT2 Decreased the protein levels of DNMT1 Decreased the HDAC activity, but did not alter mRNA and protein levels of HDACs Decreased the levels of methylated histone H3 on lysine 9 (H3-K9) Increased the levels of acetylated H3-K9	Xiang et al. 2008
	Not reported	AOM-induced rat colonic carcinogenesis model Quantification of ACF (aberrant crypt foci) Western blot analysis Quantitative RT-PCR	Se + green tea resulted in a significant additive inhibition of large ACF formation Se + green tea resulted in a significant additive inhibition effect on all tumor endpoints Se + green tea reduced the DNMT1 expression and induced the histone H3 acetylation, which were accompanied by restoration of *SFRP5* mRNA in normal-appearing colonic crypts Se + green tea significantly reduced β-catenin nuclear translocation, cyclin D1 expression, and cell proliferation	Hu et al. 2013
		Rat animal model of Keshan's disease MeDIP-Chip assay MSP assay RNA extraction, RT-PCR, and quantitative PCR Western blot analysis	Increased the TLR2 and ICAM1 (inflammatory-related genes) promoter methylation Suppressed the TLR2 and ICAM1 expression Reduced the infiltration of myocardial inflammatory cells Se + 5-aza-dC reduced mRNA and protein levels of DNMT1 regardless of TLR2 and ICAM1 promoter methylation status and expression levels of these genes Suppressed the expression of the Gadd45α, TLR2, and ICAM1 in a concentration-dependent manner	Yang et al. 2014

cell lines (CL-48 and Hep3B). These epigenetic modifications were correlated with a considerable reactivation of growth arrest and DNA damage-inducible β (GADD45β) gene expression only in HepG2 cells (Jiang et al. 2007). GADD45 plays a role in the regulation of normal cell apoptosis. However, overexpression could inhibit apoptosis in many cells such as multiple myeloma cells (Zhang et al. 2020). In contrast, the combination of depsipeptide with 5-aza-2′-deoxycytidine or 5-azacytidine, known to be potent inhibitors of DNA methylation, produced important impacts on many epigenetic marks. In fact, a sequential treatment of depsipeptide (2 nM for 24 h) followed by 5-azacytidine (5 μM for 24 h) showed a synergistic effect on HAT3 and GADD4β gene expression in HepG2 cell lines. However, the simultaneous and sequential (5-azacytidine followed by depsipeptide) treatments had no impact on GADD4β gene reactivation (Jiang et al. 2007). Moreover, the combination of depsipeptide with 5-aza-2′-deoxycytidine showed a great upregulation of gelsolin and maspin (metastasis suppressor genes) in the MDA-MB-231 and MCF-7 cell lines as compared to depsipeptide alone (Primeau et al. 2003).

S-Allylcysteine

S-Allylcysteine has antitumor activity on human ovarian cancer A2780 cells. Epigenetically, this compound may decrease 5-methylcytosine levels and the expression of mRNA and DNMT1 protein. Consequently, due to these epigenetic modifications, the expression of proteins and mRNA of CDKN1A (tumor suppressor gene) was upregulated and the expression

of cell division control 2 (CDC2) was decreased (Xu et al. 2018).

Selenite

Selenite could prevent prostate and colorectal oncogenesis through an epigenetic mechanism. Selenium used alone or in combination with green tea as a dietary supplement showed a preventive effect on colonic carcinogenesis in rats, with a great impact on the combinatorial diet. Thus, it has been shown that the treatments significantly inhibit the formation of aberrant crypt foci (ACF), the incidence and multiplicity of tumors, as well as the decrease in tumor size. Furthermore, the combined diet induced a remarkable decline of DNMT1 expression and an increase in histone H3 acetylation, with reactivation of SFRP5 mRNA expression and decrease in β-catenin nuclear and cyclin D1 expression, resulting in suppression of colorectal oncogenesis (Hu et al. 2013). In prostate cancer, selenite treatment produced partial demethylation of the glutathione-S-transferase P1 (GSTP1) promoter and re-expression of GSTP1 gene, which was shown to be hypermethylated in human prostate cancer. The epigenetic modifications caused by this treatment induced downregulation of DNA methyltransferases (DNMT1, DNMT3A, and DNMT3B) mRNA expression, protein levels of DNMT1, histone deacetylase enzyme activity, and levels of methylated histone H3 on lysine 9 (H3-K9), with increased levels of acetylated H3-K9 (Xiang et al. 2008). On the other hand, it is also reported that selenium deficiency represents the main cause of Keshan's disease (fatal dilated cardiomyopathy), where selenium deficiency is more probably related to DNA hypermethylation. Actually, selenium deficiency enhances the levels of mRNA and protein expression of TLR2, ICAM1 (inflammatory-related genes), and GADD45α. In this case, selenite treatment induces a downregulation of TLR2, ICAM1, and Gadd45α gene expression (Yang et al. 2014).

Vitamin C (ascorbate, ascorbic acid)

Vitamin C, mainly at pharmacological doses, showed promising action in cancer prevention and treatment via epigenetic modifications. In fact, vitamin C could induce DNA demethylation by downregulating DNMT activity and upregulating ten-eleven translocation (TET) expression in many cancer cell lines (Venturelli et al. 2014; Mikirova and C. Scimeca 2016; Amirabad et al. 2017; Shenoy et al. 201; Gerecke et al. 2020; Gerecke et al. 2018). Vitamin C increased the mRNA and protein expression of the tumor suppressor p16 and p21 in human epidermoid carcinoma A431 cells, as it may protect these cells against UV-induced apoptosis (Lin et al. 2014). Vitamin C treatment also produced modifications in the expression of 151 miRNAs, with the reactivation of 32 miRNAs involved in tumor suppression, metastasis, and drug resistance in BLM melanoma cells (Venturelli et al. 2014). The downregulation of DNMT1 expression in the mouse sarcoma 180 cell line following vitamin C treatment was accompanied by decreased expression of HIF (tumor-promoting gene) and an enhancement of p53 (tumor suppressor gene) and gene expression by the transcription factor Nrf2 (Mikirova and C. Scimeca 2016). On the other hand, increased TET activity by vitamin C treatment was correlated with the upregulation of SMAD1 (tumor suppressor gene) expression in DLBCL and PTCL cell lines (Shenoy et al. 2017) and also to the reactivation of the tumor suppressor p21 (CDKN1A) in human colorectal carcinoma (HCT11) cell line (Gerecke et al. 2018). Vitamin C could improve the activity of 5-aza-2′-deoxycytidine and azacytidine (DNA-demethylating agents), where the combined treatment induced a significant increase in CDKN1A expression (Gerecke et al. 2018). Moreover, although vitamin C has a low impact on 2-HG, oncometabolite caused by mutations in the enzyme isocitrate dehydrogenase 1 (IDH1), the combinatorial treatment of vitamin C with ML309 (inhibitor of mutant IDH enzymes) produced a significant decrease of 2-HG in the colon cancer cell line $HCT116^{IDH1R132H/+}$ (Gerecke et al. 2020).

Vitamin D_3/all-trans retinoic acid/resveratrol

In MCF-7 breast cancer cells, where RARβ2 was found to be partially methylated, treatments with vitamin D_3 or all-trans retinoic acid (ATRA) improved the expression of RARβ2 gene. This action was more effective when these compounds were used in combination with 2-chloro-2′-deoxyadenosine (2CdA) (Stefanska et al. 2010). Furthermore, treatments with vitamin D_3, ATRA, and resveratrol, used alone, showed a great ability to decline the promoter methylation of phosphatase and tensin homolog (PTEN) tumor suppressor gene in MCF-7 cell line. In addition, vitamin D_3 and resveratrol could also increase PTEN and p21 activity, as well as reduce DNMT activity. The combinatorial treatment of vitamin D_3 and 2CdA significantly increases the expression of PTEN in MCF-7 cells. However, only vitamin D_3 has been shown to be able to decrease the PTEN methylation and enhance its expression in MDA-MB-231 cell line (Stefanska et al. 2012).

Folic acid (folate, vitamin B_9) and vitamin E

Epigenetic modifications caused by DNA hypomethylation could also lead to the development of cancer as in the case of glioma tumor. Folic acid (vitamin B_9) was found to be effective in increasing the DNA methylation by upregulating the expression of DNMT3a and DNMT3b proteins, which leads to enhanced methylation and then inactivation of numerous genes (PDGF-B, MGMT, survivin, and bcl-w) implicated in gliomagenesis (Hervouet et al. 2009). However, high folic acid levels, associated with excessive hypermethylation, could

lead to the development of cancer by inactivating tumor suppressor genes (Lubecka-Pietruszewska et al. 2013). Vitamin E and folic acid supplements showed excellent results in regulating the disruption of uterine function and fertility caused by diabetes in rats via epigenetic pathway. These two compounds, used alone, could decline via hypermethylation process of the expression of TGF-β-1 and ESR-1 (upregulated due to diabetes disorder). While the expression of CDH-1, supposed to be downregulated by diabetes, was upregulated by vitamin E and folic acid supplementation in diabetic rats (Tabebordbar et al. 2020). Moreover, vitamin E showed a great impact on oxidative stress caused by obesity or diabetes by neutralizing the H_2O_2-induced lipid peroxidation. As well, vitamin E at lower concentrations could increase the global DNA methylation and DNMT1 gene expression in colorectal cancer cell line Caco-2, with an increase in MutL-homolog 1 (MLH1) gene expression, a protein implicated in the repair of DNA mismatches in humans (Zappe et al. 2018).

Arsenic trioxide

Arsenic trioxide (As_2O_3) is considered as a potent anticancer agent and DNA methylation inhibitor, with noticeable abilities to reactivate the silenced tumor suppressor genes, induce cell cycle arrest, and inhibit angiogenesis and metastasis (Cui et al. 2006; Du et al. 2012; Hassani et al. 2018). This compound also exhibits synergistic demethylation activity in correlation with 5-aza-2′-deoxycytidine (DAC) in some cancer cell lines (Peng et al. 2018; Ren et al. 2015). In liver cancer cell lines, the inhibition of DNMT activity by As_2O_3 was concomitant with CpG island demethylation of tumor suppressor genes, $p16^{INK4a}$, *RASSF1A*, E-cadherin, and GSTP1, leading to reactivation of the expression of these genes (Cui et al. 2006). In the case of estrogen receptor-negative breast cancer, loss of ER expression is mainly due to hypermethylation of CpG islands in the promoter regions of the ER gene. Therefore, the upregulation of ER is among the effective strategies followed in the therapy of this type of cancer. As_2O_3 possesses a positive activity on the re-expression of estrogen receptor α (Erα) in ER-negative breast cancer cells in vitro and in vivo through demethylation of the Era promoter (Du et al. 2012; Zhang et al. 2011). This molecule induces cell cycle arrest through modifications of transcriptional activity of many cell cycle regulatory genes in colorectal cancer cell lines. Thus, As_2O_3 could induce an enhancement of cyclin E1, D1, *GADD45A*, and p21 and a decline of cyclin B1 gene expressions. Furthermore, As_2O_3 declined DNA demethylation of RBL1, CHFR, and p16 genes resulted in reactivation of their expression (Eyvani et al. 2016). As_2O_3 induces apoptosis and a significant reactivation of TMS1 expression, a tumor suppressor gene that was found to be completely methylated in the K562 cell line. In addition, As_2O_3 could decrease the expression of Bcl-2 (anti-apoptotic protein) and increase the expression of Bax (proapoptotic protein) (Li et al. 2015). Arsenic trioxide also showed excellent results in the treatment of acute promyelocytic leukemia (APL) via epigenetic mechanisms (Peng et al. 2010; Hassani et al. 2018). This compound induced demethylation of CpG island at promoter region of many cell cycle-related genes: CCND1, CCNE1, CCNF, CDKN1A, GADD45α, and RBL1 in the APL cell line NB4, which led to cell cycle arrest and apoptosis. Among these genes demethylated by this compound, the expression of mRNA of CCND1, CCNE1, and GADD45α was upregulated with an increase of the protein expression only in CCND1 and CCNE1. However, expression of the CCNF and CDKN1A genes was found to be suppressed (Hassani et al. 2018). Regarding the anti-angiogenesis activity of As_2O_3, this compound could attenuate angiogenic effect in human prostate cancer cells (in vitro and in vivo) by improving the expression of microRNA-155 (miR-155), resulting in inhibition of transforming growth factor beta (TGF-β)/SMAD signal pathway and vascular endothelial growth factor secretion (Ji et al. 2014). For the anti-metastasis activity, As_2O_3 effectively decreased the migration and invasive capacity of human breast cancer cell lines (MDA-MB-231 and BT-549) through the mesenchymal to epithelial transition process. This effect was related to the ability of this compound to increase miR-200c expression, then upregulate the expression of E-cadherin (epithelial marker) and downregulate N-cadherin and vimentin (mesenchymal markers) (Si et al. 2015). Moreover, arsenic trioxide could reverse the multiple drug resistance (MDR) in hepatocellular carcinoma (HCC). In fact, this compound enhances the expression of miR-148a and declines both NF-κB pathway and cancer stem-like cells (CSCs) properties in multiple drug resistant Bel-7402 cells (Wang et al. 2020). On the other hand, As_2O_3 showed a potent inhibitory effect against hMOF activity by decreasing global H4K16 acetylation, caused by arsenic, and subsequently increasing deacetyltransferase HDAC4 expression (Liu et al. 2015).

Conclusions and perspectives

The development of drugs acting on epigenetic pathways could offer major therapeutic avenues for cancer treatment, which could lead to definitive therapy by converting transformed cells into normal cells. In this review, the epigenetic modulatory effects against cancer of some natural compounds such as cannabinoids, carotenoids, fatty acids, lignans, polysaccharides, saponins, secoiridoids, steroids, tannins, and tanshinones are discussed. These compounds showed remarkable effects as epidrug modulators and can be therefore a source of anticancer epidrugs by targeting epigenetic modifications. However, these compounds should be investigated in clinical trials to confirm these pharmacokinetic effects.

Moreover, toxicological studies should also be carried out to validate the safety of these natural epidrugs.

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Declarations

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