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Nanotechnology: a powerful therapeutic strategy for naturally derived anticancer agents

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Outline

Introduction

The term nanotechnology was first used in 1974 by Norio Taniguchi to define the production of structures around 1 nm in size [1]. Nowadays, nanotechnology represents the science and engineering involved in the development, characterization and application of functionalized materials and devices with dimensions of about 1 nm to 100 nm [1-3]. The practical applications of nanotechnology contemplate several areas such as communications, engineering, physics, chemistry, biology, robotics, medicine and food [4-10].

In medical sciences nanotechnology offers new therapeutic opportunities which contribute to the advancement of human health by improving medical care or discovering new alternatives for treatments [11-14]. The use of nanotechnology on pharmaceuticals has been responsible for the development of intelligent drug delivery devices designed to become more efficient and minimize toxic effects [11, 15-20]. The advantages of the drug delivery systems over the conventional dosage forms allows better protection of the drug against oxidation, hydrolysis, pH variations; potential drug targeting towards the desired sites of action (e.g. tumors, diseased tissues) through the use of signaling molecules such as antibodies, peptides, and polysaccharides, or by using biomaterials that directly respond to different pathophysiological conditions, increased bioavailability and cellular uptake, reduced number of doses and the amount of drug administered, sustained and prolonged drug release, lower fluctuations of the drug plasma concentration, maintenance of constant therapeutic concentration, minimization of toxic and subtherapeutic drug levels, and also better patient adherence to drug therapy with reduction of costs in health care [21-31].

Pharmaceutical nanotechnology plays a key role in the optimization of drug therapy in various diseases, especially cancer, as it can improve physicochemical, release rate, pharmacokinetics, biologic and therapeutic properties of drugs as compared to conventional cancer therapy, which eventually minimizes the unintended cytotoxic effects on healthy tissues. Additionally, enhances drug efficacy and patient compliance by providing drug levels within the optimum range over a longer period [17, 31-35]. However, some negative factors have been appointed, such as high cost and difficulty of scaling up processes [13, 31, 36, 37].

Nanotechnology-Based Drug Delivery Systems Applied to Cancer Treatment

Advances in cancer nanotherapies have been made in order to solve problems related to the limited tumor-targeting ability of drugs, intolerable toxicity of most anti-cancer agents due to high dose requirements and multidrug resistance. All these factors contribute to low success rates of conventional cancer therapies, where pharmaceutical nanotechnology represents a promising strategy to increase the safety and efficacy of cancer treatments [31, 38-41]. Moreover, according to World Health Organization (WHO) report, cancer is responsible for about 13% of all deaths worldwide and it was appointed as second most common cause of disease-related deaths in humans [40].

In recent years, the successful progress of drug delivery systems applied to cancer treatment has resulted in a better understanding of cancer biology (e. g. microenvironment, signal pathways and metastasis) and the discovering of strategies on delivering drugs encapsulated in nanostructures by using passive and bioactive targeting nanoparticles (Figure 10.1) [37, 42, 43].

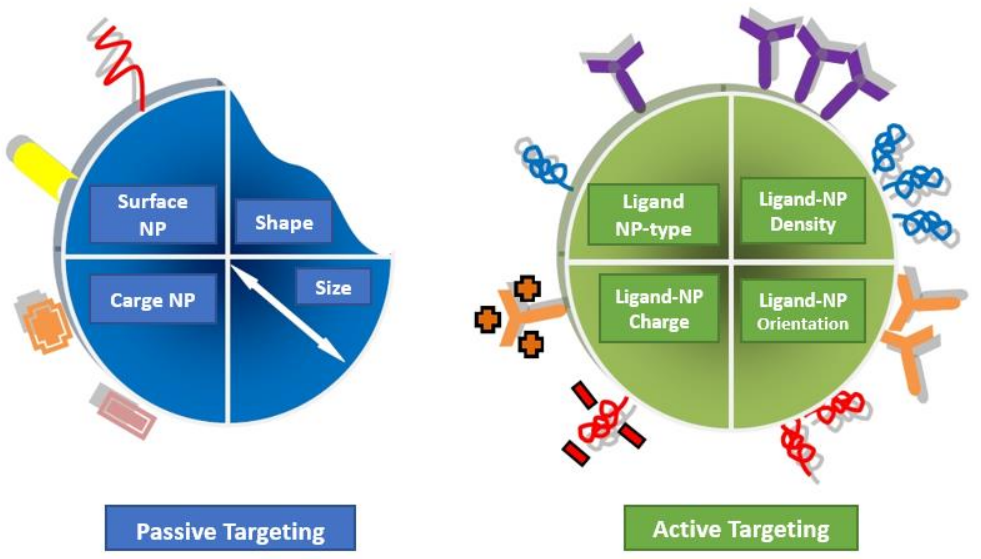

FIGURE 10.1
Characteristics of passive and active targeting mechanisms for nanoparticles delivery at the specific tumor tissue. Source: by author adapted from Aydin, 2014 [42].

Nanovehicles applied to cancer therapy may have a preferred distribution towards tumor cells through mechanisms of passive and active targeting. In the passive targeting mechanism, the typical anatomical and functional differences between the normal and tumor vasculature, combined with the lack of effective lymphatic drainage on tumor site, allow a selective accumulation of drug carries at the tumor tissue. This process is known as enhanced permeability and retention (EPR) effect [43].

The effective accumulation of nanocarries at the tumor site has been influenced by other factors such as the sizes of the *inter*-endothelial gap junctions and the *trans*-endothelial channels for extravasation of nanoparticles across tumor tissue (about 200 nm to 400 nm), as well the circulation time, tumor size, degree of tumor vascularization and angiogenesis [37].

In passive targeting a better understanding of the tumor biology such as the EPR effect, is fundamental in order to improve the effectiveness of nanomedicines applied to cancer management. Studies have shown that the size, shape, nature and surface charge of the nanoparticles directly influence their circulation time along with the distribution extent into tumor tissue [42, 44].

The particle size is an important parameter in low permeability tumors where nanoparticles with less than 50 nm in diameter are shown to be more easily accumulated in the tissue. In addition, elongated nanostructures such as nanotubes and nanorods induces strong diffusion into tumors. In other hand, nanoparticles with no surface charge can diffuse deeper in the tumoral interstitium, although its access to the tumor endothelium become hindered. The presence of hydrophilic polymers such as polyethylene glycol (PEG) and methacrylamide (HPMA) on the surface of the nanoparticles also induce the EPR effect [44].

A longer circulation time is required for nanostructures which can be achieved by modification approaches such as the stealth nanoparticles that besides increasing the blood circulation time, increasing the selective accumulation of carries at the tumor site [45]. The most usual modifications occur with the hydrophilic polymer polyethylene glycol (PEG) due to its poor immunogenicity,

antigenicity and non-toxicity. PEG can be grafted, conjugated or absorbed on the nanoparticle surfaces providing chemical steric stabilization favoring protection against mononuclear phagocytic system uptake [46]. However, there are some drawbacks related to passive targeting carriers such as insufficient drug concentration delivered to the tumor site and multiple drug resistance (MDR), which correspond to the cancer cells resistance for multiple different drugs due to the overexpression of transporter P-glycoprotein (P-gp) on the surface of these cells [47].
Nowadays, studies have shown that bioactive targeting nanoparticles are more advantageous than the passive ones. For the active targeting specific interactions occurs between receptors on the tumor tissue and a ligand or a biomarker on the nanocarrier, which includes monoclonal antibodies, peptides, antibody fragments, and small molecules that favors a preferred accumulation. Thus, the advantage of the active targeting nanoparticles is the specific recognition of receptors that are uniquely expressed on certain cancer cells contributing to a selective delivery of cytotoxic drug to the targeted cancer cells, while expose minimal toxicity to healthy cells [48].

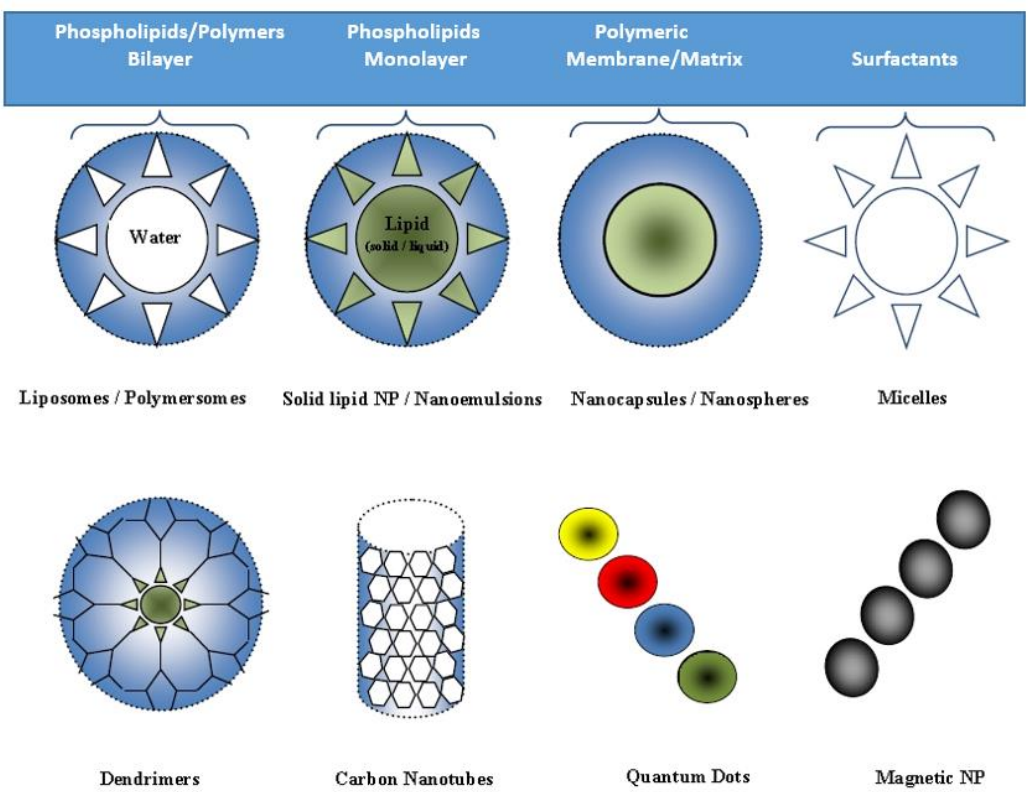

FIGURE 10.2
Different types of nanoparticulate systems used as carriers for several anticancer drugs.
Source: by author.

Therefore, the choice of ligand conjugated to the surface of the nanosystem is a crucial step to define the effectiveness of these devices in the cancer treatment. Effective ligand-surface interaction depends on factors such as the nature of the surface of the nanosystem (organic or inorganic), the type of chemical bond (covalent or ionic) and if the ligand-surface interaction took

place before or after the nanosystem formation. Characteristics such as density, spatial orientation and electrostatic charge of the ligand influence the cellular uptake, since there is a cooperative effect saturation equilibrium between the ligand and the receptor site on the tumor cell, in which a promoter, competitive or deleterious effect can occur from this interaction [44].

Significant progress in cancer nanotechnology has been achieved through the development of several carriers, such as polymeric nanoparticles, micelles, conjugates, solid lipid nanoparticles, liposomes, immunoliposomes, polymersomes, dendrimers, carbon nanotubes, inorganic and metallic nanoparticles, quantum dots and exosomes (Figure 10.2) all containing various anticancer molecules (e.g. drugs, proteins, nucleic acids, genes and other) [31, 33, 49-53]. Table 10.1 specify some examples of drug release systems applied to cancer therapy.

TABLE 10.1
Examples of drug release systems applied to cancer therapy.

System (Product name / Status)	Description	Composition
Polymeric nanoparticles (Livitag/ Clinic studies Phase III)	Colloidal particles measuring 10 nm to1000 nm [54]	Polyisohexylcryano-acrylate containing doxorubicin [55]
Liposomes (Lipoplatin/ Clinic studies Phase III)	Spherical vesicles based on lipid bilayers [56]	Pegylated liposomes with cisplatin [57]
Solid lipid nanoparticles (*In vitro* studies)	Submicron particles based on solid lipid monolayers at body or room temperature [58]	Paclitaxel [59]
Polymersomes (*In vitro* studies)	Spherical vesicles based on bilayer polymers [14]	Paclitaxel and doxorubicin [60]
Dendrimers (Preclinical studies)	Macromolecules with symmetrical structure based on polymers [58]	Folic acid-modified dendrimer-doxorubicin [61]

Carbon nanotubes (Preclinical studies)	Tubular hydrophobic networks of carbon atoms with diameter 1 nm to 4 nm [62]	Cisplatin and epidermal growth factor (EGF) conjugated to the sidewall of single-walled nanotubes (SWNTs) [63]
Exosomes (*In vivo* studies)	Nano-sized membrane vesicles (30 nm to100 nm) that are shed from living cells [64]	Cyclophosphamide and polyinosinic-polycytidylic [65]
Graphene oxide (*In vitro* studies)	Oxidation derivative of graphene [66]	PEG-Paclitaxel [53]

Source: by author.

According to online research conducted with the keywords “nanotechnology” and “cancer” on the Web of Science database from January/2015 to January/2016, the polymeric, inorganic and magnetic systems were the most prevalent types of nanoparticles that have been investigated (Figure 10.3A), where they have been mainly applied to breast, liver, brain and pancreas cancers, even though other cancer-types such as lung, gastric, cervical, ovarian, prostate and colorectal cancers (Figure 10.3B).

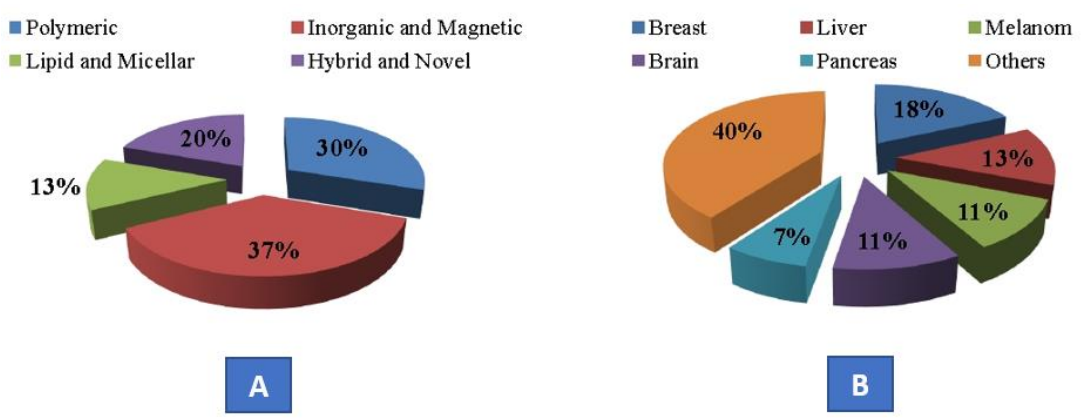

FIGURE 10.3
Main types of nanoparticles (A) that have been mostly investigated for the treatment of different cancers (B) according to research conducted on the Web of Science database from Jan/2015 to Jan/2016. Source: by author.

As depicted on Table 10.1, among the several drug delivery systems applied to cancer treatment, the liposomes and polymeric nanoparticles stand out for clinical requirements by Food and Drug Administration. PLD, DOXIL or Caelyx (pegylated liposomal doxorubicin) and Abraxane (paclitaxel protein-bound particles) are considered the first-line treatments for various types of cancer [31, 33, 38, 52]. For instance, non-pegylated liposomal doxorubicin (NPLD; Myocet) and liposomal daunorubicin (DaunoXome) are clinically approved. In addition, polymeric nanoparticles of paclitaxel (Genexol-PM), which is composed of PEG and poly-(D,L-lactic acid) (PLGA) block copolymers, has been approved for the treatment of breast and lung cancers (Table 1).

The biodistribution profile of nanosystems can be modulated by passive and active targeting strategies. Highlighting an example of passive targeting nanostructure available on market, Doxil is the one approved (in 1995) to treat SIDA-related Kaposi's sarcoma, ovarian cancer, and other cancer-types. CALAA-01 is an example of siRNA-containing active targeting system using human transferrin (Tf) as the target ligand of melanoma cells [53].

The choice of the route administration as well the type of nanocarrier may also influence the distribution of these systems in the body. Studies have shown that intravenous administration of liposomes and solid lipidic nanoparticles led to distribution preferentially to the liver followed to the lungs. On the other hand, a greater selectivity for the lung cancer therapy can be achieved through administration of nanosystems by inhalation [67].

Emerging research in engineering advanced delivery systems has also been developed in order to achieve successful cancer treatments, in which nanosystem-type and its coating, size, surface charge and shape, have been performed with the purpose of improving treatment outcomes. For instance, polymer-based nanomaterials such as polyurethane coated polycaprolactone (PCL) nanofibers containing the drug cyclopamine have been used to move tumors along a different path towards a chosen destination [68]. Gold and iron oxide inorganic nanoparticles enhanced drug delivery capabilities as a result of heating by infrared light and using magnetic fields, respectively [54].

In this scenario, carbon based materials such as carbon nanotubes and graphene have been investigated as carriers for antitumor drugs, showing promising results due to its high surface area and ability to be used in photothermal therapies. Rod shaped nanoparticles conjugated with the targeting antibody trastuzumab showed higher specific uptake by breast cancer cell lines in comparison to spherical nanoparticles. Nanoscale carries with hydrodynamic diameter beyond 200 nm are taken up preferentially by macrophages and reticuloendothelial system due to blood opsonization process [69]. In addition, the uptake of neutral or positively charged nanoparticles by macrophages/lymphocytes is drastically lower than that of the negatively charged nanoparticles [70].

Nanotechnological Improvement of Naturally Derived Drugs in the Treatment of Cancer

Historically, the potential uses of natural products as a source of anticancer agents is unquestionable. Natural products as anticancer agents can be classified by the chemical structure of its molecules, and can be categorized in vitamins, carotenoids, alkaloids, selenium derivatives, organosulfurs, fatty acids, polyphenols and flavonoids (incluing chalcones, flavones, quercetin, resveratrol, curcumin, genistein) compounds class. Indeed, more than 400 anticancer agents from African flora have been investigated, covering three major classes: flavonoids, alkaloids and

terpenoids. Among the flavonoids are quercetin, catechin and apigenin. Erythralinekaousine and *trans*-fagaramide are the most commonly studied alkaloids, whereas lupeol, α-amyrin and englerin A are the main terpenoids [33, 71-78].

The natural products applied to the cancer can be also classified by its origin: natural, synthetic or semisynthetic. According Newman and Cragg, the 206 anticancer drugs approved worldwide (1940s to 12/31/2010) were obtained by biological (13%); natural product (13%); derived from a natural product and is usually a semisynthetic modification (28%); totally synthetic drug (21%); made by total synthesis, but the pharmacophore is/ was from a natural product (10%), among other [79]. Previously, on cancer area over the time frame from 1946 to 1980, of the 75 small molecules, 40% or 53.3%, are natural product (NP) or derived from a natural product (NPD). In the 1981 to date time frame the equivalent figures for the NP compounds of the 185 small molecules are 62% or 33.5%, though to these can be added the 58 totally synthetic drug and 64.9% made by total synthesis, but the pharmacophore is/ was from a natural product [80]. Although combinatorial chemistry techniques have succeeded as methods of optimizing structures and are successfully applied in the optimization of many recently approved anticancer agents, from which only two new combinatorial compounds were approved as drugs in this 39 years (and one of it should be speculative). Though there is also one drug that was developed by using the "fragment-binding methodology", approved in 2012. Newman and Cragg, also consider a significant number of natural product drugs/leads produced by microbes and/or microbial interactions which indeed, should be expanded significantly [80].

Anticancer agents derived from natural products are obtained from different sources which may be also directly or indirectly associated with its millennial and popular use. Comprehensively, the usual classification of plant-derived anticancer agents is based on four classes: 1) vinca alkaloids (vinblastin, vincristine and vindesine), 2) epipodophyllotoxins (etoposide and teniposides), 3) texanes (paclitaxel and docetaxel), and 4) camptothecin (camptothecin and irinotecan) [33, 79-84].

The nanotechnology applied to natural products has been focused on the development of anticancer agents with both chemotherapeutic and chemopreventive effects. Natural nanochemopreventive drugs are usually used during carcinogenesis (i.e., initiation, promotion and progression of the tumor) and proved to be effective in reducing cell proliferation or inducing apoptosis in human cancer cells, while nanochemotherapeutic agents directly affect the cancerous cells [81].

The concept of chemoprevention was proposed in 1970 and referred to the prevention of cancer using naturally occurring compounds or analogs compounds, in which experimental carcinogenesis models have been used to investigate their efficacy in a stage-specific manner. Resveratrol, selenium, vitamins A, C, D and E, organosulfurs, and curcumin become as chemopreventive agents, and could be used in the prophylaxis of cancer, as well in combination with chemotherapeutic agents for treating several cancer-types [85, 86].

The chemopreventive agents can be delivered in food-derived products, while the chemotherapeutic ones are classified as controlled use drugs due to their serious side effects, although they are considered as having tolerable toxicity, especially when the risk-benefit ratio associated with cancer therapy is taken into account [87].

Highlighting some examples to the side effects associated with chemotherapeutic natural compounds: i) etoposide caused pain through a continuous IV infusion; ii) myelosuppression, diarrhea and mucositis were related to 5-fluorouracil; iii) hypersensitivity reactions, nephrotoxicity and neurotoxicity were associated to paclitaxel [88]; and iv) doxorubicin is another important anticancer drug which has been extensively used in major organs carcinoma (e.g., breast, stomach, lung, ovary, and bladder) and soft tissue sarcoma. Nevertheless, the severe cardiotoxicity

associated with DOX limits its clinical applications [89]. Meanwhile, nanotechnology minimize the toxicity and enhance the chemotherapy efficacy, and also improve some of the properties of bioactive molecules (synthetic or isolated from natural sources) in terms of bioavailability, pharmacokinetic and biodistribution. In addition, nanotechnology has been used to enhance the selectivity of chemopreventive agents towards tumor cells. For instance, resveratrol and epigallocatechin gallate (EGCG) encapsulated into solid lipid nanoparticles and polymer-based nanoparticles, showed selective delivered to tumor cells, reducing the overall toxicity [89-92]. Other naturally derived anticancer drugs, such as paclitaxel, doxorubicin, 5-fluorouracil, curcumin and diospyrin have been incorporated in nanoparticulate systems with promising results [80, 87, 89, 90-94].

Paclitaxel (Taxol®) is a potent anticancer agent from natural source that has shown to be effective against leukemia and a number of solid tumors including breast, lung, ovary, gastric, brain, and prostate cancer. The use of nanotechnology applied to the new clinically validated formulation of Paclitaxel protein (albumin)-bound nanoparticles (Abraxane®) was able to reduce the side effects associated with cremophor EL, which was the solvent of choice for these preparations [87].

Various nanosystems have been developed as strategies to minimize the toxicity and to enhance the chemotherapy efficacy of doxorubicin (DOX). For instance, polymeric micelles based on alginate-g-poly(N-*iso*-propylacrylamide) (PNIPAAm) in which DOX-loaded micelles accumulated selectively in a tumor-bearing mouse model acting as a passive target [90]. Magnetic gold nanoparticles (MGNPs) functionalized with thiol-terminated polyethylene glycol (PEG) was applied to incorporate DOX, aiming at to reduce its random distribution on various tissues and also its related side effects [91], with significant results. Doxorubicin-loaded cisplatin crosslinked polysaccharide-based nanoparticles (Dex-SA-DOX-CDDP) was developed by using the principles of selective biodistribution and release. The results showed that the Dex-SA-DOX-CDDP nanoparticles were more effective than non-entrapped DOX in colorectal carcinoma and metastasis of mammary carcinoma at different *in vivo* models [92].

5-Fluorouracil is an anticancer drug that has been extensively used in the treatment of some of the most frequently occurring malignant tumors such as breast, colon, and skin cancer. However, this drug exhibits a high toxicity and low tumor affinity, which decreases its effectiveness and contributes to the appearance of severe side effects [94]. In order to overcome these problems, 5-fluorouracil was incorporated in polymeric nanoparticles [95, 96] liposomes [97, 98] and solid lipid nanoparticles [99]. *Trans*-ferrin-coupled liposomes have been used to enhance the brain uptake of 5-fluorouracil. The *in vivo* studies showed the selective uptake of the *trans*-ferrin-coupled liposomes from the brain capillary endothelial cells, in which a 10-fold increase in the brain uptake of the drug was observed after liposomal delivery against a 17-fold increase in that with the transferrin-coupled liposomes compared with that of free-drug [97].

According to Thomas et al. [98], 5-fluorouracil tends to be poorly retained in the aqueous compartment of liposomes. These authors overcame this problem by developing liposomes based on a ternary complex comprising copper, low molecular weight polyethylenimine and 5-fluorouracil. Plasma concentrations of 5-fluorouracil were 7- to 23-fold higher when the drug was administered intravenously to mice as ternary complex, when compared with the non-load 5-fluorouracil (free-drug). In addition, the therapeutic effects of the ternary formulation, as determined in a HT-29 subcutaneous colorectal cancer model, showed to be greater than that achieved to the free-drug administered at equivalent doses [98].

5-Fluorouracil has been incorporated into chitosan nanoparticles for controlled release, in which a fast release during the first hour followed by a slow drug release during a 24 h period was observed [95]. In other hand, a promising drug delivery system developed by using nanoparticles formed

with hydrophobic core polymer and triblock copolymers Poly(DL-lactic acid), Poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol) copolymer (PLA/PEG-PPG-PEG) and Poly(D,L-lactide–co-glycolide)/Poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol) copolymer (PLGA/PEG-PPG-PEG), had sizes ranging from 145 nm to 198 nm, favor the formation of passive targeting against tumor cells due to the enhanced permeability and retention effect. These nanoparticles could release up to 94.4% of 5-fluorouracil at the end of 72 h and their low cell viability values indicated a much lower toxicity of the encapsulated 5-fluorouracil, when compared with the free- drug [96].

Curcumin (diferuloylmethane) a low-molecular-weight polyphenol, extracted from the perennial herb *Curcuma longa* (known as turmeric), showed to able on suppress the proliferation of a wide variety of tumor cells [100, 101]. Although, shows promising potential as a natural antitumor agent, its poor aqueous solubility is a barrier for a reasonable bioavailability and clinical efficacy. So, its encapsulation in alginate-chitosan-pluronic composite nanoparticles proved to be an efficient alternative to improve the dissolution of this lipophilic drug. The curcumin-composite nanoparticles showed to be nontoxic to HeLa cells at a concentration of 50 μg/mL [102]. In another study, curcumin was encapsulated in liposomes for intravenous administration. The results showed a potent NF-kB inhibitory activity that was associated to the antiproliferative and proapoptotic effects. So, the liposomal curcumin successfully inhibited pancreatic cell growth in murine xenograft models. Moreover, this study evidenced the potent anti-angiogenic response of liposomal curcumin with no sign of toxicity, even when maximal volumes of curcumin-composite nanoparticles were administered to mice [103].

Diospyrin (bis-naphthoquinonoid) is a plant-derived compound that has emerged as a potential molecule for development of anticancer drugs. However, diospyrin showed significant cytotoxic due to its ability to generate reactive oxygen species and to induce apoptosis in human cancer cell lines [104]. Hazra et al. (2005) demonstrated that the liposomal encapsulation of diospyrin was able to enhance its antitumor activity against mice bearing Ehrlich ascites carcinoma in comparison with the non-encapsulated drug [105]. The liver is the main organ affected by Ehrlich ascites carcinoma and therefore, the liver function enzymes were used in this study to evaluate liver tissue damage. The results showed that the glycolytic and liver enzymes were restored to near normal levels in the mice treated with diospyrin encapsulated in liposomes.

Similarly, Siripong at al. [106] using nanotechnology to improve the bioavailability of a medicinal plant called *Rhinacanthus nasutus* Kurz (Acanthaceae), showed the herbal antitumor effectiveness. Indeed, its encapsulation into liposomes enabled its injection in BALB/c mice, and showed strong antiproliferative activity against HeLaS3 cells, suppressing the solid tumor growth in Meth-A sarcoma at the dose of 5.0 mg/kg/d for 10 days.

Crocus sativus L., commonly known as saffron, is a raw material extracted from the red gold spice that has been largely used in folk medicine. *In vivo* studies have shown the antitumor and cancer preventive properties of saffron, where it has been demonstrated to have significant anticancer activity in skin, breast, lung, pancreatic and leukemic cells [107]. Vijayakumar et al. [108] reported the biosynthesis of gold nanoparticles using the leaf extract of saffron through a clean, non-toxic and environmentally friendly route (green chemistry). The authors could successfully produce monodispersed spherical and triangular gold nanoparticles with dimensions ranging from 11 nm to 20 nm. Besides its antitumor activity, saffron was able to stabilize the gold nanoparticles.

The natural compounds *trans*-dehydrocrotonin and usnic acid as isolated natural molecules, should be new potential natural products for cancer therapies, from which nanoencapsulations have been used to improve their anticancer activities [17, 33, 109].

***Trans*-dehydrocrotonin as a potential anticancer natural product**

The diterpene *trans*-dehydrocrotonin (Figure 10.4) is a natural drug isolated from the stem barks of *Croton cajucara* Benth (Euphorbiaceae) [33, 71- 74]. This vegetal species represents a medical source of great importance in the Brazilian Amazon region and because of its foremost pharmacological results become recommended by the Brazilian Unified Health System (SUS).

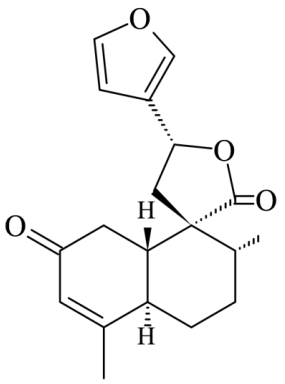

FIGURE 10.4
Molecular structure of *trans*-dehydrocrotonin as the major compound isolated from the stem barks of *Croton cajucara* Benth. Source: by author.

An extensive phytopharmacological research of *C. cajucara* revealed that its stem barks showed to be a rich source of diterpene clerodane-type compounds, being the 19-*nor*-clerodane *trans*-dehydrocrotonin (DCTN) the major isolated constituent. Among the minor compounds, the triterpene acetyl aleuritolic acid (AAA) and the other furano-clerodane-type diterpenes: *trans*-crotonin (CTN), *trans*-cajucarin B (*t*-CJC-B), *cis*-cajucarin B (*c*-CJC-B), *trans*-cajucarin A (CJC-A), and *iso*-sacacarin, and also two butenolide-clerodane-type diterpenes cajucarinolide (CJCR) and *iso*-cajucarinolide (ICJCR) (Figure 10.5). These compounds proved to be biological effective against several diseases [74, 110-112].

The clerodanes DCTN as well as CTN (natural and semi-synthetic), *t*-CJC-B, *c*-CJC-B, CJC-A, CJCR, and *iso*-CJCR were studied for their effects against human K562 leukemia and ascitic Ehrlich carcinoma cells [112]. The inhibitory effects on the cell growth were dose dependent on Ehrlich carcinoma cells assays with IC_{50} values of 166 μM for DCTN, 164 μM for CTN, 65 μM for CJCR and 10 μM for ICJCR. The flavonoid quercetin (44 μM) was used as positive control, where the tested clerodanes (DCTN and ICJCR) were the lowest citotoxic agents. Both natural and semisynthetic clerodanes showed significant cytotoxic activity against human K562 leukemia cells with IC_{50} of 38 μM (*t*-CJC-B), 33 μM (*t*-CJC-A), 36 μM (CJCR) and 43 μM (ICJCR) [112].

The therapeutic action of *C. cajucara* is largely correlated with the bioactive clerodane *t*-DCTN [74, 113] which has the following pharmacological activities: hypoglycemic [114, 115], hypolipidemic [116-117], antigenotoxic [118, 119], antiulcerogenic [120-122], anti-inflammatory and antinociceptive [123-125], antiestrogen [126], cardiovascular [127] and antitumor [128]. The use of *Croton cajucara* in the popular medicine has been based not only on its pharmacological properties, but upon a medicinal history of safe use of this plant. On the other hand, during the 1990s, several cases of toxic hepatitis correlated to this plant were notified in public hospitals

around the North region of Brazil. This disease has been associated with the abusive use (large quantities and prolonged treatments) of its leaf and stem bark [72-74].

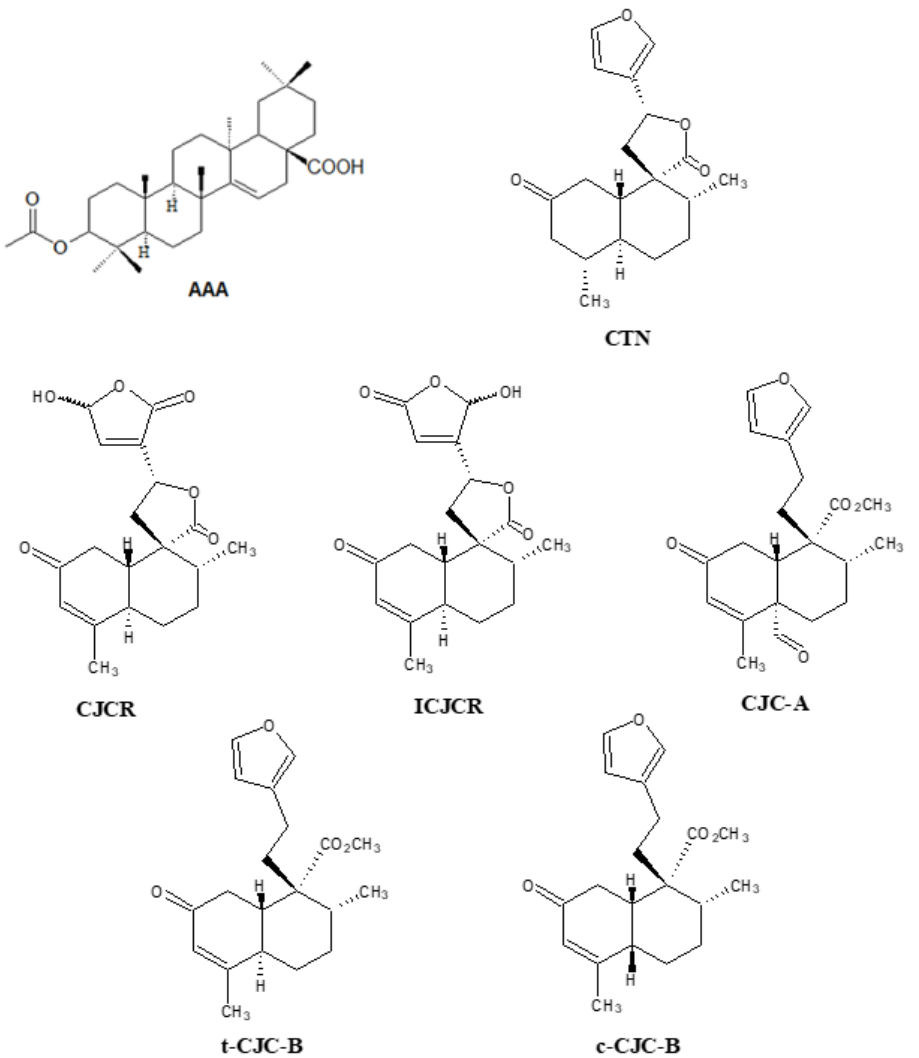

FIGURE 10.5
Molecular structures of minor compounds isolated from the stem barks of *Croton cajucara* Benth. Source: by author.

Aspects related to cytotoxicity and toxicity of DCTN must be considered in the development of a DCTN-drug release system. Therefore, cytotoxicity of this bioactive compound has been evaluated by parameters such as cell morphology changes, measurements of cell viability and inhibition of cellular metabolism as well as through IC_{50} values. In this context, several tumor cell lines have been studied: fibroblastic lung cells of Chinese hamsters (V79) [129-131], rat hepatocytes [130, 131], Ehrlich tumor cells [112, 128], *Escherichia coli* [128], and promyelocytic leukemic cells (HL60 and K562) [112, 132, 133].

The clerodane DCTN produced changes in ascitic Ehrlich carcinoma cells with IC_{50} of 16 mM [112, 128] as well as in Chinese hamster lung fibroblast cells (V79) at concentrations of 80 to 400 mM with IC_{50} of 240 mM [129-131]; in leukemic promyelocytic cells (HL60) inducing apoptosis; in human blood mononuclear cells causing low toxicity [112, 132, 133] and in rat hepatocytes with selective toxicity after subchronic treatment with 8 mM [130, 131].

The antigenotoxicity and antimutagenic activities of DCTN have been reported. Indeed, Santos et al. [134, 135] observed the absence of mutagenicity of the stem bark methanolic extract of *C. cajucara* (ME-CC) in bone marrow cells of Swiss albino mice. Moreover, Agner et al. [118, 119] investigated the mutagenic effect of DCTN in Swiss mice (under acute treatments, via gavage and intraperitoneal route). The performed assays showed that DCTN does not present any mutagenic effect. Encouraged by these results and considering the wide use of *Croton cajucara* in the traditional medicine on the Amazon region of Brazil, Agner et al. [119] and Santos et al. [134] evaluated the chemoprotective activity of both ME-CC and DCTN, respectively, correlating the results with cyclophosfamide, a cytotoxic chemotherapeutic agent with known DNA alkylation action. The animals underwent a subchronic treatment with the ME-CC extract (312.5, 625.0 and 1,250.0 mg/kg) for 4 consecutive weeks. This extract diluted in DMSO or water was administered to the animals via gavage an hour before the administration of cyclophosfamide (150 mg/kg; via intraperitoneal). The results revealed that the animals treated with the lowest extract concentration (312.5 mg/kg) showed a significant reduction in the micronuclei cell frequency on the last two weeks of treatment. On the other hand, at higher concentrations (625 and 1,250.0 mg/kg) ME-CC extract revealed a protective effect during the entire period of treatment, with a significant reduction of micronuclei cell frequency induced by the cyclophosfamide.

The cyclophosfamide effect of DCTN was evaluated in bone marrow cells of Swiss mice, using chromosome and micronucleus aberration tests. The animals underwent intraperitoneal treatment or gavage injection with a concomitant use of DCTN and cyclophosfamide. For animals treated via gavage, the 25% dose of the LD_{50} showed antimutagenic effect in relation to cyclofosfamide. In the two types of cytogenetic tests (intraperitoneal or gavage administration) DCTN at 50% and 75% doses of the LD_{50}, reduced the genotoxicity due to cyclophosphamide. These above described results confirmed both DCTN and the ME-CC as antimutagenic agents in blood and bone marrow cells [119, 134-136].

The clastogenic, apoptotic and cytotoxic effects of DCTN have been investigated *in vitro* experimental model. The results showed that this drug is not clastogenic or cytotoxic and that it does not induce apoptosis [136].

Decreased cytotoxicity of DCTN was observed in V79 cells and hepatocyte culture when DCTN was complexed with β-cyclodextrin [130]. On the other hand, in subacute toxicity studies (35 days), DCTN was administered daily through the oral route (25, 50 and 100 mg/kg) causing a reduction in the plasma levels of alkaline phosphate and cholesterol, as well as histopathological changes in the liver (cloudy swelling, microvacuolar degeneration and nuclear changes) [121]. Indeed, at higher doses, a significant increase in liver weight and in the levels of gamma-glutamyl transpeptidase (female rats) was observed.

Acute toxicity studies (14 days) of DCTN in male Swiss mice through oral (125, 250, 500, 750 and 1000 mg/Kg) and intraperitoneal routes (25, 31, 62.5, 125, 250 and 500 mg/kg) demonstrated the low toxicity of this compound, with a DL_{50} value of 876 mg/kg (12 h) and 47.2 mg/kg (14 days) for both routes of administration [129]. Meanwhile, acute toxicity studies (72 h) of DCTN through oral route revealed no toxic symptoms in the central nervous system, such as stereotyping, ataxia, and convulsions. Its DL_{50} was found to be 555 mg/kg, as determined in mice through oral route [122].

Nonetheless, nanotechnology has been successfully applied to incorporate DCTN into polymeric nanoparticles functionalized with L-ascorbic acid 6-stearate (AAS) using active-targeting pathway towards HL60 tumoral cells. The DCTN-loaded into target nanoparticles showed a more effective antitumoral activity when compared to free-DCTN [137].
In another study DCTN was encapsulated into liposomes, where an enhancement of its antitumor activity, together with a reduced hepatotoxicity effect, were observed. In this study it was investigated the formation of the inclusion complexes of DCTN with hydropropyl-β-cyclodextrin (HP-β-CD), with further encapsulation on liposomal carriers. The anticancer activity of the liposomes formulations was evaluated against Sarcoma 180-bering mice, with histopathological and hematological analysis. Animals treated with both DCTN-loaded lipossomes and DCTN-HP-β-CD-loaded lipossomes had a substantial increase in the efficacy of tumor treatment in comparison with the non-entrapped drug (free-DCTN), resulting in tumor inhibitions of 79.4 ± 9.6% and 63.5 ± 5.5%, respectively. Moreover, no significant hematological toxicity and only a little decrease of plaque levels were observed using lipossomal formulation containing DCTN [17, 138].
The complexation of DCTN with HP-β-CD also led to an increase in the drug aqueous solubility which enhanced its entrapment efficiency in the aqueous phase of liposomes [139]. It was evidenced that the toxicity of DCTN was reduced when complexed with β-cyclodextrin (β-CD) [138]. In other study, DCTN loaded into PLGA enhanced its hypoglycemiant effect [140]. It was found that the bioactive *trans*-dehydrocrotonin (DCTN) loaded into a colloidal nanosystem for oral use, was patented for immunomodulatory therapeutic process associated with its hypolipidemic and hypoglycemic effects [141].

Usnic acid as a potential anticancer natural product

Usnic acid (Figure 10.6) is a dibenzofuran derivative exclusively found in lichens in genera such as Alectoria, Cladonia, Usnea, Lecanora, Ramalina and Evernia [142]. Usnic acid has shown promising anticancer properties, as well as several other pharmacological activities such as antimicrobial against human and plant pathogens, antiviral, antiprotozoal, anti-inflammatory and analgesic.

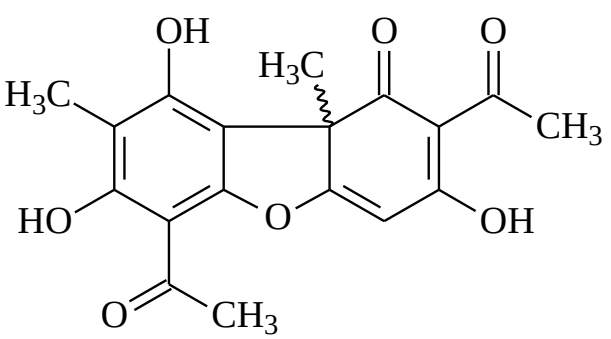

FIGURE 10.6
Molecular structure of (+)-(9b-R)- and (-)-(9b-S)-usnic acid. Source: by author.

The antitumour activity of usnic acid was first reported in 1975 against Lewis lung carcinoma [143]. This drug showed to be a non-genotoxic antineoplastic agent with a p53-independent mechanism. Among several lichen constituents, the (+)-usnic acid enantiomer exhibited the highest antitumour effect induced by Epstein–Barr virus when compared to the (-)-usnic acid enantiomer [144]. On a study performed with a fat-burner called lipokinetix containing usnic acid, the data showed a

reduction in the ATP production on liver and cell mitochondria in cancer cells, inhibiting the cell proliferation [145]. Additionally, usnic acid and another typical secondary metabolite of lichens (parietin, atranorin and gyrophoric acid) were applied towards different human cancer cell lines (A2780, HeLa, MCF-7, SK-BR-3, HT-29, HCT-116 $p53^{+/+}$, HCT-116 $p53^{-/-}$,HL-60 and Jurkat). The data showed that usnic acid was the most efficient at equitoxic doses and correlated more strongly with an increased number of floating cells or a higher apoptotic index and accumulation of cells in S-phase [146]. It was found that usnic acid is an activator of programmed cell death by inducing a massive loss in the mitochondrial membrane potential and phosphatidylserine externalization in two tested cell lines (A2780 and HT-29 cells) [147]. Regarding to its toxicity in humans, it has been demonstrated that usnic acid has unwanted dermatological effects such as: local irritation and allergic contact dermatitis, whereas in other animal's toxic effects were observed at high doses, with i.v. LD_{50} doses of 25 mg/kg in mice, 30 mg/kg in rats and rabbits, and 40 mg/kg in dogs [148].

Although showing promising results as a natural antitumor drug, usnic acid has a poor solubility in water [149] and some hepatotoxicity [150], which have limited its therapeutic use. So, nanotechnology has been applied as an alternative to reduce hepatotoxicity and improve its antitumour activity, by encapsulation into PLGA nanoparticles. The antitumor activity of load-usnic acid nanoparticles was carried out on an ascitic tumor (Sarcoma 180) in Swiss mice, where the encapsulated usnic acid produced a 26.4% increase in tumor inhibition when compared with the non-encapsulated drug. Moreover, no histological changes were observed in the kidneys or spleen of animals treated with either free-usnic acid or loaded-usnic acid nanoparticles [151].

Bioactive Natural Products in the Treatment of Oral Cancer and its Technological Improvement

The multidisciplinary approach of the natural compounds represents an important field of study, enabling the development of novel therapeutic alternatives for cancer treatments [79, 80, 152]. Retinoic acid, green tea polyphenols, curcumin, resveratrol and lycopene are few examples of bioactive natural products that have shown potential applications for treating head and neck cancers [153]. Head and neck squamous cell carcinoma (HNSCC) have high levels of morbidity and mortality and causes nearly 550,000 deaths worldwide per year [154]. Most HNSCC tumors are diagnosed in late stages of development, presenting nonspecific symptoms. The current treatment options available are surgery, radiotherapy and chemotherapy, isolated or in combination, depending on the disease stage. However, these treatments have significant side effects, functional impairment and unfavorable cosmetic results [155, 156]. In addition, such therapies are not completely effective and do not improve the prognosis of some HNSCC patients [157, 158]. Therefore, the need for new treatment strategies with fewer side effects for HNSCC tumors is evident and the use of nanotechnology represents an obvious alternative.

Head and Neck Squamous Cell Carcinoma (HNSCC)

Epidemiology

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer worldwide, including primary tumors derived from the epithelial lining of the oral cavity mucous membranes,

pharynx and larynx [154]. About 30,000 deaths caused by HNSCC have been reported in the US in 2015 [155]. More than 90% of head and neck cancers are squamous cell carcinomas (SCC). In the oral cavity, most tumors often affect the ventrolateral surface of the tongue, the floor of the mouth and the soft palate [156].

Although the mechanisms of pathogenesis and the molecular biology of tumors are better understood nowadays, and several new prognostic markers have been identified in the last decade, five-years-survival rate of patients affected by HNSCC has not changed accordingly, which is still around 50% [157, 158]. The HNSCC diagnosed in stage III and IV has the worst prognosis [159, 160]. The longer the time between diagnosis and the beginning of treatment the smaller the survival of patients. Delay of treatment has been a serious problem that doctors, and health care policy makers have to tackle [160].

The major risk factors of HNSCC are tobacco smoking, alcohol consumption and environmental exposures. Carcinogenesis of head and neck is associated with formation of DNA adducts by numerous chemical compounds already identified in tobacco smoke [161-164]. Additionally, HPV and EBV infection are emerging as causative factors of oropharynx and esophagus carcinomas [165, 166]. HPV-positive tumors have a better prognosis than HPV-negative tumors, although the same treatment protocol is still suggested for both tumors [167].

Etiology of HNSCC

Oral carcinogenesis is a complex multifocal process that comprises the malignant transformation of epithelial cells. The properties of these cells include proliferation, cornification, and apoptosis, and these events are fundamental for homeostasis of oral mucosa [168]. In the basal layer, cells have a better proliferation capacity, whereas in the upper layers the cells acquire differentiation capacity (cornification) and at the end of the differentiation process, the cells suffer apoptosis [169, 170].

Several cancers and many systemic diseases are related with tobacco smoking, including epithelial tumors of respiratory system and oral mucosa [171-173]. Other risk factors include alcohol consumption, immune deficiency and family history of squamous cells carcinoma (SCC) [174, 175]. Tobacco use causes alterations in genetic material and is associated with malignant phenotype acquisition, where the major genetic alterations in the epithelial cells are mutations in apoptosis [176, 177], migration [178] and gene invasion [179]. The p53 mutation is commonly known in epithelial tumors, including in oral SCC [180].

A group of compounds, produced while tobacco is smoked and from the constituents of the environmental pollutants, is denominated polycyclic aromatic hydrocarbons and shows relevant carcinogenic activity in keratinocytes and other epithelial cells [181]. Despite the current knowledge about the effects of chemical compounds of tobacco in oral carcinogenesis, the effects of nicotine in human epithelial cells remain less explored. However, Michcik et al. [182] studied the effects of nicotine in epithelial cells of smokers and nonsmokers, and concluded that keratinocytes from former subjects showed fewer cells with early and late apoptotic features than those of latter ones, but no effect on the oral keratinocyte cell cycle. These alterations occur due to DNA adducts formation in genetic material of the cells. Covalent adducts of DNA initiate the process of oral carcinogenesis in epithelial cells [183, 184]. This critical event contributes to DNA instability and genes mutation, which results in cells with invasive phenotype [185].

Current therapy for HNSCC

Currently, the treatment options for primary and secondary HNSCC tumors are surgery,

radiotherapy and/or chemotherapy [186, 187]. In a recent meta-analysis, the benefits of chemotherapy in terms of the overall survival on HNSCC cases were reviewed [188-190]. All current treatment options result in significant side effects, where these complications affect the quality of life of most patients [191, 192]. The main side effects of chemotherapy are listed in Table 10.2. The use of Cisplatin/Carboplatin alone or in combination with 5-fluorouracil, methotrexate, or paclitaxel may result in tumor size control and prevention of distant metastases. However, the increased risk of myocardial ischemia and infarction with the use of these combinations have limit its clinical application [193-195, 189]. Substantial toxicity of chemotherapy drugs has been reported as cause of delays and cessation of cancer treatment [191, 196].

Increasing doses or combining drugs treatments not necessarily end up with better results, especially in advanced stages of the disease [177, 178]. The understanding of deregulated signaling pathways in carcinogenesis and in HNSCC tumor development may lead to therapies with fewer side effects and targeted molecular therapies.

TABLE 10.2
Treatments options for HNSCC tumours and its side effects.

Treatment	Side effects	Reference
Surgery	Taste and smell disorders, Breathing and hearing difficulties, Impairment of the oral function and appearance, Poor diet/nutrition	[197-199]
Chemotherapy: Platinum agents (Cisplatin/Carboplatin)	Nephrotoxicity, ototoxicity Neurotoxicity, myelosuppression Nausea, vomiting, Electrolyte disturbances	[200-202]
Chemotherapy: 5-Flurouracil	Cardiac toxicity Nausea, vomiting, ulcers Myelosuppression, Thrombophlebitis, dermatologic disorders (rash)	[200-202]

Chemotherapy: Taxanes Paclitaxel/Docetaxel	Hypotension, EKG changes Gastrointestinal mucositis, nausea, vomiting, neutropenia, leukopenia, thrombocytopenia, neurotoxicity, Hepatotoxicity	[200-202]
Chemotherapy: Cetuximab	Infusion reaction, acneiform rash, pruritus, abdominal pain, constipation, diarrhea, nausea, vomiting, dyspnea, cough, Neuromuscular weakness	[203-206]
Radiotherapy	Mucositis, oral candidiasis, loss of taste and xerostomia, osteoradionecrosis, salivary glands, oral mucosa, bone, dentition, masticatory muscles and temporomandibular joints damage	[197, 207, 208]

Source: by author.

These therapies have targeted important pathways regulated by epidermal growth factor receptor (EGFR) and vascular endothelial growth factor (VEGF) [209-214]. Developing new chemotherapy drugs derived from medicinal plants or others biomolecules with similar efficacy but less toxicity should be a priority for future research.

Chemotherapy and chemopreventive effects of natural compounds in HNSCC

The term “cancer chemoprevention” has been used for description of the use of natural or synthetic substances to reverse, suppress or prevent the initiation, promotion, or progression of cancer. The cancer chemoprevention has benefited from technological advances in several areas [214, 215]. However, the use of natural compounds in cancer therapy has not developed significantly, especially in HNSCC [216, 217]. Several medicinal plants and natural compounds have been suggested as chemotherapy and chemopreventive agents and although the number of studies in HNSCC is small, they have shown to be effective and to show fewer side effects when compared with the conventional chemotherapy drugs. Examples of naturally occurring compounds studied in HNSCC include retinoids, green tea polyphenols, curcumin, resveratrol, lycopene and luteolin, where the most satisfactory results have been obtained with the use of retinoids and curcumin [171].

The first reports of natural compounds used to prevent and/or treat HNSCC include 13-cis-retinoic acid (13-cRA) or *iso*-treonin, which is an analog of vitamin A [218, 219]. Retinoic acid is a natural compound that regulates the cell proliferation, differentiation and apoptosis process [220, 221]. Hong et al. [218] reported that the use of 13-cRA is effective in the treatment of oral leukoplakia and others premalignancies. However, subsequent trials demonstrated no significant improvement in survival for HNSCC patients who were at high risk of recurrence when treated with low or high doses of isotretinoin for up to 3 years [214, 219, 222, 223].

Some clinical trials and *in vitro* or *in vivo* studies evaluated the effects of 13-cRA in increasing the survival of HNSCC patients. 103 HNSCC patients, who were disease-free at the time of enrollment, were treated with 13-cRA for the prevention of Second Primary Tumors (SPTs). Reduction of SPTs recurrence was observed in patients who used 13-cRA [219, 222]. Other phase III clinical trial evaluated the effect of low dose 13-cRA (30 mg/day for 3 years) in early stage of HNSCC patients. The authors concluded that low-doses of isotretinoin were not effective in reducing the rate of second primary tumor development, as well as death and smoking-related diseases, when compared with the placebo group [223]. Other study performed by Lee et al. (2011) [224] identified 9,465 single nucleotide polymorphisms including RXRA (retinoid X receptor), JAK2 (Janus kinase 2), and CDC25C (cell division cycle 25 homolog C phosphatase) associated with HNSCC patients at high risk of SPT/recurrence. These authors also predicted a favorable response to 13-cRA, depending on the genotype of the patient. They concluded that biomarkers could be used to select patients who would be in the most need for 13-cRA chemoprevention, as well as the ones who would best respond to the use of this compound.

Most biocompounds derived from natural products shows poor oral bioavailability, which limits its clinical applications. For instance, 13-cRA is extensively metabolized by cytochrome P450s in the liver, which reduces its bioavailability especially when administered by oral route. To overcome such limitation, Park et al. [225] developed microspheres containing both 13-cRA and celecoxib, which were designed to treat 4-NQO-induced oral carcinogenesis. The authors stated that the use of celecoxib could maintain the 13-cRA plasma concentration at higher levels while reducing its metabolism by preventing inflammatory responses, thereby improving their chemopreventive effects against 4-nitroquinolone-1-oxide (4-NQO)-induced oral carcinogenesis [225].

The PI3K/Akt/mTOR signaling pathway plays a key role in many processes of cancer cell proliferation. It also regulates cell survival processes, initiation and maintenance of the malignant phenotype. Since it has been shown to be altered in several types of cancer, it may represent a novel therapeutic target [226-229]. Some carcinogens are associated with loss of function of the tumor suppressor PTEN and PI3K, Akt mutation [230-235]. Thus, activation of the PI3K/Akt/mTOR pathway has been associated with resistance to conventional cancer treatment and poor prognosis in some cancers [230, 236]. High levels of p-Akt expression in HNSCC patients may contribute to tumor growth, regional lymph nodes metastasis and shorter survival time [234, 237, 238].

Curcuma longa is a perennial plant of the Zingiberacae (ginger) family and is native to Southeast Asia. Curcumin is a hydrophobic polyphenol isolated from rhizomes of this plant [239]. Several studies have demonstrated that curcumin use is associated with cytotoxicity, reduced inflammation, antioxidant effect, immunomodulation, anti-angiogenic effect, cytokine release and apoptosis [240-243], turning it into a promising therapeutic agent for HNSCC treatment. *In vitro* studies showed that curcumin inhibits the NF-kB pathway in various oral squamous cell carcinoma lines. Moreover, curcumin treatment has shown to suppress the growth and survival of these cell lines, as well as to downregulate several molecular targets including COX-2, HER2, EGFR, Akt and VEGF [244, 245]. Although curcumin has shown clear beneficial effects in some *in vitro* studies, it is not suitable for clinical use due to its rapid metabolism in the liver and intestinal wall [246]. Despite

its beneficial use in patients with colorectal cancer through oral route, the treatment resulted in low bioavailability [247]. Nanotechnology-based drug delivery systems may optimize the bioavailability of curcumin and improve cancer treatment.

In HNSCC treatment, curcumin has demonstrated *in vitro* and *in vivo* growth suppressive effects using nude mouse xenograft models [248]. However, most of its bioactive compounds are lipophilic and showed low aqueous solubility, resulting in low bioavailability when administered orally, therefore, justifying the use of nanotechnology as an effective way to improve the chemotherapeutic effect of these compounds.

Wang et al. [248] evaluated the effect of intravenous liposomal curcumin in mouse xenograft tumors of the oral cancer cell lines CAL27 and UM-SCC-1. Three groups were used: xenograft mouse tumors with no treatment, treatment with non-loaded liposomes and treatment with curcumin encapsulated liposomes. 3.5 weeks of treatment with i.v. liposomal curcumin was able to suppress the tumors growth, where no sign of toxicity was observed upon autopsy. Immunohistochemical analysis of tumor samples revealed decreased expression of cyclin D1, cyclooxygenase-2, matrix metalloproteinase-9, Bcl-2, Bcl-xL, Mcl-1L, and Mcl-1S in liposomal curcumin-treated tumors, indicating its effects on the NF-kappaB pathway.

4-NQO administration is a carcinogenesis model based in formation of DNA adducts and has been applicated for upper aerodigestive tract cancer promotion, simulating effects of tobacco in keratinocytes. Is a very efficient method for evaluating the compounds therapeutic effect or chemopreventive in HNSCC [226].

In addition, Clark et al. [249] have demonstrated the chemopreventive effects of curcumin in HNSCC, where the administration of 15 mg of this compound significantly increased the survival rate (286 ± 37 vs. 350 days) in the 4NQO carcinogenic model survival study. The authors have attributed the effects of curcumin on carcinogenesis suppression to the inhibition of the AKT/mTOR pathway, as indicated in the analysis of treated and untreated tumor cell extracts, associated with a significant decrease in MMP-9.

Nanotechnology and treatment of HNSCC

Despite recent advances in multidisciplinary applications in the nanotechnology field, its use in HNSCC has been concentrated in novel methods of laboratory-based diagnostics, such as development of biosensors based on nanomechanical systems and *in vivo* clinical diagnostic imaging [250-254]. Application of nanotechnology as drug delivery systems has been poorly investigated as carrier for conventional chemotherapy drugs in HNSCC treatment. Nanotechnology-based drug delivery systems (NB-DDS) have improved the stability of drugs and have controlled their targeted delivery resulting in enhanced specificity and efficacy of drugs towards specific tumor sites. The encapsulation of drugs into nanostructures allows reducing side effects when compared with the conventional treatment [255, 256]. In HNSCC treatment, few clinical trials have evaluated the efficacy of NB-DDS. Table 10.3 shows the main nanotechnology-based systems (polymeric nanoparticles, hydrogels, liposomes and cyclodextrins) as well as the main results of the use of these systems *in vitro* and in murine *in vivo* models. Despite being a relatively unexplored field of research, conventional chemotherapy drugs loaded in liposomes showed satisfactory results [257, 258]. In addition, other studies have evaluated the use of more sophisticated drug delivery systems based on magnetic nanoparticles and carbon nanotubes [259, 260].

TABLE 10.3
In vitro and *in vivo* studies that used nanotechnology-based drug delivery system in HNSCC and its improvements of chemotherapy efficacy.

Drug	NBDDS	Study design	Improvement of chemotherapy efficacy	Reference
Cisplatin	PNP	*In vitro* and *in vivo* (nude mice bearing OSC-19)	Noticeable antitumor activity, lymphatic drug delivery and reduced nephrotoxicity	[261]
Cisplatin	Hydrogel	*In vivo* (mice with OSCC cell xenografts)	Enhanced the therapeutic effects and could diminished the side effects	[262]
Paclitaxel	PNP	Clinical trial – preliminary study	Paclitaxel in albumin nanoparticles is reproducible and effective as induction chemotherapy before definitive treatment of advanced tumors of the tongue, with a view to organ preservation. Reduction of toxicity.	[263]
Paclitaxel	LC	*In vivo* (oral squamous cell carcinoma - OSCC - xenotransplanted into nude mice)	Reduced the immunohistochemical expression of VEGF and CD31 and VEGF mRNA. Inhibitory effect on the growth of transplanted human OSCC.	[264]

NBDDS: Nanotechnology-based Drug Delivery Systems
PNP: Polymeric Nanoparticle
LC: Liquid Crystal
Source: by author.

Rosental et al. [256] evaluated the use of cisplatin in liposomal formulation for HNSCC treatment. Twenty patients (median 55.9 years) with stage IVa/b HNSCC were treated with Stealth® liposomal cisplatin (SPI-077) concurrent with radiotherapy (60-72 Gy in 6-7 weeks), where the usual side effects attributed to cisplatin, such as ototoxicity, neurotoxicity and nephrotoxicity, were not detected. In addition, this work showed that SPI-077 liposomal chemotherapy preparations demonstrated promising capacity to deliver adequate amounts of drug to the tumor.

The potential use of cationic liposomes towards selectively targeted angiogenic endothelial cells has been studied in experimental tumors and human HNSCC [260, 265]. Strieth et al. [260] investigated the use of placitaxel loaded in liposomes for HNSCC treatment. Seven patients with non-resectable therapy-refractory HNSCC were recruited and treated with two doses of vascular targeting cationic liposomes encapsulating paclitaxel (EndoTAG-1 [ET]). The tumor volume measurements revealed stable disease in 4 out of 5 cases. This study demonstrated the efficacy of EndoTAG-1 [ET] for HNSCC treatment.

Cancer treatment has been changed in the last decades, and nanotechnology will modify it even further. The use of nanoparticles will facilitate drug delivery towards the targeted tissue in the treatment of oral cancer [252, 254]. Magnetic nanoparticles (ferrofluids) bound to chemotherapeutic drugs and subjected to an external magnetic field are of special relevance. The effect of magnetic nanoparticles carrying mitoxantrone for the treatment of oral cavity cancer in rabbits. They evaluated the mechanism of nanoparticles distribution by measuring their amount in the tumor, peritumoral area, various organs and body fluids (e.g. blood and urine), with and without magnetic drug targeting. The authors concluded that mitoxantrone showed higher concentration when encapsulated in magnetic nanoparticles, whose therapeutic efficacy was demonstrated in the treatment of squamous cell carcinoma in rabbits.

Other drug delivery system that has shown promising results for improving cancer therapy outcomes is the carbon nanotube-based drug delivery [266, 267]. Bhirde et al. [259] demonstrated the use of single wall carbon nanotubes (SWNT)-Cisplatin-EGF bioconjugates for killing cancer cells *in vitro* and *in vivo* by using EGF-EGFR interactions. This study showed that SWNTs bioconjugated with cisplatin and specific receptor ligand EGF were able to selectively and efficiently target squamous cancer cells that overexpress EGFR as demonstrated by *in vivo* and *in vitro* imaging and cancer cell viability.

In addition, other biomolecules extracted from medicinal plants have shown to have promising chemopreventive activity of HNSCC. However, some biomolecules are lipophilic and show low bioavailability following oral administration. The challenge of developing effective delivery systems for chemotherapeutic agents has not been fully overcome and more comprehensive efforts still are required to achieve a true measure of improvement on the current treatment of HNSCC.

Final Comments

Although the anticancer agents obtained from natural sources have shown promising results in terms of efficacy on preclinical and clinical studies, where they can have both chemopreventive and chemotherapeutic effects, problems associated to their poor solubility, bioavailability and biodistribution, as well as to their high cytotoxicity to diferent organs (heart, liver, spleen and others) have limited the application of these compounds as chemotherapeutic agents. However, the use of nanocarries for drug delivery in cancer therapy has improved these physicochemical and pharmacokinetics limitations, which have been achieved through a better control of the release kinetics as well as through an increase in the selective cellular uptake by mechanisms of passive and active targeting.

Among the nanosystems that have been used to carry and deliver anticancer drugs, particular attention has been devoted to liposomes and polymeric nanoparticles with non-stealth and stealth characteristics, where some pharmaceutical products have already been approved by FDA for human use. Recent advances in nanotechnology applied to natural products have favored the use

of magnetic and inorganic nanoparticles as promising alternatives for cancer therapy, where the size, shape and charge of the nanoparticle have influenced on the cellular uptake.
Plant-derived anticancer drugs such as *iso*-treonin, paclitaxel, curcumin, DCTN, and usnic acid, whose main classes of compounds are vitamins, alkaloids, polyphenols, flavonoids and terpenes, have been extensively used with some promising results. The natural products go on to be as an important source of highly potent anticancer drugs, whose problems of pharmacokinects and toxicity may to be solved by nanotechnology. Thus, a better understanding of cancer biology, an easier access to novel nanotechnological tools and the development of novel functional biomaterials might contribute to an improvement in the efficacy of natural anticancer drugs.

Conflicts of interest

The authors wish to confirm that there are no known conflicts of interest associated with this publication.

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