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Current Status and Contemporary Approaches to the Discovery of Antitumor Agents from Higher Plants

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Abstract

Higher plant constituents have afforded clinically available anticancer drugs. These include both chemically unmodified small molecules and their synthetic derivatives currently used or those in clinical trials as antineoplastic agents, and an updated summary is provided. In addition, botanical dietary supplements, exemplified by mangosteen and noni constituents, are also covered as potential cancer chemotherapeutic agents. Approaches to metabolite purification, rapid dereplication, and biological evaluation including analytical hyphenated techniques, molecular networking, and advanced cellular and animal models are discussed. Further, enhanced and targeted drug delivery systems for phytochemicals, including micelles, nanoparticles and antibody drug conjugates (ADCs) are described herein.

Keywords

Plant natural products; Botanical dietary supplements; Cancer chemotherapeutic agents; Dereplication analytical methods; Molecular networking; 2D and 3D cell cultures; Micelles; Nanoparticles; Antibody-drug conjugates (ADCs)

1. Introduction

Cancer is the second-leading cause of fatalities in the U.S.A. and it is estimated that this disease will be responsible for more than 600,000 deaths in 2018 (Siegel et al., 2018). There are multiple oncology drugs approved for chemotherapy, although these differ in their efficacy as single-agent therapeutics and in their degree of inherent toxicity. Over the past 50 years, more than 200 new chemical entities (NCEs) have been approved to combat this multifaceted disease of which about 50% are either unmodified natural product molecules, their semi-synthetic derivatives, or synthetic biological mimics (Butler et al., 2014; Newman and Cragg, 2016). Typically, these compounds have reached the market after the initial collection of plants, terrestrial microbes, and marine organisms, followed by laboratory screening programs as carried out by the pharmaceutical or biotech industries, as well as in academic, government, and private research institute environments. Examples of some

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anticancer natural products of plant origin include paclitaxel (Taxol®), topotecan (Hycamtin®), etoposide phosphate (Etopophos®), and homoharringtonine (Synribo®) (Atanasov et al., 2015; Newman and Cragg, 2016). Newer approaches have been used to discover potential plant natural product drugs inclusive of anticancer agents, incorporating enhanced dereplication methods like molecular networking, hyphenated analytical techniques, in addition to the application of better *in vitro* and *in vivo* bioassay models, which together have enabled more targeted discovery of novel compounds from natural sources.

This review article summarizes the small organic molecules obtained from higher plants and their derivatives currently on the market and in clinical trials as single chemical entity (SCE) anticancer agents. Examples of the constituents of botanical dietary supplements that have shown potential anticancer properties, in particular those from noni and mangosteen, are given. Also discussed are new approaches to the purification of biologically active plant extracts containing thousands of individual compounds and the use of hyphenated analytical techniques and molecular networking in rapid dereplication procedures. The application of biotechnological strategies, including different formulations, such as micelles, and nanoparticles, is covered. Finally, antibody-drug conjugates (ADCs) and their role in enhancing targeted drug delivery using natural product-derived molecules are also described.

2. Antitumor compounds from natural sources

2.1. Phytochemicals and their derivatives on the market as approved anticancer agents and in clinical trials

There has been extensive work done to discover and develop potent cancer chemotherapeutic agents, and a substantial number are now available, but some have shown toxic side effects and non-specificity to cancer cells (Khazir et al., 2014; Sporn and Liby, 2005). In a continual search for new active anticancer agents, biomedical scientists have explored several avenues, inclusive of chemical synthesis, biotechnological tools, immunotherapy, and the systematic investigation of organisms (Atanasov et al., 2015). Medicinal plants have played a significant role in providing therapeutic agents for different disease states. They have afforded several lead compounds, which either have been developed into drugs themselves or have served as “pharmacophores” for the chemical synthesis of analogs with better physicochemical properties and enhanced potencies (Katz and Baltz, 2016). Several anticancer drugs available in the USA that are FDA- approved and others in clinical trials are either unmodified natural products, or their semi-synthetic analogs, or biological mimics, as summarized in Table 1.

The first class of plant-derived small molecules utilized were the two bisindole alkaloids, vincristine (Figure 1, **1**) and vinblastine (Figure 1, **2**), which were approved by the U.S. FDA in the 1960s under the trade names Oncovin® and Velban®, respectively, and used for the treatment of various types of solid tumors and lymphomas (Khazir et al., 2014). These compounds were both isolated from the Madagascar periwinkle, *Catharanthus roseus* G. Don (Apocynaceae), and demonstrated to work by acting as tubulin polymerization inhibitors. Since then, semi-synthetic analogs including vindesine, vinorelbine, and vinflunine have been developed and later approved either or both by FDA and the European

Medicines Agency (EMA). Vinflunine is one such example that was approved by the EMA only in 2009 under the trade name Javlor® for the treatment of metastatic urothelial carcinoma (Jordan and Wilson, 2004; Lucas et al., 2010; Ng, 2011). More recently, in 2012, a nanoparticle-liposomal injection of vincristine sulfate, Marqibo®, was approved that has not only reduced toxicity but also enhanced efficacy, as seen in patients with acute lymphoblastic leukemia (Spectrum Pharmaceuticals Inc., 2017). Some other bisindole derivatives, such as anhydrovinblastine, vintafolide, vinglycinate, and vintriptol have been evaluated in several clinical trials for their efficacy against different cancer types (Khazir et al., 2014).

Another important class of plant-derived compounds to have been studied extensively for their anticancer activity are derivatives of the plant lignan, podophyllotoxin (Figure 2, **15**), from *Podophyllum peltatum* L. (Berberidaceae), with its analogs etoposide (Figure 1, **3**), approved in 1983 for testicular cancer, and teniposide (Figure 1, **4**), approved a decade later. These compounds have been subjected to earlier review by our group (Lucas et al., 2010). The latter compound is more potent than etoposide, and has shown positive results in combinatorial therapeutic approaches against different cancer types (Lee and Xiao, 2012).These lignans are known to express their antineoplastic effects by acting as inhibitors of tubulin polymerization and DNA topoisomerase II, thereby arresting cancer cells in their initial cell division phase. Both these small molecules (**3** and **4**) are undergoing phase II and IV combinatorial studies against cancer types including but not limited to CNS lymphoma, leukemia, and squamous cell carcinoma (U.S. National Library of Medicine, 2018b).

Seminal work occurred because of the isolation of paclitaxel (Figure 1, **5**), a nitrogen-containing diterpenoid, from *Taxus brevifolia* Nutt. (Taxaceae). Paclitaxel exerts its anticancer properties by inhibiting microtubule polymerization during cell division, and, after extensive research demonstrating its effectiveness in cancer patients, it was initially approved in 1992 under the trade name Taxol® for ovarian cancer, given as an injection. At present, paclitaxel is a first-line treatment offered to patients with breast, ovarian, lung, prostate and other solid tumor cancers (Lucas et al., 2010). Despite its potent activity, paclitaxel has shown suboptimal drug properties, like low bioavailability and high toxicity, leading to the development of two approved semi-synthetic derivatives thus far, namely, docetaxel (Figure 1, **6**) and cabazitaxel (Figure 1, **7**). Several other taxane derivatives inclusive of paclitaxel poliglumex (Xyotax™, CT-2103), 7-DHA-taxol (Taxoprexin, Phase II and III), larotaxel (XPR9881, Phase I-III), docetaxel nanoparticles (BIND-014, Phase I and II),TPI-287 (Phase I and II), and the polymeric micelle of docetaxel nanoparticle (CriPec®, Phase I) are at different stages of development in order to potentially overcome the observed resistance to the lead compound in cancer types like NSCLC and breast cancer (Butler et al., 2014; Khazir et al., 2014). Abraxane®, an injectable nanoparticle-albumin (nab-paclitaxel) suspension, has been approved with reduced toxicity and increased overall survival rate as compared to the parent compound (Khazir et al., 2014).

The same scientific team at Research Triangle Institute in North Carolina, who first obtained paclitaxel, also isolated camptothecin (Figure 2, **16**), a topoisomerase-I inhibitor, from *Camptotheca acuminata* Decne. (Nyssaceae), which has garnered much interest due to its novel structure and activity (Rahier et al., 2012). Despite showing positive activities *in vitro*

and *in vivo*, camptothecin in the unmodified chemical form did not receive official approval due to its low solubility and severe toxicity (Khazir et al., 2014). To explore this compound further and overcome its physicochemical limitations, two semi-synthetic derivatives, topotecan and irinotecan have been approved for clinical use in the U.S. (Khazir et al., 2014). Topotecan (Hycamtin®, Figure 1**, 8**) was the first-in-class topoisomerase I poison approved by FDA initially for the treatment of ovarian cancer, then in 2007 for recurrent small-cell lung cancer, and in 2011 for cervical cancer in combination with cisplatin (Rahier et al., 2012). Another water-soluble derivative, irinotecan (Figure 1, **9**, hydrochloride salt), was approved in 1996, under the trade name Camptosar®, for colorectal cancer (Rahier et al., 2012). After administration, irinotecan acts as a prodrug and gets converted into its active metabolite SN-38 (Figure 2**, 17**) which, in a similar manner to **9,** triggers cell death by inhibiting topoisomerase-I based DNA replication (Rahier et al., 2012). A liposomal injectable formulation of **9**, Onivyde® (Ipsen Inc.), was approved in 2015 to be administered with flurouracil to treat pancreatic cancer (U.S. Food & Drug Administration, 2015). This particular formulation was shown to work better and have fewer side effects compared to the parent compound **9**. Several other camptothecin derivatives are also undergoing clinical development against different cancer types (Khazir et al., 2014). A nanoparticle formulation of camptothecin, CRLX-101, in combination with olaparib, is currently being explored in Phase I and II studies against different solid tumors and lung cancer (Young et al., 2011). Furthermore, 9-aminocamptothecin, along with other derivatives such as karenitecin, DRF-1042, exatecan mesylate, rubitecan, have shown promising effects against cancer cell types like pancreatic, small-cell lung, and epithelial, ovarian, and fallopian tube cancers, and are at different study phases (Khazir et al., 2014; Rahier et al., 2012). Moreover, DS-8201a, an antibody-drug conjugate (ADC), is a modified version of the camptothecin derivative, exatecan (Figure 2, **18**), which targets HER2 expressing cancer cells, has shown better results compared to other ADCs like T-DM1, approved for breast cancer, mentioned below (Katz and Baltz, 2016; Leal et al., 2014). Several clinical trials (phase I/II) have been undertaken evaluating the efficacy of DS-8201a either independently or in combination with drugs like gemcitabine (Henkin et al., 2018).

Omacetaxine mepesuccinate (Figure 1, **10**, homoharringtonine), discovered from *Cephalotaxus harringtonii* Kitam. (Taxodiaceae) is a protein translation inhibitor and was approved in 2012, under the trade name Synribo® for chronic myeloid leukemia (Butler et al., 2014; Kinghorn et al., 2016). Several studies have corroborated the ability of omacetaxine mepesuccinate to reversibly inhibit protein synthesis and induce cell apoptosis (Itokawa et al., 2012). This alkaloidal drug is also currently undergoing several clinical studies for different cancer types, such as colorectal carcinoma, acute and chronic myeloid leukemia, and other solid tumor (Itokawa et al., 2012; Winer and DeAngelo, 2018).

As mentioned earlier, ado-trastuzumab emtansine (TDM-1, trade name: Kadcyla®), was approved by FDA in 2013 against HER2-positive breast cancer, and is derived from the potently cytotoxic agent maytansine (Figure 2, **19**). Belonging to the family of maytansinoids, maytansine was isolated from the Ethiopian shrub *Maytenus ovatus* Loes. (Celastraceae), and acts by inhibiting microtubule assembly, like the taxoids and podophyllotoxins (Amiri-Kordestani et al., 2014; Cassady et al., 2004; Katz and Baltz, 2016;

Kupchan et al., 1972). There are other ADCs of maytansinoids that are currently undergoing phase I/II clinical trials for several types of cancer (Yu et al., 2012). These and other examples of ADCs are discussed further in sub-section 4.3.3.

Another interesting compound, found in the sap of *Euphorbia peplus* L. (Euphorbiaceae), ingenol mebutate (Figure 1, **11**), was approved as a topical agent for the treatment of actinic keratosis as seen in patients with sun-related squamous cell carcinoma (Lebwohl et al., 2012). Masoprocol (Figure 1, **12**), isolated from *Larrea tridentata* (DC.) Coville (Zygophyllaceae) (Luo et al., 1998), is approved under the trade name Actinex® as a topical agent also for actinic keratosis and acts as a transcriptional inhibitor. Masoprocol decreases tumor cell susceptibility by inhibiting the activation of the insulin-growth factor receptor (IGF-1R) and the HER2 receptor, receptors involved in cell proliferation (Lu et al., 2010). There are several other nordihydroguaiaretic acid derivatives under development (Lu et al., 2010). In one such study performed, synergistic effectiveness was shown for terameprocol (Figure 2, **20**), a semi-synthetic tetra-methylated derivative of nordihydroguaiaretic acid, with antiseizure drugs in patients with recurrent high-grade glioma (brain and central nervous system tumors) (Grossman et al., 2012). Masoprocol is also in multiple phase I and II studies against prostate cancer (Atanasov et al., 2015; Smolewski, 2008).

Napabucasin (Figure 1, **13**), a naphthaquinone, was first derived from the stem bark of *Tabebuia cassinoides* (Lam.) DC. (Bignoniaceae) as an antileukemic agent (Rao and Kingston, 1982). It is a small-molecule cancer stemness inhibitor that acts by blocking STAT-3 driven gene transcription and spherogenesis of cancer stem cells (Hubbard and Grothey, 2017; Sonbol and Bekaii-Saab, 2018). Several assays have shown napabucasin to not only inhibit prostate cancer growth but also block the sphere formation of prostate cancer stem cells, thereby demonstrating it as a novel approach to cancer treatment (Li et al., 2015; Zhang et al., 2016)). After exhibiting successful antitumor activity in a phase IB trial in combination with nab-paclitaxel and gemcitabine, napabucasin has recently been assigned orphan drug status for both gastric and pancreatic cancer (U.S. Food & Drug Administration, 2016). It is currently in phase III studies for the potential treatment of advanced colorectal, lung, gastric and gastro-esophageal cancers and in early phase trials for multiple tumor types, including tumors of the liver, pancreas, and brain (Hubbard and Grothey, 2017) and in combination therapy with paclitaxel for carcinoma (Figure 1, **5**). DSP-0337, is an oral prodrug of napabucasin, and presently is in phase I clinical development for patients with advanced solid tumors (U.S. National Library of Medicine, 2018c).

CA-1P (OXi4503), a phosphate derivative of combretastatin (Figure 2, **21**), also recently acquired orphan designation, in this case for the treatment of patients with acute myeloid leukemia (Khazir et al., 2014). Other combretastatin analogs, also isolated from the bark of the African tree *Combretum caffrum* Kuntz (Combretaceae), such as combretastatin A4 and A1 [CA-4 (Figure 2, **22**) and CA-1 (Figure 2, **23**)] have been studied extensively for their structure-activity relationships, and are under development clinically for several cancer types (Khazir et al., 2014).

Flavopiridol (Alvocidib, Figure 1, **14**) is a synthetic molecule inspired from a natural product lead compound, rohitukine (Figure 2, **24**). First isolated from *Dysoxylum*

binectariferum (Roxb.) Hook. (Meliaceae) (Naik et al., 1988), rohitukine has found to target cyclin dependent kinases (CDKs), and Mcl-1 and other proteins involved either in the elongation process of transcription or in cell death (Khazir et al., 2014). Flavopiridol was the first in the class of CDKs taken to clinical trials against different types of cancer cells, and, for example, it is currently in phase I and II lymphoma and in combination with docetaxel against stage IV pancreatic cancer (U.S. National Library of Medicine, 2018a). In 2014, alvocidib was accorded orphan drug designation for patients with chronic lymphocytic leukemia by the U.S. FDA and in 2015 by the EMA, for the same indication (Wiernik, 2016).

2.2. Other potential chemotherapeutic agents from plant sources

Secondary metabolites of plant origin, as demonstrated in the previous section, have shown great potential to be developed directly or semi-synthetically as anticancer drugs. Another strategy for obtaining new anticancer lead compounds is drug repurposing. Latterly, this strategy has gained increasing attention and this involves retesting existing drugs and their close structural analogs, mostly with non-oncological clinical applications, for their potential cancer-related activity based on their structural parameters or mechanism of action (Pantziarka et al., 2014). Accordingly, drugs and their analogues belonging to different pharmacological classes such as antidepressants, anti-infectives, antipsychotics, antivirals, and cardiovascular agents have been investigated as possible oncological drugs (Cragg et al., 2014). One such compound is dihydroartemisinin (DHA, Figure 2, **25**), a derivative of the antimalarial drug artemisinin, a sesquiterpene lactone from *Artemisia annua* L. (Asteraceae). DHA has shown apoptosis via growth-inhibitory ROS-dependent autophagy (Sleire et al., 2017) and down-regulation of the protein transferrin receptor 1 (TfR1) (Ba et al., 2012). Artesunate (Figure 2, **26**) is another semi-synthetic water-soluble artemisinin derivative that has been linked to autophagy by enhancing the activity of caspase-3, a pro-apoptopic protein (Sleire et al., 2017). It has shown promising results in *in vitro* and *in vivo* assays against lymphoma, myeloma, and hepatocellular cancer types both individually and in combination with drugs like sorafenib (Augustin et al., 2015). These antimalarial drug derivatives are currently undergoing several phase I and II clinical trials against different cancer types (Sleire et al., 2017). In certain forms of cancer, elevated insulin levels have been observed, which has led to cancer-related investigations on metformin (Figure 2, **27**), a type-II diabetes drug (Thomas and Gregg, 2017). Structurally, this biguanide is a derivative of galegine (Figure 2, **28**), a lead active constituent of *Galega officinalis* L. (Fabaceae, French lilac), obtained along with isoprenylguanidine (Fischer et al., 2010). These bioactive plant constituents showed a reduction in blood sugar levels when injected in test rabbits but with associated toxicity, which led to the later development of the derivative metformin (Fischer et al., 2010). Metformin acts by inhibiting protein translation by AMP-activated protein kinase (AMPK) and, owing to its ability to induce energetic stress, several epidemiological studies have related it as a possible therapy for treating cancer, in particular prostate, colorectal, and HER2-positive breast cancer (Sleire et al., 2017). Its mechanism of action causes protein unfolding and ER stress thereby resulting in cellular apoptosis. In a phase II pancreatic cancer study, no specific benefit was seen in patients administered with metformin as compared to a standard, but compound **28** showed positive results in diabetic women with HER2+ breast tumors (He et al., 2012; Kordes et al., 2015). Metformin is

currently being developed in several clinical trials both synergistically with other drugs like taxanes and also individually against various cancer types (Sleire et al., 2017). Table 2 provides other examples of such plant-derived small molecules that show a possibility of being repurposed as anticancer drugs.

In addition, there are several additional plant-derived compounds, representative of a wide range of different structural types not mentioned earlier in this contribution, which are currently being subjected to clinical trials as potential anticancer agents that have been reviewed earlier (Butler et al., 2014; Henkin et al., 2018; Newman and Cragg, 2016)

3. Examples of botanical dietary supplements as potential sources of cancer chemotherapeutic agents

3.1. Introductory remarks on botanical dietary supplements

Medicinal plants have provided innumerable drugs over the years (Gertsch, 2009), but despite much available relevant scientific knowledge, only in the mid-1990s did the U.S. market see a significant growth in the use of dietary supplements, inclusive of botanical products, through the passage of the Dietary Supplements Health and Education Act by the U.S. Congress (Obolskiy et al., 2009). Botanical and other dietary supplements can be sold in the form of pills, tablets, tinctures, and liquids for oral use (National Institute of Health O.D.S., 1994). Though there have been several concerns regarding the safety, efficacy and quality of some of the supplements on the market, a recent report showed the U.S. dietary supplement market size to have grown substantially and estimated this to represent a market size worth $220 billion by 2022 (Bailey et al., 2013). To be classified as a dietary supplement, a botanical must be a "*product intended to supplement the diet that bears or contains one or more dietary ingredients…or a concentrate, metabolite, extract or combination of any aforementioned ingredients*" (National Institute of Health O.D.S., 1994). The sales of botanical dietary supplements has increased by 7.7% in 2016 compared with the previous years, with horehound, cranberry, and echinacea being the top three most sold supplements in this category in the U.S. (Benatrehina et al., 2018; Smith et al., 2017). Some of the other top-selling botanicals include black cohosh (*Actaea racemosa* L.), flaxseed (*Linum usitatissimum* L.), milk thistle (*Silybum marianum* L. (Gaertn.)), and aloe (*Aloe vera* (L.) Burm. f.) (Smith et al., 2017). For some botanical supplements scientific literature reports have appeared, supporting their potential use in treating various diseases (Benatrehina et al., 2018). In addition, supplements such as turmeric (*Curcuma longa* L.), saw palmetto (*Serenoa repens* (W. Bartram) Small), and garlic (*Allium sativum* L.) have been used by some patients with, for example, cancer or cardiovascular problems, owing to their potentially beneficial supportive effects (Gonzalez-Vallinas et al., 2013). Since a discussion of all botanical dietary supplements linked to cancer support is beyond the scope of this review article, focus has been placed on two well-established dietary supplements, *Garcinia mangostana* L. (mangosteen) and *Morinda citrifolia* L. (noni), which are mentioned in turn in the following two sub-sections. These two botanical dietary supplements have been subjected to extensive prior work by the current authors, in relation to cancer, as reviewed recently (Benatrehina et al., 2018). Other examples of widely used herbal supplements in terms of their potential anticarcinogenic effects are shown in Table 3.

3.2. Mangosteen (*Garcinia mangostana*)

The so-called “queen of fruits”, *Garcinia mangostana* L. (Clusiaceae), is a juicy, slightly sweet and sour tasting fruit native to Southeast Asia and grown primarily in India, Malaysia, Indonesia, and Thailand (Benatrehina et al., 2018; Ramage et al., 2004). The purple mangosteen has been cultivated in these tropical areas for the past two centuries not just for commercial purposes but also owing to the medicinal properties of its fruits. In folkloric medicine, various plant parts of *G. magostana* have been used to treat various conditions, such as mucus accumulation, dysentery, diarrhea, and fever, among other ailments (Chin and Kinghorn, 2008). More recently, mangosteen dietary supplements (in powder, capsule, tablet, cream, and fruit juice form) have attracted much attention in the U.S. and elsewhere, due to being promoted as a “superfruit”, despite relatively limited human and clinical studies having been performed (Benatrehina et al., 2018; Gutierrez-Orozco and Failla, 2013). Moreover, mangosteen pericarps may be blended with the juices of several berries, cherry, grapes, apples and pears (Gutierrez-Orozco and Failla, 2013).

The genus *Garcinia* consists of more than 400 species and has been studied extensively for potential therapeutic effects, such as their antibacterial, antifungal, anti-inflammatory, antineoplastic, and antioxidant activities (Pedraza-Chaverri et al., 2008). The phytochemical constituents responsible for such properties include xanthones, flavonoids, pyrroles, benzophenones, and benzofurans (Chin and Kinghorn, 2008). In particular, prenylated and oxygenated xanthones, like α-, β- and γ-mangostin [α-MG (Figure 3, **29**), β-MG (Figure 3, **30**) and γ-MG (Figure 3, **31**)], mangostinone (Figure 3, **32**), 8-hydroxycudraxanthone, caloxanthone A, garcinones A-E, esmeatxanthone A have been isolated from the pericarp of the fruits of mangosteen. *In vitro* and *in vivo* studies have been conducted to determine their antiproliferative and pro-apoptotic activities, particularly against colon cancer cell lines (Gutierrez-Orozco and Failla, 2013; Han et al., 2009; Pedraza-Chaverri et al., 2008). Several articles have documented the pharmacological and biological effects of mangosteen and its secondary metabolites, in particular for α-MG, the major fruit constituent (Benatrehina et al., 2018; Obolskiy et al., 2009; Ovalle-Magallanes et al., 2017; Wang et al., 2017). For instance, a positive effect of α-MG has been demonstrated against tumor growth in a prostate cancer xenograft model. It was further observed by the same group that α-MG induces cell arrest by inhibiting the activity of caspase-3 and cyclin dependent kinase 4 (CDK 4), resulting in apoptosis in an *in vitro* prostate cancer cell line (Johnson et al., 2012). A study by Jung et al. (2006) demonstrated the ability of α-MG to inhibit 7,12-dimethylbenz[α]anthracene (DMBA)-induced preneoplastic lesions in a mouse mammary organ culture assay. Another group showed the antitumor effects of panaxanthone, comprised of 75–85% of α-MG and 5–15% γ-MG, in a mouse metastatic mammary cancer model. Panaxanthone led to cell cycle arrest, via decreased phosphorylation of the Akt pathway, and caused reduced metastasis in a breast cancer *in vivo* model (Shibata et al., 2013). In an *in vitro* HT-29 human colon adenocarcinoma study, α-MG showed antiproliferative potential by decreasing Bcl-2 and β-catenin at a 6–15 μM dose range (Chitchumroonchokchai et al., 2013). To further substantiate the mechanism of action of α-MG, its therapeutic potential was studied in a non-small cell lung cancer cell line (NSCLC) and in normal lung fibroblasts (Zhang et al., 2017). Apoptosis was observed in the case of NSCLC due to the increased generation of reactive oxygen species (ROS), with little or no

cytotoxic effects shown towards the non-cancerous cells studied, thereby leading to a dose-dependent ROS-induced apoptosis mediated by α-MG in NSCLC cells. In the same study, also observed was a decreased level of B-cell lymphoma 2 protein (Bcl-2) and increased levels of Bcl-2 associated x protein (Bax), as possible causes of cell death (Zhang et al., 2017). γ-MG, on the other hand induced apoptosis via loss of mitochondrial membrane potential and enhanced intracellular peroxide levels as observed with a 60 μM dose in the hepatic carcinoma cell (HCC) line (Chang et al., 2013). An earlier study identified garcinone E (Figure 3, **33**), a further xanthone derivative, as having a potent cytotoxic effect in a HCC cell line along with lung and gastric cancer cells in an *in vitro* 15-cell line panel (Ho et al., 2002).

Furthermore, quite extensive research has also been done on determining xanthone metabolism and bioavailability when mangosteen is consumed as a botanical dietary supplement in animal models. In general, mangosteen xanthones have been shown to be safe and selective to tumor cells in animal models, but have been found to have a low oral bioavailability when administered to mice (Gutierrez-Orozco and Failla, 2013). In order to increase the bioavailability of mangosteen xanthones, several research groups have turned to nuanced biotechnological tools of drug delivery such as the use of micelles and nanoparticle formulations. In one such study, poly(ethylene glycol)-*b*-poly[*N*-(2-hydroxypropyl)methacrylamide-dilactate] [mPEG-*b*-p(HPMAm-Lac_2)] micelles of α-MG were prepared and were tested in doxorubicin-sensitive and-resistant cancer cell lines and in normal peripheral blood mononuclear cells (Khonkarn et al., 2012). With a loading capacity of approximately 19% in micelles (similar to that of paclitaxel micelles), a slow *in vitro* release of this xanthone was observed. The cytotoxic effect of the micellar formulation was observed to be 50-fold higher than that of the positive control substance, doxorubicin, against cancer cells, along with increased normal cell viability on exposure to the mPEG-*b*-p(HPMAm-Lac_2) xanthone micelle (Khonkarn et al., 2012). A similar study involved microemulsions of α-MG that were administered orally to C57/BL6 mice, which showed enhanced oral bioavailability and increased distribution in lymphatic organs (Xu et al., 2017). Several groups have also prepared nanoparticle formulations of xanthone mixtures (both α- and γ-MG) and α-MG by itself. While some studies showed increased cytotoxicity of the nano formulation against colon and prostate cancer cell lines (Ovalle-Magallanes et al., 2017), other xanthone nanoparticle formulations were not so successful but raised the possibility of the controlled release of these formulations (Aisha et al., 2015).

3.3. Noni (*Morinda citrifolia*)

Noni (*Morinda citrifolia* L., Rubiaceae), primarily found in Asia and the Pacific region, is used as both food and medicine (Mian-Ying et al., 2002). Traditionally, various parts of this small shrubby tree, like the roots, fruits and leaves have been used to treat the common cold, diarrhea, tuberculosis, bacterial infections, oral and stomach ulcers (Benatrehina et al., 2018; McClatchey, 2002). A considerable amount of work has been done on analyzing the chemical composition of the roots, bark, leaves, and particularly, the fruits of noni (Obolskiy et al., 2009).

So far, more than 200 phytochemicals have been identified from *M. citrifolia* including phenols (for example, damnacanthal, morindin, and scopoletin), lignans (americanin A, morindolin, and isoprincepin), flavonol glycosides (rutin, narcissoside, and nicotifloroside), small organic acids (caproic and caprylic acids), and other miscellaneous compounds such as triterpenoids and sterols (Chan-Blanco et al., 2006; Mian-Ying et al., 2002; Potterat and Hamburger, 2007). Several studies have been published exploring the potential activity of noni juice and its constituents specifically in the overall fields of cancer, inflammation and metabolic disease (Potterat and Hamburger, 2007). The ability of a noni fruit juice precipitate to disrupt cell adhesion of cancerous cells, was demonstrated using Lewis lung carcinoma (LLC) and sarcoma 180 ascites cultures (Liu et al., 2000; Potterat and Hamburger, 2007). Another study evaluated damnacanthal (Figure 4, **34**) as an inhibitor of tumor formation by inhibiting the ras gene activation and cell apoptosis in human colorectal cancer cell lines (Abou Assi et al., 2017). Americanin A (Figure 4, **35**), a neolignan that was also isolated from the seeds of *Phytolacca americana* L. (Phytolaccaceae) has also been demonstrated as a noni constituent with potent antioxidant activity (Kinghorn et al., 2011a; Su et al., 2005). In a recent study, americanin A from *P. americana* was found to be effective in *in vivo* in a mouse xenograft study with human colon cancer cells. Mechanistically, americanin A induced G_2/M cell cycle arrest followed by apoptosis. The same investigators further examined the mechanism of action, with the ATM/ATR signaling pathways and Skp2-p27 modulated, leading to a low micromolar cytotoxic activity (Jung et al., 2015). Another bioactive noni constituent is 2-methoxy-1,3,6-trihydroxyanthraquinone (Figure 4, **36**), which in a quinone reductase (QR) *in vitro* assay showed a lack of cytotoxicity and 40-fold increased activity on comparison with the positive control, L-sulforaphane (Pawlus et al., 2005). Two glycosides isolated from noni fruits, 6-*O*-(β-D-glucopyranosyl)-1-*O*-octanoyl-β-D-glucopyranose (Figure 4, **37**) and asperulosidic acid (Figure 4, **38**), were tested *in vitro* against the mouse epidermal JB6 cell line. These two compounds suppressed 12-*O*-tetradecanoylphorbol-13-acetate (TPA)- and epidermal growth factor (EGF)- induced cell transformation and activator protein (AP)-1 associated activity, which plays a key role in tumorigenesis (Liu et al., 2001). Hence, previous work on a diverse range of different noni constituents, particularly of the fruits, are supportive of a potential role in cancer chemotherapy or chemoprevention, but additional studies of this type are required.

4. Contemporary techniques in the discovery and targeted delivery of small-molecule natural products from plants

4.1. Dereplication strategies in plant natural product purification procedures

4.1.1. Current dereplication methods—Since the dawn of human history, natural products contained in organisms have been an indispensable part of human life, representing both day-to-day assets to important medicines (Cragg and Newman, 2001a, b). From the medicinal perspective, up until the early 19th century, almost all natural products were used as extracts of whole organisms, with their chemistry or therapeutic constituent(s) little known prior to this (Atanasov et al., 2015). The isolation of morphine in 1805 from the crude drug opium obtained from poppy capsules (*Papaver somniferum* L.; Papaveraceae) paved the way for the isolation of numerous natural products, mostly from plants (Li and Vederas, 2009). For some of the early plant natural products from the 17th and 18th

centuries, subsequently used in medicine and pharmacy, the timeline of their initial purification has been reviewed by Drobnik and Drobnik (2016). Many of these compounds were found to be pharmacologically active and some still have current clinical utilization. Furthermore, the accidental discovery of penicillin by Alexander Fleming opened the "Golden Age of Antibiotics" that led to an extensive search of the microbial world for important medicines (Cragg and Newman, 2001a; Cragg et al., 2014; Newman et al., 2000). This led to the isolation and clinical application of drugs that are still the mainstay of treatment including aminoglycosides, avermectins, cephalosporins, clavulanic acid, cyclosporins, glycopeptides, polyene macrolides, rapamycin, statins and the tetracyclines (Cragg and Newman, 2013; Katz and Baltz, 2016). The overall value and contribution of natural products to modern medicine were reflected in a recent analysis of the sources of all drugs from 1981 to 2014. Of the 1562 drugs approved in western countries during this time period, 619 (~40%) were natural products or their derivatives (Newman and Cragg, 2016).

Despite such tremendous success, in the past two decade several major pharmaceutical industries including Bristol Myers Squibb, Merck, Johnson and Johnson, Pfizer and GlaxoSmithKline have abandoned their natural product drug discovery research units (David et al., 2015; Li and Vederas, 2009; Ortholand and Ganesan, 2004). The contributing factors to this are various and can be related to factors such as cost, time involved, compound novelty, synthetic feasibility, quantities obtained and intellectual property considerations (Ortholand and Ganesan, 2004). Furthermore, difficult access to source organisms, the presence of most of the bioactive compounds in low amounts, and the continued re-isolation of already known compounds were also major considerations that have led to a decline in the use of natural products in the drug discovery process in the pharmaceutical industry (Kingston, 2011; Lam, 2007; Mohamed et al., 2016). Some of these problems were recognized early, for example by Hanka et al. (1978) and Suffness and Douros (1981), and a recent review highlights how these remain continued limitations in the natural products drug discovery process (Kurita and Linington, 2015). One major issue, the repeated re-isolation of known compounds, is the main topic of dereplication, a term probably introduced first in the antibiotic field (van Middlesworth and Cannell, 1998) and that is discussed further in the present part of this review.

Beutler et al. (1990) defined the term "dereplication" to refer to those processes used *"…to identify [the] samples containing known active compounds or compound classes in a rapid fashion, without investing time in traditional bioassay-guided fractionation and full structure elucidation",* when they were attempting to process a large number of bioactive plant extracts using a phorbol dibutyrate (PDBu) receptor-binding assay. In general, dereplication involves the screening of natural product mixtures and biological sources prior to isolation for their chemistry and bioactivity, not only to prioritize the isolation of novel bioactive compounds but also to exclude previously isolated compounds from further investigation. Dereplication may be further facilitated by comparing experimental data with information available in-house and/or commercial databases (Sarker and Nahar, 2012). An example of the early recognition of the importance of dereplication from a pharmaceutical industry perspective was highlighted by Borris (1996). In short, the main aim of dereplication is the saving of time and resources by avoiding the re-isolation of already characterized and documented compounds from candidate organisms of interest, and this involves the

elimination, grouping and/or prioritization of plant and other extracts from organisms for further investigation (Borris, 1996; Butler, 2004).

4.1.2. Dereplication methods and characteristics—As indicated above, for a given dereplication procedure in natural products drug discovery to be effective, it should involve the combined use of a separation method and a spectroscopic detection procedure, and the subsequent data analysis and interpretation may be facilitated by online database searching (Sarker and Nahar, 2012). In addition, the process can be further supported by integration with a biological assay, or indeed the biological assay can be used alone as a dereplication strategy (Yang et al., 2013). Whatever the overall approach, the appropriate selection and integration of efficient dereplication methods will impact the length of time from the initial identification of results of biological or pharmacological studies to the final characterization of the new bioactive compounds (Jia, 2003). Most dereplication processes involve the use of spectroscopic techniques to guide the isolation process (mostly also using chromatographic methods) and this on-line combination with an “interface” is termed a “hyphenated technique” (Hirschfeld, 1980). Of the chromatographic separation methods available, HPLC and gas chromatography are among the most commonly used (Seger et al., 2013). Owing to its ability to be combined with numerous detectors and availability of various columns, LC (both HPLC and UHPLC) is the most common separation method used in the various hyphenated techniques available (Seger et al., 2013). Furthermore, LC can be integrated with bioassay methods as “high-resolution screening” to facilitate the dereplication process by pinpointing novel bioactive compounds (Shi et al., 2009). In addition, the introduction of ultrahigh-performance liquid chromatography (UHPLC) with sub-2 μm particle-sized stationary phases to increase the resolution and/or shorten analysis times has made LC a method of choice among numerous available hyphenated techniques (Jorgenson, 2010). Thus, some of the common hyphenated techniques based on LC include LC-DAD, LC-ESLD (Megoulas and Koupparis, 2005), LC-MS (Korfmacher, 2005), LC-NMR (Exarchou et al., 2005; Jaroszewski, 2005a) and LC-IR (Kuligowski et al., 2010).

It is also possible to use two or more spectroscopic methods in combination, allowing for the separation and acquisition of all spectral data at once, particularly for samples available only in limited amounts (Wilson and Brinkman, 2007). In this regard, a widely used dereplication technique in drug discovery is LC-MS-NMR (Corcoran and Spraul, 2003), with many variants available such as LC-PDA-HRMS-SPE-NMR, where solid-phase extraction (SPE) is intended to resolve the technical issues associated with using in combination two detection methods (i.e., NMR and MS) with different degrees of sensitivity (Jaroszewski, 2005b; Lima et al., 2017). This procedure offers the ability of the investigator to elucidate the structure of individual natural products when present in mixtures (i.e., crude extracts of organisms and chromatographic fractions), thereby facilitating early dereplication without isolation, in addition to allowing preparative-scale isolation, and has been applied for the isolation of numerous secondary metabolites (Clarkson et al., 2006; Johansen et al., 2011; Johansen et al., 2013; Kesting et al., 2011; Staerk et al., 2009). Furthermore, LC-PDA-HRMS-SPE-NMR has been integrated with various biological assays, with many of these being enzyme-inhibition assays, such as protein tyrosine phosphatase-1B and α-glucosidase (Wubshet et al., 2015; Wubshet et al., 2016), hyaluronidase (Liu et al., 2015), H^+−ATPase

(Kongstad et al., 2014), α-amylase (Okutan et al., 2014), and antioxidant assays (Wubshet et al., 2013). Excellent reviews on the methodologies and informatics involved in dereplication in general have been published (Gaudencio and Pereira, 2015; Hubert et al., 2017; Ito and Masubuchi, 2014; Mohamed et al., 2016; Perez-Victoria et al., 2016; Wolfender et al., 2006; Wolfender et al., 2015). Accordingly, in the following paragraphs, the discussion will mainly focus on a newer dereplication technique based on (LC-) MS, namely, "molecular networking".

Mass spectrometry and tandem mass spectrometry advantages and their limitations: Mass spectrometry (MS) is one the most sensitive (nanogram range) analytical techniques available. Along with its simplicity of operation and robustness, most of the dereplication methods used in natural products isolation chemistry involve mass spectrometry integrated with a separation method, most commonly HPLC, as in the hyphenated technique LC-MS (Bouslimani et al., 2014; Wolfender et al., 2015). Furthermore, data acquisition and analysis using mass spectrometry is rapid with a high degree of resolution. In recent years, significant instrumental developments in the separation, sampling and ionization techniques have simplified the analysis of complex mixtures and made mass spectrometry a technique of choice for many applications (Garg et al., 2015). When incorporating high-resolution mass spectrometry (HRMS) (Marshall and Hendrickson, 2008), LC-MS gives accurate masses of compounds being analyzed that can be used for database searching, and also the identification of molecules present can be facilitated further by analysis of fragmentation patterns and isotopic ratios, retention times, and may also include chemotaxonomic information (Nielsen et al., 2011). However, the number of candidate molecular formulas for a given mass (e.g. a molecular ion) can be large and daunting (Yang et al., 2013). For example, with the molecular formula of $C_{15}H_{22}O_3$, there were 113 candidates in the microbial natural products database Antibase2008, all of which were in the terpene class of natural products (Nielsen et al., 2011). In contrast, since each molecule has its own unique fragmentation pattern that is solely dependent on its chemical architecture, tandem mass spectrometry (MS/MS) of compounds will usually provide a better insight into its chemical structure. Thus, information obtained from MS/MS can be used to increase the reliability of mass spectrometry-based dereplication (Allard et al., 2016). However, when it comes to mixtures, this generates thousands of MS/MS spectra, or "big data", as is the case in natural products research involving metabolomics, which makes manual analysis, and the interpretation and presentation of data difficult (Garg et al., 2015). Furthermore, identification of previously unknown compounds, where there is no data in the spectral libraries, within mixtures can be difficult in case of "untargeted" MS/MS (Hartmann et al., 2017)

4.1.3. Molecular networking methods

4.1.3.1. Principles of molecular networking: Molecular networking (sometimes abbreviated as MN) is a computational algorithm that was developed to tackle the above-mentioned problems of tandem mass spectrometry and may be applied to enhance the efficiency of fractionation procedures for bioactive plant natural products in crude extracts from organisms (Garg et al., 2015). First reported in 2012 to investigate the chemical profiles of microbial colonies, molecular networking is based on the principle that small-

organic molecular constituents of organisms with a similar mass spectrometric chemical architecture will exhibit similar fragmentation patterns (Watrous et al., 2012). Most of the examples covered thus far do not refer to compounds with potential anticancer activity, so various different types of biological effects will be referred to in the discussion below. In molecular networking, this level of similarity is determined by a vector-based cosine score that takes the relative intensities of fragment ions and mass difference of the matched precursor ions into account in the calculation of a "similarity score". This score ranges from 0 to 1, with a score of 1 indicating identical spectra (Watrous et al., 2012). To avoid the redundancy of identical ions (i.e., molecules) appearing more than once, identical spectra, usually with scores of more than 0.95, typically are merged to give a consensus spectrum. After determination of the score and necessary pre-networking make-ups (e.g., the removal of solvent spectra and low-intensity ions), a molecular network showing the level of similarity between the individual MS/MS parameters (depicted by nodes) is constructed. In the network, each consensus spectrum that corresponds to a particular precursor ion (and thus the MS/MS data) is represented by a node and connected by lines (called "edges") to other nodes having similar MS/MS profiles. The thickness of the edges indicates the level of similarity between the precursor ions based on the cosine score set up by the user (for example, it could be set at 0.7) (Quinn et al., 2017; Watrous et al., 2012; Yang et al., 2013). Various attributes can be designated with regard to the shape and color of the nodes such as the relative amount of the precursor ion, the source organism for the particular precursor ion, the chromatographic fraction number, and bioactivity data and database matches (Garg et al., 2015; Kleigrewe et al., 2015; Nothias et al., 2018). Isolated nodes that are not connected to any other nodes and molecular families (see below) may represent compounds with structural novelty (Duncan et al., 2015). However, care should be taken not to interpret the total number of nodes (= ions detected) visualized in molecular networking as being equal to the total number of unique compounds present in the sample, because of adduct formation for the same compound, among other reasons (Crusemann et al., 2017). Furthermore, since chromatographic retention times are not usually considered, isomers can cluster into single nodes in natural product samples (Olivon et al., 2017b). For example, in a study conducted to isolate anti-chikungunya virus diterpenoids from the aerial parts of the plant *Euphorbia pithyusa* L. (Euphorbiaceae) using "targeted" MS/MS, dideoxyphorbol ester isomers were clustered into the same node due to the clustering mechanism of the algorithm (Esposito et al., 2017). This could be solved by application of appropriate preprocessing parameters using MZmine before submitting to GNPS (Global Natural Product Social Molecular Networking; see below) (Olivon et al., 2017b) or by deactivation of the "MS-Cluster" feature of the GNPS Data Analysis workflow (Nothias et al., 2018). Other documented limitations of molecular networking result from the inherent limitations of mass spectrometry such as ionization problems and an inability to discriminate isomers (Yang et al., 2013).

4.1.3.2. Uses of molecular networking: The real utility of molecular networking, besides overall organization and visualization, lies in the fact that molecules with similar chemical structures and hence similar mass spectrometric fragmentation patterns tend to cluster together to form "molecular families". In other words, each molecular family may represent molecules with unique chemical scaffolds that may or may not be related to other in the

dataset (Nguyen et al., 2013). As such, molecular networking can be used for linking biosynthetic gene clusters (BGCs) of natural product samples with molecular families (Nguyen et al., 2013). In addition, nodes in a family can be assumed to possess similar bioactivities as dictated by their structural differences (Olivon et al., 2017a). Furthermore, a large number of nodes in a cluster indicates that most of molecules are structurally related while those with only a few nodes might indicate a degree of novelty that is worthy of a follow-up investigation (Nguyen et al., 2013). In addition, once one of the nodes is annotated, this can be used to annotate other nodes and even extend the annotation to other families by looking at common fragment losses such as 14 amu for loss of CH_2, and 16 amu for oxygen, and so on (Nothias et al., 2018). With the ability of molecular networking to organize, analyze and visualize very large datasets of MS/MS spectra in a convenient manner, this technique has garnered considerable interest from different natural product research groups. Thus, the applications of molecular networking are diverse and some of these include the study of intra- and/or inter-species microbial interactions (Shi et al., 2017; Vallet et al., 2017), the isolation of bioactive secondary metabolites through the study of BGCs (Duncan et al., 2015; Kleigrewe et al., 2015; Schorn et al., 2014; Wu et al., 2016), in investigations of drug discovery and metabolism, clinical diagnostics and precision medicine (Quinn et al., 2017), and to organize and visualize the chemistry of samples of unrelated origin (Garg et al., 2015). For example, by using molecular networking of extracts derived from 146 *Salinispora* and *Streptomyces* species, Crusemann et al. (2017) showed that secondary metabolite production (i.e., the number of compounds and their diversity) was dependent on the species and strain, duration of culturing, the location where the strain and species were collected, and the type and composition of the growth medium. Furthermore, it has been shown that the type and number of solvents used for extraction directly affects the number and diversity of compounds extracted (Crusemann et al., 2017). Thus, since its first description, molecular networking has been used as a major dereplication tool as described in several natural products publications (Chervin et al., 2017; de Oliveira et al., 2017; Nothias et al., 2017; Nothias et al., 2018; Remy et al., 2018), as will be discussed in more detail below.

4.1.3.3. Molecular networking as a dereplication tool: As pointed out earlier, dereplication is usually facilitated by database searches that contain spectra of known compounds. Similarly, successful and reliable dereplication by molecular networking may be achieved through comparison with MS/MS spectra of standards or libraries (databases) (Kleigrewe et al., 2015; Olivon et al., 2017b). The clustering of similar compounds within a network, as determined by their fragmentation pattern, followed by analysis, will help in determining whether that cluster is worthy of further investigation, thereby facilitating the dereplication process (Allard et al., 2016). For example, a molecular family containing the cytotoxic dolastatin 10 and its analogs was excluded from further investigation from the bioactive extracts and fractions from a *Symploca* species (Naman et al., 2017). Furthermore, the similarity of the spectra of previously unknown compounds to those with available reference spectra facilitates their structural characterization and the categorization process (Naman et al., 2017). Depending on the capacity of the database used for searching the spectral library, the absence of spectral matches during dereplication might be enough to warrant further investigation of an extract or fraction from an organism, as this might

suggest chemical novelty. For instance, inspired by the lack of reference spectra matches in the MarinLit or AntiMarin databases, Liaw and associates isolated ten new amino-polyketides, named as vitroprocines A-J, from the active ethyl acetate extract of the marine-derived *Vibrio* sp. QWI-06, in which three of them were found to be active against *Acinetobacter baumannii* (Liaw et al., 2015).

The use of molecular networking as a dereplication strategy has been successfully articulated effectively earlier, and successful dereplication using molecular networking involves three main steps: (i) acquisition of tandem mass spectra, (ii) the generation of molecular networks (iii) and analyses using various mechanisms (Yang et al., 2013). In particular, it has been emphasized that reference LC-MS/MS spectra of known compounds and/or previously isolated and well-characterized compounds can be generated in the same manner as the sample to aid in the dereplication process by serving as a "seed" (Fox Ramos et al., 2017; Yang et al., 2013). However, it should be noted that application of molecular networking is not strictly dependent on databases or reference compounds (Yang et al., 2013). A dual purpose of molecular networking has been explained by Quinn et al. (2017) as either an "ab initio paradigm" (with no need to know the chemistry of the sample for annotation) or as an "incremental paradigm" (where the spectra of the reference or purified compounds can used to annotate the chemistry of molecular families). Based on the results of either of these methods, the cluster to pursue for isolating bioactive compounds can be then selected (Quinn et al., 2017). In the case of the inavailability of reference compounds and/or the lack of spectral matches in the available in-house, commercial databases and public libraries (GNPS; see below), "virtual" spectra can be generated by *in silico* modeling (Allard et al., 2016). For example, an *in silico* model was used for the targeted isolation of acetylcholinesterase inhibitory monoterpene indole alkaloids from the leaves of *Palicourea sessilis* (Vell.) C.M. Taylor (Rubiaceae) (Klein-Junior et al., 2017). The generation of molecular networks can be performed by using the Global Natural Product Social Molecular Networking (GNPS) Web platform and final visualization of the whole network by Cytoscape® or any visualization method (Quinn et al., 2017). The GNPS platform is a community-based web platform for sharing, and a repository for the visualization and analysis of tandem MS/MS datasets (Wang et al., 2016). With regard to dereplication, the advantage of GNPS is the availability of reference spectra from both the community and third-parties such as MassBank, ReSpect and NIST (Wang et al., 2016).

The power of "seeding" reference spectra during generation of molecular networks can be elaborated upon as follows. Since clustering is based on spectral similarity, and hence structural similarity, examination of the cluster in which the "seed" spectra is located will help in the partial or total identification of the chemical structure or class of the other nodes within that cluster. This can be done by closer analysis of the individual MS/MS data for common fragment losses between the reference node and node(s) of interest (Nothias et al., 2018; Yang et al., 2013). For example, tandem mass spectra of known jatrophane esters were "seeded" in the molecular networks to aid the annotation of nodes that led to isolation of new jatrophane ester analogs from the whole part of *Euphorbia semiperfoliata* Viv. (Euphorbiaceae) (Nothias et al., 2017). The use of an in-house MS/MS spectral database for those natural products with unavailable public or commercial spectra was demonstrated for targeted isolation of monoterpene indole alkaloids from the bark of *Geissospermum laeve*

(Vell.) Miers (Apocynaceae) (Fox Ramos et al., 2017). As mentioned in the preceding paragraph, another viable and powerful option is to use the spectral library (i.e., reference spectra) of the GNPS to search for similar spectra to the experimental spectra. Furthermore, the variable dereplication feature of GNPS will also help in identifying possible analogs with different substitution or modification even if the compounds have unrelated basic skeletons (Wang et al., 2016). This is an analog search option, in which the annotation of nodes once any of the other nodes structure is solved or the annotation and isolation of analogs based on the "seed spectra" could be important; as it is known that simple structural variation might be enough to switch bioactivity from inactive to extremely potent or *vice versa* or even switch targets (Pye et al., 2017). As an example of the value of molecular networking in this perspective, the reference spectra of various diterpenoids facilitated the annotation of several analogs, that were excluded from further investigation since the reference diterpene esters were found to be inactive in the anti-chikungunya virus assay used (Nothias et al., 2018).

Comprehensive databases (on-line, *in silico* or public like GNPS) are important in order for molecular networking to be reliable and to succeed as a dereplication tool (Fox Ramos et al., 2017). Major limitations with the currently available databases are their lack of chemodiversity and the restricted access to reference spectra. Furthermore, the procedures used to extract and analyze the plant and other secondary metabolites as well as the methods and acquisition parameters used in acquiring the LC-MS/MS data might have an effect on the spectral comparison between standards and the sample MS/MS parameters (Garg et al., 2015). The quality of MS/MS data used for constructing the molecular networks might also affect the comparison process (Olivon et al., 2017c). It is also indicated that in molecular networks generated using GNPS, information regarding the relative abundance of compounds might be missing as GNPS's major purpose is for spectral comparison (Nothias et al., 2018).

4.1.3.4. Solving some pitfalls of bioassay-guided isolation with molecular networking: Bioassay-guided isolation, in which proceeding to the next step of compound purification is dictated by the bioactivity data of the previous step used, has been the main means of isolation of bioactive natural products (Gomes et al., 2018). However, the re-isolation of already characterized compounds along with the costly and time-consuming nature of the process has been the major challenge in bioassay-guided isolation (Atanasov et al., 2015; Olivon et al., 2017a). Thus, it has been suggested that the classical iterative bioassay-guided fractionation and isolation procedures can be significantly shortened by integration of molecular networking with a bioassay (Naman et al., 2017). For example, after the necessary sample clean-up and preparation, chromatographic methods can used to make major fractions from an extract and submitted for bioassay as well as subjected to LC-MS/MS. Then, molecular networks can be constructed with node color and/or size correlating with bioactivity level and/or the fraction number in which the compounds of interest are present. Comparison with in-house, *in silico* or reference spectra in the available databases (e.g., GNPS) might indicate whether the compounds are known or new. As an example, this was applied in the isolation of anti-chikungunya virus diterpenoids from *Euphorbia pithyusa* L. (Euphorbiaceae) (Esposito et al., 2017). Furthermore, once the LC-

MS/MS, bioactivity and molecular networking steps are conducted, various exclusion/inclusion criteria (e.g., bioactivity level, and the presence or absence compounds with defined chemistry and activity) may be applied in order to guide the selection of fraction for further investigation, as was shown in the isolation of a cytotoxic cyclic peptide, samoamide A, from the marine cyanobacterial species *Symploca* (Naman et al., 2017).

Thus, the integration of molecular networking with a bioassay will help in concentrating the isolation protocol on the isolation of novel bioactive compounds without wasting time and resources on often long and tedious traditional bioassay-guided fractionation and isolation steps (Naman et al., 2017). In another example, bioactivity data obtained from WNT signaling assay and chikungunya virus assays were used to prioritize clusters in a molecular networking derived from 292 Euphorbiaceae species extracts collected from New Caledonia (Olivon et al., 2017a). In this study, the nodes were tagged with various bioactivity levels (EC_{50} or IC_{50}) of the extracts, which enabled selection of potent sub-networks for further investigations. Furthermore, to aid in the targeted isolation of the bioactive compounds, molecular networks were tagged with taxonomic information. This tagging of molecular networks with bioactivity and taxonomic data followed by targeted isolation led to isolation of new and known WNT signaling and chikungunya virus inhibitors from two Euphorbiaceae species, *Bocquillonia nervosa* Airy Shaw and *Neoguillauminia cleopatra* Baill., some of which were active in both assays. The annotation of some of the nodes in order to search for analogues (i.e., variable dereplication) was aided by *in silico*-generated MS/MS spectra targeted to secondary metabolites specific to the plant species Euphorbiaceae (Olivon et al., 2017a). Finally, to facilitate the dereplication and bioassay-guided isolation process, the concept of “bioactive molecular networking” has been introduced recently (Nothias et al., 2018). In this proof of concept analysis, bioactive molecular networking was applied for isolation of minor but potent and selective anti-chikungunya virus compounds from the latex of *Euphorbia dendroides* L. (Euphorbiaceae). To locate and isolate the most potent and selective compounds, bioactivity scores were calculated for each ion detected by LC-MS using the Pearson correlation coefficient (r) that correlated the relative abundance of an ion from its LC-MS peak and the selectivity index of fraction from the anti-chikungunya virus assays. Then, bioactive molecular networks were generated using GNPS and visualized by Cytoscape®, in which large node size indicated high antiviral activity in direct relation to a high r, while relative abundance in a certain fraction was indicated by a pie chart. With this tool, the researchers were able to isolate four new phorbol esters, two of which were found have some selectivity against the virus investigated. Further, investigators were able to dereplicate known compounds and analogs (using the “analogs search function” feature of the GNPS) through seeding of spectra of known compounds available in the GNPS (Nothias et al., 2018).

4.2. Biological assays

The therapeutic potential of any solvent-produced extract of an organism depends on the active secondary metabolites present. Additive or synergistic effects can be observed sometimes for mixtures of active constituents. Either single chemical entities (SCEs) or groups of compounds can be developed as chemotherapeutic agents with the aid of bioassays. Several approaches can be applied to determine the potential of these chemical

entities. In the laboratory, the utilization of both cellular and animal models has been key in the development of new drug leads in recent years. The general types of bioassays utilized in natural product anticancer drug development include cell-based and cell-free assays for the study of biochemical interactions as well as the use of animal models for efficacy studies. Additionally, these lead compounds have needed to be optimized for proper bioavailability and selective delivery, in order to reduce side effects and adverse events (Afsar et al., 2016; Muñoz-Acuña et al., 2013; Ren et al., 2012; Singh et al., 2016; Wang et al., 2012).

4.2.1. Cell culture—Cells have been used in natural product drug discovery procedure since the early 1900's, and subsequently have become indispensable tools that are utilized for a variety of basic and clinical studies (Sawant and Torchilin, 2010). Two different systems of cell culture have been utilized over the years including 2D and 3D cell culture arrangements. The main advantages of 2D cell culture-based systems include facile cell observation, environmental control, and bioactivity measurement. However, these systems also exhibit limitations such as diminished compatibility with *in vivo* models, augmented drug sensitivity, and, due to the required exposed surface, their use in both clinical and laboratory investigations is also limited. The traditional model used consists of a static dish culture system, which generates mainly adherent two-dimensional (2D) cell monolayers. Hence, most cell types used in 2D cultures are adherent and cannot be grown in flotation without a mechanical provision, except for blood cells (e.g., leukemia cells). All newly isolated and immortalized culture cells have been grown in tissue culture using appropriate plastic flasks to generate localized adhesions. To maintain healthy conditions of the cells passing between generations, they are monitored and controlled. A classic example of this type of system include the so-called cell cytotoxicity assays commonly used to evaluate cell proliferation and cell cytotoxicity levels, such as methyl-thiazolyl-tetrazolium (MTT) proliferation and sulforhodamine B (SRB) assays (Mosmann, 1983; Ren et al., 2012).

Using a similar format, collection of a substantial amount of data gathered over the years using 2D cultures has helped to answer essential questions and will continue to be used to respond to fundamental biological questions for the foreseeable future. This general method is inexpensive compared to other culture models, has a large database of prior results available for comparative analysis and thus is better understood, and also yields data that can be more easily analyzed with traditionally accepted methods than most three-dimensional (3D) cell culture systems (see below), which require more advanced technologies. However, it has been found that cells, specifically cells in 2D cultures, act structurally and functionally different to *in vivo* conditions due to environmental exchanges, which would not be the same because of the lack of a 3D structure that a tumor would exhibit intrinsically (Riedl et al., 2017). Thus, 2D cell cultures have some basic disadvantages but even so they are currently used widely in natural products research directed towards the discovery of new anticancer agents (Figure 5) (Kapałczyńska et al., 2018).

A previous example of plant natural products of interest studied using typical 2D monolayer cells is the cyclopenta[*b*]benzofuran, silvestrol (Figure 10A, **41**) isolated first from *Aglaia foveolata* Pannell (Meliaceae), a species collected in Indonesia (Hwang et al., 2004; Pan et al., 2010). Silvestrol, was isolated in the laboratory of the current author group, and this compound has shown potential in inhibiting B-cell malignancies (Hwang et al., 2004; Lucas

et al., 2009; Pan et al., 2010; Pan et al., 2014). The 2D and normal cancer cell lines used were lung cancer (Lu1), hormone-dependent human prostate cancer (LNCaP), human breast cancer (MCF-7), and human umbilical vein endothelial cells (HUVEC), showing IC_{50} values of 1.2, 1.5, 1.5, and 4.6 nM, respectively, when compared to paclitaxel (2.3, 4.7, 0.7, 105.5 nM) (Hwang et al., 2004; Pan et al., 2010). Silvestrol is discussed in additional detail in sub-sections 4.2.2.1 and 4.3.3.2 of this review article.

The 3D cancer cell model alternatives are key in solving 2D matrix shortcomings that can essentially change the behavior of cells (Riedl et al., 2017). Recent studies have shown that cells closely mimic their natural environment when grown as a thick layer of polymeric 3D molecules, because the structure tends to maintain a balanced state, making it similar to tissues and organs, and more physiologically appropriate for human applications (Imamura et al., 2015). The 2D culture systems referred to above lack the conditions where cells grow within a complex 3D microenvironment with vascularization that supplies and eliminates metabolites and catabolites as well as additional traits found in the body such as an extracellular matrix. A 3D culture would exhibit extracellular matrix (ECM) interactions similar to that of the native tumor ECM, increasing resistance. In contrast, a 2D culture would have the ECM missing and resistance to compound treatment would not be the same. Recent developments have studied complex systems on how diverse sorts of cells interact and therefore 3D cultures allow for the simulation of conditions found in a living organism. As an example, spheroids derived from patients were used to identify the best therapy for different stages of HER2-negative breast cancer. The results, using this method, revealed that the spheroids for HER2-negative breast cancer were according to current treatment guideline recommendations (Halfter et al., 2016). As a result, the data sets collected from 3D models are more predictable than those obtained from 2D models (Figure 6). Thus, 3D cultures have been introduced in order to simulate conditions as in the living organism.

On the other hand, 3D cultures show more structural complexity, and so the laboratory techniques used with these 3D culture can be cumbersome and time-consuming. Additionally, certain 3D models may contain undesirable elements, such as growth factors and viruses. Another difficulty is that 3D cell cultures are larger in size when compared to 2D systems, making difficult some visualization and quantification analytical procedures. The increased size and layers cause differences in the distribution of nutrients and oxygen throughout the system, which would not be ideal in a living system. Overall, it is felt that cell culture in a 3D format is a better representation of the tissues and their external environment, even with the complications that arise from their use (Brancato et al., 2018; Riedl et al., 2017).

Among the different types of 3D cultures available are: (a) reaggregate or sphere cultures, (b) hydrogel/scaffold cultures, (c) rotary bioreactor cultures with cell aggregates or microcarriers, as well as (d) organotypic slice cultures (Fang and Eglen, 2017). These systems are characterized by differences in cell dispersion and preservation of tissue function. Re-aggregate cultures are induced to stimulate the formation of a sphere. Regardless of the weaknesses of reaggregate cultures, these systems can assist in the development of useful models to study interactions between cells. Organotypic cultures maintain architecture and preserve network. The scaffold techniques are founded on the

presence of millipores for proper nutrition and environment of the cells. Scaffold-free systems, on the other hand, classically use techniques such as hanging drop templates, magnetic levitation, and magnetic 3D bioprinting. Despite all advantages, these scaffold 3D systems are less common and less well-developed (Figure 6) (Fang and Eglen, 2017).

Presently, 3D cultures continue to be under development as they seem to have high promise in anticancer drug discovery and development (Imamura et al., 2015; Nath and Devi, 2016). The use of 3D spheroid tumor models has also been applied to the active constituents of botanical dietary supplements, such as α-mangostin (α-MG, Figure 3, **29**) from mangosteen (see sub-section 3.2 of this review). Thus, the 3D spheroid experiments for MDA-MB-231 melanoma cells had a less potent effect in reducing spheroid volume and density at 24 and 48 hours (30 μg/ml). However, in an MCF-7 breast cancer spheroid experiment, although α-MG showed the highest potency in reducing spheroid volume at both 24 and 48 hours (<5 μg/ml), the spheroid density had an inverse relationship to concentration changes of α-MG (Scolamiero et al., 2018).

There is an increased effort to develop models that mimic the microenvironment of tumors in the body (Yip and Cho, 2013). A recently developed 3D cell microenvironment technology that is used in natural products anticancer drug research is that involving 3D breast cancer microtissue (3D-μTP). This newly developed assay is used for determining *in vitro* cytotoxicity. The main advantage of this 3D model as opposed to others, like spheroids, is that the microenvironment of tumors is better represented due to the formation of an extracellular matrix (ECM). The ECM is an essential component of tumors as this regulates tumor progression. Although it has been used with doxorubicin, a non-plant-derived natural product, its application could be extended to other compounds isolated from natural sources (Brancato et al., 2018). Moreover, the comparative analysis of 2D and 3D tumor models for drug discovery has been under study as a promising approach for drug development (Stock et al., 2016).

4.2.2. Animal models—The use of animal models in natural products drug discovery research has led to an understanding that there are many compounds that are potent in monolayer cell cytotoxicity assays but not in animal models. Additionally, the compound could be non-specific, have extreme toxic effects, limited solubility, and other unexpected limitations (Carter et al., 2014; Cekanova and Rathore, 2014). There are a variety of animal models used in natural products cancer research and each of these may have a different application for the particular disease state being studied. The two animal species that will be mentioned in this review are those involving mice and zebrafish (Carter et al., 2014; Cekanova and Rathore, 2014).

4.2.2.1. Murine: A murine assay that has been established by the U.S. National Cancer Institute (NCI) and is currently in wide use for natural products anticancer research is the *in vivo* hollow fiber assay (Casciari et al., 1994; Hollingshead et al., 1995; Mi et al., 2002; Mi et al., 2009; Seo et al., 2001). The hollow fiber assay involves polyvinylidene fluoride hollow fibers containing human tumor cells that may be implanted intraperitoneally (i.p.) or subcutaneously (s.c.) in immunodeficient mice. Silvestrol (Figure 10A, **41**) has been tested using *in vitro*, as mentioned in sub-section 4.2.1, and *in vivo* using the hollow fiber assay,

with the KB, LNCaP, and Col2 cancer cell lines. Both i.p. and s.c., respectively, *in vivo* administration in mice displayed inhibition on all three cancer cell lines used KB (11.6–63.2%, 0–26.8%), LNCaP (14.9–82.5%, 12.4–15.7%) and Col2 (20.5–76.9%, 4.7–23.4%), although the inhibitory effects were more pronounced on i.p. administration. The main benefit of this model is that it allows that cancer cells to be in an environment that is similar to that of a tumor in the body and, therefore, on compound administration, the effect of a test natural product on the tumor would mimic that of the disease state (Hwang et al., 2004; Mi et al., 2009). The hollow fiber *in vivo* model may be followed by a standard mouse xenograft assay using implanted P-388 murine leukemic cells (e.g., Hwang et al., 2004).

Pancreatic cancer is in the top four most leading causes of cancer-related death. As a result, much effort in this area of natural product research has been put forth. α-Mangostin (Figure 3, **29**) from *Garcinia mangostana* (see sub-section 3.2 of this review) was tested against two cancer cell lines, ASPC1 and PL-45, representing a metastatic site-derived and a primary human pancreatic cancer, respectively. Using an ASPC1 ectopically xenografted athymic nude mice, at three days after preparing a xenograft, α-MG was administered (6 mg/kg/bodyweight i.p., 5 days a week). The compound displayed inhibitory activity by a significant difference in tumor volume ($p = 0.0033$) at week eight between the control (1000 m^3) and α-MG treated (500 m^3) groups. When using pancreatic PL-45 orthotopic xenograft tumors in athymic nude mice, α-MG-treated mice displayed an inhibitory activity of orthotopic tumors. At 9 weeks, the tumor weight decreased by around half the size using the same administration mentioned above (Hafeez et al., 2014).

Therefore, natural products cancer research has used murine animal models as a tool and as shown above has displayed positive results against cancer. Murine models can be used to measure inhibitory activity against cancer in various ways, with some being shown from the examples mentioned above.

4.2.2.2. Zebrafish: The use of this animal model with zebrafish (*Danio rerio*) has now become an essential part of natural products drug research. Among the less-expensive animal models that are being used increasingly as a substitute for murine animal models, zebrafish have been demonstrated to have a high homology of ~70% with human genes (Howe et al., 2013). An additional point that makes zebrafish as a model beneficial for anticancer drug research is its flexibility. Fish in general have a low occurrence of natural cancer formation but an elevated rate of tumorigenesis has been observed in the presence of carcinogenic compounds. Different areas of cancer can be researched and include, but are not limited to, apoptosis, tubulin binding, angiogenesis, microangiography, transgenesis, toxicity and others (Berghmans et al., 2005; Mandrekar and Thakur, 2009; Rubinstein, 2006).

An example of how zebrafish have been used in plant natural products anticancer drug research is that of the edible *Rhynchosia viscosa* (Roth.) DC. (Fabaceae) plant. A methanol extract of this legume was subjected to *in vivo* bioassay-guided fractionation to determine the active compounds present. The zebrafish model used exhibited intersegmental vessel (IVS) inhibition when treated with the crude methanol fraction (50 μg/mL). This crude extract afforded five compounds that were also tested *in vivo* using zebrafish. The two most

active, namely, genistein and licoisoflavone, displayed IC_{50} values of 24.2 μM and 16.7 μM, respectively, when tested for antiangiogenic activity two days post-fertilization (2 dpf). Another experiment that used zebrafish demonstrated the anti-inflammatory effects of the compounds isolated from *R. viscosa.* Moderate leukocyte migration inhibition was observed when the zebrafish larvae (4 dpf) were tested with genistein and sophoraisoflavone (12.5 and 25 μM, respectively), when compared to indomethacin (100 μM) as the positive control. The integration of zebrafish in *in vivo* bioassay-guided isolation procedures shows the potential versatility of this animal model since it can be incorporated to the research workflow effectively (Bohni et al., 2013). However, additional studies are required for the better utilization of this animal model in studies on plant anticancer drug discovery research.

4.3. Drug bioavailability and delivery systems

Assuring adequate compound bioavailability is another challenge in natural product drug development. A specific example of a natural product with bioavailability issues is the diarylheptanoid curcumin from *Curcuma longa* L. (Zingiberaceae), which has been found to be active in many biological studies. To improve its bioavailability, liposomes, nanotechnology, micelles, phospholipid complexes, and various coating materials have been applied to also enhance the water solubility of curcumin. Moreover, representation of the diverse library of compounds obtained from natural sources does not satisfy Lipinski's "rule of five", regardless of bioactivity and ADMET profile (absorption, distribution, metabolism, excretion, and toxicity). Hence, natural products overall lack of the Lipinski's drug likeness criteria for drug leads, requiring a molecular weight less than 500 daltons, a maximum of five partition coefficient (log P) and hydrogen donors, and a maximum of twice as many hydrogen bond acceptors as the minimum amount of hydrogen donors. Adherence to Lipinski's rules is recommended so that the highest bioavailability may be reached for a selected molecule. However, some plant-derived anticancer drugs such as paclitaxel (Figure 1, **6**) do not satisfy the Lipinski's requirements, for example, by having greater than ten hydrogen bond acceptors and a large log P. Therefore, additional standard features are constantly under development for natural product lead compounds (Sawant and Torchilin, 2010; Singh et al., 2016; Wang et al., 2012).

4.3.1. Micelles—Micelles are nanounits used as carriers for targeted delivery and are self-assembled colloidal particles that contain two components: a lipophilic center and a lipophobic shell (Figure 7). Micelles composed of polyethylene glycol-phosphatidyl ethanolamine (PEG-PE) have drawn considerable attention for clinical applications because of key properties such as having durability and the capacity to amass in selective areas with an irregular vasculature via improved retention effects and permeability (Prompruk et al., 2005). Moreover, these micelles can be optimized to target specific ligand molecules to the micelle surface. Adding a surfactant or hydrophobic material to the micelle will improve solubility of the aggregates without affecting their stability. Micelles can also be modified so as to carry various contrast agents and then imaged using different modalities (Prompruk et al., 2005).

Micelles can serve as drug carriers in aqueous media by solubilizing drugs with poor solubility within the micelle core. These can also be designed so that molecules with high

polarity are bound to the micelle exterior and those with intermediate polarity are dispersed among surfactant molecules in the central positions. The use of micelles to increase solubilization for drug candidates improves water solubility of slightly soluble active principles, lowers adverse effects, improves bioavailability, enhances permeability, reduces toxicity, and affords positive changes in biodistribution (Kabanov et al., 2002; Kwon and Okano, 1999; Lin and Kawashima, 1987; Maeda et al., 2000; Yokoyama et al., 1990). Due to the difference in structural complexity, solubility, and their targets, different block copolymers have been used to make the nanoparticles for a drug lead compound. Examples of plant antineoplastic agents modified using micelles are paclitaxel (Figure 1, **5**) from *Taxus brevifolia* and camptothecin (Figure 2, **16**) from *Camptotheca acuminata*. For paclitaxel, poly(D,L-lactide)-*b*-methoxy-polyethylene glycol and for camptothecin, polyethylene glycol-phosphatidyl ethanolamine (PEG-PE) have been employed (Sawant and Torchilin, 2010). There are a variety of different types of micelle formulations and their application is an active area of research that should influence greatly the use and efficacy of additional natural product anticancer agents in the future.

4.3.2. Nanoparticles—Nanotechnology is defined as the "*engineering, characterization, and application of man-made structures on the scale of 1–100 nm [nanometers] in at least one dimension*" (Grobmyer et al., 2010). Nanotechnology has been found to potentially improve current methods for disease diagnosis, disease-state imaging, and treatment. The use of nanotechnology has also been able to reduce toxicities associated with therapies in current use through the application of selective delivery. Nanoparticles used include carbon-based materials, dendrimers, lipids, organometallic substances, and polymers (Alexis et al., 2008; Cuenca et al., 2006; Pelaz et al., 2017). Selection of a particular type of nanoparticle will depend on biocompatibility, toxicity, size, surface chemistry, and properties of the biological system to be used in the evaluation. Nanoparticles may lead to improved effectiveness and minimal toxicity when compared with current therapeutic treatments, as they exhibit properties such as selectivity, specificity and affinity for both the target and the therapeutic agent. These small entities can be used combinatorially with a variety of anticancer agents, making them a desirable choice of drug formulation (Heath and Davis, 2008).

4.3.2.1. Role of nanoparticles in drug development: Liposomes were used initially as nanoscale vehicles to deliver chemotherapeutic agents (Moghimi et al., 2005). Liposome preparations permit the improved delivery of therapeutic agents while reducing toxicity. Moreover, liposomes are responsive to conjugation with surfaces and selective targeting for specific sites (McCarthy and Weissleder, 2008; Park et al., 2004). Other nanoscale delivery vehicles that are currently in development include "smart polymers" that are pH and temperature sensitive, dendrimers, viral nanoparticles, carbon-based nanostructures, and polymers (Pope-Harman et al., 2007; Portney and Ozkan, 2006; Wilczewska et al., 2012). Combinatorial drug delivery systems have also been made possible through nanotechnology using nanocarriers for targeted-biological ligands, providing better efficacy, while decreasing toxicity, with improved localized concentrations of synergistic therapeutics (Sengupta et al., 2005). An example from a plant source is the stilbenoid, *trans*-resveratrol, from *Vitis vinifera* L. (Vitaceae). This substance, a potential anticarcinogenic agent, but having only

limited bioavailability, has been investigated so as to deliver it using planetary ball milled (PBM) nanoparticles in combination with an approved anticancer agent, docetaxel, used to treat prostate cancer. The study included prostate cancer cells and docetaxel-resistant prostate cancer cells, and a combination of folic acid, resveratrol, docetaxel, and the nanoparticle. The formulation of folic acid,resveratrol and docetaxel-nanoparticle (3 μM + 0.01 μM) led to an increase of 47.3% and 18.6% in early and late apoptotic cells, respectively, compared to folic acid-resveratrol-nanoparticle alone (in turn, 21.3% and 9.6%) against resistant prostate cancer cells. In normal prostate cancer cells, folic acid-resveratrol + docetaxel-nanoparticle led to a value of 50.6% in early and late apoptosis while the apoptosis level remained at 21.1% with resveratrol-nanoparticle alone (Singh et al., 2018). This study has exemplified the potential of nanoparticle formulations to significantly boost efficacy when compared to current modalities.

4.3.2.2. Localized delivery of nanomaterials: Imaging plays a key role for *in vivo* nanotechnology in cancer therapy, as nanomaterials are delivered to localized sites for different disease states such as cancer. Imaging helps to optimize applications for selective delivery of nanoparticles to target sites. Two general methods have been applied to achieve this: (i) passive targeting and (ii) active targeting (Cho et al., 2008).

The first delivery system is passive targeting that in cancer drug discovery and development depends on irregular gap connections found in the endothelium of the tumor blood vasculature to support the accumulation of nanoparticles in tumors (Maeda and Matsumura, 1989). Overall, a small particle size favors intratumoral vessel fluid leakage (Kong et al., 2000; Yuan et al., 1995). Hence, both particle composition and shape determine particle uptake and the final outcome of drug efficacy (De Jong and Borm, 2008). Passive accumulation of particles is better accomplished by engineering particles with long half-lives in circulation [e.g., polyethylene glycol (PEG)] (Cho et al., 2008; Lammers et al., 2008). A recent natural product example is that of nanoparticle delivery of the plant-derived carotenoid, lycopene. Oligomerized (−)-epigallocatechin-3-*O*-gallate (a further plant secondary metabolite), along with chitosan, has been used to self-assemble a nanoparticle containing lycopene. This nanoparticle allowed lycopene blood concentrations to reach higher levels when compared to lycopene alone (Li et al., 2017).

A second delivery system, which is more precise in delivery, is active targeting. Active targeting of nanoparticles depends on appropriate attachment to the ligand for targeted distribution in the tumor (Allen, 2002; Black et al., 2008). Examples of precision delivery units previously studied are antibodies, aptamers, ligands found on the cell surface, and peptides (Cho et al., 2008; Lammers et al., 2008). Targets of tumors that have been discovered *in vivo* include antigens found in the tumor, cell surface receptors that have been assimilated, and tumor vessel formation (Sahoo et al., 2004; Santra et al., 2005; Smith et al., 2008). To date, precision delivery has been has not been translated successfully into the clinic even though it has been studied *in vivo*, so it may be necessary to re-evaluate how nanoparticles are being used (Lammers et al., 2008; Zhao and Liu, 2018). Hence, directed drug delivery without an increase in overall localized drug accumulation is an important area of development for selective targeting strategies to safely deliver the drug at the target tissue, particularly for cancer treatment (Kirpotin et al., 2006; Park et al., 2004).

The complex interactions between nanoparticles and the host in addition to their general delivery at present is poorly understood (Carugo et al., 2016; Hood, 2004; Jabr-Milane et al., 2008; Powers et al., 2006). Different environments could affect particle features, so measuring conditions is of utmost importance and should be done in an environment similar to that of the location of the application (Powers et al., 2006). Thus, it is essential to understand the principles involved in order to safely use and reproducibly utilize nanomaterials for human applications in the management of different illnesses, particularly cancer (Powers et al., 2006).

4.3.3. Antibody-drug conjugates (ADCs)

4.3.3.1. Overview of antibody-drug conjugates (ADCs): Since the initial success of DNA-alkylating agents such as nitrogen mustards in the early 20th century to treat cancer, cancer chemotherapy has been a mainstay of treatment for this disease. The main working principle of cancer chemotherapy is the selective killing of cancerous cells without affecting, or having minimal impact, on normal cells, by targeting those processes (e.g., the cell cycle) and components of cells that modulate growth (Chari et al., 2014; Iyer and Kadambi, 2011). However, selectivity itself is not enough to differentiate normal cells from malignant cells, and hence chemotherapeutic drugs are very commonly associated with severe types of toxicity. Rapidly dividing normal cells such those of the gastrointestinal tract and the bones are the major off-targets (Chari, 1998). Moreover, the dose required to elicit a therapeutic response of a cancer chemotherapeutic agent may be close to that of its toxic dose. As a result of this, most of the available anticancer drugs have a narrow therapeutic index (Chari et al., 2014). In addition, some of the most potent and effective natural product lead compound antineoplastic agents such as maytansine (Widdison et al., 2006), dolastatin 10 (Maderna and Leverett, 2015), and calicheamicin γ_1^I (Hamann et al., 2002), each had to be set aside very early in their development process because of their individual unacceptable severe toxicity and narrow therapeutic window.

4.3.3.2. Targeted delivery to increase selectivity and reduce side effects: A means of selective delivery of highly cytotoxic natural products as “prodrugs” to tumor cells has proven necessary in order to reduce off-target effects and increase therapeutic outcomes (Ducry and Stump, 2010). The discovery of a mass production method for monoclonal antibodies (mAbs) in 1975 paved the way to produce mAbs to selectively target antigens that are expressed specifically on the surface of cancer cells (Chari et al., 2014). Since then, many mAbs have been introduced to the clinic for the treatment of a variety of cancers and some of them with their targets include trastuzumab (Herceptin®; targets the antigen HER2), alemtuzumab (Campath®; targets the antigen CD52), cetuximab (Erbitux®; targets the EGF receptor) and panitumumab (Vectibix®; targets the EGF receptor) (Reichert and Valge-Archer, 2007). However, such mAbs are not effective in eradicating tumor cells alone, and thus have to be used in combination with anticancer drugs (Sievers and Senter, 2013). For example, standard therapies for HER2-positive breast cancer involve the combined use of the mAb trastzumab and various anticancer drugs such as paclitaxel, doxorubicin, or cisplatin (Slamon et al., 2001; Verma et al., 2012). On the other hand, it has been determined reliably that the specificity of mAbs can be used for the targeted delivery of cytotoxic drugs to tumor cells (Liu et al., 1996). This involves linking each cytotoxic drug concerned with a

mAb by a linker group to produce a tripartite drug called an “antibody-drug conjugate” (ADCs) (Figure 8) (Peters and Brown, 2015). Also known as “payload” or “warhead”, the cytotoxic drug can be a large molecule such as a protein or a small one including a radioisotope (Chari, 1998). Since the active cytotoxic drug is attached to a large carrier protein molecule and thus has to be released once inside the cell, ADCs can be considered as “macromolecular prodrugs” (Rautio et al., 2018).

4.3.3.3. The importance of conjugation: The attachment and delivery of a cytotoxic drug in the form of an ADC together impact three important parameters: the plasma half-life, specificity, and therapeutic window of the cytotoxic drug (Rautio et al., 2018). Owing to the conjugation and selective delivery to the tumor site, the cytotoxic payloads of the ADCs are relatively safe compared to when used alone, a form in which they are generally not utilized because of their extreme systemic toxicity (de Goeij and Lambert, 2016). Furthermore, this conjugation may also dramatically increase the half-life of the cytotoxic drug from a few hours to several days (Chari, 2008). Thus, ADCs have been developed with the aim of delivering a target specific lethal dose of a cytotoxic drug by taking advantage of the specificity, intrinsic activity, and favorable pharmacokinetics of antibodies (Chari, 1998; Lambert and Morris, 2017).

4.3.3.4. Important characteristics of each component of an ADC: To meet the desired level of selectivity, it is important that the antigen, linker, antibody and the cytotoxic payload of an ADC be selected carefully in each case (Figure 8). Thus, as much as possible the target antigens should be either tumor-specific or tumor-associated, and hence should be solely or preferentially expressed in high amounts on the tumor cells when compared to normal cells, respectively (Peters and Brown, 2015). There should also be a fine balance in the design of the linker to avoid spontaneous/premature release in the bloodstream but also to facilitate a facile and effective release of the payload once inside of the cancer cell (Chari, 2008). Similarly, the antibodies must have a reasonable affinity and selectivity for the target antigen, as high-affinity antibodies do not always guarantee therapeutic success, and, for example, these may cause poor penetration of the ADC into solid tumors (Tsuchikama and An, 2018). It is also important that the cytotoxic agent concerned should possess an *in vitro* activity in the picomolar or sub-nanomolar range (Chari, 2016; Lambert and Morris, 2017). In fact, one of the hallmarks of the success of ADCs has emanated from the extreme potencies of their cytotoxic payloads (Lambert and Morris, 2017). One reason for the requirement of extreme potency is due to fact that only a limited number (approximately 1.5% of an administered dose) of the cytotoxic payload can reach the target (Teicher and Chari, 2011). In addition to their extreme potency, the cytotoxic lead compounds concerned or, in some cases approved drugs, should possess functional groups for the direct linkage to the antibody or should tolerate the addition of such groups by partial synthesis. Furthermore, the payload should have sufficient stability and optimum solubility in conditions where mAbs and ADCs are being synthesized and formulated (Chari, 2008).

4.3.3.5. Natural products as components of ADCs: For more than half a century, natural products and their derivatives have been among the major sources of drugs for cancer chemotherapy, and have been subjected to numerous reviews (Chin et al., 2006; Cragg and

Newman, 2000; Cragg et al., 2009; Cragg and Pezzuto, 2016; Kinghorn et al., 2009; Kinghorn et al., 2016). As discussed in sub-section 2.1, several anticancer drugs are plant-derived natural products or their derivatives (Newman and Cragg, 2016). Furthermore, natural products and their derivatives are the major sources of ADCs that are used currently in the clinical setting (Table 4) as well as many of those in clinical trials (Mack et al., 2014). Most of those investigated belong to the enediyne and anthracycline anticancer antibiotics, the ansamitocin maytansinoid derivatives, the dolastatin and CC-1065 peptide analogs, and the taxane diterpenes (Lambert, 2005). Other classes include derivatives of the antitumor antibiotic anthramycin, the pyrrolobenzodiazepines (PBD) (Mantaj et al., 2017), and amantinin and camptothecin analogs (Chari et al., 2014).

In the following paragraphs, brief descriptions of each of the four currently U.S. FDA-approved ADCs are provided. All of these contain a natural product-derived cytotoxic agent component. With regard to the mechanism of action, the warheads are either DNA-damaging agents (e.g., a calicheamicin) or tubulin disruptors (a maytansinoid or an auristatin). Those based on calicheamicin exhibit unique chemical features wherein three distinct but connected components play a crucial role in the mechanism of action: an oligosaccharide unit with hexasubstituted benzene ring, a trisulfide moiety, and an enediyne warhead. While the oligosaccharide unit serves as a delivery system by binding to the minor grove of DNA in a basic-specific manner, the trisulfide unit acts as a trigger that causes the enediyne warhead to release a benzyne diradical that attacks DNA, leading to DNA strand breaks (Nicolaou et al., 2009; Smith and Nicolaou, 1996). On the other hand, the maytansinoids and auristatins, including dolastatin 10, are potent antimitotic agents that inhibit polymerization of tubulin (Hamel, 1992).

The first ADC to receive FDA approval was gemtuzumab ozogamicin (Mylotarg®; Pfizer, Inc.) in 2000, for the treatment of acute myeloid leukemia (Bross et al., 2001). Gemtuzumab ozogamicin is a conjugate of the humanized IgG4 antibody hP67.6 (that targets the antigen CD33) with a derivative (*N*-acetyl gamma-calicheamicin dimethyl hydrazide; CalichDMH) of the extremely potent enediyne antibiotic calicheamicin γ_1^I, linked by an acid-labile bifunctional hydrazone linker (Hamann et al., 2002). Calicheamicin γ_1^I was isolated initially from the bacterium *Micromonospora echinospora* ssp. *calichensis* (Lee et al., 1987a; Lee et al., 1987b) and has been shown to cause site-specific DNA double-strand breaks by binding to DNA minor groves (Zein et al., 1988). However, after a decade of clinical use, gemtuzumab ozogamicin was removed voluntarily from the market by Pfizer, Inc. in 2010, because of a lack of clinical efficacy in confirmatory clinical trials and hepatotoxicity associated with a dose of 9 mg/m^2 (Clarke and Marks, 2010). Later, it was re-approved by the U.S. FDA in September 2017, after studies indicating that a low divided dose (3 mg/m^2) of the drug was effective with minimal hepatotoxicity (Norsworthy et al., 2018).

The second ADC approved, in 2011 (by an “accelerated approval” process), was brentuximab vedotin (Adcetris®, Seattle Genetics, Inc.), for the treatment of relapsed Hodgkin lymphoma and relapsed systemic anaplastic large-cell lymphoma (de Claro et al., 2012). Brentuximab vedotin (also known as cAC10−vcMMAE and SGN-35) is a conjugate of the chimeric antibody cAC10 that targets the antigen CD30, with monomethyl auristatin E (MMAE; vedotin) linked via the enzyme cleavable dipeptide linker, valine-citrulline (vc)

and the spacer, *p*-aminobenzyloxycarbonyl (PABC) (Senter and Sievers, 2012). MMAE (Doronina et al., 2003), as well as other auristatins (Maderna and Leverett, 2015), are synthetic derivatives of the extremely potent cytotoxic pentapeptide, dolastatin 10, first isolated from the sea hare *Dolabella auricularia* (Aplysiidae) collected from the Indian Ocean (Pettit et al., 1987). MMAE and its congeners monomethyl auristatin F (MMAF) and PF-06380101, are the most common auristatins used as components of experimental ADCs (Maderna and Leverett, 2015).

The third ADC approved in 2013 was ado-trastuzumab emtansine (Kadcyla®, Genentech, Inc.; Figure 9B), also known as T-DM1 and trastuzumab emtansine, used for the treatment of human epidermal growth factor receptor 2 (HER2)-positive metastatic breast cancer in those patients having been treated previously with a single or combined regimen of trastuzumab and a taxane derivative (Amiri-Kordestani et al., 2014). T-DM1 is derived from the monoclonal antibody trastuzumab (Herceptin®) that targets the antigen HER2 when covalently linked by a nonreducible thioether with DM1 (Lambert and Chari, 2014). The cytotoxic payload, DM1 (mertansine; $N^{2'}$-deacetyl-$N^{2'}$-(3-mercapto-1-oxopropyl)maytansine, Figure 9A, **39**), is a semisynthetic analog (Widdison et al., 2006) of the ansamitocin, maytansine (Figure 2, **19**), which was initially isolated from the Ethiopian shrub *Maytenus ovatus* Loes. (Celastraceae) (Kupchan et al., 1972). However, it has now been established that maytansine is biosynthesized by endophytic bacteria that co-exist in the plant roots (Kusari et al., 2014). Another maytansinoid analogue, DM4 (ravtansine; $N^{2'}$-deacetyl-$N^{2'}$-(4-mercapto-4-methyl-1-oxopentyl)maytansine, Figure 9A, **40**), which contains a sterically hindered thiol that makes it more stable and potent, is also of interest as a component of some ADCs (Widdison et al., 2006).

Finally, the fourth and the most recent ADC is inotuzumab ozogamicin (Besponsa®; Pfizer, Inc.), which was approved in 2017 for relapsed or refractory B-cell precursor acute lymphoblastic leukemia (ALL) (U.S. Food & Drug Administration, 2017). The approval was based on the results of an INO-VATE ALL trial, a phase III trial designed to evaluate the safety and efficacy of inotuzumab ozogamicin. In this randomized two-group trial (i.e., inotuzumab ozogamicin *vs*. an investigator's choice of standard therapy, such as fludarabine, cytarabine, mitoxantrone and granulocyte colony-stimulating factor), the percentage of complete remission shown in the inotuzumab ozogamicin-treated group was significantly greater than that using the standard therapy (80.7% *vs*. 29.4%; $p < 0.001$). Furthermore, other outcomes such as progression-free survival, overall survival, and minimal residual disease were superior in those groups treated with inotuzumab ozogamicin than with the standard group (Kantarjian et al., 2016). Previously known as CMC-544, inotuzumab ozogamicin is produced by conjugation of the humanized IgG4 mAb G544 to target the antigen CD22 with a derivative of calicheamicin γ_1^I, CalichDMH, via an acid labile hydrazone linker (DiJoseph et al., 2004).

These successful applications of natural products as components of approved ADCs rely on the fact that they meet most of the requirement of cytotoxic payloads mentioned above, particularly their extreme potency, unusual mechanisms of action, and possession of functional groups such as amino, hydroxy and thiol moieties for facile attachment to antibodies (Gerber et al., 2013). All of the major natural product classes that are the main

components of ADC are known to have biological potencies comparable to known toxins such as ricin and diphtheria (Lambert, 2005). For example, it was the extreme potency of maytansine that made it attractive as a payload, although it was found in very small amounts in the plant of occurrence (~0.2 mg/kg) and was associated with severe toxicities (Cassady et al., 2004). The other advantage of using natural products as components of ADCs is that they can be manipulated to add groups that aid their attachment to the antibodies using various methods such biosynthetic engineering (Gerber et al., 2013).

4.3.3.6. Plant natural products as components of developmental ADCs: Small-molecule constituents of plants have been used to derive potential therapeutic agents for many years (Balunas and Kinghorn, 2005; Cragg and Newman, 2003, 2005; Kinghorn et al., 2011b; Kinghorn et al., 2016) and several of these plant metabolites have been developed as anticancer drugs prescribed to patients (see sub-section 2.1 of this review). Furthermore, in addition to their therapeutic uses, plant-derived natural product molecules also serve pharmacological tools in the elucidation of important biological pathways and targets (Bahar et al., 2011; Pan et al., 2009). For example, the pyrroloquinoline alkaloid camptothecin was pivotal in the elucidation of topoisomerase I (Topo I) as an important and viable molecular target (Cragg and Newman, 2004).

As referred to earlier, several derivatives of camptothecin (Figure 2, **16**) are important components of experimental ADCs. One example is exatecan (DX-8951f, used as the mesylate salt; Figure 2, **18**) (Mitsui et al., 1995), of which its derivative, DXd, is conjugated with the mAb, trastuzumab, via a protease cleavable peptide linker, to produce the ADC trastuzumab deruxtecan or DS-8201a (Ogitani et al., 2016b). DS-8201a contains a high (7.7:1) drug-antibody ratio and was shown to be active in T-DM1-resistant tumors (Takegawa et al., 2017) in addition to exhibiting a bystander cytotoxic effect (Ogitani et al., 2016a). Furthermore, in a small-scale phase I trial, DS-8201a showed promising antitumor activities against breast and gastric or gastroesophageal cancers (Doi et al., 2017).

The active metabolite of irinotecan, SN-38 (7-ethyl-10-hydroxycamptothecin; Figure 2, **17**), is the component of the ADC sacituzumab govitecan (also known as IMMU-132 and hRS7-CL2A-SN-38; Immunomedics, Inc.) which was shown to be effective *in vitro* using cancer cells and in *in vivo* xenograft models (Cardillo et al., 2011; Cardillo et al., 2015; Sharkey et al., 2015) and clinical trials (Bardia et al., 2017; Faltas et al., 2016; Gray et al., 2017; Heist et al., 2017; Ocean et al., 2017; Starodub et al., 2015). IMMU-132 is a conjugated product of SN-38 with the humanized mAb, hRS7, which targets the human trophoblastic cell-surface antigen (Trop-2) (Cardillo et al., 2011). Interestingly, when compared to other ADCs, IMMU-132 comprises a moderately toxic payload (nM *vs.* pM or sub-nM) and a moderately stable pH-sensitive benzylcarbonate linker that also stabilizes the lactone of SN-38 and high drug-antibody ratio (7.6:1 *vs.* 4:1) (Goldenberg et al., 2015). A similar camptothecin-derived ADC that uses the same linker and payload with the humanized IgG mAb hMN-14 (labetuzumab) that targets the antigen CEACAM5 (carcinoembryonic antigen-related cell adhesion molecule-5) is labetuzumab govitecan (IMMU-130 and hMN-14-CL2A−SN-38), which has shown promising preclinical (Govindan et al., 2015) and clinical (Dotan et al., 2017; Sharkey et al., 2018) results.

Another example of a potent antineoplastic molecule from a plant source that may be mentioned is silvestrol (Figure 10A, **41**), first isolated and structurally characterized from the fruits and twigs of *Aglaia foveolata* Pannell (Meliaceae) in 2004 (Hwang et al., 2004; Pan et al., 2010). This compound with an unprecedented dioxanyl ring structure has shown promising results both *in vitro* and *in vivo* against several B-cell malignancy-related assays (Kinghorn et al., 2016; Lucas et al., 2009; Pan et al., 2014). Silvestrol and its close analog, 5‴-episilvestrol, each exert their cytotoxic property by inhibiting the eukaryotic initiation factor eIF4A downstream in the transcription pathway (Cencic et al., 2009; Pan et al., 2010). This mechanism of action is similar to another cyclopenta[*b*]benzofuran, rocaglamide, which is involved in the inhibition of MEK-REK and eIF4E, upstream of eIF4A in the transcription pathway (Ebada et al., 2011; Pan et al., 2014). Very recently, several experimental silvestrol-antibody drug conjugates (silvestrol-ADCs) targeting B-cell cancer have been developed (Pillow et al., 2017). Silvestrol ADCs were prepared by linking different antibodies (anti-HER2, anti-CD22, anti-CD33 and others) to two different sites on silvestrol (i.e., the C-2 and C-6‴ positions) (Pillow et al., 2017). The results depicted dose-dependent and target-specific antitumor activities in xenograft models. Of those tested ADCs, ADC-104 (containing the anti-CD22 antibody, Figure 10B) showed comparable tumor stasis at 6 mg/kg and 10 mg/kg in xenograft mice models using CD22 cells expressing BJAB-luc. The other silvestrol ADCs, ADC-103 (target: HER2; HER2 expressing KPL4 xenograft mouse model), ADC-105 (target: CD22, Figure 10C) and ADC-108 (target: CD22, Figure 10C), showed complete reduction of tumor size at 6 mg/kg, 3 mg/kg and 3 mg/kg, respectively (Pillow et al., 2017).

5. Future prospects

Natural products research, inclusive of using plants as source organisms, has developed considerably over the approximately last three centuries of formal scientific investigation. Higher plants continue to be a rich source of novel anticancer drugs and lead molecules, currently either on the market as U.S. FDA approved drugs or at different development stages, i.e., in *in vitro* and *in vivo* preclinical studies, and clinical trials. Moreover, drugs like certain botanical dietary supplements also offer promise of yielding new cancer chemotherapeutic agents, as demonstrated in this review using the two examples of mangosteen and noni. Furthermore, the application of contemporary techniques, such as hyphenated analytical techniques, like LC-MS, and LC-MS-NMR, along with molecular networking, has proven to be effective in the targeted identification and purification of novel compounds from plant sources in a systematic and cost-effective way. The use of these methodologies has garnered much interest lately owing to their versatility, ease of access, and applicability. In addition, enhanced cell (2D and 3D culture systems) and animal models have aided in improved biological evaluation and better clinical translation of plant secondary molecules to human studies. Major obstacles in natural product research and their development as anticancer drugs have been their low bioavailability, limited water solubility, and potential toxicity. Drug formulations like micelles, liposomes, and nanoparticles have not only improved compound solubility but have also played a key role in the enhancement of compound bioavailability. Another advanced strategy that has garnered much interest from the biomedical community is development of antibody-drug conjugates (ADCs), which

permit the targeted delivery of highly toxic substances inclusive of phytochemicals in a form suitable for patient use. This merging of traditional drug discovery methods with modern biotechnological techniques has stimulated a renewed interest in this field of isolation, purification, characterization and development of natural product molecules from plants. The increasing advances in biotechnological methods allow for the most efficient development and identification of antineoplastic drug leads and for better utilization of novel compounds that can be delivered efficiently to the patient.

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Abbreviations:

ADC	antibody-drug conjugate
ADMET	absorption, distribution, metabolism, excretion, and toxicity
ALL	acute lymphoblastic lymphoma
BGCs	biosynthetic gene clusters
CD22/33	cluster differentiation-22/33
CDK	cyclin dependent kinase
CNS	central nervous system
DAD	diode array detection
ECM	extracellular matrix
eIF4	eukaryotic initiation factor 4
ELSD	electron light scattering detector
EMA	European Medicines Agency
FDA	U.S. Food and Drug Administration
GNPS	Global Natural Product Social molecular networking
HER2	human epidermal growth factor receptor 2
HPLC	High-performance liquid chromatography
HTS	high-throughput screening
HUVEC	human umbilical vein endothelial cells
IR	infrared spectrometry
LC	liquid chromatography

LNCaP	hormone-dependent human prostate cancer
Lu1	lung cancer cell line
mAbs	monoclonal antibodies
MCF-7	human breast cancer cell line
MMAE	monomethyl auristatin E
MMAF	monomethyl auristatin F
MS	mass spectrometry
NCE	new chemical entity
NMR	nuclear magnetic resonance
NSCLC	non-small-cell lung cancer cell line
PBS	phosphate buffer solution
PDA	photodiode array detector
PEG-PE	polyethylene glycol-phosphatidyl ethanolamine
ROS	reactive oxygen species
SCE	single chemical entity
SPE	solid-phase extraction
SRB	sulforhodamine B

References

Abou Assi R., Darwis Y, Abdulbaqi IM, Khan AA, Vuanghao L, Laghari MH, 2017 Morinda citrifolia (noni): a comprehensive review on its industrial uses, pharmacological activities, and clinical trials. Arabian J. Chem 10, 691–707.

Afsar T, Trembley JH, Salomon CE, Razak S, Khan MR, Ahmed K, 2016 Growth inhibition and apoptosis in cancer cells induced by polyphenolic compounds of Acacia hydaspica: involvement of multiple signal transduction pathways. Sci. Rep 6, 23077. [PubMed: 26975752]

Aisha AFA, Abdulmajid AMS, Ismail Z, Alrokayan SA, Abu-Salah KM, 2015 Development of polymeric nanoparticles of Garcinia mangostana xanthones in eudragit RL100/RS100 for anti-colon cancer drug delivery. J. Nanomater 2015, 701979.

Alexis F, Rhee J-W, Richie JP, Radovic-Moreno AF, Langer R, Farokhzad OC, 2008 New frontiers in nanotechnology for cancer treatment. Urol. Oncol.: Semin. Orig. Invest 26, 74–85.

Allard P-M, Peresse T, Bisson J, Gindro K, Marcourt L, Pham VC, Roussi F, Litaudon M, Wolfender J-L, 2016 Integration of molecular networking and in-silico MS/MS fragmentation for natural products dereplication. Anal. Chem 88, 3317–3323. [PubMed: 26882108]

Allen T, 2002 Ligand-targeted therapeutics in anticancer therapy. Nat. Rev. Cancer 2, 705–763. [PubMed: 12209159]

Amiri-Kordestani L, Blumenthal GM, Xu QC, Zhang L, Tang SW, Ha L, et al., 2014 FDA approval: ado-trastuzumab emtansine for the treatment of patients with HER2-positive metastatic breast cancer. Clin. Cancer Res 20, 4436–4441. [PubMed: 24879797]

Atanasov AG, Waltenberger B, Pferschy-Wenzig EM, Linder T, Wawrosch C, Uhrin P, et al., 2015 Discovery and resupply of pharmacologically active plant-derived natural products: a review. Biotechnol. Adv 33, 1582–1614. [PubMed: 26281720]

Augustin Y, Krishna S, Kumar D, Pantziarka P, 2015 The wisdom of crowds and the repurposing of artesunate as an anticancer drug. ecancer 9, ed50.

Ba Q, Zhou N, Duan J, Chen T, Hao M, Yang X, et al., 2012 Dihydroartemisinin exerts its anticancer activity through depleting cellular iron via transferrin receptor-1. PLoS One 7, e42703. [PubMed: 22900042]

Bahar M, Deng Y, Fletcher JN, Kinghorn AD, 2011 Plant-derived natural products in drug discovery and development: an overview. In: Ikan R (Ed.) Selected Topics in the Chemistry of Natural Products World Scientific Publishing Co., Singapore, pp. 11–48.

Bailey RL, Gahche JJ, Miller PE, Thomas PR, Dwyer JT, 2013 Why US adults use dietary supplements. JAMA Intern. Med 173, 355–361. [PubMed: 23381623]

Balunas MJ, Kinghorn AD, 2005 Drug discovery from medicinal plants. Life Sci 78, 431–441. [PubMed: 16198377]

Bardia A, Mayer IA, Diamond JR, Moroose RL, Isakoff SJ, Starodub AN, et al., 2017 Efficacy and safety of anti-trop-2 antibody drug conjugate sacituzumab govitecan (IMMU-132) in heavily pretreated patients with metastatic triple-negative breast cancer. J. Clin. Oncol 35, 2141–2148. [PubMed: 28291390]

Benatrehina PA, Pan L, Naman CB, Li J, Kinghorn AD, 2018 Usage, biological activity, and safety of selected botanical dietary supplements consumed in the United States. J. Tradit. Complement. Med 8, 267–277. [PubMed: 29736381]

Berghmans S, Jette C, Langenau D, Hsu K, Stewart R, Look T, Kanki JP, 2005 Making waves in cancer research: new models in the zebrafish. Biotechniques 39, 227–237. [PubMed: 16116796]

Beutler JA, Alvarado AB, Schaufelberger DE, Andrews P, McCloud TG, 1990 Dereplication of phorbol bioactives: Lyngbya majuscula and Croton cuneatus. J. Nat. Prod 53, 867–874. [PubMed: 2128889]

Black KC, Kirkpatrick ND, Troutman TS, Xu L, Vagner J, Gillies RJ, Barton JK, Utzinger U, Romanowski M, 2008 Gold nanorods targeted to delta opioid receptor: plasmon-resonant contrast and photothermal agents. Mol. Imaging 7, 50–57. [PubMed: 18384724]

Bohni N, Cordero-Maldonado ML, Maes J, Siverio-Mota D, Marcourt L, Munck S, et al., 2013 Integration of microfractionation, qNMR and zebrafish screening for the *in vivo* bioassay-guided isolation and quantitative bioactivity analysis of natural products. PLoS One 8, e64006. [PubMed: 23700445]

Borris RP, 1996 Natural products research: perspectives from a major pharmaceutical company. J. Ethnopharmacol 51, 29–38. [PubMed: 9213624]

Bouslimani A, Sanchez LM, Garg N, Dorrestein PC, 2014 Mass spectrometry of natural products: current, emerging and future technologies. Nat. Prod. Rep 31, 718–729. [PubMed: 24801551]

Brancato V, Gioiella F, Imparato G, Guarnieri D, Urciuolo F, Netti PA, 2018 3D breast cancer microtissue reveals the role of tumor microenvironment on the transport and efficacy of free-doxorubicin in vitro. Acta Biomater 75, 200–212. [PubMed: 29864516]

Bross PF, Beitz J, Chen G, Chen XH, Duffy E, Kieffer L, et al., 2001 Approval summary: gemtuzumab ozogamicin in relapsed acute myeloid leukemia. Clin. Cancer Res 7, 1490–1496. [PubMed: 11410481]

Butler MS, 2004 The role of natural product chemistry in drug discovery. J. Nat. Prod 67, 2141–2153. [PubMed: 15620274]

Butler MS, Robertson AA, Cooper MA, 2014 Natural product and natural product derived drugs in clinical trials. Nat. Prod. Rep 31, 1612–1661. [PubMed: 25204227]

Cardillo TM, Govindan SV, Sharkey RM, Trisal P, Goldenberg DM, 2011 Humanized anti-trop-2 IgG-SN-38 conjugate for effective treatment of diverse epithelial cancers: preclinical studies in human cancer xenograft models and monkeys. Clin. Cancer Res 17, 3157–3169. [PubMed: 21372224]

Cardillo TM, Govindan SV, Sharkey RM, Trisal P, Arrojo R, Liu D, Rossi EA, Chang CH, Goldenberg DM, 2015 Sacituzumab govitecan (IMMU-132), an anti-trop-2/SN-38 antibody-drug conjugate:

characterization and efficacy in pancreatic, gastric, and other cancers. Bioconjug. Chem 26, 919–931. [PubMed: 25915780]

Carter LG, D'Orazio JA, Pearson KJ, 2014 Resveratrol and cancer: focus on *in vivo* evidence. Endocr. Relat. Cancer 21, 209–225.

Carugo D, Bottaro E, Owen J, Stride E, Nastruzzi C, 2016 Liposome production by microfluidics: potential and limiting factors. Sci. Rep 6, 25876. [PubMed: 27194474]

Casciari JJ, Hollingshead MG, Alley MC, Mayo JG, Malspeis L, Miyauchi S, Grever MR, Weinstein JN, 1994 Growth and chemotherapeutic response of cells in a hollow-fiber *in vitro* solid tumor. J. Natl. Cancer Inst 86, 1846–1852. [PubMed: 7990159]

Cassady JM, Chan KK, Floss HG, Leistner E, 2004 Recent developments in the maytansinoid antitumor agents. Chem. Pharm. Bull 52, 1–26. [PubMed: 14709862]

Cekanova M, Rathore K, 2014 Animal models and therapeutic molecular targets of cancer: utility and limitations. Drug Des. Devel. Ther 8, 1911–1921.

Cencic R, Carrier M, Galicia-Vazquez G, Bordeleau ME, Sukarieh R, Bourdeau A, et al., 2009 Antitumor activity and mechanism of action of the cyclopenta[*b*]benzofuran, silvestrol. PLoS One 4, e5223. [PubMed: 19401772]

Chan-Blanco Y, Vaillant F, Mercedes Perez A., Reynes M, Brillouet J-M, Brat P, 2006 The noni fruit (Morinda citrifolia L.): a review of agricultural research, nutritional and therapeutic properties. J. Food Compost. Anal 19, 645–654.

Chang HF, Wu CH, Yang LL, 2013 Antitumour and free radical scavenging effects of γ-mangostin isolated from *Garcinia mangostana* pericarps against hepatocellular carcinoma cell. J. Pharm. Pharmacol 65, 1419–1428. [PubMed: 23927480]

Chari RV, 1998 Targeted delivery of chemotherapeutics: tumor-activated prodrug therapy. Adv. Drug Deliv. Rev 31, 89–104. [PubMed: 10837619]

Chari RV, 2008 Targeted cancer therapy: conferring specificity to cytotoxic drugs. Acc. Chem. Res 41, 98–107. [PubMed: 17705444]

Chari RV, Miller ML, Widdison WC, 2014 Antibody-drug conjugates: an emerging concept in cancer therapy. Angew. Chem. Int. Ed. Engl 53, 3796–3827. [PubMed: 24677743]

Chari RV, 2016 Expanding the reach of antibody-drug conjugates. ACS Med. Chem. Lett 7, 974–976. [PubMed: 27882193]

Chervin J, Perio P, Martins-Froment N, Pharkeovilay C, Reybier K, Nepveu F, et al., 2017 Dereplication of natural products from complex extracts by regression analysis and molecular networking: case study of redox-active compounds from *Viola alba* subsp. dehnhardtii. Metabolomics 13, 96.

Chin YW, Balunas MJ, Chai H, Kinghorn AD, 2006 Drug discovery from natural sources. AAPS J 8, E239–E253. [PubMed: 16796374]

Chin YW, Kinghorn AD, 2008 Structural characterization, biological effects, and synthetic studies on xanthones from mangosteen (Garcinia mangostana), a popular botanical dietary supplement. Mini Rev. Org. Chem 5, 355–364. [PubMed: 21562610]

Chitchumroonchokchai C, Thomas-Ahner JM, Li J, Riedl KM, Nontakham J, Suksumrarn S, Clinton SK, Kinghorn AD, Failla ML, 2013 Anti-tumorigenicity of dietary α-mangostin in an HT-29 colon cell xenograft model and the tissue distribution of xanthones and their phase II metabolites. Mol. Nutr. Food Res 57, 203–211. [PubMed: 23239542]

Cho K, Wang X, Nie S, Chen Z, Shin DM, 2008 Therapeutic nanoparticles for drug delivery in cancer. Clin. Cancer Res 14, 1310. [PubMed: 18316549]

Clarke WT, Marks PW, 2010 Gemtuzumab ozogamicin: is there room for salvage? Blood 116, 2618–2619. [PubMed: 20930085]

Clarkson C, Staerk D, Hansen SH, Smith PJ, Jaroszewski JW, 2006 Identification of major and minor constituents of Harpagophytum procumbens (Devil's Claw) using HPLC-SPE-NMR and HPLC-ESIMS/APCIMS. J. Nat. Prod 69, 1280–1288. [PubMed: 16989520]

Corcoran O, Spraul M, 2003 LC-NMR-MS in drug discovery. Drug Discov. Today 8, 624–631. [PubMed: 12867148]

Cragg GM, Newman DJ, 2000 Antineoplastic agents from natural sources: achievements and future directions. Expert Opin. Investig. Drugs 9, 2783–2797.

Cragg GM, Newman DJ, 2001a Medicinals for the millennia: the historical record. Ann. N. Y. Acad. Sci 953, 3–25. [PubMed: 11795420]

Cragg GM, Newman DJ, 2001b Natural product drug discovery in the next millennium. Pharm. Biol 39, 8–17. [PubMed: 21554167]

Cragg GM, Newman DJ, 2003 Plants as a source of anti-cancer and anti-HIV agents. Ann. Appl. Biol 143, 127–133.

Cragg GM, Newman DJ, 2004 A tale of two tumor targets: topoisomerase I and tubulin. The Wall and Wani contribution to cancer chemotherapy. J. Nat. Prod 67, 232–244. [PubMed: 14987065]

Cragg GM, Newman DJ, 2005 Plants as a source of anti-cancer agents. J. Ethnopharmacol 100, 72–79. [PubMed: 16009521]

Cragg GM, Grothaus PG, Newman DJ, 2009 Impact of natural products on developing new anti-cancer agents. Chem. Rev 109, 3012–3043. [PubMed: 19422222]

Cragg GM, Newman DJ, 2013 Natural products: a continuing source of novel drug leads. Biochim. Biophys. Acta 1830, 3670–3695. [PubMed: 23428572]

Cragg GM, Grothaus PG, Newman DJ, 2014 New horizons for old drugs and drug leads. J. Nat. Prod 77, 703–723. [PubMed: 24499205]

Cragg GM, Pezzuto JM, 2016 Natural products as a vital source for the discovery of cancer chemotherapeutic and chemopreventive agents. Med. Princ. Pract 25, 41–59. [PubMed: 26679767]

Crusemann M, O'Neill EC, Larson CB, Melnik AV, Floros DJ, da Silva RR, Jensen PR, Dorrestein PC, Moore BS, 2017 Prioritizing natural product diversity in a collection of 146 bacterial strains based on growth and extraction protocols. J. Nat. Prod 80, 588–597. [PubMed: 28335604]

Cuenca AG, Jiang H, Hochwald SN, Delano M, Cance WG, Grobmyer SR, 2006 Emerging implications of nanotechnology on cancer diagnostics and therapeutics. Cancer 107, 459–466. [PubMed: 16795065]

Cui H, Li TL, Guo HF, Wang JL, Xue P, Zhang Y, Fan JH, Li ZP, Gao YJ, 2018 Silymarin-mediated regulation of the cell cycle and DNA damage response exerts antitumor activity in human hepatocellular carcinoma. Oncol. Lett 15, 885–892. [PubMed: 29399153]

David B, Wolfender J-L, Dias DA, 2015 The pharmaceutical industry and natural products: historical status and new trends. Phytochem. Rev 14, 299–315.

de Claro RA, McGinn K, Kwitkowski V, Bullock J, Khandelwal A, Habtemariam B, et al., 2012 U.S. Food and Drug Administration approval summary: brentuximab vedotin for the treatment of relapsed Hodgkin lymphoma or relapsed systemic anaplastic large-cell lymphoma. Clin. Cancer Res 18, 5845–5849. [PubMed: 22962441]

de Goeij BE, Lambert JM, 2016 New developments for antibody-drug conjugate-based therapeutic approaches. Curr. Opin. Immunol 40, 14–23. [PubMed: 26963132]

De Jong WH, Borm PJA, 2008 Drug delivery and nanoparticles: applications and hazards. Int. J. Nanomed 3, 133–149.

de Oliveira GG, Carnevale Neto F., Demarque DP, de Sousa Pereira-Junior JA, Sampaio Peixoto Filho R.C., de Melo SJ, da Silva Almeida JRG, Lopes JLC, Lopes NP, 2017 Dereplication of flavonoid glycoconjugates from *Adenocalymma imperatoris-maximilianii* by untargeted tandem mass spectrometry-based molecular networking. Planta Med 83, 636–646. [PubMed: 27806406]

DiJoseph JF, Armellino DC, Boghaert ER, Khandke K, Dougher MM, Sridharan L, et al., 2004 Antibody-targeted chemotherapy with CMC-544: a CD22-targeted immunoconjugate of calicheamicin for the treatment of B-lymphoid malignancies. Blood 103, 1807–1814. [PubMed: 14615373]

Doi T, Shitara K, Naito Y, Shimomura A, Fujiwara Y, Yonemori K, et al., 2017 Safety, pharmacokinetics, and antitumour activity of trastuzumab deruxtecan (DS-8201), a HER2-targeting antibody-drug conjugate, in patients with advanced breast and gastric or gastro-oesophageal tumours: a phase 1 dose-escalation study. Lancet Oncol 18, 1512–1522. [PubMed: 29037983]

Doronina SO, Toki BE, Torgov MY, Mendelsohn BA, Cerveny CG, Chace DF, et al., 2003 Development of potent monoclonal antibody auristatin conjugates for cancer therapy. Nat. Biotechnol 21, 778–784. [PubMed: 12778055]

Dotan E, Cohen SJ, Starodub AN, Lieu CH, Messersmith WA, Simpson PS, et al., 2017 Phase I/II trial of labetuzumab govitecan (anti-CEACAM5/SN-38 antibody-drug conjugate) in patients with refractory or relapsing metastatic colorectal cancer. J. Clin. Oncol 35, 3338–3346. [PubMed: 28817371]

Drobnik J, Drobnik E, 2016 Timeline and bibliography of early isolations of plant metabolites (1770–1820) and their impact to pharmacy: a critical study. Fitoterapia 115, 155–164. [PubMed: 27984164]

Ducry L, Stump B, 2010 Antibody-drug conjugates: linking cytotoxic payloads to monoclonal antibodies. Bioconjug. Chem 21, 5–13. [PubMed: 19769391]

Duncan KR, Crusemann M, Lechner A, Sarkar A, Li J, Ziemert N, et al., 2015 Molecular networking and pattern-based genome mining improves discovery of biosynthetic gene clusters and their products from Salinispora species. Chem. Biol 22, 460–471. [PubMed: 25865308]

Ebada SS, Lajkiewicz N, Porco JA Jr., Li-Weber M, Proksch P, 2011 Chemistry and biology of rocaglamides (= flavaglines) and related derivatives from Aglaia species (Meliaceae). In: Kinghorn AD, Falk H, Kobayashi J (Eds.), Progress in the Chemistry of Organic Natural Products Springer-Verlag, Vienna, pp. 1–58.

Esposito M, Nothias L-F, Retailleau P, Costa J, Roussi F, Neyts J, et al., 2017 Isolation of premyrsinane, myrsinane, and tigliane diterpenoids from *Euphorbia pithyusa* using a chikungunya virus cell-based assay and analogue annotation by molecular networking. J. Nat. Prod 80, 2051–2059. [PubMed: 28671832]

Exarchou V, Krucker M, van Beek TA, Vervoort J, Gerothanassis IP, Albert K, 2005 LC-NMR coupling technology: recent advancements and applications in natural products analysis. Magn. Reson. Chem 43, 681–687. [PubMed: 16049952]

Faltas B, Goldenberg DM, Ocean AJ, Govindan SV, Wilhelm F, Sharkey RM, et al., 2016 Sacituzumab govitecan, a novel antibody-drug conjugate, in patients with metastatic platinum-resistant urothelial carcinoma. Clin. Genitourin. Cancer 14, e75–e79. [PubMed: 26541586]

Fang Y, Eglen RM, 2017 Three-dimensional cell cultures in drug discovery and development. SLAS Discov 22, 456–472. [PubMed: 28520521]

Fischer J, Ganellin CR, Ganesan A, Proudfoot J, 2010 Standalone drugs. In: Fischer J, Ganellin CR (Eds.), Analogue-based Drug Discovery II Wiley-VCH, Weinheim, pp. 29–60.

Fox Ramos AE, Alcover C, Evanno L, Maciuk A, Litaudon M, Duplais C, et al., 2017 Revisiting previously investigated plants: a molecular networking-based study of Geissospermum laeve. J. Nat. Prod 80, 1007–1014. [PubMed: 28282127]

Garg N, Kapono C, Lim YW, Koyama N, Vermeij MJ, Conrad D, Rohwer F, Dorrestein PC, 2015 Mass spectral similarity for untargeted metabolomics data analysis of complex mixtures. Int. J. Mass Spectrom 377, 719–727. [PubMed: 25844058]

Gaudencio SP, Pereira F, 2015 Dereplication: racing to speed up the natural products discovery process. Nat. Prod. Rep 32, 779–810. [PubMed: 25850681]

Gerber HP, Koehn FE, Abraham RT, 2013 The antibody-drug conjugate: an enabling modality for natural product-based cancer therapeutics. Nat. Prod. Rep 30, 625–639. [PubMed: 23525375]

Gertsch J, 2009 How scientific is the science in ethnopharmacology? Historical perspectives and epistemological problems. J. Ethnopharmacol 122, 177–183. [PubMed: 19185054]

Goldenberg DM, Cardillo TM, Govindan SV, Rossi EA, Sharkey RM, 2015 Trop-2 is a novel target for solid cancer therapy with sacituzumab govitecan (IMMU-132), an antibody-drug conjugate (ADC). Oncotarget 6, 22496–22512. [PubMed: 26101915]

Gomes NGM, Pereira DM, Valentao P, Andrade PB, 2018 Hybrid MS/NMR methods on the prioritization of natural products: applications in drug discovery. J. Pharm. Biomed. Anal 147, 234–249. [PubMed: 28826951]

Gonzalez-Vallinas M, Gonzalez-Castejon M, Rodriguez-Casado A, Ramirez de Molina A, 2013 Dietary phytochemicals in cancer prevention and therapy: a complementary approach with promising perspectives. Nutr. Rev 71, 585–599. [PubMed: 24032363]

Govindan SV, Cardillo TM, Rossi EA, Trisal P, McBride WJ, Sharkey RM, Goldenberg DM, 2015 Improving the therapeutic index in cancer therapy by using antibody-drug conjugates designed with a moderately cytotoxic drug. Mol. Pharm 12, 1836–1847. [PubMed: 25402018]

Gray JE, Heist RS, Starodub AN, Camidge DR, Kio EA, Masters GA, et al., 2017 Therapy of small cell lung cancer (SCLC) with a topoisomerase-I-inhibiting antibody-drug conjugate (ADC) targeting trop-2, sacituzumab govitecan. Clin. Cancer Res 23, 5711–5719. [PubMed: 28679770]

Grobmyer SR, Iwakuma N, Sharma P, Moudgil BM, 2010 What is cancer nanotechnology? In: Grobmyer SR, Moudgil BM (Eds.), Cancer Nanotechnology, Methods and Protocols Humana Press, New York, pp. 1–10.

Grossman SA, Ye X, Peereboom D, Rosenfeld MR, Mikkelsen T, Supko JG, Desideri S, 2012 Phase I study of terameprocol in patients with recurrent high-grade glioma. Neuro Oncol 14, 511–517. [PubMed: 22323663]

Gupta SC, Sung B, Prasad S, Webb LJ, Aggarwal BB, 2013 Cancer drug discovery by repurposing: teaching new tricks to old dogs. Trends Pharmacol. Sci 34, 508–517. [PubMed: 23928289]

Gutierrez-Orozco F, Failla ML, 2013 Biological activities and bioavailability of mangosteen xanthones: a critical review of the current evidence. Nutrients 5, 3163–3183. [PubMed: 23945675]

Hafeez BB, Mustafa A, Fischer JW, Singh A, Zhong W, Shekhani MO, et al., 2014 α-Mangostin: a dietary antioxidant derived from the pericarp of *Garcinia mangostana* L. inhibits pancreatic tumor growth in xenograft mouse model. Antioxid. Redox Signaling 21, 682–699.

Halfter K, Hoffmann O, Ditsch N, Ahne M, Arnold F, Paepke S, Grab D, Bauerfeind I, Mayer B, 2016 Testing chemotherapy efficacy in HER2 negative breast cancer using patient-derived spheroids. J. Transl. Med 14, 112. [PubMed: 27142386]

Hamann PR, Hinman LM, Hollander I, Beyer CF, Lindh D, Holcomb R, et al., 2002 Gemtuzumab ozogamicin, a potent and selective anti-CD33 antibody-calicheamicin conjugate for treatment of acute myeloid leukemia. Bioconjug. Chem 13, 47–58. [PubMed: 11792178]

Hamel E, 1992 Natural products which interact with tubulin in the vinca domain: maytansine, rhizoxin, phomopsin A, dolastatins 10 and 15 and halichondrin B. Pharmacol. Ther 55, 31–51. [PubMed: 1287674]

Han A-R, Kim J-A, Lantvit DD, Kardono BS, Riswan S, Chai H-B, et al., 2009 Cytotoxic xanthone constituents of the stem bark of Garcinia mangostana (mangosteen). J. Nat. Prod 72, 2028–2031. [PubMed: 19839614]

Hanka LJ, Kuentzel SL, Martin DG, Wiley PF, Neil GL, 1978 Detection and assay of antitumor antibiotics. Recent Results Cancer Res 63, 69–76. [PubMed: 705015]

Haque MU, Ferdiousi N, Sajon SR, 2016 Anti-cancer agents derived from plant and dietary sources: a review. Int. J. Pharmacogn 3, 55–66.

Hartmann AC, Petras D, Quinn RA, Protsyuk I, Archer FI, Ransome E, et al., 2017 Meta-mass shift chemical profiling of metabolomes from coral reefs. Proc. Natl. Acad. Sci. U.S.A 114, 11685–11690. [PubMed: 29078340]

He X, Esteva FJ, Ensor J, Hortobagyi GN, Lee MH, Yeung SC, 2012 Metformin and thiazolidinediones are associated with improved breast cancer-specific survival of diabetic women with HER2+ breast cancer. Ann. Oncol 23, 1771–1780. [PubMed: 22112968]

Heath JR, Davis ME, 2008 Nanotechnology and cancer. Annu. Rev. Med 59, 251–265. [PubMed: 17937588]

Heist RS, Guarino MJ, Masters G, Purcell WT, Starodub AN, Horn L, et al., 2017 Therapy of advanced non-small-cell lung cancer with an SN-38-anti-trop-2 drug conjugate, sacituzumab govitecan. J. Clin. Oncol 35, 2790–2797. [PubMed: 28548889]

Henkin JM, Ren Y, Soejarto DD, Kinghorn AD, 2018 The search for anticancer agents from topical plants. In: Kinghorn AD, Falk H, Gibbons S, Kobayashi J, Asakawa Y, Liu J-K (Eds.), Progress in the Chemistry of Organic Natural Products Springer-International, Cham, Switzerland, pp. 1–94.

Hirschfeld T, 1980 The hy-phen-ated methods. Anal. Chem 52, 297A–312A.

Ho C-K, Huang Y-L, Chen C-C, 2002 Garcinone E, a xanthone derivative, has potent cytotoxic effect against hepatocellular carcinoma cell lines. Planta Med 68, 975–979. [PubMed: 12451486]

Hollingshead MG, Alley MC, Camalier RF, Abbott BJ, Mayo JG, Malspeis L, Grever MR, 1995 *In vivo* cultivation of tumor cells in hollow fibers. Life Sci 57, 131–141. [PubMed: 7603295]

Hood E, 2004 Nanotechnology: looking as we leap. Environ. Health Perspect 112, A740–749. [PubMed: 15345364]

Howe K, Clark MD, Torroja CF, Torrance J, Berthelot C, Muffato M, et al., 2013 The zebrafish reference genome sequence and its relationship to the human genome. Nature 496, 498–503. [PubMed: 23594743]

Hubbard JM, Grothey A, 2017 Napabucasin: an update on the first-in-class cancer stemness inhibitor. Drugs 77, 1091–1103. [PubMed: 28573435]

Hubert J, Nuzillard J-M, Renault J-H, 2017 Dereplication strategies in natural product research: how many tools and methodologies behind the same concept? Phytochem. Rev 16, 55–95.

Hwang BY, Su BN, Chai H-B, Mi Q, Kardono BS, Afriastini JJ, et al., 2004 Silvestrol and episilvestrol, potential anticancer rocaglate derivatives from *Aglaia* silvestris. J. Org. Chem 69, 3350–3358; ibid, 69, 6156. [PubMed: 15132542]

Imamura Y, Mukohara T, Shimono Y, Funakoshi Y, Chayahara N, Toyoda M, et al., 2015 Comparison of 2D- and 3D-culture models as drug-testing platforms in breast cancer. Oncol. Rep 33, 1837–1843. [PubMed: 25634491]

Ito T, Masubuchi M, 2014 Dereplication of microbial extracts and related analytical technologies. J. Antibiot 67, 353–360. [PubMed: 24569671]

Itokawa H, Hitotsuyanagi Y, Lee K-H, 2012 Homoharringtonine and related compounds. In: Cragg GM, Kingston DG, Newman DJ (Eds.), Anticancer Agents from Natural Products CRC Press, Boca Raton, Florida, pp. 65–94.

Iyer U, Kadambi VJ, 2011 Antibody drug conjugates-trojan horses in the war on cancer. J. Pharmacol. Toxicol. Methods 64, 207–212. [PubMed: 21843648]

Jabr-Milane LS, van Vlerken LE, Yadav S, Amiji MM, 2008 Multi-functional nanocarriers to overcome tumor drug resistance. Cancer Treat. Rev 34, 592–602. [PubMed: 18538481]

Jaroszewski JW, 2005a Hyphenated NMR methods in natural products research, Part 1: Direct hyphenation. Planta Med 71, 691–700. [PubMed: 16142630]

Jaroszewski JW, 2005b Hyphenated NMR methods in natural products research, Part 2: HPLC-SPE-NMR and other new trends in NMR hyphenation. Planta Med 71, 795–802. [PubMed: 16206031]

Jia QI, 2003 Generating and screening a natural product library for cyclooxygenase and lipoxygenase dual inhibitors. In: Atta-ur-Rahman (Ed.) Studies in Natural Products Chemistry Elsevier B.V., Amsterdam, pp. 643–718.

Johansen KT, Wubshet SG, Nyberg NT, Jaroszewski JW, 2011 From retrospective assessment to prospective decisions in natural product isolation: HPLC-SPE-NMR analysis of *Carthamus* oxyacantha. J. Nat. Prod 74, 2454–2461. [PubMed: 22060189]

Johansen KT, Wubshet SG, Nyberg NT, 2013 HPLC-NMR revisited: using time-slice high-performance liquid chromatography-solid-phase extraction-nuclear magnetic resonance with database-assisted dereplication. Anal. Chem 85, 3183–3189. [PubMed: 23432092]

Johnson JJ, Petiwala SM, Syed DN, Rasmussen JT, Adhami VM, Siddiqui IA, Kohl AM, Mukhtar H, 2012 α-Mangostin, a xanthone from mangosteen fruit, promotes cell cycle arrest in prostate cancer and decreases xenograft tumor growth. Carcinogenesis 33, 413–419. [PubMed: 22159229]

Jordan MA, Wilson L, 2004 Microtubules as a target for anticancer drugs. Nat. Rev. Cancer 4, 253–265. [PubMed: 15057285]

Jorgenson JW, 2010 Capillary liquid chromatography at ultrahigh pressures. Annu. Rev. Anal. Chem 3, 129–150.

Jung C, Hong JY, Bae SY, Kang SS, Park HJ, Lee SK, 2015 Antitumor activity of americanin A isolated from the seeds of *Phytolacca americana* by regulating the ATM/ATR signaling pathway and the skp2-p27 axis in human colon cancer cells. J. Nat. Prod 78, 2983–2993. [PubMed: 26595875]

Jung H-A, Su B-N, Keller WJ, Mehta RG, Kinghorn AD, 2006 Antioxidant xanthones from the pericarp of Garcinia mangostana (mangosteen). J. Agric. Food Chem 54, 2077–2082. [PubMed: 16536578]

Kabanov AV, Batrakova EV, Alakhov VY, 2002 Pluronic block copolymers as novel polymer therapeutics for drug and gene delivery. J. Control. Release 82, 189–212. [PubMed: 12175737]

Kantarjian HM, DeAngelo DJ, Stelljes M, Martinelli G, Liedtke M, Stock W, et al., 2016 Inotuzumab ozogamicin versus standard therapy for acute lymphoblastic leukemia. N. Engl. J. Med 375, 740–753. [PubMed: 27292104]

Kapałczyńska M, Kolenda T, Przybyła W, Zajączkowska M, Teresiak A, Filas V, et al., 2018 2D and 3D cell cultures – a comparison of different types of cancer cell cultures. Arch. Med. Sci 14, 910–919. [PubMed: 30002710]

Katz L, Baltz RH, 2016 Natural product discovery: past, present, and future. J. Ind. Microbiol. Biotechnol 43, 155–176. [PubMed: 26739136]

Kesting JR, Olsen L, Staerk D, Tejesvi MV, Kini KR, Prakash HS, Jaroszewski JW, 2011 Production of unusual dispiro metabolites in *Pestalotiopsis virgatula* endophyte cultures: HPLC-SPE-NMR, electronic circular dichroism, and time-dependent density-functional computation study. J. Nat. Prod 74, 2206–2215. [PubMed: 21942847]

Khazir J, Mir BA, Pilcher L, Riley DL, 2014 Role of plants in anticancer drug discovery. Phytochem. Lett 7, 173–181.

Khonkarn R, Mankhetkorn S, Talelli M, Hennink WE, Okonogi S, 2012 Cytostatic effect of xanthone-loaded mPEG-b-p(HPMAm-Lac2) micelles towards doxorubicin sensitive and resistant cancer cells. Colloids Surf. B 94, 266–273.

Kinghorn AD, Chin YW, Swanson SM, 2009 Discovery of natural product anticancer agents from biodiverse organisms. Curr. Opin. Drug Discov. Devel 12, 189–196.

Kinghorn AD, Chai HB, Sung CK, Keller WJ, 2011a The classical drug discovery approach to defining bioactive constituents of botanicals. Fitoterapia 82, 71–79. [PubMed: 20804827]

Kinghorn AD, Pan L, Fletcher JN, Chai H, 2011b The relevance of higher plants in lead compound discovery programs. J. Nat. Prod 74, 1539–1555. [PubMed: 21650152]

Kinghorn AD, Carcache de Blanco E.J., Lucas DM, Rakotondraibe HL, Orjala J, Soejarto DD, et al., 2016 Discovery of anticancer agents of diverse natural origin. Anticancer Res 36, 5623–5637. [PubMed: 27793884]

Kingston DG, 2011 Modern natural products drug discovery and its relevance to biodiversity conservation. J. Nat. Prod 74, 496–511. [PubMed: 21138324]

Kirpotin DB, Drummond DC, Shao Y, Shalaby MR, Hong K, Nielsen UB, Marks JD, Benz CC, Park JW, 2006 Antibody targeting of long-circulating lipidic nanoparticles does not increase tumor localization but does increase internalization in animal models. Cancer Res 66, 6732–6740. [PubMed: 16818648]

Kleigrewe K, Almaliti J, Tian IY, Kinnel RB, Korobeynikov A, Monroe EA, et al., 2015 Combining mass spectrometric metabolic profiling with genomic analysis: a powerful approach for discovering natural products from cyanobacteria. J. Nat. Prod 78, 1671–1682. [PubMed: 26149623]

Klein-Junior LC, Cretton S, Allard PM, Genta-Jouve G, Passos CS, Salton J, et al., 2017 Targeted isolation of monoterpene indole alkaloids from Palicourea sessilis. J. Nat. Prod 80, 3032–3037. [PubMed: 29120642]

Ko AH, LoConte N, Tempero MA, Walker EJ, Kate Kelley R., Lewis S, et al., 2016 A phase I study of FOLFIRINOX plus IPI-926, a hedgehog pathway inhibitor, for advanced pancreatic adenocarcinoma. Pancreas 45, 370–375. [PubMed: 26390428]

Kong G, Braun RD, Dewhirst MW, 2000 Hyperthermia enables tumor-specific nanoparticle delivery: effect of particle size. Cancer Res 60, 4440–4445. [PubMed: 10969790]

Kongstad KT, Wubshet SG, Johannesen A, Kjellerup L, Winther AM, Jager AK, Staerk D, 2014 High-resolution screening combined with HPLC-HRMS-SPE-NMR for identification of fungal plasma membrane H^+-ATPase inhibitors from plants. J. Agric. Food Chem 62, 5595–5602. [PubMed: 24830509]

Kordes S, Pollak MN, Zwinderman AH, Mathôt RA, Weterman MJ, Beeker A, Punt CJ, Richel DJ, Wilmink JW, 2015 Metformin in patients with advanced pancreatic cancer: a double-blind, randomised, placebo-controlled phase 2 trial. Lancet Oncol 16, 839–847. [PubMed: 26067687]

Korfmacher WA, 2005 Principles and applications of LC-MS in new drug discovery. Drug Discov. Today 10, 1357–1367. [PubMed: 16253874]

Kotecha R, Takami A, Espinoza JL, 2016 Dietary phytochemicals and cancer chemoprevention: a review of the clinical evidence. Oncotarget 7, 52517–52529. [PubMed: 27232756]

Kuligowski J, Quintás G, Garrigues S, Lendl B, de la Guardia M, 2010 Recent advances in on-line liquid chromatography - infrared spectrometry (LC-IR). Trends Anal. Chem 29, 544–552.

Kupchan SM, Komoda Y, Court WA, Thomas GJ, Smith RM, Karim A, Gilmore CJ, Haltiwanger RC, Bryan RF, 1972 Tumor inhibitors. LXXIII. Maytansine, a novel antileukemic ansa macrolide from Maytenus ovatus. J. Am. Chem. Soc 94, 1354–1356. [PubMed: 5062169]

Kurita KL, Linington RG, 2015 Connecting phenotype and chemotype: high-content discovery strategies for natural products research. J. Nat. Prod 78, 587–596. [PubMed: 25728167]

Kusari S, Lamshoft M, Kusari P, Gottfried S, Zuhlke S, Louven K, Hentschel U, Kayser O, Spiteller M, 2014 Endophytes are hidden producers of maytansine in *Putterlickia* roots. J. Nat. Prod 77, 2577–2584. [PubMed: 25478947]

Kwon GS, Okano T, 1999 Soluble self-assembled block copolymers for drug delivery. Pharm. Res 16, 597–600. [PubMed: 10349998]

Lam KS, 2007 New aspects of natural products in drug discovery. Trends Microbiol 15, 279–289. [PubMed: 17433686]

Lambert JM, 2005 Drug-conjugated monoclonal antibodies for the treatment of cancer. Curr. Opin. Pharmacol 5, 543–549. [PubMed: 16087399]

Lambert JM, Chari RV, 2014 Ado-trastuzumab emtansine (T-DM1): an antibody-drug conjugate (ADC) for HER2-positive breast cancer. J. Med. Chem 57, 6949–6964. [PubMed: 24967516]

Lambert JM, Morris CQ, 2017 Antibody-drug conjugates (ADCs) for personalized treatment of solid tumors: a review. Adv. Ther 34, 1015–1035. [PubMed: 28361465]

Lammers T, Hennink WE, Storm G, 2008 Tumour-targeted nanomedicines: principles and practice. Br. J. Cancer 99, 392–397. [PubMed: 18648371]

Leal M, Sapra P, Hurvitz SA, Senter P, Wahl A, Schutten M, Shah DK, Haddish-Berhane N, Kabbarah O, 2014 Antibody-drug conjugates: an emerging modality for the treatment of cancer. Ann. N.Y. Acad. Sci 1321, 41–54. [PubMed: 25123209]

Lebwohl M, Swanson N, Anderson LL, Melgaard A, Xu Z, Berman B, 2012 Ingenol mebutate gel for actinic keratosis. N. Engl. J. Med 366, 1010–1019. [PubMed: 22417254]

Lee K-H, Xiao Z, 2012 Podophyllotoxins and analogs. In: Cragg GM, Kingston DG, Newman DJ (Eds.), Anticancer Agents from Natural Products CRC Press, Boca Raton, Florida, pp. 95–122.

Lee MD, Dunne TS, Siegel MM, Chang CC, Morton GO, Borders DB, 1987a Calichemicins, a novel family of antitumor antibiotics. 1. Chemistry and partial structure of calichemicin γ1I. J. Am. Chem. Soc 109, 3464–3466.

Lee MD, Dunne TS, Chang CC, Ellestad GA, Siegel MM, Morton GO, McGahren WJ, Borders DB, 1987b Calichemicins, a novel family of antitumor antibiotics. 2. Chemistry and structure of calichemicin γ1I. J. Am. Chem. Soc 109, 3466–3468.

Li JW, Vederas JC, 2009 Drug discovery and natural products: end of an era or an endless frontier? Science 325, 161–165. [PubMed: 19589993]

Li W, Yalcin M, Lin Q, Ardawi M-SM, Mousa SA, 2017 Self-assembly of green tea catechin derivatives in nanoparticles for oral lycopene delivery. J. Control. Release 248, 117–124. [PubMed: 28077264]

Li Y, Rogoff HA, Keates S, Gao Y, Murikipudi S, Mikule K, et al., 2015 Suppression of cancer relapse and metastasis by inhibiting cancer stemness. Proc. Natl. Acad. Sci. U.S.A 112, 1839–1844. [PubMed: 25605917]

Liaw CC, Chen PC, Shih CJ, Tseng SP, Lai YM, Hsu CH, Dorrestein PC, Yang YL, 2015 Vitroprocines, new antibiotics against *Acinetobacter baumannii*, discovered from marine *Vibrio* sp. QWI-06 using mass-spectrometry-based metabolomics approach. Sci. Rep 5, 12856. [PubMed: 26238555]

Lima RCL, Gramsbergen SM, van Staden J, Jager AK, Kongstad KT, Staerk D, 2017 Advancing HPLC-PDA-HRMS-SPE-NMR analysis of coumarins in *Coleonema album* by use of orthogonal reversed-phase C_{18} and pentafluorophenyl separations. J. Nat. Prod 80, 1020–1027. [PubMed: 28248501]

Lin S-Y, Kawashima Y, 1987 Pluronic surfactants affecting diazepam solubility, compatibility, and adsorption from i.v. admixture solutions. PDA J. Pharm. Sci. Technol 41, 83–87.

Liu C, Tadayoni BM, Bourret LA, Mattocks KM, Derr SM, Widdison WC, et al., 1996 Eradication of large colon tumor xenografts by targeted delivery of maytansinoids. Proc. Natl. Acad. Sci. U.S.A 93, 8618–8623. [PubMed: 8710920]

Liu G, Bode A, Ma W-Y, Sang S, Ho C-T, Dong Z, 2001 Two novel glycosides from the fruits of Morinda citrifolia (noni) inhibit AP-1 transactivation and cell transformation in the mouse epidermal JB6 cell line. Cancer Res 61, 5749–5756. [PubMed: 11479211]

Liu J-M, Haroun-Bouhedja F, Boisson-Vidal C, 2000 Analysis of the *in vitro* inhibition of mammant adenocarcinoma cell adhesion by sulphated polysaccharides. Anticancer Res 20, 3265–3272. [PubMed: 11062752]

Liu Y, Staerk D, Nielsen MN, Nyberg N, Jager AK, 2015 High-resolution hyaluronidase inhibition profiling combined with HPLC-HRMS-SPE-NMR for identification of anti-necrosis constituents in Chinese plants used to treat snakebite. Phytochemistry 119, 62–69. [PubMed: 26386983]

Lu J-M, Nurko J, Weakely SM, Jiang J, Kougias P, Lin PH, Yao Q, Chen C, 2010 Molecular mechanisms and clinical applications of norhydroguaiaretic acid (NDGA) and its derivative: an update. Med. Sci. Monit 16, RA93–RA100. [PubMed: 20424564]

Lucas DM, Edwards RB, Lozanski G, West DA, Shin JD, Vargo MA, et al., 2009 The novel plant-derived agent silvestrol has B-cell selective activity in chronic lymphocytic leukemia and acute lymphoblastic leukemia *in vitro* and *in vivo*. Blood 113, 4656–4666. [PubMed: 19190247]

Lucas DM, Still PC, Bueno-Perez L, Grever MR, Kinghorn AD, 2010 Potential of plant-derived natural products in the treatment of leukemia and lymphoma. Curr. Drug Targets 11, 812–822. [PubMed: 20370646]

Luo J, Chuang T, Chrung J, Quan J, Tsai J, Sullivan C, et al., 1998 Masoprocol (nordihydroguaiaretic acid): a new antihyperglycemic agent isolated from the creosote bush (Larrea tridentata). Eur. J. Pharmacol 346, 77–79. [PubMed: 9617755]

Mack F, Ritchie M, Sapra P, 2014 The next generation of antibody drug conjugates. Semin. Oncol 41, 637–652. [PubMed: 25440608]

Maderna A, Leverett CA, 2015 Recent advances in the development of new auristatins: structural modifications and application in antibody drug conjugates. Mol. Pharm 12, 1798–1812. [PubMed: 25697404]

Maeda H, Matsumura Y, 1989 Tumoritropic and lymphotropic principles of macromolecular drugs. Crit. Rev. Ther. Drug Carrier Syst 6, 193–210. [PubMed: 2692843]

Maeda H, Sawa T, Matsumura Y, Hori K, 2000 Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review. J. Control. Release 65, 271–284. [PubMed: 10699287]

Mandrekar N, Thakur NL, 2009 Significance of the zebrafish model in the discovery of bioactive molecules from nature. Biotechnol. Lett 31, 171–179. [PubMed: 18931972]

Mantaj J, Jackson PJ, Rahman KM, Thurston DE, 2017 From anthramycin to pyrrolobenzodiazepine (PBD)-containing antibody-drug conjugates (ADCs). Angew. Chem. Int. Ed. Engl 56, 462–488. [PubMed: 27862776]

Marshall AG, Hendrickson CL, 2008 High-resolution mass spectrometers. Annu. Rev. Anal. Chem 1, 579–599.

Mazumder A, Cerella C, Diederich M, 2018 Natural scaffolds in anticancer therapy and precision medicine. Biotechnol. Adv, 10.1016/j.biotechadv.2018.04.009.

McCarthy JR, Weissleder R, 2008 Multifunctional magnetic nanoparticles for targeted imaging and therapy. Adv. Drug Delivery Rev 60, 1241–1251.

McClatchey W, 2002 From Polynesian healers to health stores: Changing perspective of Morinda citrifolia (Rubiaceae). Integr. Cancer Ther 1, 110–120. [PubMed: 14664736]

Megoulas NC, Koupparis MA, 2005 Twenty years of evaporative light scattering detection. Crit. Rev. Anal. Chem 35, 301–316.

Mi Q, Lantvit DD, Reyes-Lim E, Chai H-B, Zhao W, Lee I-S, et al., 2002 Evaluation of the potential cancer chemotherapeutic efficacy of natural product isolates employing in vivo hollow fiber tests. J. Nat. Prod 65, 842–850. [PubMed: 12088425]

Mi Q, Pezzuto JM, Farnsworth NR, Wani MC, Kinghorn AD, Swanson SM, 2009 Use of the in vivo hollow fiber assay in natural products anticancer drug discovery. J. Nat. Prod 72, 573–580. [PubMed: 19161316]

Mian-Ying W, West BJ, Jensen CJ, Nowicki D, Chen S, Palu AK, Anderson G, 2002 Morinda citrifloia (noni): a literature review and recent advances in noni research. Acta Pharmacol. Sin 23, 1127–1141. [PubMed: 12466051]

Miola AC, Ferreira ER, Abbade LPF, Schmitt JV, Miot HA, 2018 Randomized clinical trial testing the efficacy and safety of 0.5% colchicine cream versus photodynamic therapy with methyl aminolevulinate in the treatment of skin field cancerization: study protocol. BMC Cancer 18, 340. [PubMed: 29587662]

Mitsui I, Kumazawa E, Hirota Y, Aonuma M, Sugimori M, Ohsuki S, et al., 1995 A new water-soluble camptothecin derivative, DX-8951f, exhibits potent antitumor activity against human tumors *in vitro* and in vivo. Jpn. J. Cancer Res 86, 776–782. [PubMed: 7559102]

Moghimi SM, Hunter AC, Murray JC, 2005 Nanomedicine: current status and future prospects. FASEB J 19, 311–330. [PubMed: 15746175]

Mohamed A, Nguyen CH, Mamitsuka H, 2016 Current status and prospects of computational resources for natural product dereplication: a review. Brief. Bioinform 17, 309–321. [PubMed: 26153512]

Mosmann T, 1983 Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods 65, 55–63. [PubMed: 6606682]

Muñoz-Acuña U, Shen Q, Ren Y, Lantvit DD, Wittwer JA, Kinghorn AD, Swanson SM, Carcache de Blanco E.J., 2013 Goyazensolide induces apoptosis in cancer cells *in vitro* and in vivo. Int. J. Cancer Res 9, 36–53. [PubMed: 25621077]

Naik RG, Kattige SL, Bhat SV, Alreja B, de Souza NJ, Rupp RH, 1988 An antiinflammatory cum immunomodulatory piperidinylbenzopyrnose from *Dysoxylum binectariferum*: isolation, structure and total synthesis. Tetrahedron 44, 2081–2086.

Naman CB, Rattan R, Nikoulina SE, Lee J, Miller BW, Moss NA, et al., 2017 Integrating molecular networking and biological assays to target the isolation of a cytotoxic cyclic octapeptide, samoamide A, from an American Samoan marine cyanobacterium. J. Nat. Prod 80, 625–633. [PubMed: 28055219]

Nath S, Devi GR, 2016 Three-dimensional culture systems in cancer research: focus on tumor spheroid model. Pharmacol. Ther 163, 94–108. [PubMed: 27063403]

National Institute of Health O.D.S., 1994 Dietary Supplement Health and Education Act (DSHEA) of 1994 https://ods.od.nih.gov/About/dshea_wording.aspx. (Accessed May 25, 2018).

Newman DJ, Cragg GM, Snader KM, 2000 The influence of natural products upon drug discovery. Nat. Prod. Rep 17, 215–234. [PubMed: 10888010]

Newman DJ, Cragg GM, 2016 Natural products as sources of new drugs from 1981 to 2014. J. Nat. Prod 79, 629–661. [PubMed: 26852623]

Ng JS, 2011 Vinflunine: review of a new vinca alkaloid and its potential role in oncology. J. Oncol. Pharm. Pract 17, 209–224. [PubMed: 20584744]

Nguyen DD, Wu C-H, Moree WJ, Lamsa A, Medema MH, Zhao X, et al., 2013 MS/MS networking guided analysis of molecule and gene cluster families. Proc. Natl. Acad. Sci. U.S.A 110, E2611–E2620. [PubMed: 23798442]

Nicolaou KC, Chen JS, Dalby SM, 2009 From nature to the laboratory and into the clinic. Bioorg. Med. Chem 17, 2290–2303. [PubMed: 19028103]

Nielsen KF, Mansson M, Rank C, Frisvad JC, Larsen TO, 2011 Dereplication of microbial natural products by LC-DAD-TOFMS. J. Nat. Prod 74, 2338–2348. [PubMed: 22026385]

Norsworthy KJ, Ko CW, Lee JE, Liu J, John CS, Przepiorka D, Farrell AT, Pazdur R, 2018 FDA approval summary: Mylotarg for treatment of patients with relapsed or refractory CD33-positive acute myeloid leukemia. Oncologist 23, 1103–1108. [PubMed: 29650683]

Nothias L-F, Boutet-Mercey S, Cachet X, De La Torre E, Laboureur L, Gallard JF, et al., 2017 Environmentally friendly procedure based on supercritical fluid chromatography and tandem mass spectrometry molecular networking for the discovery of potent antiviral compounds from Euphorbia semiperfoliata. J. Nat. Prod 80, 2620–2629. [PubMed: 28925702]

Nothias L-F, Nothias-Esposito M, da Silva R, Wang M, Protsyuk I, Zhang Z, et al., 2018 Bioactivity-based molecular networking for the discovery of drug leads in natural product bioassay-guided fractionation. J. Nat. Prod 81, 758–767. [PubMed: 29498278]

Obolskiy D, Pischel I, Siriwatanametanon N, Heinrich M, 2009 *Garcinia mangostana* L.: a phytochemical and pharmacological review. Phytother. Res 23, 1047–1065. [PubMed: 19172667]

Ocean AJ, Starodub AN, Bardia A, Vahdat LT, Isakoff SJ, Guarino M, et al., 2017 Sacituzumab govitecan (IMMU-132), an anti-trop-2-SN-38 antibody-drug conjugate for the treatment of diverse epithelial cancers: safety and pharmacokinetics. Cancer 123, 3843–3854. [PubMed: 28558150]

Ogitani Y, Hagihara K, Oitate M, Naito H, Agatsuma T, 2016a Bystander killing effect of DS-8201a, a novel anti-human epidermal growth factor receptor 2 antibody-drug conjugate, in tumors with human epidermal growth factor receptor 2 heterogeneity. Cancer Sci 107, 1039–1046. [PubMed: 27166974]

Ogitani Y, Aida T, Hagihara K, Yamaguchi J, Ishii C, Harada N, et al., 2016b DS-8201a, a novel HER2-targeting ADC with a novel DNA topoisomerase I inhibitor, demonstrates a promising antitumor efficacy with differentiation from T-DM1. Clin. Cancer Res 22, 5097–5108. [PubMed: 27026201]

Okutan L, Kongstad KT, Jager AK, Staerk D, 2014 High-resolution α-amylase assay combined with high-performance liquid chromatography-solid-phase extraction-nuclear magnetic resonance spectroscopy for expedited identification of α-amylase inhibitors: proof of concept and α-amylase inhibitor in cinnamon. J. Agric. Food Chem 62, 11465–11471. [PubMed: 25368916]

Olivon F, Allard P-M, Koval A, Righi D, Genta-Jouve G, Neyts J, et al., 2017a Bioactive natural products prioritization using massive multi-informational molecular networks. ACS Chem. Biol 12, 2644–2651. [PubMed: 28829118]

Olivon F, Grelier G, Roussi F, Litaudon M, Touboul D, 2017b MZmine 2 data-preprocessing to enhance molecular networking reliability. Anal. Chem 89, 7836–7840. [PubMed: 28644610]

Olivon F, Roussi F, Litaudon M, Touboul D, 2017c Optimized experimental workflow for tandem mass spectrometry molecular networking in metabolomics. Anal. Bioanal. Chem 409, 5767–5778. [PubMed: 28762069]

Ortholand JY, Ganesan A, 2004 Natural products and combinatorial chemistry: back to the future. Curr. Opin. Chem. Biol 8, 271–280. [PubMed: 15183325]

Ovalle-Magallanes B, Eugenio-Perez D, Pedraza-Chaverri J, 2017 Medicinal properties of mangosteen (Garcinia mangostana L.): a comprehensive update. Food Chem. Toxicol 109, 102–122. [PubMed: 28842267]

Pan L, Carcache de Blanco E.J., Kinghorn AD, 2009 Plant-derived natural products as leads for drug discovery. In: Osbourn AE, Lanzotti V (Eds.), Plant-derived Natural Products: Synthesis, Function, and Application Springer, New York, pp. 547–567.

Pan L, Kardono LBS, Riswan S, Chai H-B, Carcache de Blanco E.J., Pannell CM, et al., 2010 Isolation and characterization of minor analogues of silvestrol and other constituents from a large-scale re-collection of Aglaia foveolata. J. Nat. Prod 73, 1873–1878. [PubMed: 20939540]

Pan L, Woodard JL, Lucas DM, Fuchs JR, Kinghorn AD, 2014 Rocaglamide, silvestrol and structurally related bioactive compounds from Aglaia species. Nat. Prod. Rep 31, 924–939. [PubMed: 24788392]

Pantziarka P, Bouche G, Meheus L, Sukhatme V, Sukhatme VP, Vikas P, 2014 The repurposing drugs in oncology (ReDO) project. Ecancermedicalscience 8, 442. [PubMed: 25075216]

Park JW, Benz CC, Martin FJ, 2004 Future directions of liposome- and immunoliposome-based cancer therapeutics. Semin. Oncol 31, 196–205. [PubMed: 15717745]

Pawlus AD, Su B-N, Keller WJ, Kinghorn AD, 2005 An anthraquinone with potent quinone reductase-inducing activity and other constituent of the fruits of Morinda citrifolia (noni). J. Nat. Prod 68, 1720–1722. [PubMed: 16378361]

Pedraza-Chaverri J, Cardenas-Rodriguez N, Orozco-Ibarra M, Perez-Rojas JM, 2008 Medicinal properties of mangosteen (Garcinia mangostana). Food Chem. Toxicol 46, 3227–3239. [PubMed: 18725264]

Pelaz B, Alexiou C, Alvarez-Puebla RA, Alves F, Andrews AM, Ashraf S, et al., 2017 Diverse applications of nanomedicine. ACS Nano 11, 2313–2381. [PubMed: 28290206]

Perez-Victoria I, Martin J, Reyes F, 2016 Combined LC/UV/MS and NMR strategies for the dereplication of marine natural products. Planta Med 82, 857–871. [PubMed: 27002401]

Peters C, Brown S, 2015 Antibody-drug conjugates as novel anti-cancer chemotherapeutics. Biosci. Rep 35, e00225. [PubMed: 26182432]

Pettit GR, Kamano Y, Herald CL, Tuinman AA, Boettner FE, Kizu H, et al., 1987 The isolation and structure of a remarkable marine animal antineoplastic constituent: dolastatin 10. J. Am. Chem. Soc 109, 6883–6885.

Pillow T, Polson AG, Zheng B Silvestrol antibody-drug conjugates and methods of use. U.S. Pat. Publ 20170348422A1, 2017.

Polachi N, Bai G, Li T, Chu Y, Wang X, Li S, et al., 2016 Modulatory effects of silibinin in various cell signaling pathways against liver disorders and cancer - a comprehensive review. Eur. J. Med. Chem 123, 577–595. [PubMed: 27517806]

Pope-Harman A, Cheng MM-C, Robertson F, Sakamoto J, Ferrari M, 2007 Biomedical nanotechnology for cancer. Med. Clin. North Am 91, 899–927. [PubMed: 17826110]

Portney NG, Ozkan M, 2006 Nano-oncology: drug delivery, imaging, and sensing. Anal. Bioanal. Chem 384, 620–630. [PubMed: 16440195]

Potterat O, Hamburger M, 2007 Morinda citrifolia (noni) fruit - phytochemistry, pharmacology, safety. Planta Med 73, 191–199. [PubMed: 17286240]

Powers KW, Brown SC, Krishna VB, Wasdo SC, Moudgil BM, Roberts SM, 2006 Research strategies for safety evaluation of nanomaterials. Part VI. Characterization of nanoscale particles for toxicological evaluation. Toxicol. Sci 90, 296–303. [PubMed: 16407094]

Priyadarsini RV, Nagini S, 2012 Cancer chemoprevention by dietary phytochemicals: promises and pitfalls. Curr. Pharm. Biotechnol 13, 125–136. [PubMed: 21466433]

Prompruk K, Govender T, Zhang S, Xiong CD, Stolnik S, 2005 Synthesis of a novel PEG-block-poly(aspartic acid-stat-phenylalanine) copolymer shows potential for formation of a micellar drug carrier. Int. J. Pharm 297, 242–253. [PubMed: 15907607]

Pye CR, Bertin MJ, Lokey RS, Gerwick WH, Linington RG, 2017 Retrospective analysis of natural products provides insights for future discovery trends. Proc. Natl. Acad. Sci. U.S.A 114, 5601–5606. [PubMed: 28461474]

Quinn RA, Nothias L-F, Vining O, Meehan M, Esquenazi E, Dorrestein PC, 2017 Molecular networking as a drug discovery, drug metabolism, and precision medicine strategy. Trends Pharmacol. Sci 38, 143–154. [PubMed: 27842887]

Rahier NJ, Thomas CJ, Hecht SM, 2012 Camptothecin and its analogs. In: Cragg GM, Kingston DG, Newman DJ (Eds.), Anticancer Agents form Natural Products CRC Press, Boca Raton, Florida, pp. 5–26.

Ramage CM, Sando L, Peace CP, Carroll BJ, Drew RA, 2004 Genetic diversity revealed in the apomictic fruit species Garcinia mangostana L. (mangosteen). Euphytica 136, 1–10.

Rao MM, Kingston DGI, 1982 Plant anticancer agents XII. Isolation and structure elucidation of new cytotoxic quinones from Tabebuia cassinoides. J. Nat. Prod 45, 600–604. [PubMed: 7153777]

Rautio J, Meanwell NA, Di L, Hageman MJ, 2018 The expanding role of prodrugs in contemporary drug design and development. Nat. Rev. Drug Discov. Article in press DOI: 10.1038/nrd.2018.46.

Reichert JM, Valge-Archer VE, 2007 Development trends for monoclonal antibody cancer therapeutics. Nat. Rev. Drug Discov 6, 349–356. [PubMed: 17431406]

Remy S, Olivon F, Desrat S, Blanchard F, Eparvier V, Leyssen P, et al., 2018 Structurally diverse diterpenoids from Sandwithia guyanensis. J. Nat. Prod 81, 901–912. [PubMed: 29493237]

Ren Y, Acuña UM, Jiménez F, García R, Mejía M, Chai H, et al., 2012 Cytotoxic and NF-κB inhibitory sesquiterpene lactones from Piptocoma rufescens. Tetrahedron 68, 2671–2678. [PubMed: 22685350]

Rida PC, LiVecche D, Ogden A, Zhou J, Aneja R, 2015 The noscapine chronicle: a pharmaco-historic biography of the opiate alkaloid family and its clinical applications. Med. Res. Rev 35, 1072–1096. [PubMed: 26179481]

Riedl A, Schlederer M, Pudelko K, Stadler M, Walter S, Unterleuthner D, et al., 2017 Comparison of cancer cells in 2D vs. 3D culture reveals differences in AKT–mTOR–S6K signaling and drug responses. J. Cell Sci 130, 203–218. [PubMed: 27663511]

Roy A, Jauhari N, Bharadvaja N, 2018 Medicinal plants as a potential source of chemopreventive agents. In: Akhtar MS, Swamy MK (Eds.), Anticancer Plants: Natural Products and Biotechnological Implements Springer Nature, Singapore, pp. 109–140.

Rubinstein AL, 2006 Zebrafish assays for drug toxicity screening. Expert Opin. Drug Metab. Toxicol 2, 231–240. [PubMed: 16866609]

Sahoo SK, Ma W, Labhasetwar V, 2004 Efficacy of transferrin-conjugated paclitaxel-loaded nanoparticles in a murine model of prostate cancer. Int. J. Cancer 112, 335–340. [PubMed: 15352049]

Santra S, Liesenfeld B, Dutta D, Chatel D, Batich CD, Tan W, Moudgil BM, Mericle RA, 2005 Folate conjugated fluorescent silica nanoparticles for labeling neoplastic cells. J. Nanosci. Nanotechnol 5, 899–904. [PubMed: 16060150]

Sarker SD, Nahar L, 2012 Hyphenated techniques and their applications in natural products analysis. In: Sarker SD, Nahar L (Eds.), Natural Products Isolation Humana Press, Totowa, NJ, pp. 301–340.

Sawant RR, Torchilin VP, 2010 Polymeric micelles: polyethylene glycol-phosphatidylethanolamine (PEG-PE)-based micelles as an example. In: Grobmyer SR, Moudgil BM (Eds.), Cancer Nanotechnology, Methods and Protocols Humana Press, New York, pp. 131–150.

Schorn M, Zettler J, Noel JP, Dorrestein PC, Moore BS, Kaysser L, 2014 Genetic basis for the biosynthesis of the pharmaceutically important class of epoxyketone proteasome inhibitors. ACS Chem. Biol 9, 301–309. [PubMed: 24168704]

Scolamiero G, Pazzini C, Bonafe F, Guarnieri C, Muscari C, 2018 Effects of α-mangostin on viability, growth and cohesion of multicellular spheroids derived from human breast cancer cell lines. Int. J. Med. Sci 15, 23–30. [PubMed: 29333084]

Seger C, Sturm S, Stuppner H, 2013 Mass spectrometry and NMR spectroscopy: modern high-end detectors for high resolution separation techniques - state of the art in natural product HPLC-MS, HPLC-NMR, and CE-MS hyphenations. Nat. Prod. Rep 30, 970–987. [PubMed: 23739842]

Sengupta S, Eavarone D, Capila I, Zhao G, Watson N, Kiziltepe T, Sasisekharan R, 2005 Temporal targeting of tumour cells and neovasculature with a nanoscale delivery system. Nature 436, 568–572. [PubMed: 16049491]

Senter PD, Sievers EL, 2012 The discovery and development of brentuximab vedotin for use in relapsed Hodgkin lymphoma and systemic anaplastic large cell lymphoma. Nat. Biotechnol 30, 631–637. [PubMed: 22781692]

Seo E-K, Kim N-C, Mi Q, Chai H, Wall ME, Wani MC, et al., 2001 Macharistol, a new cytotoxic cinnamylphenol from the stems of Machaerium aristulatum. J. Nat. Prod 64, 1483–1485. [PubMed: 11720542]

Sharkey RM, McBride WJ, Cardillo TM, Govindan SV, Wang Y, Rossi EA, Chang CH, Goldenberg DM, 2015 Enhanced delivery of SN-38 to human tumor xenografts with an anti-trop-2-SN-38 antibody conjugate (sacituzumab govitecan). Clin. Cancer Res 21, 5131–5138. [PubMed: 26106073]

Sharkey RM, Govindan SV, Cardillo TM, Donnell J, Xia J, Rossi EA, Chang CH, Goldenberg DM, 2018 Selective and concentrated accretion of SN-38 with a CEACAM5-targeting antibody-drug conjugate (ADC), labetuzumab govitecan (IMMU-130). Mol. Cancer Ther 17, 196–203. [PubMed: 29079710]

Shi S-Y, Zhang Y-P, Jiang X-Y, Chen X-Q, Huang K-L, Zhou H-H, Jiang X-Y, 2009 Coupling HPLC to on-line, post-column (bio)chemical assays for high-resolution screening of bioactive compounds from complex mixtures. Trends Anal. Chem 28, 865–877.

Shi Y, Pan C, Wang K, Chen X, Wu X, Chen CA, Wu B, 2017 Synthetic multispecies microbial communities reveals shifts in secondary metabolism and facilitates cryptic natural product discovery. Environ. Microbiol 19, 3606–3618. [PubMed: 28714207]

Shibata M-A, Matoba Y, Tosa H, Iinuma M, 2013 Effects of mangosteen pericarp extracts against mammary cancer. Alt. Int. Med 2, 139.

Siegel RL, Miller KD, Jemal A, 2018 Cancer statistics, 2018. CA Cancer J. Clin 68, 7–30. [PubMed: 29313949]

Sievers EL, Senter PD, 2013 Antibody-drug conjugates in cancer therapy. Annu. Rev. Med 64, 15–29. [PubMed: 23043493]

Singh S, Sharma B, Kanwar SS, Kumar A, 2016 Lead phytochemicals for anticancer drug development. Front. Plant Sci 7, 1667. [PubMed: 27877185]

Singh SK, Lillard JW, Singh R, 2018 Reversal of drug resistance by planetary ball milled (PBM) nanoparticle loaded with resveratrol and docetaxel in prostate cancer. Cancer Lett 427, 49–62. [PubMed: 29678549]

Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, et al., 2001 Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N. Engl. J. Med 344, 783–792. [PubMed: 11248153]

Sleire L, Forde HE, Netland IA, Leiss L, Skeie BS, Enger PO, 2017 Drug repurposing in cancer. Pharmacol. Res 124, 74–91. [PubMed: 28712971]

Smith AL, Nicolaou KC, 1996 The enediyne antibiotics. J. Med. Chem 39, 2103–2117. [PubMed: 8667354]

Smith BR, Cheng Z, De A, Koh AL, Sinclair R, Gambhir SS, 2008 Real-time intravital imaging of RGD-quantum dot binding to luminal endothelium in mouse tumor neovasculature. Nano Lett 8, 2599–2606. [PubMed: 18386933]

Smith T, Kawa K, Eckl V, Morton C, Stredney R, 2017 Herbal supplement sales in U.S. increase 7.7% in 2016. Consumer preferences shifting toward ingredients with general wellness benefits, driving growth of adaptogens and digestive health products. Herbalgram 115, 56–65.

Smolewski P, 2008 Terameprocol, a novel site-specific transription inhibitor with anticancer activity. IDrugs 11, 204–214. [PubMed: 18311658]

Sonbol MB, Bekaii-Saab T, 2018 A clinical trial protocol paper discussing the BRIGHTER study. Future Oncol 14, 901–906. [PubMed: 29297698]

Spectrum Pharmaceuticals Inc., 2017 Marqibo (vincristine sulpfate liposome injection) http://www.marqibo.com/. (Accessed May 30, 2018).

Sporn MB, Liby KT, 2005 Cancer chemoprevention: scientific promise, clinical uncertainty. Nat. Clin. Pract. Oncol 2, 518–525. [PubMed: 16205771]

Staerk D, Kesting JR, Sairafianpour M, Witt M, Asili J, Emami SA, Jaroszewski JW, 2009 Accelerated dereplication of crude extracts using HPLC-PDA-MS-SPE-NMR: quinolinone alkaloids of Haplophyllum acutifolium. Phytochemistry 70, 1055–1061. [PubMed: 19540540]

Starodub AN, Ocean AJ, Shah MA, Guarino MJ, Picozzi VJ Jr., Vahdat LT, et al., 2015 First-in-human trial of a novel anti-trop-2 antibody-SN-38 conjugate, sacituzumab govitecan, for the treatment of diverse metastatic solid tumors. Clin. Cancer Res 21, 3870–3878. [PubMed: 25944802]

Stock K, Estrada MF, Vidic S, Gjerde K, Rudisch A, Santo VE, et al., 2016 Capturing tumor complexity *in vitro*: comparative analysis of 2D and 3D tumor models for drug discovery. Sci. Rep 6, 28951. [PubMed: 27364600]

Su BN, Pawlus AD, Jung HA, Keller WJ, McLaughlin JL, Kinghorn AD, 2005 Chemical constituents of the fruits of Morinda citrifolia (noni) and their antioxidant activity. J. Nat. Prod 68, 592–595. [PubMed: 15844957]

Suffness M, Douros JD, 1981 Discovery of antitumor agents from natural sources. Trends Pharmacol. Sci 2, 307–310.

Takegawa N, Nonagase Y, Yonesaka K, Sakai K, Maenishi O, Ogitani Y, et al., 2017 DS-8201a, a new HER2-targeting antibody-drug conjugate incorporating a novel DNA topoisomerase I inhibitor, overcomes HER2-positive gastric cancer T-DM1 resistance. Int. J. Cancer 141, 1682–1689. [PubMed: 28677116]

Teicher BA, Chari RV, 2011 Antibody conjugate therapeutics: challenges and potential. Clin. Cancer Res 17, 6389–6397. [PubMed: 22003066]

Thomas I, Gregg B, 2017 Metformin; a review of its history and future: from lilac to longevity. Pediatr. Diabetes 18, 10–16. [PubMed: 28052534]

Tsuchikama K, An Z, 2018 Antibody-drug conjugates: recent advances in conjugation and linker chemistries. Protein Cell 9, 33–46. [PubMed: 27743348]

U.S. Food & Drug Administration, 2015 FDA approved drug products https://www.accessdata.fda.gov/scripts/cder/daf/index.cfm?event=overview.process&ApplNo=207793. (Accessed July 7, 2018).

U.S. Food & Drug Administration, 2016 FDA grants orphan drug status for napabucasin. Oncology Times 38, 25.

U.S. Food & Drug Administration, 2017 FDA approves inotuzumab ozogamicin for relapsed or refractory B-cell precursor ALL https://www.fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/ucm572133.htm. (Accessed June 6, 2018).

U.S. National Library of Medicine, 2018a 63 studies found for flavopiridol https://www.clinicaltrials.gov/ct2/results?cond=&term=flavopiridol&cntry=&state=&city=&dist=. (Accessed June 10, 2018).

U.S. National Library of Medicine, 2018b 75 studies found for podophyllotoxin https://clinicaltrials.gov/ct2/results?cond=&term=podophyllotoxin&cntry=&state=&city=&dist=&Search=Search&recrs=a&recrs=b&recrs=d&recrs=e. (Accessed June 10, 2018).

U.S. National Library of Medicine, 2018c A study of DSP-0337 in patients with advanced solid tumors to determine the safety and the pharmacokinetic profile https://clinicaltrials.gov/ct2/show/NCT03416816?term=DSP-0337&rank=1. (Accessed June 25, 2018).

Vallet M, Vanbellingen QP, Fu T, Le Caer J-P, Della-Negra S, Touboul D, et al., 2017 An integrative approach to decipher the chemical antagonism between the competing endophytes Paraconiothyrium variabile and Bacillus subtilis. J. Nat. Prod 80, 2863–2873. [PubMed: 29139291]

van Middlesworth F, Cannell RJP, 1998 Dereplication and partial identification of natural products. In: Cannell RJP (Ed.) Natural Products Isolation Humana Press, Totowa, NJ, pp. 279–327.

Verma S, Miles D, Gianni L, Krop IE, Welslau M, Baselga J, et al., 2012 Trastuzumab emtansine for HER2-positive advanced breast cancer. N. Engl. J. Med 367, 1783–1791. [PubMed: 23020162]

von Hagens C, Walter-Sack I, Goeckenjan M, Osburg J, Storch-Hagenlocher B, Sertel S, et al., 2017 Prospective open uncontrolled phase I study to define a well-tolerated dose of oral artesunate as add-on therapy in patients with metastatic breast cancer (ARTIC M33/2). Breast Cancer Res. Treat 164, 359–369. [PubMed: 28439738]

Wang H, Khor TO, Shu L, Su Z-Y, Fuentes F, Lee J-H, Kong A-NT, 2012 Plants vs. cancer: a review on natural phytochemicals in preventing and treating cancers and their druggability. Anticancer Agents Med. Chem 12, 1281–1305. [PubMed: 22583408]

Wang M-H, Zhang K-J, Gu Q-L, Bi X-L, Wang J-X, 2017 Pharmacology of mangostins and their derivatives: a comprehensive review. Chin. J. Nat. Med 15, 81–93. [PubMed: 28284429]

Wang M, Carver JJ, Phelan VV, Sanchez LM, Garg N, Peng Y, et al., 2016 Sharing and community curation of mass spectrometry data with global natural products social molecular networking. Nat. Biotechnol 34, 828–837. [PubMed: 27504778]

Watrous J, Roach P, Alexandrov T, Heath BS, Yang JY, Kersten RD, et al., 2012 Mass spectral molecular networking of living microbial colonies. Proc. Natl. Acad. Sci. U.S.A 109, E1743–E1752. [PubMed: 22586093]

Widdison WC, Wilhelm SD, Cavanagh EE, Whiteman KR, Leece BA, Kovtun Y, et al., 2006 Semisynthetic maytansine analogues for the targeted treatment of cancer. J. Med. Chem 49, 4392–4408. [PubMed: 16821799]

Wiernik PH, 2016 Alvocidib (flavopiridol) for the treatment of chronic lymphocytic leukemia. Expert Opin. Investig. Drugs 25, 729–734.

Wilczewska AZ, Niemirowicz K, Markiewicz KH, Car H, 2012 Nanoparticles as drug delivery systems. Pharmacol. Rep 64, 1020–1037. [PubMed: 23238461]

Wilson ID, Brinkman UAT, 2007 Hype and hypernation: multiple hyphenation of column liquid chromatography and spectroscopy. Trends Anal. Chem 26, 847–854.

Winer ES, DeAngelo DJ, 2018 A review of omacetaxine: a chronic myeloid leukemia treatment resurrected. Oncol. Ther 6, 9–20.

Wolfender J-L, Queiroz EF, Hostettmann K, 2006 The importance of hyphenated techniques in the discovery of new lead compounds from nature. Expert Opin. Drug Discov 1, 237–260. [PubMed: 23495845]

Wolfender J-L, Marti G, Thomas A, Bertrand S, 2015 Current approaches and challenges for the metabolite profiling of complex natural extracts. J. Chromatogr. A 1382, 136–164. [PubMed: 25464997]

Wu C, Medema MH, Lakamp RM, Zhang L, Dorrestein PC, Choi YH, van Wezel GP, 2016 Leucanicidin and endophenasides result from methyl-rhamnosylation by the same tailoring enzymes in Kitasatospora sp. MBT66. ACS Chem. Biol 11, 478–490. [PubMed: 26675041]

Wubshet SG, Nyberg NT, Tejesvi MV, Pirttila AM, Kajula M, Mattila S, Staerk D, 2013 Targeting high-performance liquid chromatography-high-resolution mass spectrometry-solid-phase extraction-nuclear magnetic resonance analysis with high-resolution radical scavenging profiles - bioactive secondary metabolites from the endophytic fungus Penicillium namyslowskii. J. Chromatogr. A 1302, 34–39. [PubMed: 23827469]

Wubshet SG, Brighente IM, Moaddel R, Staerk D, 2015 Magnetic ligand fishing as a targeting tool for HPLC-HRMS-SPE-NMR: α-glucosidase inhibitory ligands and alkylresorcinol glycosides from Eugenia catharinae. J. Nat. Prod 78, 2657–2665. [PubMed: 26496505]

Wubshet SG, Tahtah Y, Heskes AM, Kongstad KT, Pateraki I, Hamberger B, Moller BL, Staerk D, 2016 Identification of PTP1B and α-glucosidase inhibitory serrulatanes from *Eremophila* spp. by combined use of dual high-resolution PTP1B and α-glucosidase inhibition profiling and HPLC-HRMS-SPE-NMR. J. Nat. Prod 79, 1063–1072. [PubMed: 26960032]

Wurth R, Thellung S, Bajetto A, Mazzanti M, Florio T, Barbieri F, 2016 Drug-repositioning opportunities for cancer therapy: novel molecular targets for known compounds. Drug Discov Today 21, 190–199. [PubMed: 26456577]

Xu WK, Jiang H, Yang K, Wang YQ, Zhang Q, Zuo J, 2017 Development and *in vivo* evaluation of self-microemulsion as delivery system for α-mangostin. Kaohsiung J. Med. Sci 33, 116–123. [PubMed: 28254113]

Yang JY, Sanchez LM, Rath CM, Liu X, Boudreau PD, Bruns N, et al., 2013 Molecular networking as a dereplication strategy. J. Nat. Prod 76, 1686–1699. [PubMed: 24025162]

Yip D, Cho CH, 2013 A multicellular 3D heterospheroid model of liver tumor and stromal cells in collagen gel for anti-cancer drug testing. Biochem. Biophys. Res. Commun 433, 327–332. [PubMed: 23501105]

Yokoyama M, Miyauchi M, Yamada N, Okano T, Sakurai Y, Kataoka K, Inoue S, 1990 Characterization and anticancer activity of the micelle-forming polymeric anticancer drug adriamycin-conjugated poly(ethylene glycol)-poly(aspartic acid) block copolymer. Cancer Res 50, 1693–1700. [PubMed: 2306723]

Young C, Schluep T, Hwang J, Eliasof S, 2011 CRLX101 (formerly IT-101)-A novel nanopharmaceutical of camptothecin in clinical development. Curr. Bioact. Compd 7, 8–14. [PubMed: 22081768]

Yu T-W, Floss HG, Cragg GM, Newman DJ, 2012 Ansamitocins (maytansinoids). In: Cragg GM, Kingston DG, Newman DJ (Eds.), Anticancer Agents from Natural Products CRC Press, Boca Raton, Florida, pp. 407–428.

Yuan F, Dellian M, Fukumura D, Leunig M, Berk DA, Torchilin VP, Jain RK, 1995 Vascular permeability in a human tumor xenograft: molecular size dependence and cutoff size. Cancer Res 55, 3752–3756. [PubMed: 7641188]

Zein N, Sinha AM, McGahren WJ, Ellestad GA, 1988 Calicheamicin γ1I: an antitumor antibiotic that cleaves double-stranded DNA site specifically. Science 240, 1198–1201. [PubMed: 3240341]

Zhang C, Yu G, Shen Y, 2017 The naturally occurring xanthone α-mangostin induces ROS-mediated cytotoxicity in non-small scale lung cancer cells. Saudi J. Biol. Sci 25, 1090–1095. [PubMed: 30174507]

Zhang Y, Jin Z, Zhou H, Ou X, Xu Y, Li H, Liu C, Li B, 2016 Suppression of prostate cancer progression by cancer cell stemness inhibitor napabucasin. Cancer Med 5, 1251–1258. [PubMed: 26899963]

Zhao M, Liu M, 2018 New avenues for nanoparticle-related therapies. Nanoscale Res Lett 13, 136. [PubMed: 29740711]

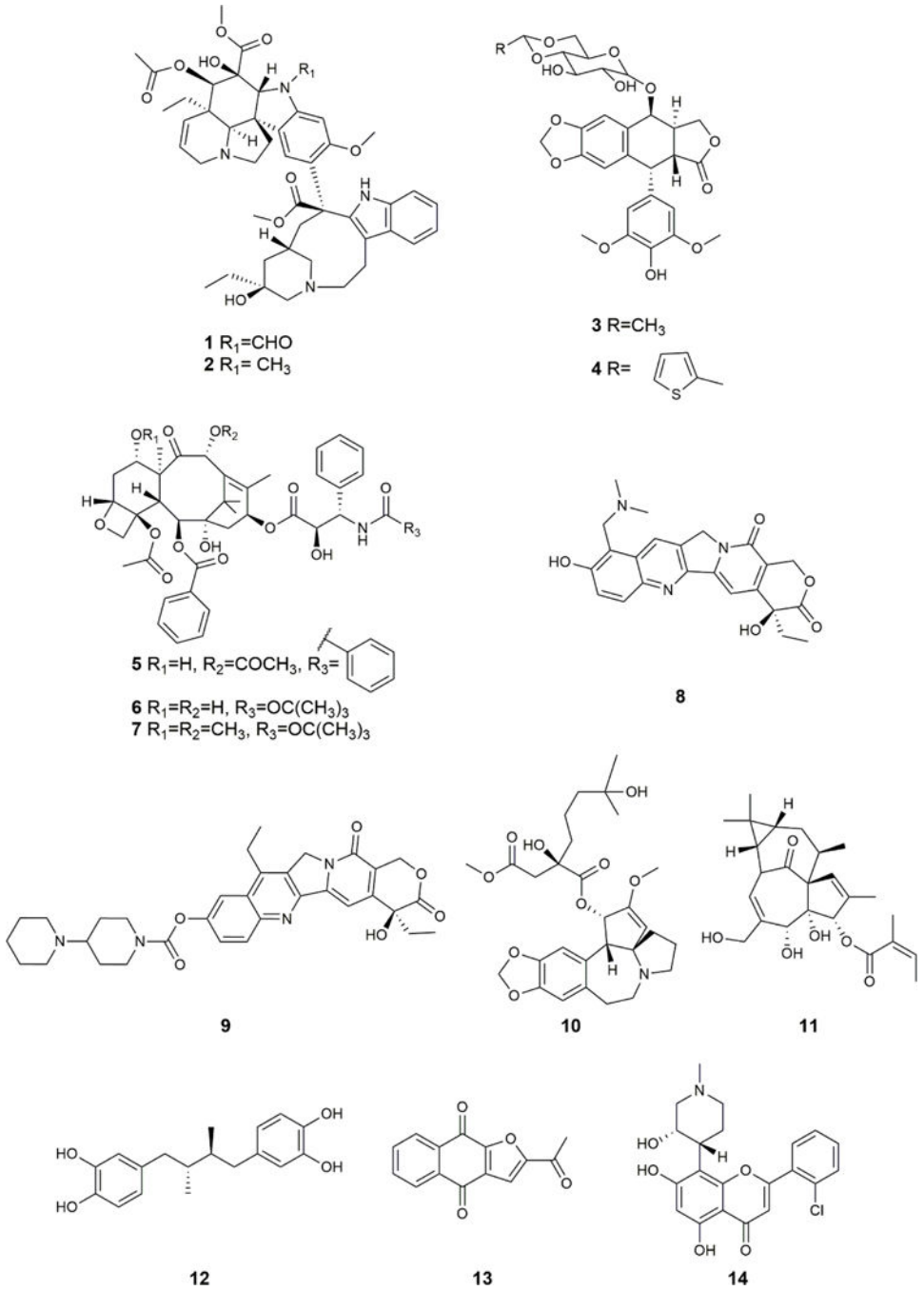

Figure 1.
Plant-derived natural products used as antitumor drugs

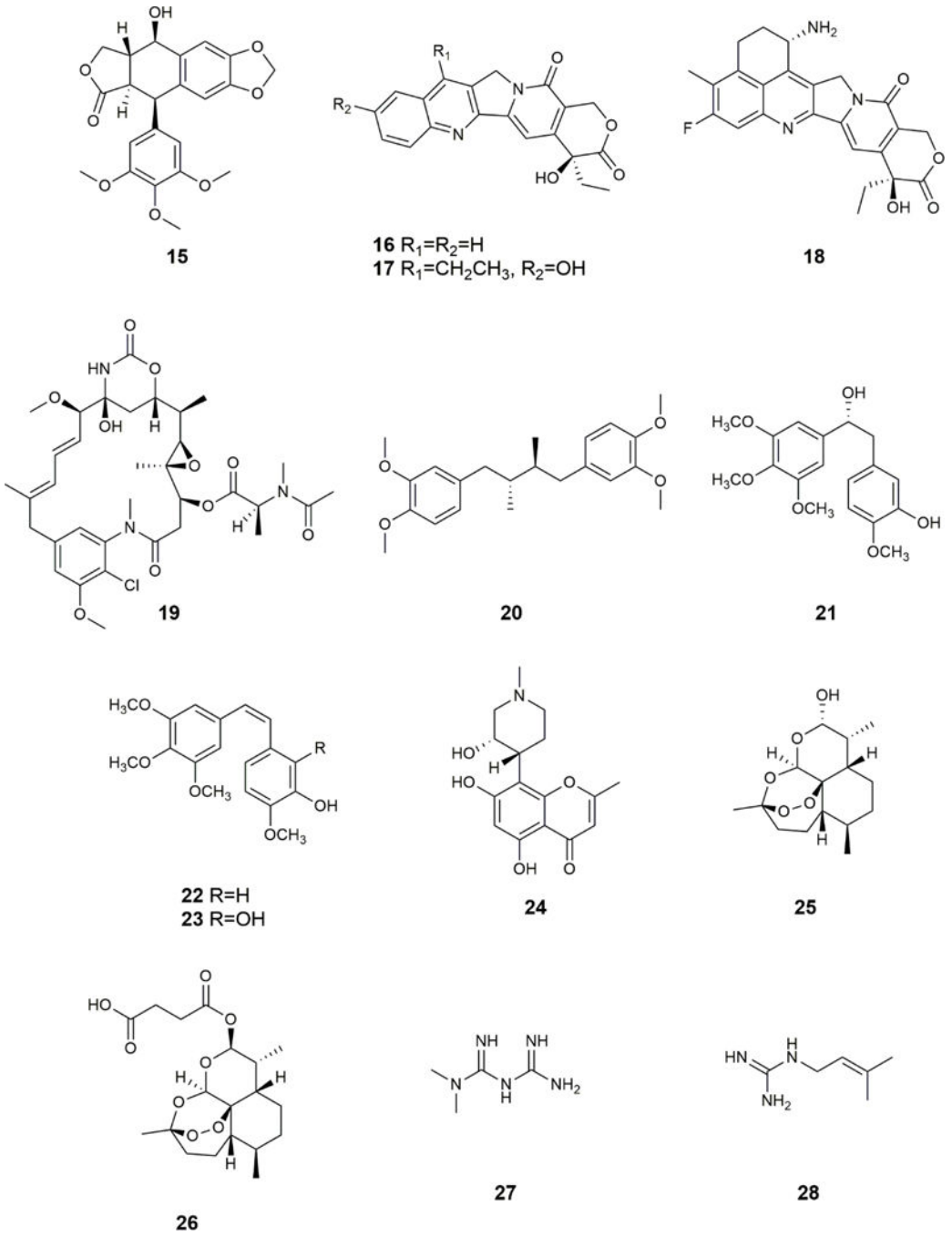

Figure 2.
Examples of other plant-derived secondary metabolites and selected derivatives with antitumor activity

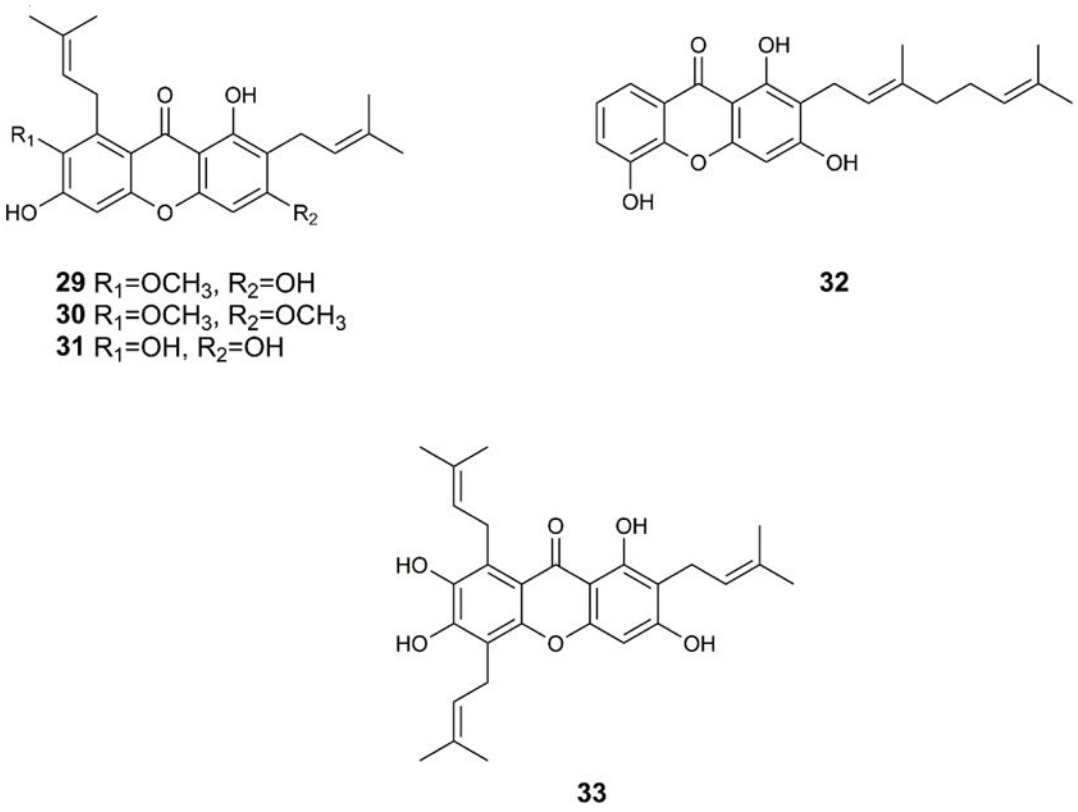

Figure 3.
Examples of bioactive xanthones from mangosteen (*Garcinia mangostana*)

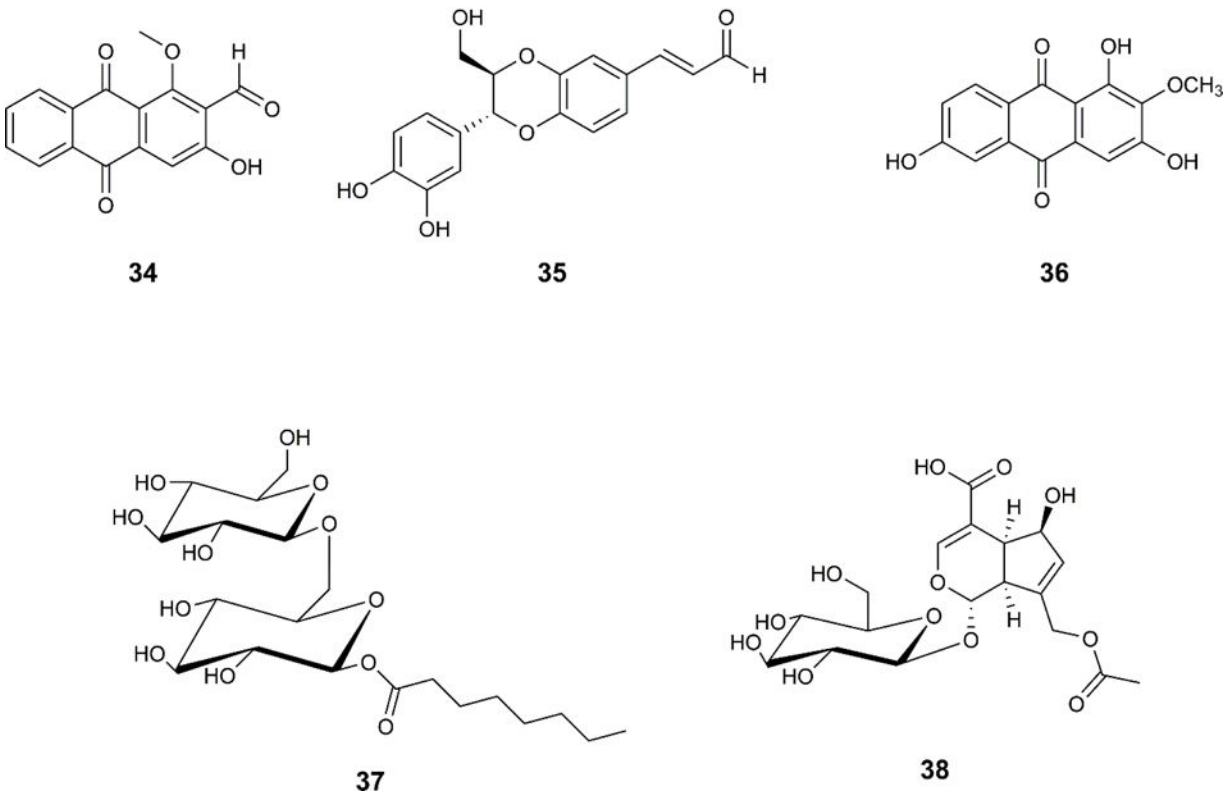

Figure 4.
Selected chemical constituents of noni (*Morinda citrifolia*)

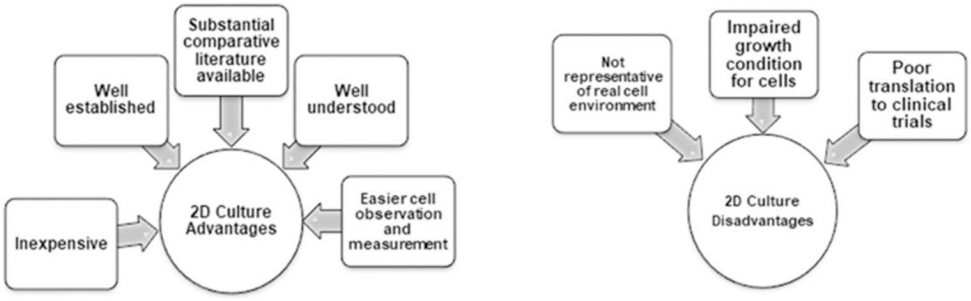

Figure 5.
Summary of advantages and disadvantages of using 2D cell culture

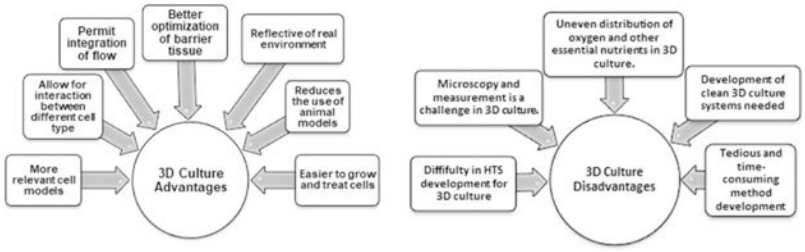

Figure 6.
Summary of advantages and disadvantages of using 3D cell culture

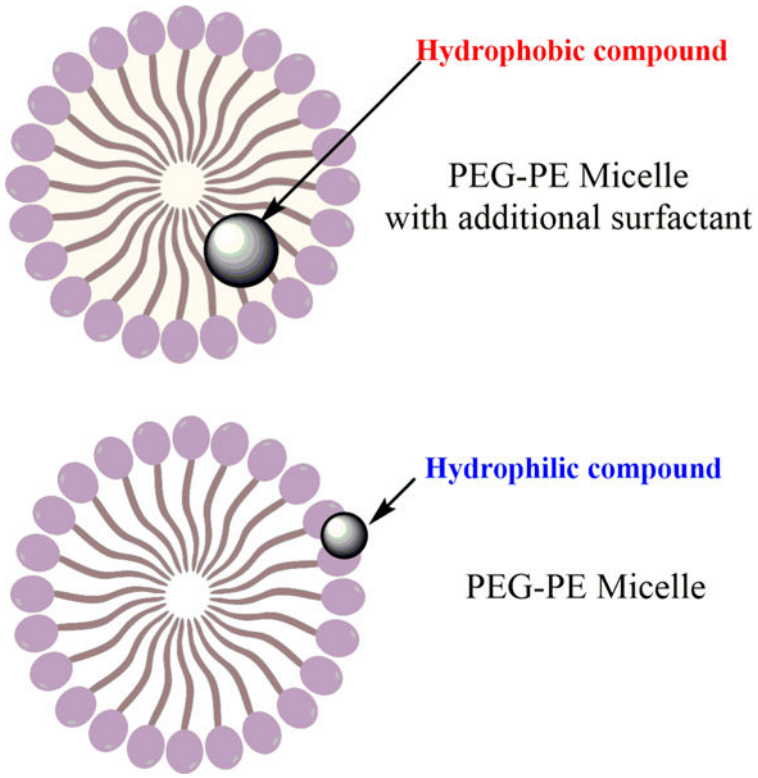

Figure 7.
Examples of micelle designs

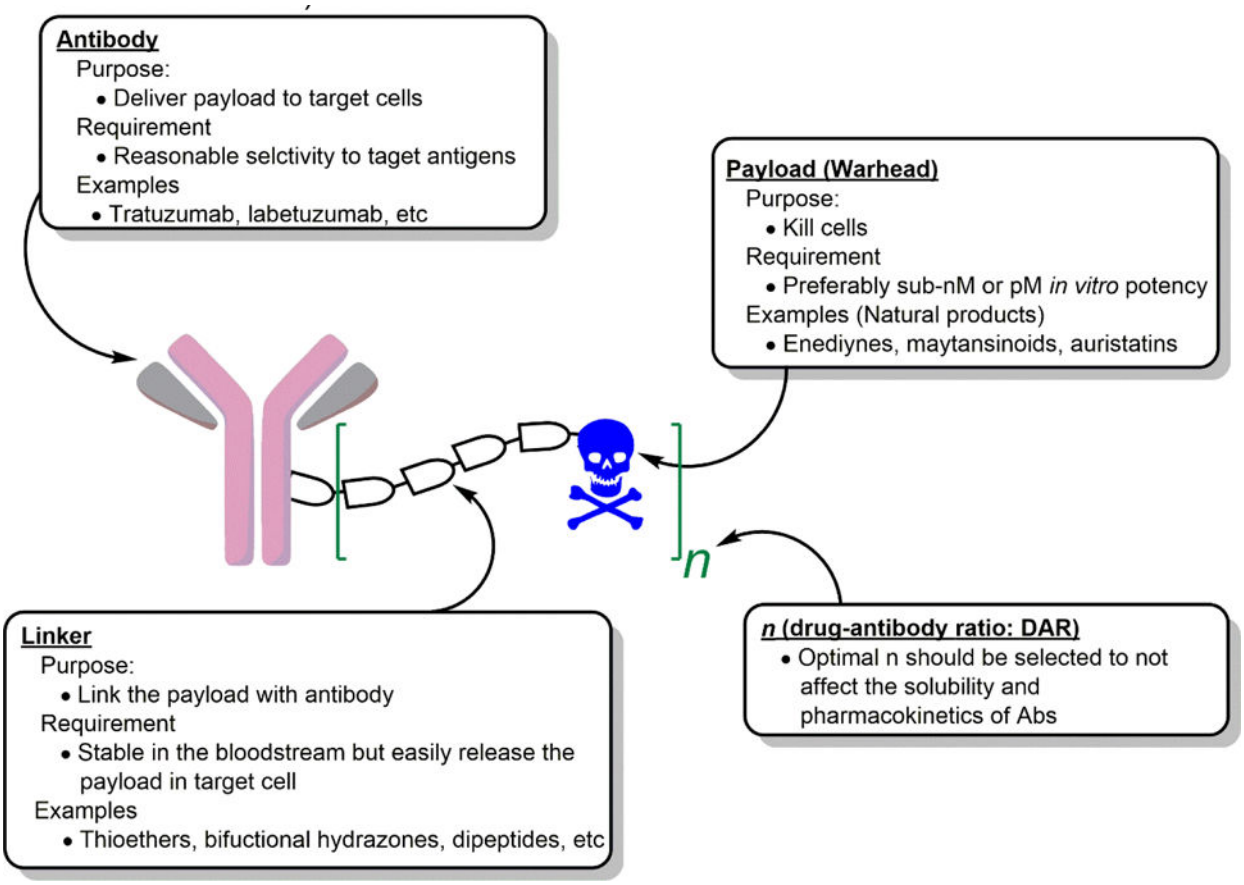

Figure 8.

Major components of ADCs. Included is each component's main purpose, requirements to be a candidate for an ADC, and some examples based on approved ADCs (Note: the purpose, requirements and examples stated are not comprehensive but only representative of the most common ones; see the cited references for additional information).

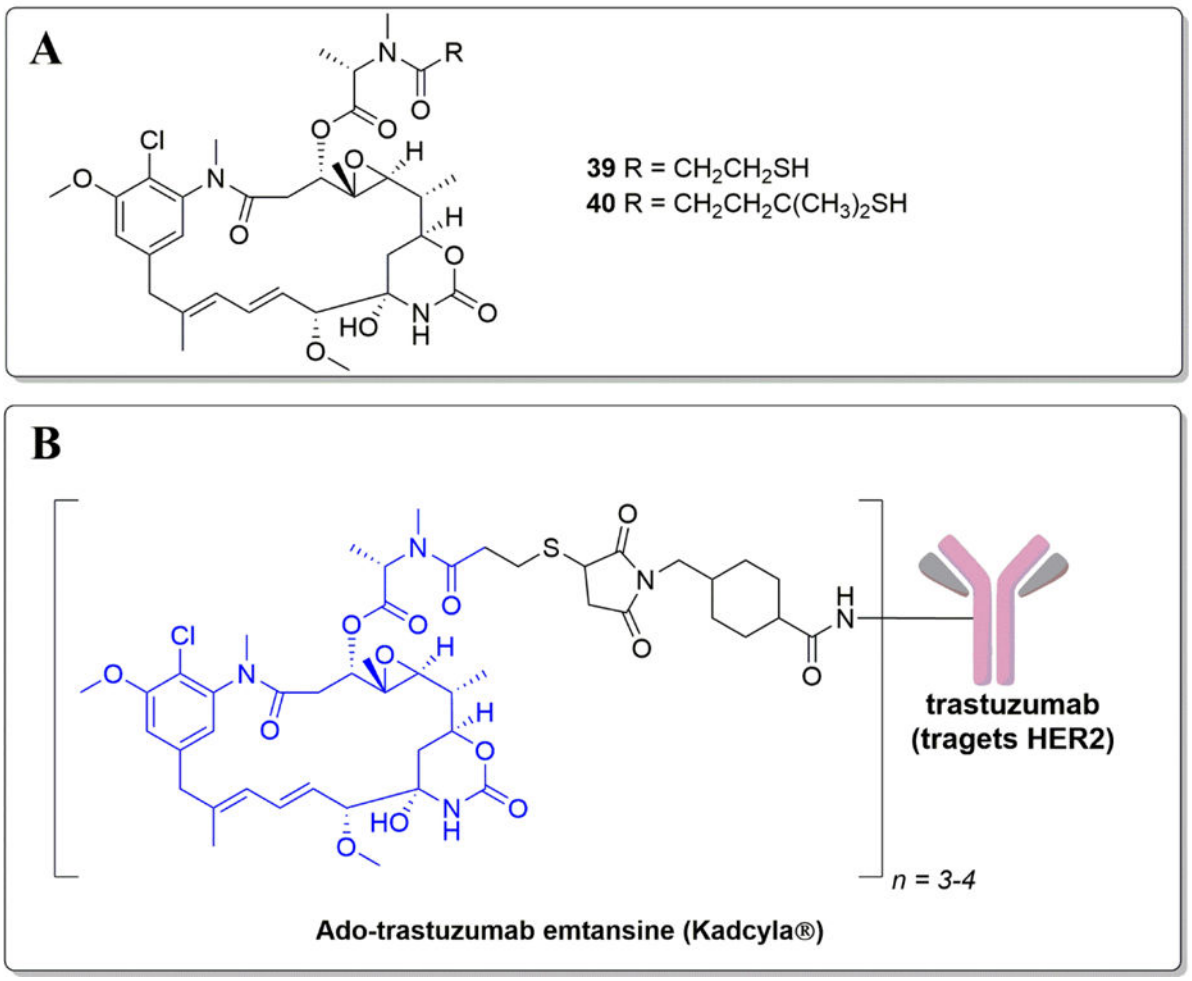

Figure 9.

A) Structure of maytansine and analogues (DM1 and DM4) common in ADCs. B) Structure of ado-trastuzumab emtansine (Kadcyla®) (Note: the n, i.e., DAR, given is an approximate value and may vary).

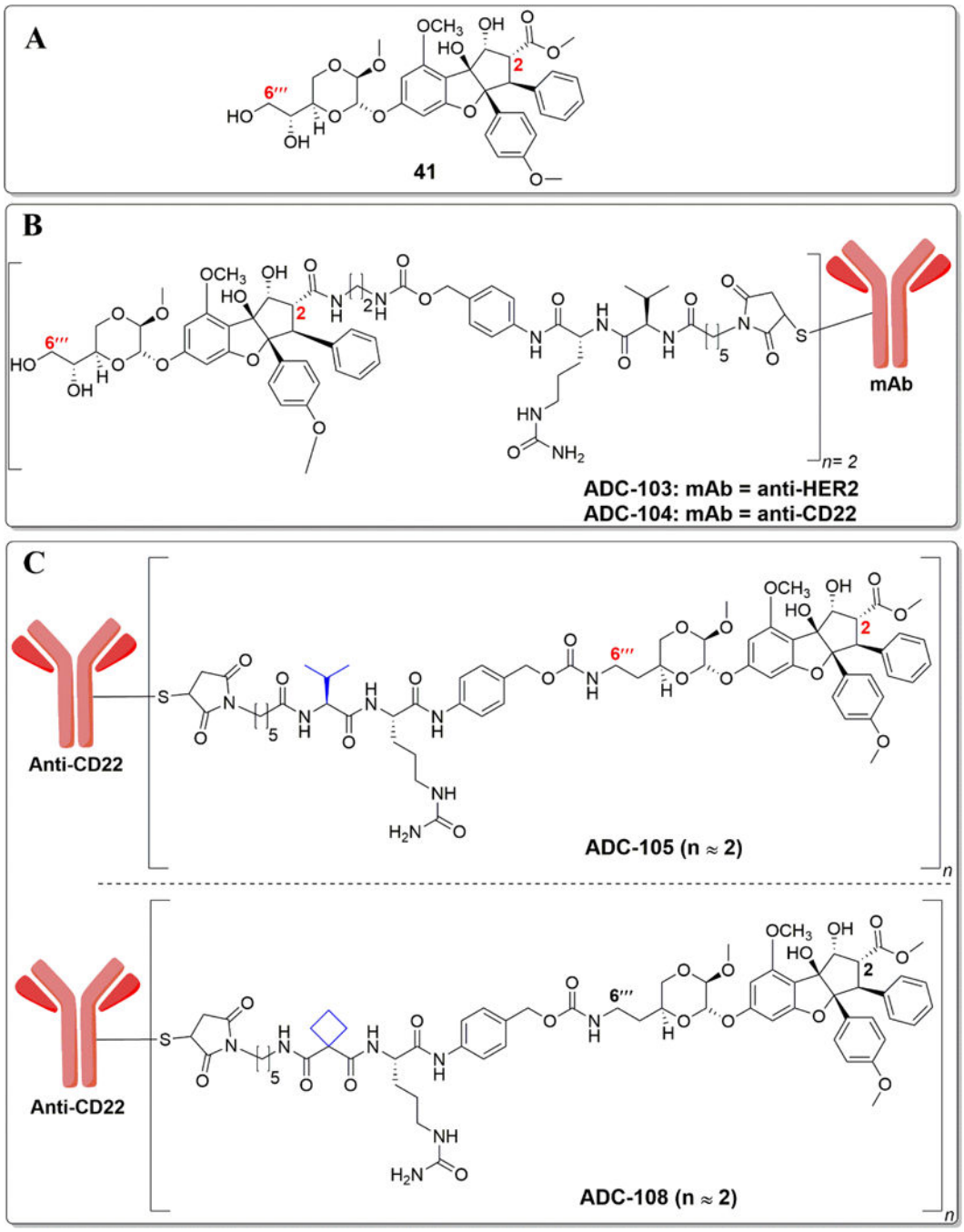

Figure 10.

A) Structure of silvestrol. B) ADCs (ADC-103 and ADC-104) conjugated at position 2 of silvestrol. C) ADCs (ADC-105 and ADC-108) conjugated at position 6′′′ of silvestrol.

Table 1:

Examples of anticancer agents of plant origin (natural, semi-synthetic derivatives, and modified formulations) on the U.S. market and in clinical trials (Information taken from www.clinitrials.gov and www.accessdata.fda.gov)

Drug Name	Source	Status	Formulation	Primary Indication
Vincristine (Oncovin®)	*Catharanthus roseus* G. Don	Approved (1963)	Injection	Solid tumor
Vincristine sulfate (Marqibo®, Vincasar PFS®)		Approved (2012)	Nanoparticle liposomal injection	
Teniposide (Vumon®)	Semi-synthetic derivative of podophyllotoxin (*Podophyllum peltatum* L.)	Approved (1993)	Injection	Lung, testicular cancer, and lymphoma
Paclitaxel (Taxol®)	*Taxus brevifolia* Nutt.	Approved (1992)	Injection	Solid tumor cancer
Abraxane®		Approved (2005)	Nanoparticle albumin injection	Breast cancer
Docetaxel		Phase III	Injection	Non-small cell lung cancer
CriPec® docetaxel	Polymeric nanoparticle of docetaxel	Phase II	Injection	Ovarian cancer
Topotecan (Hycamtin®)	Semi-synthetic derivative of camptothecin (*Camptotheca acuminata* Decne.)	Approved (1996)	Injection	Ovarian cancer
		Approved (2007)	Capsule	Small-cell lung cancer
Irinotecan (Camptosar®)		Approved (1996)	Injection	Colorectal cancer
Irinotecan hydrochloride (Onivyde®)		Approved (2015)	Liposomal injection	Pancreatic cancer
CRLX-101		Phase I and II	Nanoparticle formulation of camptothecin	Solid tumor and small cell lung carcinoma
DS-8201a	ADC of exatecan (camptothecin derivative)	Phase II and III	Injection (ADC aqueous solution)	Breast cancer and colorectal neoplasm
Omacetaxine mepesuccinate (Synribo®)	*Cephalotaxus harringtonii* Kitam.	Approved (2012)	Injection (powder)	Chronic myeloid leukemia
Ado-tratuzamab emtansine (Kadcyla®)	ADC of emtansine (derivative of maytansine (*Maytenus ovatus* Loes.)	Approved (2013)	Injection (ADC aqueous solution)	HER2-positive breast cancer
Napabucasin (GB201)	*Tabebuia cassinoides* (Lam.) DC.	Phase III	Capsule	Metastatic colorectal cancer

Table 2:

Examples of repurposed plant natural product drugs or their derivatives with antitumor activity (Cragg et al., 2014; Gupta et al., 2013; Sleire et al., 2017; Wurth et al., 2016)[a]

Drug	Source	Presently approved drug use	Cancer type[a]	Phase[a]	Reference
Artesunate (derivative of artemisinin)	*Artemisia annua* L. (sweet wormwood)	Antimalarial	Breast Colorectal Hepatocellular carcinoma	1 2 1	von Hagens et al. (2017)
IPI-926 (derivative of cyclopamine)	*Veratrum californicum* Durand (corn lily)	None	Pancreatic Head and neck	1/2 1	Ko et al. (2016)
Digoxin	*Digitalis purpurea* L. (foxglove)	Congestive heart failure	Prostate AML NSCLC	2 1/2 2	Wurth et al. (2016)
Noscapine	*Papaver somniferum* L. (opium poppy)	None (in U.S.A.)	Refractory multiple myeloma CLL	1 1/2	Rida et al. (2015)
Metformin	*Galega officinalis* L. (French lilac)	Antidiabetic (type-2 diabetes)	Breast Lung Prostate Brain	1/2 2 1/2 1/2/3	Wurth et al. (2016)
Colchicine	*Colchicum autumnale* L. (meadow saffron)	Familial Mediterranean fever and gout flares	Prostate Skin Hepatocellular carcinoma	2 2 4	Miola et al. (2018)

[a]More information on these clinical trials is available at www.clinicaltrials.gov

Table 3:

Examples of botanical dietary supplements in U.S. clinical trials as cancer chemotherapeutic agents

Supplement	Major active constituent	Mechanisms of action	In clinical trials for cancer type[b]	Phase[b]	References
Allium sativum L. (garlic)	Allicin, diallyl disulfide [a]	Suppression/ activation of multiple factors involved in cancer cell progression	Breast, pancreatic, and follicular lymphoma	1/2/3	Haque et al. (2016), Priyadarsini and Nagini (2012)
Curcuma longa L. (turmeric)	Curcumin	Pleiotropic modulation of factors such as NF-κB, TNF-α, COX-2, and STAT-3	Prostate, lung, and colon cancer	Early phase 1/1/2/3	Kotecha et al. (2016)
Panax ginseng C.A. Mey. (ginseng)	Ginsenosides	Stimulation of apoptosis via inhibition of mitochondrial mediated-pathway	Breast, colorectal, NSCLC, and prostate cancer	Early phase 1/2/3	Roy et al. (2018)
Glycine max (L.) Merr. (soybean)	Genistein and daidzein	Induce apoptosis by targeting NF-κB and Akt pathways	Prostate, laryngeal and hypopharyngeal squamous cell carcinoma,	1/2/3	Mazumder et al. (2018)
Silybum marianum (L.) Gaertn. (milk thistle)	Silymarin (silibinin)	Inhibits NF-κB and induces TNF, balancing transcription cascade and modulator of STAT3	Upper GI cancer, leukemia, and hepatocellular carcinoma	1/2	Polachi et al. (2016), Cui et al. (2018)
Vitis vinifera L. (grape vine)	*trans*-Resveratrol	Induction of natural killer (NK) cells, in particular NKG2D.	Liver, and colon cancer	1/2	Kotecha et al., (2016)

[a] Information taken from www.drugs.com

[b] Clinical trial examples obtained from www.clinicaltrials.gov

Table 4.

Currently approved ADCs based on natural products

ADC (Trade name, company)	Year approved (USA)	Payload		Linker type	Target antigen	Antibody	Primary indication (based on first approval)
		Natural product (Source, year of first isolation)	Derivative in the ADC				
Gemtuzumab ozogamicin (Mylotarg®, Pfizer Inc.)	• First approved: 2000 • Withdrawn: 2010 • Re-approved: 2017	Calicheamicin γ_1^I (*Micromonospora echinospora* ssp. *calichensis*; 1987)	*N*-Acetyl gamma calicheamicin dimethyl hydrazide (CalichDMH)	Acid labile bifunctional hydrazone linker	CD33	hP67.6	Acute myeloid leukemia
Brentuximab vedotin (Adcetris®, Seattle Genetics Inc.)	2011	Dolastatin 10 (*Dolabela auricularia*; 1987)	MMAE	Enzyme cleavable valine-citrulline (vc) linker	CD30	cAC10	Relapsed Hodgkin lymphoma and relapsed systemic anaplastic large-cell lymphoma
Ado-trastuzumab emtansine (Kadcyla®, Genentech Inc.)	2013	Maytansine (*Maytenus ovatus*; 1972)	DM1	Nonreducible thioether	HER2	Trastuzumab	HER2-positive metastatic breast cancer
Inotuzumab ozogamicin (Besponsa®, Pfizer Inc.)	2017	Calicheamicin γ_1^I (*Micromonospora echinospora* ssp. *calichensis*; 1987)	*N*-Acetyl gamma calicheamicin dimethyl hydrazide (CalichDMH)	Acid labile bifunctional hydarzone linker	CD22	G544	Relapsed or refractory B-cell precursor acute lymphoblastic leukemia (ALL)