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REVIEW

Natural compounds for pediatric cancer treatment

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Abstract There is a tremendous need in clinics to impair cancer progression through noninvasive therapeutic approaches. The use of natural compounds to achieve this is of importance to improve the quality of life of young patients during their treatments. This review will address the "status of the art" related to the potential of natural compounds that are undergoing investigation in combination with standard therapeutic protocols in preclinical and clinical studies and their importance for pediatric cancer treatment. The early studies of drug discovery of these natural compounds discussed here include the main targets, the cellular signaling pathways involved, and the potential modes of action. We also focus on some promising natural compounds that have shown excellent results in vitro and in vivo: Chebulagic acid, Apigenin, Norcantharidin, Saffron/Crocin, Parthenolide, Longikaurin E, Lupeol, Spongistatin 1, and Deoxy-variolin B. Additionally, we introduce the effects of several compounds from nutraceutical and functional foods, to underline their potential use as adjuvant therapies to improve therapeutic benefits. For this purpose, we have selected several compounds: Agaritine, Ganoderma and GL6 peptide, Diallyl trisulfide and Ajoene from garlic, Epigallocatechin gallate from green tea, Curcumin, Resveratrol, and Quercetin.

Keywords Anti-cancer drugs · Natural compounds · Functional foods · Nutraceuticals · Pediatric tumors · Side effects · Tumorigenic pathways

Abbreviations

EGCG	Epigallocatechin gallate
IAPs	Inhibitor-of-apoptosis proteins
RGD	Arginine–lysine–aspartate

Introduction

Advances in therapeutic strategies based on small synthetic molecules and/or monoclonal humanized antibodies designed for inhibition of signaling pathways can often produce toxicity in vivo. Thus, the development of non-toxic molecules that target specific genes or proteins should be the strategy for the near future.

Natural compounds are small molecules that are produced by a biological source, from plants, animals, marine organisms, and microorganisms (Watkins et al. 2015). The introduction of natural agents into cancer treatments has been helpful for many types of malignancies. Indeed, over 60 % of approved anti-tumorigenic drugs derive from natural sources (da Rocha et al. 2001, Mann 2002). The anti-tumorigenic agents derived from plants and microorganisms currently used in clinics in pediatric oncology are listed in Table 1.

However, not all of the characterization studies of natural compounds identified worldwide follow the regulation guidelines. Indeed, only those natural compounds that show a specific biologically active ingredient in, for example, a plant

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Q2 t1.1 **Table 1** Chemotherapeutic products derived from plant and microbial extracts currently used as anti-cancer drugs. The main features of natural compounds used in clinical trials for childhood tumors are shown

t1.2 Name	Molecular formula	Target	Toxicity	Clinical trials	Doses	Reference	
t1.3 Vincristine	$C_{46}H_{56}N_4O_{10}$	Mitotic spindle	Granulocytopenia Thrombocytopenia Neurotoxicity	Hematological neoplasms Neuroblastoma Medulloblastoma	1–2 mg/m^2	(Rubie, De Bernardi et al. 2011) (De Moerloose, Suciu et al. 2010)	Q3/Q4
t1.4 Vinblastine	$C_{46}H_{58}N_4O_9$	Mitotic spindle	Hematotoxicity Hypertension Neurotoxicity	Hodgkin's disease Lymphomas Sarcomas Intracranial germ cell tumors	2.5–6 mg/m^2	(Bates, Danilov et al. 2013) (Salerni, Bates et al. 2010)	Q5/Q6
t1.5 Paclitaxel	$C_{47}H_{51}NO_{14}$	Mitotic spindle	Neutropenia Neurotoxicity	Sarcoma Neuroblastoma	250–350 mg/m^2	(Geller, Wall et al. 2009)	Q7
t1.6 Irinotecan	$C_{33}H_{38}N_4O_6$	DNA topoisomerase I	Neutropenia Gastrointestinal disorders Hypersensibility	Rhabdomyosarcoma Neuroblastoma Medulloblastoma Ependymoma	25–130 mg/m^2	(Turner, Gururangan et al. 2002) (Ma and McLeod 2003) (Kim, Kang et al. 2013)	Q8/Q9 Q10
t1.7 Topotecan	$C_{23}H_{23}N_3O$	DNA topoisomerase I	Hematotoxicity Gastrointestinal disorders Dermatotoxicity	Neuroblastoma Rhabdomyosarcoma	0.75–1.5 mg/m^2	(Feng, Tang et al. 2013)	Q11
t1.8 Etoposide	$C_{29}H_{32}O_{13}$	Cell cycle	Myelosuppression Alopecia Gastrointestinal disorders	Neuroblastoma Lymphomas Leukemia Rhabdoid tumor	60–150 mg/m^2	(Najar and Johri 2014) (Wagner, Hill et al. 2002) (Kushner, Modak et al. 2013)	Q12/Q13 Q14
t1.9 Daunorubicin	$C_{27}H_{29}NO_{10}$	DNA topoisomerase II	Bone marrow depression Gastrointestinal disorders Cardiotoxicity	Leukemia Hodgkin's/non-Hodgkin's diseases Sarcomas	25–45 mg/m^2	(Nickel, Keller et al. 2014)	Q15
t1.10 Idarubicin	$C_{26}H_{27}NO_9$	DNA topoisomerase II	Myelosuppressions Cardiotoxicity	Acute myeloid leukemia Relapsed acute lymphoblastic leukemia Medulloblastoma	5–12 mg/m^2	(Dreyer, Kadota et al. 2003) (Sekine, Morais et al. 2014)	Q16 Q17
t1.11 Actinomycin D	$C_{62}H_{86}N_{12}O_{16}$	Protein synthesis	Myelosuppression Hypersensitivity Gastrointestinal disorders	Rhabdomyosarcoma Ewing's sarcoma	15–600 μg/m^2	(Graf, van Tinteren et al. 2012)	Q18

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extract, rather than the crude extract, and those for which clear molecular signaling and biological effects are evident should be considered. Moreover, the identification of novel natural extracts must always provide the correct botanical plant and family names from which it is derived, the methods for its extraction, and its physicochemical properties (Michel et al. 2005). Taking all of these considerations into account, for the selection of the most promising natural compounds here, we have used this description to include only those that have followed these strict rules. One additional important point is their use in combinatorial therapies, to reduce side effects of defined drugs, with the awareness that they can improve a child's outcome during treatment.

However, the vast majority of the anti-tumor drugs are only approved for the treatment of adults and not for pediatric cancers. The main reason for this is that targeting pediatric cancers is a "small market" for investment (Hirschfeld et al. 2003). Moreover, pediatric cancers can be driven by molecular mechanisms that are different from those responsible for adult tumors (Smith and Reaman 2015); this makes it more difficult to find specific drugs for childhood tumor treatment.

There is a growing body of literature that shows the importance of natural compounds. Indeed, a large number of natural products have been found to inhibit different pathways that contribute to tumorigenesis, angiogenesis, and metastasis in childhood tumors (including those that originate from brain, bone, and liver), both in vitro and in vivo. Their main mechanisms of action involve disruption of the mitotic spindle assembly, inhibition of DNA topoisomerase, arrest of cell-cycle progression, induction of apoptosis, decrease of telomerase activity, and elimination of the "tumor-initiating cells" that are often responsible for chemotherapeutic resistance and tumor relapse.

Taking all of these observations into account, pediatric tumors are generally treated equally following standard protocols after surgery, using combined radiotherapy and chemotherapy. However, restricted therapeutic choices and multidrug resistance have limited the benefit of many treatments for children because of their devastating side effects, which can include non-selective mechanisms of cell killing (e.g., young patients have frequently been left with severe myelosuppression); gastrointestinal toxicity; neurological deficit; endocrine dysfunction; and cognitive difficulties that can lead to postsurgery-related psychological problems (Kinahan et al. 2012); Anderson 2003); (Brydoy et al. 2007). Moreover, complete tumor resection is often difficult, and the responses to radiotherapy and chemotherapy are not always efficient because of the resistance acquired by tumorigenic cells.

Another problem is related to the ability of current chemotherapeutics to pass the blood–brain barrier. Despite aggressive and multimodal therapies that have recently been developed, many tumors remain unaffected by these treatments and continue to have poor outcomes for children with metastatic or recurrent disease, and thus natural compounds might have the potential to fill these gaps (Giangaspero, Perilongo et al. 1999; Schwab, Westermann et al. 2003; Blonski, Taillandier et al. 2012; Taylor, Northcott et al. 2012; Urbanska, Sokolowska et al. 2014; (da Rocha et al. 2001, Litten and Tomlinson 2008, HaDuong et al. 2015). Through their remarkable chemical and structural diversity, agents derived from natural products continue to be of relevance as sources of drug leads that act as templates for the construction of novel molecules, with the challenge being to reduce their side effects (Koehn and Carter 2005).

In this review, we discuss nature-derived drugs that are emerging in the treatment of pediatric tumors, through literature searches of PubMed (http://www.ncbi.nlm.nih.gov/pubmed) for the year 2015, with the scheme applied described in Supplementary Figure S1. Most importantly, these compounds are considered along with their mechanisms of action in tumorigenic cells, for their application as pediatric therapies.

Natural compounds in plants applied to adult clinical cancer trials that are of interest for pediatric trials

The approval of novel anti-cancer drugs for pediatric patients usually occurs after their approval for treating adult cancers. An ongoing challenge today is to define which agents in development for treating adult cancers have potential therapeutic value for childhood cancers. On this basis, several plant-derived compounds are currently successfully used in the treatment of different types of cancers in adult patients. Among these, various natural compounds are proposed as candidates for adjuvant treatment of childhood cancers (Table 2).

One of the most promising plant-derived chemopreventive agents is Ursolic acid, which is an ursane-type pentacyclic triterpenic acid that belongs to the cyclosqualenoid family of triterpenoids (Shanmugam et al. 2013). Ursolic acid has been reported in leaves and berries of several medicinal plants, including *Arctostaphylos uva-ursi* (L.) Spreng (bearberry), *Vaccinium macrocarpon* Air. (cranberry), *Rhododendron hymenanthes* Makino, *Rosmarinus officinalis*, *Eriobotrya japonica*, *Calluna vulgaris*, *Ocimum sanctum*, and *Eugenia jambolana*. Ursolic acid can have valuable effects in a variety of human diseases, including inflammation-driven cancers (Shanmugam et al. 2013), and it is well tolerated by adult patients with advanced tumors (Zhu et al. 2013). Therefore, the idea that Ursolic acid could become a powerful chemopreventive agent in childhood tumors has been developed. Its molecular mechanisms have been studied in hepatoblastoma (Son et al. 2013) because of its pro-apoptotic effects in HepG2 cells (Satomi et al. 2005) and in doxorubicin-resistant HepG2 cells (Yang et al. 2010). The HepG2 cell line has for several years been confused in the literature, with its use as a model of

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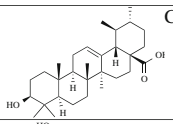
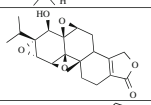
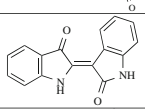
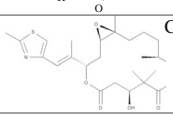
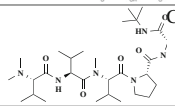
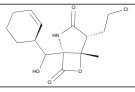
t2.1 **Table 2** Promising natural compounds for pediatric malignancies in clinic trials as anti-cancer agents in adult tumors. The main features of the natural compounds are given, with reference to their use in adult cancers

Compound	Molecular structure	Molecular formula	Molecular weight (g/mol)	Tumorigenic cells and/or mice models	Evidence *in vitro* and *in vivo*	Dose *in vitro* (μM)	Dose *In vivo* (mg/kg)	References
Ursolic acid		$C_{30}H_{48}O_3$	456.70	Hepatoblastoma Leukemia	*In vitro*	20-30	–	(Yang, Liu et al. 2010) (Son, Kwon et al. 2013) (Deng, Zhang et al. 2014)
Triptolide		$C_{20}H_{24}O_6$	360.40	Leukemia	*In vitro*	20-100	–	(Liu, Li et al. 2013) (Huang, Zhang et al. 2013)
Indirubin		$C_{16}H_{10}N_2O_2$	262.26	Leukemia Glioblastoma	*In vitro*	10	–	(Nam, Scuto et al. 2012) (Williams, Nowicki et al. 2011)
Epothilone B		$C_{27}H_{41}NO_6S$	507.68	Neuroblastoma Rhabdomyosarcoma	*In vitro* and in vivo (animal models)	0.010-0.100	1.5	(Scherzinger-Laude, Schonherr et al. 2013)
Tasidotin		$C_{32}H_{58}N_6O_5$	606.84	Rhabdomyosarcoma Ewing's Sarcoma Synovial Sarcoma Osteosarcoma	*In vitro* and *in vivo* (animal models)	0.2-0.3	90	(Garg, Zhang et al. 2007)
Salinosporamid A		$C_{15}H_{20}ClNO_4$	313.77	Leukemia	*In vitro*	0.0051	–	(Ruiz, Krupnik et al. 2006) (Niewerth, Jansen et al. 2014)

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hepatocellular carcinoma, which is indeed a rare tumor in adults (Aden et al. 1979). Instead, recent studies of comparative genomic hybridization and histopathology analyses (Lopez-Terrada et al. 2009) have shown that these cells are indeed epithelia hepatoblastoma, which mainly occurs during the early years of life, in children. Son et al. showed that the Ursolic acid-induced apoptosis in HepG2 cells occurs in a caspase-dependent manner and is regulated by GSK3-β (Son et al. 2013). Their study revealed that in Ursolic acid-treated cells, there was a dose-dependent increase in the inhibition of phosphorylation of GSK3-β (on serine 9), which blocked the up-regulation of the pro-apoptotic protein poly (ADP-ribose) polymerase (Son et al. 2013). Furthermore, these data also showed that this apoptosis was regulated by Ursolic acid through the GSK3-β/AKT/mTOR axis, because Ursolic acid not only increased the phosphorylation of GSK3-β but also down-regulated the Akt/mTOR signaling pathway (Son et al. 2013). Ursolic acid has recently been shown to also induce differentiation in the U937 leukemia cell line, with an increase in the levels of specific monocyte surface markers (i.e., CD14, CD11b) through the activation of the PI3K/Akt pathway (Deng et al. 2014). Moreover, Ursolic acid was less toxic than all-*trans* retinoic acid, which has already been successfully used as a therapy for acute promyelocytic leukemia (Johnson and Redner 2015). However, the differentiation-inducing effects of all-*trans* retinoic acid are more efficient than those of Ursolic acid (Deng et al. 2014). Future studies need to determine the exact mechanism of Ursolic acid-induced differentiation to promote the development of new pharmacological drugs with higher therapeutic effects and lower side effects for the treatment of leukemias (Deng et al. 2014). Thus, in conclusion, Ursolic acid represents a potential candidate in the treatment of hepatoblastoma and leukemia in children through its targeting of apoptosis and induction of cell differentiation processes.

A novel therapeutic strategy to treat hematological malignancies is seen for the diterpene triepoxide Triptolide, which is the major active component in extracts from *Tripterygium wilfordii* Hook F. Triptolide has different pharmacological functions, which include anti-tumorigenic effects both in vitro and in vivo through down-regulation of anti-apoptotic proteins (i.e., XIAP, Mcl-1) and up-regulation of pro-apoptotic proteins (i.e., p53, p21, Bax) (Liu et al. 2013). The roles of Triptolide in human leukemia cell lines and in primary human leukemia blasts have been investigated recently (Liu et al. 2013). These data show selective induction of caspase-dependent cell death and activation of Rho-associated coiled-coil-containing protein kinase ROCK1, which is responsible for stabilizing actin microfilaments and promoting cell contraction and cell substratum contact (Liu et al. 2013). Triptolide has also been demonstrated to induce apoptosis in acute lymphoblastic leukemia cells that overexpress the MDM2 oncoprotein, through inhibition of MDM2 and XIAP expression (Huang et al. 2013).

Indirubin is another promising natural therapeutic agent for hematological malignancies, and it has already been

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used for the treatment of chronic myeloid leukemia (Xiao et al. 2002). It is an ingredient of the traditional Chinese herbal medicine "*Danggui Longhui Wan*" which is made from 11 herbal ingredients (Xiao et al. 2002). Indirubin has been shown to inhibit cell growth via the cyclin-dependent kinases (CDKs) in several human cancer cells, by targeting CDK1/cyclin B, CDK2/cyclin A, CDK2/cyclin E, GSK3-β, and CDK5/p25 (Nam et al. 2013). Indirubin derivatives have been demonstrated to inhibit the Signal Transducer and Activator of Transcription member 5 (Stat-5) protein and to down-regulate the expression of its target proteins Bcl-xL and Mcl-1, which are associated with induction of apoptosis (Nam et al. 2012). This was shown in human K562 chronic myeloid leukemia cells, in imatinib-resistant human KCL-22 chronic myeloid leukemia cells expressing the T315I mutant Bcr-Abl (KCL-22 M), and in CD34-positive primary chronic myeloid leukemia cells from patients (Nam et al. 2012). Therefore, Indirubin derivatives represent a promising structural class for the development of new drugs for patients with wild-type or T315I mutant Bcr-Abl-positive chronic myeloid leukemia. Indirubin derivatives might also be useful for the treatment of pediatric solid tumors, because it can reduce the invasion of glioma cells and glioma-initiating cell-enriched neurospheres both in vitro (by modulating β-catenin levels) and in vivo (by improving survival in glioma-bearing mice) (Williams et al. 2011). These data suggest that Indirubin offers a novel therapeutic approach that might well be applicable not only in pediatric hematological malignancies.

Natural compounds from microorganisms applied to adult clinical cancer trials that are of interest for pediatric trials

Microorganisms represent an attractive and advantageous source for the production of medically useful metabolites. In contrast to plants, they can be grown in culture media on a large scale, which provides an unlimited and uninterrupted supply of the raw material needed for drug development. Several anti-tumorigenic natural compounds have been derived from microorganisms.

Epothilones were discovered as secondary macrolide metabolites that are produced by the myxobacterium *Sorangium cellulosum* (Molnar et al. 2000). Like taxanes, epothilones are responsible for microtubule stabilization in vitro (Molnar et al. 2000). Patupilone, which is also known as Epothilone B, is a potent microtubule stabilizer that belongs to this class (Molnar et al. 2000). Patupilone binds to β-tubulin with a higher affinity than taxanes and alters their spindle formation, which results in the arrest of mitotic cells, and cell death through apoptosis (Molnar et al. 2000). In comparison with taxanes, Patupilone has been shown to be more potent in its in vivo anti-cancer activity at tolerated dose levels in two pediatric tumor mice models of neuroblastoma and rhabdomyosarcoma (Scherzinger-Laude et al. 2013).

Additionally, a liposomal formulation of Patupilone was developed and characterized in both in vitro and in vivo studies (Scherzinger-Laude et al. 2013). Patupilone has been shown to have a strong anti-tumor effect in both of these pediatric cancer models, without triggering major side effects. These effects were not seen when Patupilone was delivered to integrin-expressing cells using RGD liposomes. Moreover, tumor targeting of liposomal Patupilone enhances its anti-tumor activity in rhabdomyosarcoma (Scherzinger-Laude et al. 2013). The potent anti-tumor effects and the significantly increasing cumulative survival of low-dose Patupilone-RGD-liposomes open the way to new pharmacological opportunities for targeting liposomes as part of low-dose therapeutics in pediatric oncology.

Natural compounds from marine organisms applied to adult clinical cancer trials that are of interest for pediatric trials

Marine organisms are another great source of secondary metabolites. In the near future, the exploration of marine environments will provide new potential natural compounds. To date, the new classes of anti-cancer drugs isolated from marine organisms have been shown to have cytotoxic activities against multiple tumor types by targeting different signaling pathways.

The progression of cell growth is inhibited by dolastatins, which were originally identified in the Indian Ocean sea hare *Dolabella auricularia* (Garg et al. 2007). These are a group of proteins that can inhibit the assembly of new microtubules by binding to tubulin and subsequently inhibiting tubulin-dependent GTP hydrolysis in vitro (Garg et al. 2007). Tasidotin HCl (also known as ILX651) is a synthetic pentapeptide (i.e., N,N-dimethyl-L-valyl-L-valyl-N-methyl-L-valyl-L-prolyl-Lproline-tert-butylamide hydrochloride) (Garg et al. 2007). When administered both orally and i.v., Tasidotin has shown strong activity in xenograft mice models of breast and ovarian cancer, taxane-resistant ovarian cancer, prostate carcinomas, melanoma, non-small cell lung carcinoma, colon carcinoma, and P388 murine leukemia (Mita et al. 2006). Several phase I multi-institution clinical trials of Tasidotin have recently been completed in adults (Mita et al. 2006), although Tasidotin has not yet been explored for the treatment of pediatric malignancies. With its promising anti-neoplastic activity, metabolic stability, and oral bioavailability, Tasidotin shows potential for broad therapeutic applications. Garg et al. investigated the effects of Tasidotin in xenograft mice models of pediatric tumors (e.g., rhabdomyosarcoma, Ewing's sarcoma, synovial sarcoma,

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osteosarcoma) (Garg et al. 2007). Here, Tasidotin showed significant anti-tumoral activity in all of these mice xenograft model studies. These data demonstrate that Tasidotin might have positive effects in the treatment of pediatric sarcomas (Garg et al. 2007).

The effects of marine anti-tumorigenic agents have also been tested in other pediatric tumor models, including acute myeloid leukemia, acute lymphoblastic leukemia, and glioma (Potts et al. 2011). Salinosporamide A (Marizomib) is a natural β-lactone-γ-lactam proteasome inhibitor that was derived from the marine actinobacterium *Salinispora tropica* (Potts et al. 2011). The proteasome represents an important clinical target for the treatment of malignancies, and Marizomib has high specificity for the proteasome by binding and inhibiting all three proteolytic protein subunits (i.e., β1, β2, β5) (Potts et al. 2011). A decade ago, the reversible proteasome inhibitor Bortezomib was approved for the treatment of relapsed/refractory and newly diagnosed multiple myeloma and mantle cell lymphoma (Kane et al. 2006), and it represents an emerging treatment strategy for acute leukemia (Messinger et al. 2012, Niewerth et al. 2013). The relevant clinical disadvantages of Bortezomib are related to its unsuitability for oral administration, its toxicity profile, and the emergence of drug resistance (Kale and Moore 2012). Enhanced potency of Salinosporamide A over Bortezomib has been shown in human multiple myeloma cell lines (Chauhan et al. 2005) and in specimens from patients with chronic lymphocytic leukemia (Ruiz et al. 2006). Salinosporamide A might be attractive as a proteasome inhibitor in several malignancies, including acute leukemia. Indeed, tumor cell growth inhibition by Salinosporamide A has been evaluated in human CCRF-CEM acute lymphocytic leukemia cells and in two of the Bortezomib-resistant sub-lines, CEM/BTZ7 (10-fold resistance to bortezomib) and CEM/BTZ200 (123-fold resistance to Bortezomib) (Niewerth et al. 2014). These findings show that Salinosporamide A is emerging as a potent anti-leukemic agent for both parental and bortezomib-resistant leukemia cells. At this time, further clinical evaluations of its suitability are required.

Novel promising plant-derived compounds for pediatric cancer treatment

To reduce toxicity and side effects due to current chemotherapeutic regimens, an increasing number of additional nature-derived molecules are currently under investigation to treat pediatric tumors (Table 3). Previously, we highlighted the importance of medicinal plants, which have often been investigated as sources of new drugs for treating cancer. Due of their low toxicity, plant-derived compounds can effectively reduce the toxicity that is often encountered in vivo.

Terminalia chebula is a member of the *Combretaceae* family that is native to India, and it is already being used in alternative medicine (Kumar et al. 2014). One of its major components is the benzopyran tannin Chebulagic acid, the anti-tumor effects, and molecular mechanisms of which were studied by Kumar et al. in retinoblastoma cells (Kumar et al. 2014). In recent years, a dramatic change in the management of retinoblastoma has occurred, and several methods have now been developed, including focal (e.g., cryotherapy, laser photocoagulation, transpupillary thermotherapy, plaque brachytherapy), local (e.g., external beam radiotherapy, enucleation), and systemic (e.g., chemotherapy) methods (Pandey 2014). All of these can lead to terrible complications that can include serious detachment, tearing, holding, traction and vascular retinal occlusion, and focal paraxial lens opacity (Pandey 2014). Primary enucleation and chemoreduction have become the standard care for advanced intraocular retinoblastoma. Chebulagic acid treatment decreases the proliferation of Y79 cells in a dose-dependent manner (Kumar et al. 2014). The treated cells underwent apoptosis through increased expression of BAX, decreased expression of Bcl2, release of cytochrome c, and activation of caspase 3 (Kumar et al. 2014). Moreover, Chebulagic acid also induced G1 arrest through induction of p27 expression and interference with the NFκB pathway (Kumar et al. 2014). Chebulagic acid can also exert in vitro angiogenic effects by decreasing cell sprouting in the treated rat aortic ring and down-regulation of VEGF production and expression of endothelial specific markers (i.e., CD31, E-selectin) (Athira et al. 2013). Further studies will be required to determine the efficacy of Chebulagic acid in children affected by retinoblastoma and to investigate its in vivo anti-tumorigenic effects.

Among the emerging natural compounds, the naturally occurring plant flavone (4′,5,7-trihydroxyflavone), or Apigenin, has been increasingly recognized as a cancer chemopreventive agent because of its remarkable anti-mutagenic, anti-inflammatory, anti-oxidant, and anti-carcinogenic properties (Birt et al. 2001). Apigenin is abundant in common fruit and vegetables, including parsley, onions, oranges, tea, chamomile, wheat sprouts, and some seasonings (Birt et al. 2001). Several studies have reported that Apigenin can induce apoptosis in a wide range of tumor cells, including those derived from pediatric tumors (e.g., leukemia and neuroblastoma cells), through different cell signaling and transduction pathways, like NFκB, p53, MAPK, and PI3K/Akt (Patel et al. 2007). Wang et al. demonstrated that Apigenin has the highest potency among all of the flavonoids they tested for the induction of apoptosis in a caspase-dependent manner in HL60 leukemia cells (Wang et al. 1999). Subsequently, Budhraja et al. showed that Apigenin can induce apoptosis in dose-dependent and time-dependent manners in U937 cells (Budhraja et al. 2012). Their results suggest a hierarchical model of Apigenin-induced apoptosis in human leukemia cells in which Akt inactivation

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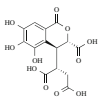
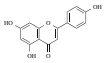
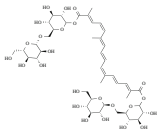
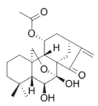
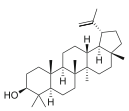
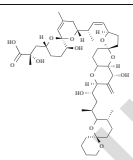
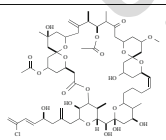
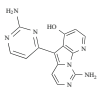
t3.1 **Table 3** Novel natural compounds that can affect several tumorigenic pathways in vitro and/or in vitro in pediatric cancer models. The main features related to the natural compounds are given, as described in the text, for the promising anti-cancer drugs in pediatric tumors

Compound	Molecular structure	Molecular formula	Molecular weight (g/mol)	Tumorigenic cells and/or mice models	Evidence *in vitro* and *in vivo*	Dose *in vitro* (μM)	Dose *in vivo* (mg/kg)	References
Chebulic acid		$C_{14}H_{12}O_{11}$	356.23	Retinoblastoma	*In vitro*	50	–	(Kumar, Gangappa et al. 2014)
Apigenin		$C_{15}H_{10}O_5$	270.23	Leukemia Neuroblastoma	*In vitro* and *in vivo* (animal models)	40-60	20-40	(Torkin, Lavoie et al. 2005) (Budhraja, Gao et al. 2012)
Norcantharidin		$C_8H_8O_4$	168.15	Medulloblastoma Glioblastoma Leukemia Hepatoblastoma	*In vitro* and *in vivo* (animal models)	25-100	1-10	(Cimmino, Scoppettuolo et al. 2012) (Zheng, Du et al. 2014) (Liao, Chen et al. 2011) (Lu, Gao et al. 2014)
Crocin		$C_{44}H_{64}O_{24}$	976.96	Hepatoblastoma Leukemia	*In vitro* and *in vivo* (animal models)	600-5000	6.25-25	(Noureini and Wink 2012) (Sun, Xu et al. 2013)
Parthenolide		$C_{15}H_{20}O_3$	248.32	Hepatoblastoma Glioblastoma	*In vitro* and	5-10	10-40	(Sun, Zhang et al. 2014) (Nakabayashi and Shimizu 2012)
				Leukemia Osteosarcoma	*in vivo* (animal models)			(Guzman, Rossi et al. 2005) (Diamanti, Cox et al. 2013) (Kishida, Yoshikawa et al. 2007)
Longikaurin E		$C_{22}H_{30}O_6$	390.47	Pancreatic tumor	*In vitro*	0,5-4	–	(Cheng, Bo et al. 2015)
Lupeol		$C_{30}H_{50}O$	426.73	Pancreatic tumor Osteosarcoma	*In vitro* and in vivo (animal models)	7.5-120	30-60	(Liu, Bi et al. 2015) (Liu, Bi et al. 2015)
Okadaic acid		$C_{44}S_{68}O_{13}$	805	Retinoblastoma Neuroblastoma	*In vitro*	0.005-0.050	–	(Di Fiore, Drago-Ferrante et al. 2013) (Edelstein and Rockwell 2012)
Spongistatin 1		$C_{65}H_{99}ClO_{20}$	1235.92	Leukemia	*In vitro*	0.0002-0.001	–	(Schyschka, Rudy et al. 2008)
Variolin b		$C_{14}H_{11}N_7O$	29328	Leukemia	*In vitro*	0.050-0.10	–	(Simone, Erba et al. 2005)

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causes activation of JNK signaling, down-regulation of Mcl-1 and Bcl-2, and caspase activation (Budhraja et al. 2012). Furthermore, they also demonstrated that this flavonoid can inhibit tumor growth in vivo, in a U937 cell mice xenograft model (Budhraja et al. 2012). However, the Akt and JNK signaling pathways are not the only targets for Apigenin, because Arango et al. identified other 160 potential targets of Apigenin using a phage display system coupled with second generation sequencing, an innovative approach that provides high-throughput discovery of small molecule–protein interactions (Arango et al. 2013). They focused on heterogeneous nuclear ribonucleoprotein A2 (hnRNPA2), which is an important factor in the progression of tumorigenesis through the regulation of splicing, messenger RNA (mRNA) stability, Q24

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and mRNA transport (Arango et al. 2013). High expression of hnRNPA2/B1 has been reported in a variety of human cancers (Zhou et al. 2001), including glioblastoma (Golan-Gerstl et al. 2011). Arango et al. established that by interacting with the C-terminal domain of hnRNPA2, Apigenin inhibits hnRNPA2 dimerization and alters the alternative splicing patterns of the hnRNPA2 substrates in breast cancer cells (Arango et al. 2013). Indeed, the addition of Apigenin to malignant cells reverts the splicing of caspase-9 and cellular FADD-like IL-1β-converting enzyme (FLICE)-inhibitory protein (c-FLIP), which are two key regulators of apoptosis, without acting against the splice variants in non-carcinogenic cells (Safa 2012). These results might help to explain how Apigenin has an anti-carcinogenic activity by decreasing the inhibition of apoptosis (Arango et al. 2013). The effects of Apigenin have also been studied in human neuroblastoma cells. Torkin et al. reported that Apigenin inhibits tumor growth and induces caspase-dependent apoptosis not only in vitro but also in a non-obese diabetic/severe combined immunodeficient (NOD/SCID) mouse xenograft model, using NUB-7 implanted tumors (Torkin et al. 2005). Apigenin induced p53 nuclear accumulation, thus leading to increased expression of its target proteins (i.e., p21, Bax) (Torkin et al. 2005). Due to its apoptotic effect being restricted to tumorigenic cells without toxicity shown to non-transformed neuronal cells, Apigenin emerged in this study as a potential compound for further development as a therapeutic agent for patients with neuroblastoma.

Cantharidin is a natural compound that was originally isolated from beetles, and it is one of the most emergent anti-tumorigenic agents because of its broad therapeutic applications across a large number of pediatric tumors (Torbeck et al. 2014). The clinical use of Cantharidin has, however, been limited, because of its severe side effects, with analogs of Cantharidin synthesized to reduce this toxicity. Cantharidin and its derivatives have been shown to have strong in vitro anti-tumor activities. Norcantharidin is a demethylated form of Cantharidin, and it has already been used as a routine anti-cancer drug in China (Chen et al. 2005). Norcantharidin showed lower toxicity toward normal cells and was more effective in anti-cancer activities (Chen et al. 2005, Liao et al. 2007). Cantharidin and Norcantharidin express their anti-cancer activities through inhibition of protein phosphatase 1 (PP1) and PP2A, which are involved in cell-cycle progression. Norcantharidin is active in vitro against several tumor cell lines, including hepatoma and leukemia cell lines, and its cytotoxic action has been demonstrated to be approximately 10-fold less than that of Cantharidin in many of these cell lines (Tarleton et al. 2012). Norcantharidin has also shown potent anti-metastatic and anti-angiogenic effects through inhibition of cell migration and capillary-like tube formation in HUVEC cells, by decreasing the amount of pro-angiogenic secreted soluble factors (e.g., angiogenin, GRO, IGF-1, IL-8, MCP-1, VEGF), and without significant toxicity (Chen et al. 2009). This inhibition was accompanied by anoikis, down-regulation of integrin β1, and breakdown of vimentin (Chen et al. 2009). Moreover, in vivo experiments have demonstrated that Norcantharidin prolonged the survival and reduced the plasma VEGF levels in mice with pulmonary metastasis without showing significant renal and liver toxicity (Chen et al. 2009). Therefore, with its ability to pass through the blood–brain barrier, Norcantharidin has been investigated for pediatric brain tumors in vitro and in vivo. In medulloblastoma cell lines, Norcantharidin treatment impaired the growth of DAOY and UW228 cells by affecting Wnt/β-catenin signaling through nuclear β-catenin loss (Cimmino et al. 2012). The efficacy of Norcantharidin to decrease medulloblastoma tumor growth was also assessed in vivo using medulloblastoma orthotopic brain-cerebellum xenograft animal models. These results showed significantly reduced tumor size in the treated mice (Cimmino et al. 2012). Norcantharidin also inhibited cell growth by impairment of the Raf/MEK/ERK pathway in a dose-dependent manner, and it induced apoptosis in glioma cells through down-regulation of the pro-apoptotic proteins Bcl-2 and Mcl-1 (Zheng et al. 2014). Liao et al. developed a model to induce cancer progression via PMAI in a human T cell leukemia cell line and then defined the mechanisms behind Norcantharidin for the inhibition of cell growth and induction of cell-cycle arrest (Liao et al. 2011). The results of this study showed that Norcantharidin can significantly inhibit the viability of these treated cells by induction of cell-cycle arrest at the G2/M phase, down-regulation of the expression of calcineurin, and attenuation of interleukin (IL)-2 production (Liao et al. 2011). These results provide important information about the possible use of Norcantharidin as a chemopreventive agent to inhibit leukemia progression (Liao et al. 2011). Recently, Norcantharidin was shown to have anti-tumor activity also through modulation of the tumor microenvironment in hepatocellular carcinoma models (Lu et al. 2014). Macrophages are known to take part in the tumor microenvironment and to have a dual role in tumor development and progression (Lamagna et al. 2006). Classically, activated M1 macrophages have anti-tumor effects through the release of pro-inflammatory cytokines. On the contrary, alternatively activated M2 macrophages facilitate the progression of tumors, through the release of anti-inflammatory cytokines (Sica et al. 2008). As a result, tumor-associated macrophages have been the target for cancer treatments. Norcantharidin inhibited tumor growth in hepatoma-bearing mice through the β-catenin signaling pathway and subsequently by shifting from M2 macrophage to M1 macrophage polarization (Lu et al. 2014).

In hepatoblastoma, in addition to Norcantharidin, the anti-tumorigenic effects of *Crocus sativus* L. which is commonly known as Saffron have been investigated. This natural compound comes from this perennial stem-less herb of the large *Iridaceae* family that has been widely studied over the last decade for its biomedical properties (Abdullaev et al. 2003).

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Chemopreventive potential of Saffron against cancer and the absence of toxicity and mutagenicity have been reported. Indeed, Saffron appears to have cytotoxic activity against different tumorigenic cells, while no inhibitory effects are seen on normal cell growth (Abdullaev et al. 2003). Crocin is the main water-soluble carotenoid of Saffron extracts, and it represents the most promising Saffron constituent as a therapeutic agent against cancers (Escribano et al. 1996). One of its anti-tumorigenic effects relates to telomerase activity. In hepatoblastoma cell lines, Crocin has been shown to decrease telomerase activity by down-regulation of the expression of hTERT, the catalytic subunit of telomerase (Noureini and Wink 2012). As the telomerase activity is high in cancer cells and almost undetectable in normal cells, it might be a selective target in pediatric cancer therapy. Inhibition of telomerase would selectively suppress tumor growth without large effects on normal cells. Crocin anti-tumor effects have also been investigated in human leukemia cells, both in vitro and in vivo. HL-60 leukemia cells treated with Crocin showed a reduced proliferation rate and induction of apoptosis (Sun et al. 2013). Moreover, daily intraperitoneal injections of Crocin inhibited the growth of leukemia cells in nude mice through modulation of the expression of the apoptosis-related molecules Bcl-2 and Bax (Sun et al. 2013). These findings suggested a potential role for Crocin in the treatment of leukemia.

Parthenolide is another promising plant-derived candidate for cancer chemoprevention. This is a sesquiterpene lactone that was originally purified from the shoots of the feverfew, *Tanacetum parthenium* (Ghantous et al. 2013), that has shown anti-cancer and anti-inflammatory activities in several cell lines derived from pediatric tumors (Gach et al. 2015). These biological properties of Parthenolide can be attributed to its strong inhibition of NFκB (Bork et al. 1997). It was also shown recently that Parthenolide can induce growth inhibition, autophagy, and caspase-mediated cell death in hepatoblastoma cells (Sun et al. 2014). Further, Parthenolide has been shown to suppress cell proliferation and invasion, and tumor-induced angiogenesis in vitro, and to inhibit neovascularity and tumor growth in vivo in glioblastoma mice models (Nakabayashi and Shimizu 2012). It has been suggested that this anti-tumor function of Parthenolide might be mediated not only by inhibition of NFκB but also by inhibition of Akt signaling (Nakabayashi and Shimizu 2012). It is of note that Parthenolide effectively eradicated acute myeloid leukemia stem cells and progenitor cells in vitro while sparing normal hematopoietic stem cells (Guzman et al. 2005). Thus, Parthenolide has been investigated as a potential chemotherapeutic agent in acute myeloid leukemia and acute B-lymphoblastic leukemia cells (Guzman et al. 2005). Treatments for pediatric patients with leukemia are now increasingly successful. However, approximately 20 % of children with acute lymphoblastic leukemia relapse because of the failure to eradicate the disease, and most of these patients do not survive. Parthenolide was reported for the first time to eradicate multiple leukemia-initiating cell populations in vivo in childhood acute lymphoblastic leukemia (Diamanti et al. 2013). For this reason, Parthenolide might have therapeutic potential in childhood acute lymphoblastic leukemia, which would provide a basis for the development of effective therapies that can eradicate leukemia-initiating cell populations, to prevent disease progression and reduce relapse (Diamanti et al. 2013). Osteosarcoma is a highly aggressive primary bone tumor that predominantly affects children and typically demonstrates significant resistance to radiotherapy (Fuchs and Pritchard 2002). Consequently, the conventional treatments do not prevent metastatic progression in 30 to 40 % of patients (Lisle et al. 2008). To obtain more effective treatments, it might be necessary to target the mechanisms used by osteosarcoma cells to acquire significant resistance to radiotherapy. This radio-resistance in the SaOS2 osteosarcoma cell line has been demonstrated to be subsequent to up-regulation of NFκB (Eliseev et al. 2005). As Parthenolide affects NFκB (Kishida et al. 2007), it can potentially provide a clinical method to decrease NFκB in patients with osteosarcoma, which would allow the radiation therapy to have an effective role in this cancer treatment. The in vitro treatment of these re-sensitized cancer stem cells and the entire cell population with radiotherapy has also been investigated in osteosarcoma. The results have indicated that Parthenolide and ionizing radiation act synergistically to induce cell death in LM7 osteosarcoma cells (Zuch et al. 2012). When used together with the standard therapeutic regimen, Parthenolide has been proposed to increase the sensitivity of these cancerous cells to radiotherapy, to provide more complete eradication of the malignant tissue. In conclusion, Parthenolide has been identified as a new therapeutic agent for glioblastoma, leukemia, and hepatoblastoma in children, although the promise that Parthenolide holds for therapy is still limited. This is because of different factors, including its off-target effects and hydrophobicity, which limits its solubility, and thus its oral bioavailability. In the near future, new strategies will be implemented involving low pharmacological doses of Parthenolide either alone or in combination with other drugs, which will define its anti-tumorigenic potential both in vitro and in vivo.

Longikaurin E is a novel natural compound that is derived from the herbal medicine *Rabdosia longituba* (Cheng et al. 2015), and that has shown anti-proliferative and pro-apoptotic effects in pancreatic cancer cell lines (Cheng et al. 2015). Pancreatic tumors are very rare at pediatric ages, and surgery remains the keystone to treat this tumor in children, as in adults (Dall'igna et al. 2010). Cheng et al. showed that Longikaurin E induces apoptosis of pancreatic cancer cells by up-regulation of Bax and down-regulation of Bcl-xL, Bcl-2, Survivin, and c-Myc (Cheng et al. 2015). They also

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reported increased p38 phosphorylation and inhibition of the PI3K/Akt pathway in the treated cells. Although the anti-tumorigenic effects of Longikaurin E were observed in pancreatic cancer cell lines derived from adult tumors (i.e., PANC1, ASPC-1, and BxPC-3 human pancreatic cancer cells), Longikaurin E has emerged as a new drug with potential applications also for pancreatic cancer in children (Cheng et al. 2015). Further studies will be necessary to test its anti-cancer effects on primary pancreatic tumorigenic cells derived from patients of pediatric ages before its clinical application.

In pancreatic cancer, in addition to Longikaurin E, the anti-tumorigenic effects of Lupeol, a dietary triterpene, have been investigated (Liu et al. 2015a, b). Lupeol is found in fruit (such as olives, mangos, strawberries, grapes, and figs), in vegetables, and in medicinal plants (Chaturvedi et al. 2008). Liu et al. showed that Lupeol has anti-proliferative and pro-apoptotic effects on pancreatic cancer both in vitro and in vivo (Liu et al. 2015a, b). Lupeol induced cell-cycle arrest by up-regulation of p21 and p27 and down-regulation of cyclin D1. Interestingly, following Lupeol treatment in vivo, they showed decreased tumor growth and down-regulation of p-ERK and p-Akt in tumor tissues (Liu et al. 2015a, b). Lupeol has also been reported to have anti-cancer efficacy against chemoresistant pancreatic cancer cells through modulation of TRAIL/cFLIP, both in vitro and in vivo in athymic mice (Murtaza et al. 2009). The anti-cancer effects of Lupeol have also been investigated in osteosarcoma, where it induced apoptosis and cell-cycle arrest of human osteosarcoma cells by targeting the PI3K/Akt/mTOR pathway (Liu et al. 2015a, b). Moreover, Lupeol administration in vivo decreased tumor growth and had no effects on the function of the liver and kidneys (Liu et al. 2015a, b). These data suggested a potential role for Lupeol in the treatment of pancreatic cancer and osteosarcoma.

Novel promising marine compounds for pediatric cancer treatment

The development of marine natural compounds is progressing actively, with a significant number of anti-tumor compounds that are entering preclinical studies and early clinical evaluation. About 3000 new compounds have been identified from marine organisms due to advances in deep-sea collection and "aquaculture" technology, which demonstrates that the sea represents a huge potential source for drug discovery (da Rocha et al. 2001).

Several phytoplanktonic species accumulated by shellfish are responsible for the production of Okadaic acid, a lipophilic toxin that causes human diarrheic shellfish poisoning, which although not lethal, causes gastrointestinal symptoms that include diarrhea (92 %), nausea (80 %), vomiting (79 %), abdominal pain (53 %), and chills (10 %) (Valdiglesias et al. 2013). These symptoms vary according to the dose of toxin ingested. The minimum dose of Okadaic acid responsible for this poisoning in human is about 40 mg (Valdiglesias et al. 2013). Several classes of protein serine/threonine phosphatases that regulate cell growth, division, and death and maintenance of the cytoskeletal structure are targets of this toxin. Indeed, Okadaic acid might cause inhibition of protein phosphatases, particularly PP1 and PP2A, but also other types, including PP4, PP5, and PP2B (Louzao et al. 2005). The most reported cytotoxic effect of Okadaic acid is apoptosis induction, while it is known to cause growth inhibition and apoptosis and to induce oxidative stress in different cell types (Valdiglesias et al. 2013). Nevertheless, the genotoxic and cytotoxic effects caused by Okadaic acid might be responsible for genomic instability that can lead to severe pathologies, including cancer (Valdiglesias et al. 2013). Despite these cytotoxic and genotoxic effects, Okadaic acid might still represent a novel candidate for neuroblastoma and retinoblastoma treatment in children. Edelstein et al. showed that in neuroblastoma cells, PPA2 inhibition by Okadaic acid induced Akt hyperphosphorylation, increased levels of ubiquitinated proteins, oxidative stress, loss of cell viability, and cell death (Edelstein and Rockwell 2012). Furthermore, they demonstrated that Rapamycin can enhance Okadaic acid-induced Akt phosphorylation, to increase the susceptibility of cells to pro-oxidant cell death caused by Okadaic acid (Edelstein and Rockwell 2012). The effects of Okadaic acid in combination with Parthenolide were investigated in human retinoblastoma cells (Di Fiore et al. 2013). These two compounds acted together to induce cytotoxicity in Y79 cells by lowering p-Akt levels, stabilizing p53, increasing reactive oxygen species, and concurrently lowering glutathione content (Di Fiore et al. 2013). These results provide strong support for a combined treatment approach to target the PTEN/Akt/mDM2/p53 pathway in this childhood tumor (Di Fiore et al. 2013). The molecular effects of Okadaic acid appear to be cell-type dependent. Valdiglesias et al. showed that Okadaic acid can produce oxidative DNA damage both in human peripheral leukocytes and human neuroblastoma cells but not in human hepatoblastoma cells (Valdiglesias et al. 2011). They concluded that the mechanism leading to DNA damage is highly dependent on cell type. Other studies will be necessary to identify the exact mechanisms of action of Okadaic acid in different tumorigenic cell lines.

Spongistatin 1 is a macrocyclic lactone that was isolated from the marine sponges *Spirastrella spinispirulifera* and *Hyrtios erecta* as a cytotoxic antimitotic compound (Bai et al. 1993). It can block the cell cycle in the mitotic phase in L120 murine leukemia cells (Bai et al. 1993). Spongistatin 1 has been shown to induce cell death in Jurkat leukemia T cells and, more importantly, in primary acute leukemic cells derived from patients (Schyschka et al. 2008). Furthermore, inhibitor-of-apoptosis proteins (IAPs) are overexpressed in cancer cells and in primary tumor biopsy samples (Salvesen

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and Duckett 2002). Drugs designed to specifically target these anti-apoptotic proteins might be valuable to overcome chemoresistance. Of note, Spongistatin 1 can induce protein degradation of the X-linked XIAP, thus impairing the XIAP-overexpressing function in Jurkat cells, and therefore targeting an important molecular key in the apoptosis resistance of tumorigenic cells (Schyschka et al. 2008).

Variolin B is a novel marine natural product that was isolated from the sponge *Kirkpatrickia variolosa*, which is found in Antarctica (Dembitsky et al. 2005). As it has been shown to have pro-apoptotic activity, Variolin B represented a new potent natural cytotoxic agent (Dembitsky et al. 2005). The use of Variolin B is limited by its low stability in solution. However, the development of a Deoxy-variolin B analog that is much more stable and soluble has overcome this limitation of the parent compound (Anderson et al. 2005). The biological activity of this deoxy analog was similar to that of Variolin B, with a slight increase in potency of the deoxy analog that is likely to be related to its greater stability and solubility (Anderson et al. 2005). The biological properties of Variolin B and its deoxy analog, and their mechanisms of action, have been investigated in detail in several tumorigenic cells, including the K562 human leukemic and Jurkat cell lines (Simone et al. 2005). Both of these compounds inhibited colony formation and caused cell-cycle perturbations through increased p53 tumor suppressor protein function and p21, thus definitively inducing apoptosis (Simone et al. 2005). However, the increase in p53 appears to be due to cellular stress induced by the dose of the treatment and does not appear to be crucial for the biological effects of Variolin B and its deoxy analog. Indeed, there were no differences seen in vitro in comparing the cytotoxicity combined with the cell-cycle perturbations induced by Variolin B and its deoxy analog in cell lines expressing wild-type p53 and within sub-lines that had no p53 expression or contained an inactivated p53 isoform (Simone et al. 2005). Moreover, Variolin B and its deoxy analog prevented the cells from entering S phase by inhibition of the CDK1/cyclin B, CDK2/cyclin A, and CDK2/cylin E complexes (Simone et al. 2005). In conclusion here, these Variolins can be considered as a new class of CDK inhibitors that activate apoptosis in a p53-independent manner, and thus they might be effective against not only pediatric tumors but also several cancers with p53 mutations or deletions.

Altogether, the data here reported support the idea that among marine and herbal-derived compounds, there is a variety of potentially effective anti-cancer agents that can act on several key targets of the tumorigenic processes. Future studies need to be performed soon to address their properties in vivo, to support new clinical trials in childhood cancers.

Nutraceutical and functional foods as anti-cancer drugs to support pediatric cancer treatment

Several natural compounds ingested in the diet are known to have anti-tumorigenic effects. As many nutraceuticals and functional foods have been demonstrated to have anti-cancer actions, they might represent novel agents for the treatment of pediatric cancers (Table 4).

Among the nutraceuticals, mushrooms contain a large number of compounds that can target different pathways that are responsible for cancer progression, which include those that modulate the tumor microenvironment. Jiang et al. provided a complete list of mushrooms that have been shown to target cancer cells and, in particular, tumorigenic cells derived from pediatric tumors (Jiang et al. 2015).

The *Basidiomycete* fungus *Agaricus blazei* Murill is an edible mushroom that is cultivated in Japan, China, and Brazil and belongs to the *Agaricaceae* family (Kim et al. 2009). It is already used in association with standard chemotherapeutics in Japan for cancer therapy (Yoshimura et al. 2005). Extracts of *A. blazei* have shown inhibition of cell growth and induction of apoptosis in several types of tumorigenic cells. Its anti-proliferative effects are partly mediated by apoptosis, as reported for different leukemic cells, although not in normal cells, which has suggested a tumor-selective action (Kim et al. 2009). These effects have been confirmed in vivo, with inhibition of tumor growth of human acute promyelocytic leukemia cells in nude mice following administration of *A. blazei* extracts (Kim et al. 2009). Later, Agaritine was discovered as one of the *A. blazei* components responsible for these anti-leukemic properties (Akiyama et al. 2011). Agaritine purified from *A. blazei* directly suppressed cell proliferation through induction of apoptosis in several leukemic cell lines and showed no effects on normal lymphatic cells (Akiyama et al. 2011). However, Agaritine is not the only interesting physicochemical agent in these *A. blazei* extracts, with the demonstration of induction of caspase-dependent apoptosis in osteosarcoma cells using the polysaccharide ABP-Ia from *A. blazei* (Wu et al. 2012). This study also showed no apoptotic effects for a normal human osteoblast cell line, highlighting again the targeted action of these agents toward tumorigenic cells (Wu et al. 2012). The effects of *A. blazei* extracts on sarcoma have been investigated in vivo using the α-(1–4)-glucan-β-(1–6)-glucan-protein complex polysaccharide alone or in association with the chemotherapeutic agent 5-fluorouracil (Gonzaga et al. 2009). These data showed strong inhibition of tumor growth and improvement of survival in sarcoma-180-bearing mice, while no changes were seen for the renal, liver, and hematological parameters, which indicated the absence of toxicological effects in the treated mice (Gonzaga et al. 2009). Of note, the

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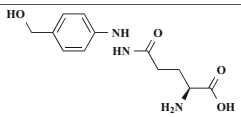
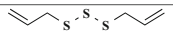
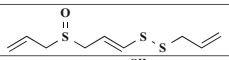
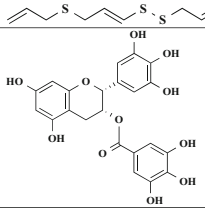
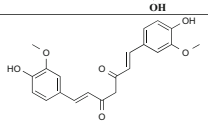
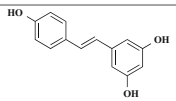
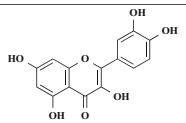
t4.1 **Table 4** Nutraceutical and functional foods that can affect several pathways in pediatric tumors. The main features of the nutraceuticals and functional foods described in the text are given

Compound	Molecular structure	Molecular formula	Molecular weight (g/mol)	Tumorigenic cells and/or mice models	Evidence *in vitro* and/or *in vivo*	Dose *in-vitro* (μM)	Dose *in-vivo* (mg/kg)	References
Agaritine		$C_{12}H_{17}N_3O_4$	267.28	Leukemia	*In vitro*	10 (μg/ml)	-	(Akiyama, Endo et al. 2011)
Diallyl trisulfide		$C_6H_{10}S_3$	178.33	Hepatoblastoma Glioma	*In vitro* and *in vivo* (animal models)	100-200	0.010-10	(Iciek, Kwiecien et al. 2012) (Wallace, Haar et al. 2013)
Ajoene		$C_9H_{14}OS_3$	234.4	Leukemia	*In vitro*	40	-	(Hassan 2004)
Epigallocatechin gallate		$C_{22}H_{18}O_{11}$	458.37	Neuroblastoma Hepatoblastoma	*In vitro*	10-100	-	(Nishimura, Hartomo et al. 2012) (Godeke, Maier et al. 2013)
Curcumin		$C_{21}H_{20}O_6$	368.38	Medulloblastoma Neuroblastoma	*In vitro* and *in vivo* (animal models)	10-40	1.0	(Lee, Krauthauser et al. 2011) (He, Li et al. 2014) (Picone, Nuzzo et al. 2014)
Resveratrol		$C_{14}H_{12}O_3$	228.24	Leukemia Neuroblastoma	*In vitro* and *in vivo* (animal models)	200	20	(Ge, Liu et al. 2013) (Soto, Hank et al. 2011)
Quercetin		$C_{15}H_{10}O_7$	302.23	Neuroblastoma	*In vitro*	20-40	-	(Sugantha Priya, Selvakumar et al. 2014)

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typical leukopenia due to the use of 5-fluorouracil was abrogated by the association with the polysaccharide derived from *A. blazei* (Gonzaga et al. 2009). It can be concluded that, together with this standard chemotherapeutic drug, this polysaccharide was not only responsible for the increment in the anti-tumor effects but also for the decrease in the side effects of the chemotherapy.

Another interesting mushroom with anti-cancer properties is *Ganoderma sinensis*, which is widely used in China as a herbal medicine (Zhou et al. 2007). Among its components, triterpenoid-enriched lipids have emerged due to their immunomodulatory and cytotoxic effects. Dose-dependent suppression of proliferation of leukemia and hepatoblastoma cells has been described for a lipid extract of this *G. sinensis* mushroom, known as GL6. Furthermore, the same GL6 was shown to induce human monocytes and immunosuppressive M2 macrophages to release pro-inflammatory cytokines (Yue et al. 2008). This feature makes this mushroom-derived compound a novel anti-cancer and immune-modulatory agent (Yue et al. 2008). However, careful clinical studies are still required to determine whether these mushrooms do indeed provide concrete benefits to patients, due to the toxicological problems associated with their use. Additionally, in vivo data will be necessary to investigate their molecular mechanisms and their safety.

Another promising anti-tumorigenic natural compound is contained in foods such as Garlic (*Allium sativum*), a bulbous plant that is easy to grown in mild climates. Among its components, Diallyl trisulfide and Ajoene are of importance not only for their remarkable anti-tumor and cancer-preventive effects, as suggested by many in vitro and in vivo studies, but also because of their many health benefits, like improvements to immune-system function, radioprotection, and protection against microbial infections (BayanBayan et al. 2014). These features make them excellent candidates for the treatment of pediatric cancers. Indeed, Diallyl trisulfide has been demonstrated to arrest HepG2 hepatoblastoma cell proliferation by inducing caspase-3 activity, enhancing H_2O_2 levels, and strongly decreasing glutathione levels (Iciek et al. 2012). Moreover,

anti-cancer effects of Diallyl trisulfide have been shown in glioblastoma, with the reduction of cancer progression in U87/MG ectopic tumors in SCID mice (Wallace, G. C. t et al. 2013). As Diallyl trisulfide has not shown any negative impact on hepatic functions, it can be considered an effective therapeutic agent for the prevention of tumor progression in glioblastoma (Wallace, G. C. t et al. 2013). Instead, Ajoene has been shown not only to inhibit cell proliferation and induce caspase-dependent apoptosis of several human myeloid leukemia cells but also to strongly enhance the apoptotic effects of two chemotherapeutic drugs: cytarabine and fludarabine (Hassan 2004). This was observed for the treatment of human CD34-positive resistant myeloid leukemia cells, through enhancement of their Bcl-2 inhibitory and caspase-3 activation activities (Hassan 2004). Thus, these food-derived natural compounds can be considered as potent agents with cancer-preventive properties.

One of the commonest popular beverages is green tea. The correlation between its consumption and inhibition of the growth of a number of cancers is already known (Kanadzu et al. 2006). Green tea contains at least four catechins: epigallocatechin gallate (EGCG), epigallocatechin, epicatechin gallate, and epicatechin (Kanadzu et al. 2006). The most abundant agent in green tea is EGCG. Its anti-cancer effects have been investigated also in neuroblastoma. Tumor-initiating cells were recently identified in neuroblastoma as spheres grown in the serum-free nonadherent culture used for neural-crest stem-cell growth. EGCG inhibited growth and induced apoptosis in BE(2)-C neuroblastoma cells in a dose-dependent manner, although it did not have the same biological effects against such spheres derived from BE(2)-C cells (Nishimura et al. 2012). However, EGCG inhibited the formation of these spheres. So, it will be important to clarify the molecular basis behind the EGCG-sensitive sphere formation in neuroblastoma cells to determine its future clinical applications in children with neuroblastoma (Nishimura et al. 2012). Moreover, EGCG has also been demonstrated to inhibit hepatoblastoma cell growth in a time-dependent and dose-dependent manner while leaving normal fibroblasts unaffected (Godeke et al. 2013). The treated hepatoblastoma cells showed impairment of the *WNT* signaling pathway (Godeke et al. 2013). This is known to enhance cell proliferation, with subsequent reduction in mRNA levels of its main target genes, c-*MYC* and *CCND1*, together with re-expression of the tumor suppressor gene *SFRP1*, which is usually silenced in these cells, thus impairing its known function: to down-regulate *WNT* signaling (Godeke et al. 2013). For these reasons, EGCG should be further investigated for its potential clinical applications in the therapy of hepatoblastoma, mainly because of its selective actions toward tumorigenic cells.

Functional foods as anti-cancer drugs for pediatric cancer treatment

The available literature has emphasized the potential advantages of functional foods as chemopreventive agents, both alone and in association with standard chemotherapeutics. Several studies have focused on the anti-tumorigenic effects of Curcumin (also known as Turmeric), a polyphenolic agent derived from the *Curcuma longa* plant. This plant has been used in Ayurvedic medicine for centuries because of its non-toxic effects and its therapeutic properties, which include anti-cancer activity (Villegas et al. 2011). Indeed, Curcumin has been shown to have anti-proliferative effects in multiple cancers, including pediatric tumors, through targeting several pathways involved in tumorigenesis, including the MAPK, PI3K, and NFκB signaling pathways, and impairing metastasis formation (Heger et al. 2014);(Villegas et al. 2011). One of the most interesting features that makes Curcumin an ideal compound to treat pediatric brain tumors is that it can cross the blood–brain barrier due to its high lipophilic properties (Marchiani et al. 2014). In addition, Curcumin has been shown to induce apoptosis in vitro in human medulloblastoma cells, and to reduce tumor growth, thus increasing survival rates in vivo in mouse models of medulloblastoma, without showing any toxic effects (Lee et al. 2011). Curcumin has been shown to bind the phosphorylated form of the protein Cdc27, and the data that have been reported indicate that due to the phosphorylated Cdc27, the fast-growing medulloblastoma cells were more susceptible to Curcumin-induced cell death than those that only had non-phosphorylated Cdc27 (Lee and Langhans 2012). These results not only provided a possible explanation for the selective mechanism of action of Curcumin, but they also suggested that the phosphorylation status of Cdc27 might be developed as a biomarker to predict which patients might respond favorably to Curcumin-based cancer therapy (Lee and Langhans 2012). Recently, the impairment of the Wnt/β-catenin signaling pathway by Curcumin in medulloblastoma cells was reported (He et al. 2014). In particular, this led to GSK-3β activation and subsequent nuclear β-catenin down-regulation (He et al. 2014). Moreover, Curcumin anti-tumorigenic effects were also shown in neuroblastoma cells (Picone et al. 2014). These treated cells showed a rapid increase in reactive oxygen species, decrease in mitochondrial membrane potential, increase in the pro-apoptotic protein Bcl-2-associated death promoter (BAD), inhibition of Akt signaling, and activation of the pro-apoptotic proteins p27, Bim, and the Fas-L tumor necrosis factor family protein (Picone et al. 2014). All of these events together suggest the induction of apoptosis in these neuroblastoma cells (Picone et al. 2014). Indeed, altogether, these

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results provide evidence for considering Curcumin as an ideal therapeutic agent for children with brain tumors.

Additional attention has been given to Resveratrol (i.e., *trans*-3, 5, 40-trihydroxystilbene), which is a phytoalexin that is found in many plants, including those often consumed by human, such as grapes, peanuts, and berries (Carter et al. 2014). It is not only found in these plants but also in processed products from them, like wine (Carter et al. 2014). Several in vitro studies have shown that Resveratrol has multiple anti-tumorigenic effects through the promotion of cell-cycle arrest and induction of apoptosis of tumorigenic cells, by inhibition of tumor growth and cell motility (Carter et al. 2014). The anti-leukemic effects of were reported by Ge et al., who showed that Resveratrol induces cell-cycle arrest, apoptosis, and autophagy in acute T-lymphoblastic leukemia cells through inhibition of the Akt/mTOR/p70S6K/4E-BP1 pathway and activation of the p38-MAPK signaling pathway (Ge et al. 2013). Furthermore, the anti-tumor effects of Resveratrol have also been described in in vivo neuroblastoma studies (Soto et al. 2011). The combination of Resveratrol and immunocytokine regimens has been reported to enhance anti-tumor activity and improve tumor-free survival in neuroblastoma mice models, while Resveratrol alone only induced tumor regression in primary sites. Indeed, the mice treated with Resveratrol alone developed tumor recurrence and metastasis (Soto et al. 2011). These results suggest a role for Resveratrol in the treatment of acute lymphoblastic leukemia and neuroblastoma.

Finally, the flavonol Quercetin is an interesting natural compound that is contained in different types of fruit (e.g., apples, berries), vegetables (e.g., brassica, onion), black tea, red wine, and many seeds, nuts, flowers, barks, and leaves (Sugantha Priya et al. 2014). As Quercetin has been shown to have many interesting biological properties, including as an anti-oxidant, anti-inflammatory, and anti-neoplastic, it appears to be a candidate for prevention and treatment of childhood tumors. Its anti-tumorigenic activity has been studied in neuroblastoma cells (Sugantha Priya et al. 2014). Here, Quercetin was shown to retain its ability to induce caspase-dependent apoptosis in murine neuroblastoma neuro2a cell lines

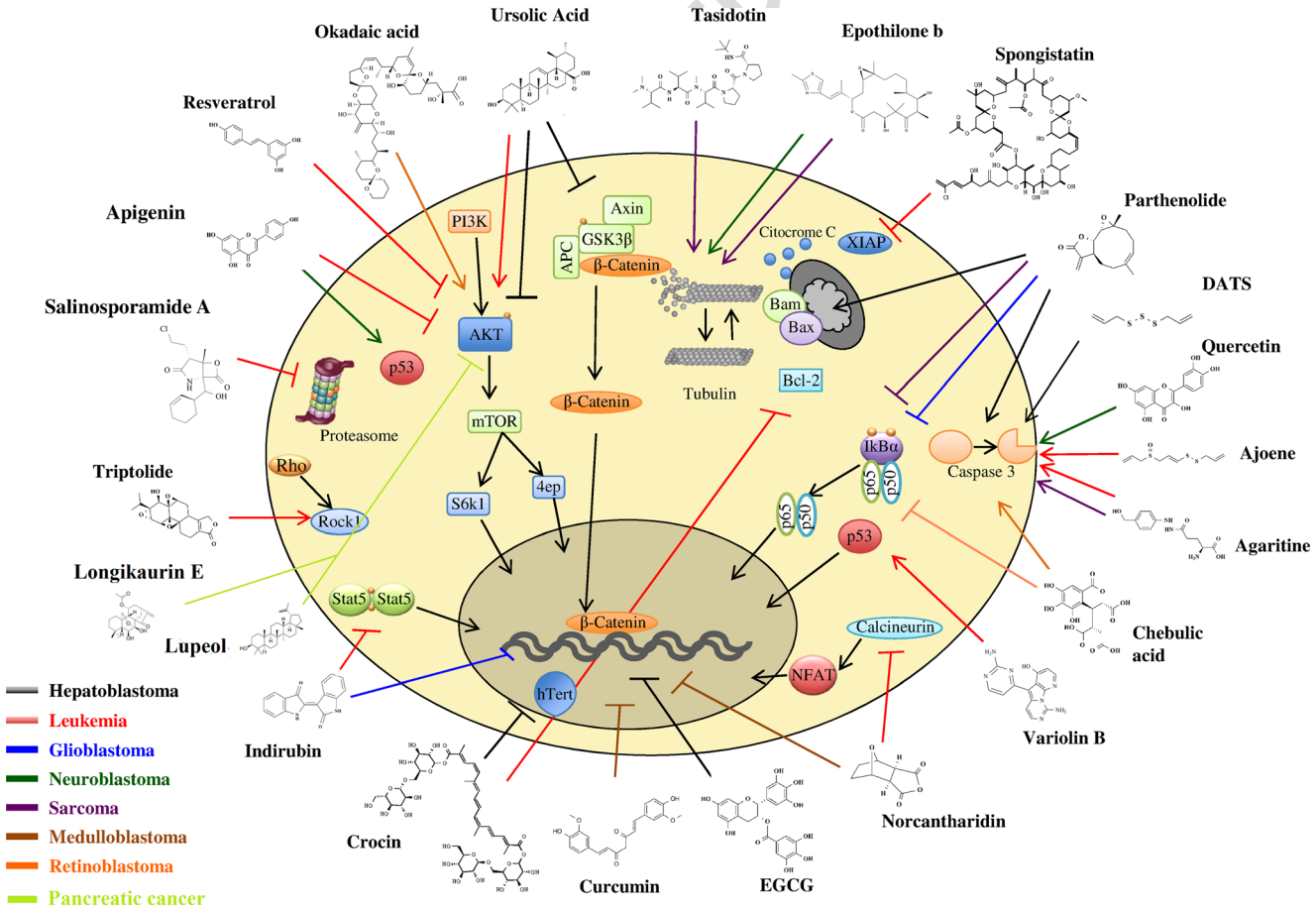

Fig. 1 Intracellular pathways altered by natural compounds in childhood malignancies. Illustration of the novel natural compounds that have been identified recently as having negative effects on intracellular signaling pathways involved in angiogenesis and metastasis formation in pediatric tumors. The *colors of the arrows* represent the types of cancer (see color legend); *dashed lines* indicate those compounds identified from literature searches into nutraceuticals and functional foods

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(Sugantha Priya et al. 2014). Further studies are required to investigate the exact mechanism of Quercetin action, toward the development of novel therapeutic approaches in neuroblastoma treatment in vivo.

Conclusions

In recent years, great contributions to childhood cancer therapy have been made through the use of natural compounds. Despite the technological progress in combinatorial chemistry, synthetic anti-cancer drugs continue to be responsible for non-selective killing of cells. As natural compounds have been shown to offer therapeutic actions with low cytotoxicity in several studies, and due to their chemical diversity, structural complexity, and biological potency, they should be considered further as major sources of drug leads, especially for the treatment of childhood cancers. These compounds are still providing important impacts on drug discovery as they act as templates for the construction of novel nature-derived molecules, with the challenge being to enhance the biological properties and reduce the side effects.

Despite recent progress in pediatric tumor treatment, cancer continues to be the major reason for childhood mortality, and conventional therapies are responsible for poor health-related quality of life and chronic health conditions in childhood cancer survivors. Due to their potential to affect every tissue, current non-selective therapies might have indeterminate long-term effects in young children. Thus, future studies should better identify and manage the functional outcomes of childhood cancer survivors because of their emotional distress (e.g., depressive symptoms, anxiety, increased somatization) and their reduced healthcare due to long-term side effects of current therapies (Kinahan et al. 2012). Thus, natural compounds, including dietary factors, nutraceuticals, and especially functional foods (e.g., Quercetin, Resveratrol, EGCG, Diallyl trisulfide, Ajoene), are of significance here. As a result of their low toxicity and targeting of a wide range of signaling pathways involved in tumorigenesis, such natural compounds can be used to decrease these therapy-related side effects in children (as shown in Fig. 1).

The introduction of such bioactive, nature-derived agents into clinics has changed the outcome of several types of pediatric cancers. Nevertheless, many efforts need to be focused on drug discovery of further new natural compounds, to identify new drugs derived from novel natural products. This will also increase their efficacy and reduce their side effects in children, with the need to also facilitate their handling and tolerability by developing oral regimens and/or liposomal formulation. Plants still represent a great source for natural compounds in drug discovery but microorganisms and marine-derived compounds will have governing roles in the future, with a large number of promising molecules that are already undergoing clinical development as anti-cancer agents for childhood tumors.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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