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REVIEW

4 **Natural compounds for pediatric cancer treatment**

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9 **Abstract** There is a tremendous need in clinics to impair  
10 cancer progression through noninvasive therapeutic ap-  
11 proaches. The use of natural compounds to achieve this is of  
12 importance to improve the quality of life of young patients  
13 during their treatments. This review will address the “status  
14 of the art” related to the potential of natural compounds that  
15 are undergoing investigation in combination with standard  
16 therapeutic protocols in preclinical and clinical studies and  
17 their importance for pediatric cancer treatment. The early stud-  
18 ies of drug discovery of these natural compounds discussed  
19 here include the main targets, the cellular signaling pathways  
20 involved, and the potential modes of action. We also focus on  
21 some promising natural compounds that have shown excellent  
22 results in vitro and in vivo: Chebulagic acid, Apigenin,  
23 Norcantharidin, Saffron/Crocin, Parthenolide, Longikaurin  
24 E, Lupeol, Spongistatin 1, and Deoxy-variolin B. Addition-  
25 ally, we introduce the effects of several compounds from nutra-  
26 ceutical and functional foods, to underline their potential use  
27 as adjuvant therapies to improve therapeutic benefits. For this  
28 purpose, we have selected several compounds: Agaritine,  
29 Ganoderma and GL6 peptide, Diallyl trisulfide and Ajoene

from garlic, Epigallocatechin gallate from green tea, 30  
Curcumin, Resveratrol, and Quercetin. 31

**Keywords** Anti-cancer drugs · Natural compounds · 32  
Functional foods · Nutraceuticals · Pediatric tumors · Side 33  
effects · Tumorigenic pathways 34

**Abbreviations** 35  
EGCG Epigallocatechin gallate 36  
IAPs Inhibitor-of-apoptosis proteins 37  
RGD Arginine–lysine–aspartate 38

**Introduction** 44

Advances in therapeutic strategies based on small synthetic 45  
molecules and/or monoclonal humanized antibodies designed 46  
for inhibition of signaling pathways can often produce toxicity 47  
in vivo. Thus, the development of non-toxic molecules that 48  
target specific genes or proteins should be the strategy for the 49  
near future. 50

Natural compounds are small molecules that are produced 51  
by a biological source, from plants, animals, marine organ- 52  
isms, and microorganisms (Watkins et al. 2015). The introduc- 53  
tion of natural agents into cancer treatments has been helpful 54  
for many types of malignancies. Indeed, over 60 % of ap- 55  
proved anti-tumorigenic drugs derive from natural sources 56  
(da Rocha et al. 2001, Mann 2002). The anti-tumorigenic 57  
agents derived from plants and microorganisms currently used 58  
in clinics in pediatric oncology are listed in Table 1. 59

However, not all of the characterization studies of natural 60  
compounds identified worldwide follow the regulation guide- 61  
lines. Indeed, only those natural compounds that show a spe- 62  
cific biologically active ingredient in, for example, a plant 63

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Q1

**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 <sub>46</sub> H <sub>56</sub> N <sub>4</sub> O <sub>10</sub>	Mitotic spindle	Granulocytopenia Thrombocytopenia Neurotoxicity	Hematological neoplasms Neuroblastoma Medulloblastoma	1–2 mg/m <sup>2</sup>	(Rubie, De Bernardi et al. 2011) (De Moerloose, Suciu et al. 2010)
t1.4	Vinblastine	C <sub>46</sub> H <sub>58</sub> N <sub>4</sub> O <sub>9</sub>	Mitotic spindle	Hematotoxicity Hypertension Neurotoxicity	Hodgkin's disease Lymphomas Sarcomas Intracranial germ cell tumors	2.5–6 mg/m <sup>2</sup>	(Bates, Danilov et al. 2013) (Salemi, Bates et al. 2010)
t1.5	Paclitaxel	C <sub>47</sub> H <sub>51</sub> NO <sub>14</sub>	Mitotic spindle	Neutropenia Neurotoxicity	Sarcoma Neuroblastoma	250–350 mg/m <sup>2</sup>	(Geller, Wall et al. 2009)
t1.6	Irinotecan	C <sub>33</sub> H <sub>38</sub> N <sub>4</sub> O <sub>6</sub>	DNA topoisomerase I	Neutropenia Gastrointestinal disorders Hypersensitivity	Rhabdomyosarcoma Neuroblastoma Medulloblastoma Ependymoma	25–130 mg/m <sup>2</sup>	(Turner, Gururangan et al. 2002) (Ma and McLeod 2003) (Kim, Kang et al. 2013)
t1.7	Topotecan	C <sub>23</sub> H <sub>23</sub> N <sub>3</sub> O	DNA topoisomerase I	Hematotoxicity Gastrointestinal disorders Dermatotoxicity	Neuroblastoma Rhabdomyosarcoma	0.75–1.5 mg/m <sup>2</sup>	(Feng, Tang et al. 2013)
t1.8	Etoposide	C <sub>29</sub> H <sub>32</sub> O <sub>13</sub>	Cell cycle	Myelosuppression Alopecia Gastrointestinal disorders	Neuroblastoma Lymphomas Leukemia	60–150 mg/m <sup>2</sup>	(Najar and Johri 2014) (Wagner, Hill et al. 2002) (Kushner, Modak et al. 2013)
t1.9	Daunorubicin	C <sub>27</sub> H <sub>29</sub> NO <sub>10</sub>	DNA topoisomerase II	Bone marrow depression Gastrointestinal disorders Cardiotoxicity	Rhabdoid tumor Leukemia Hodgkin's/non-Hodgkin's diseases Sarcomas	25–45 mg/m <sup>2</sup>	(Nickel, Keller et al. 2014)
t1.10	Idarubicin	C <sub>26</sub> H <sub>27</sub> NO <sub>9</sub>	DNA topoisomerase II	Myelosuppressions Cardiotoxicity	Acute myeloid leukemia Relapsed acute lymphoblastic leukemia Medulloblastoma	5–12 mg/m <sup>2</sup>	(Dreyer, Kadota et al. 2003) (Sekine, Morais et al. 2014)
t1.11	Actinomycin D	C <sub>62</sub> H <sub>86</sub> N <sub>12</sub> O <sub>16</sub>	Protein synthesis	Myelosuppression Hypersensitivity Gastrointestinal disorders	Rhabdomyosarcoma Ewing's sarcoma	15–600 µg/m <sup>2</sup>	(Graf, van Tinteren et al. 2012)

64 extract, rather than the crude extract, and those for which clear  
65 molecular signaling and biological effects are evident should  
66 be considered. Moreover, the identification of novel natural  
67 extracts must always provide the correct botanical plant and  
68 family names from which it is derived, the methods for its  
69 extraction, and its physicochemical properties (Michel et al.  
70 2005). Taking all of these considerations into account, for the  
71 selection of the most promising natural compounds here, we  
72 have used this description to include only those that have  
73 followed these strict rules. One additional important point is  
74 their use in combinatorial therapies, to reduce side effects of  
75 defined drugs, with the awareness that they can improve a  
76 child's outcome during treatment.

77 However, the vast majority of the anti-tumor drugs are only  
78 approved for the treatment of adults and not for pediatric can-  
79 cers. The main reason for this is that targeting pediatric can-  
80 cers is a "small market" for investment (Hirschfeld et al.  
81 2003). Moreover, pediatric cancers can be driven by molecu-  
82 lar mechanisms that are different from those responsible for  
83 adult tumors (Smith and Reaman 2015); this makes it more  
84 difficult to find specific drugs for childhood tumor treatment.

85 There is a growing body of literature that shows the impor-  
86 tance of natural compounds. Indeed, a large number of natural  
87 products have been found to inhibit different pathways that  
88 contribute to tumorigenesis, angiogenesis, and metastasis in  
89 childhood tumors (including those that originate from brain,  
90 bone, and liver), both in vitro and in vivo. Their main mech-  
91 anisms of action involve disruption of the mitotic spindle as-  
92 sembly, inhibition of DNA topoisomerase, arrest of cell-cycle  
93 progression, induction of apoptosis, decrease of telomerase  
94 activity, and elimination of the "tumor-initiating cells" that  
95 are often responsible for chemotherapeutic resistance and tu-  
96 mor relapse.

97 Taking all of these observations into account, pediatric tu-  
98 mors are generally treated equally following standard proto-  
99 cols after surgery, using combined radiotherapy and chemo-  
100 therapy. However, restricted therapeutic choices and multi-  
101 drug resistance have limited the benefit of many treatments  
102 for children because of their devastating side effects, which  
103 can include non-selective mechanisms of cell killing (e.g.,  
104 young patients have frequently been left with severe  
105 myelosuppression); gastrointestinal toxicity; neurological def-  
106 icit; endocrine dysfunction; and cognitive difficulties that can  
107 lead to postsurgery-related psychological problems (Kinahan  
108 et al. 2012); Anderson 2003); (Brydoy et al. 2007). Moreover,  
109 complete tumor resection is often difficult, and the responses  
110 to radiotherapy and chemotherapy are not always efficient  
111 because of the resistance acquired by tumorigenic cells.

112 Another problem is related to the ability of current chemo-  
113 therapeutics to pass the blood-brain barrier. Despite aggres-  
114 sive and multimodal therapies that have recently been devel-  
115 oped, many tumors remain unaffected by these treatments and  
116 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 chem-  
ical and structural diversity, agents derived from natural prod-  
ucts 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).

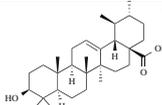
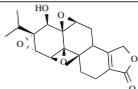
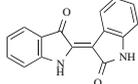
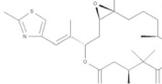
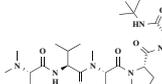
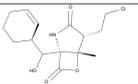
In this review, we discuss nature-derived drugs that are  
emerging in the treatment of pediatric tumors, through litera-  
ture searches of PubMed ([http://www.ncbi.nlm.nih.gov/  
pubmed](http://www.ncbi.nlm.nih.gov/pubmed)) for the year 2015, with the scheme applied  
described in Supplementary Figure 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 de-  
velopment 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 cycloqualenoid 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 chemopre-  
ventive 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

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 <i>in vitro</i> and <i>in vivo</i>	Dose <i>in vitro</i> (μM)	Dose <i>In vivo</i> (mg/kg)	References
Ursolic acid		C <sub>30</sub> H <sub>48</sub> O <sub>3</sub>	456.70	Hepatoblastoma Leukemia	<i>In vitro</i>	20-30	-	(Yang, Liu et al. 2010) (Son, Kwon et al. 2013) (Deng, Zhang et al. 2014)
Triptolide		C <sub>20</sub> H <sub>24</sub> O <sub>6</sub>	360.40	Leukemia	<i>In vitro</i>	20-100	-	(Liu, Li et al. 2013) (Huang, Zhang et al. 2013)
Indirubin		C <sub>16</sub> H <sub>10</sub> N <sub>2</sub> O <sub>2</sub>	262.26	Leukemia Glioblastoma	<i>In vitro</i>	10	-	(Nam, Scuto et al. 2012) (Williams, Nowicki et al. 2011)
Epothilone B		C <sub>27</sub> H <sub>41</sub> NO <sub>6</sub> S	507.68	Neuroblastoma Rhabdomyosarcoma	<i>In vitro</i> and <i>in vivo</i> (animal models)	0.010-0.100	1.5	(Scherzinger-Laude, Schonherr et al. 2013)
Tasidotin		C <sub>32</sub> H <sub>58</sub> N <sub>6</sub> O <sub>5</sub>	606.84	Rhabdomyosarcoma Ewing's Sarcoma Synovial Sarcoma Osteosarcoma	<i>In vitro</i> and <i>in vivo</i> (animal models)	0.2-0.3	90	(Garg, Zhang et al. 2007)
Salinosporamid A		C <sub>15</sub> H <sub>20</sub> ClNO <sub>4</sub>	313.77	Leukemia	<i>In vitro</i>	0.0051	-	(Ruiz, Krupnik et al. 2006) (Niewerth, Jansen et al. 2014)

168 hepatocellular carcinoma, which is indeed a rare tumor in  
 169 adults (Aden et al. 1979). Instead, recent studies of compara-  
 170 tive genomic hybridization and histopathology analyses  
 171 (Lopez-Terrada et al. 2009) have shown that these cells are  
 172 indeed epithelia hepatoblastoma, which mainly occurs during  
 173 the early years of life, in children. Son et al. showed that the  
 174 Ursolic acid-induced apoptosis in HepG2 cells occurs in a  
 175 caspase-dependent manner and is regulated by GSK3-β  
 176 (Son et al. 2013). Their study revealed that in Ursolic acid-  
 177 treated cells, there was a dose-dependent increase in the inhi-  
 178 bition of phosphorylation of GSK3-β (on serine 9), which  
 179 blocked the up-regulation of the pro-apoptotic protein poly  
 180 (ADP-ribose) polymerase (Son et al. 2013). Furthermore,  
 181 these data also showed that this apoptosis was regulated by  
 182 Ursolic acid through the GSK3-β/AKT/mTOR axis, because  
 183 Ursolic acid not only increased the phosphorylation of  
 184 GSK3-β but also down-regulated the Akt/mTOR signaling  
 185 pathway (Son et al. 2013). Ursolic acid has recently been  
 186 shown to also induce differentiation in the U937 leukemia cell  
 187 line, with an increase in the levels of specific monocyte sur-  
 188 face markers (i.e., CD14, CD11b) through the activation of the  
 189 PI3K/Akt pathway (Deng et al. 2014). Moreover, Ursolic acid  
 190 was less toxic than all-*trans* retinoic acid, which has already  
 191 been successfully used as a therapy for acute promyelocytic  
 192 leukemia (Johnson and Redner 2015). However, the  
 193 differentiation-inducing effects of all-*trans* retinoic acid are  
 194 more efficient than those of Ursolic acid (Deng et al. 2014).  
 195 Future studies need to determine the exact mechanism of

Ursolic acid-induced differentiation to promote the develop-  
 ment 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 repre-  
 sents 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

224 used for the treatment of chronic myeloid leukemia (Xiao  
 225 et al. 2002). It is an ingredient of the traditional Chinese  
 226 herbal medicine “*Danggui Longhui Wan*” which is made  
 227 from 11 herbal ingredients (Xiao et al. 2002). Indirubin  
 228 has been shown to inhibit cell growth via the cyclin-  
 229 dependent kinases (CDKs) in several human cancer cells,  
 230 by targeting CDK1/cyclin B, CDK2/cyclin A,  
 231 CDK2/cyclin E, GSK3-β, and CDK5/p25 (Nam et al.  
 232 2013). Indirubin derivatives have been demonstrated to  
 233 inhibit the Signal Transducer and Activator of Transcription  
 234 member 5 (Stat-5) protein and to down-regulate the  
 235 expression of its target proteins Bcl-xL and Mcl-1, which  
 236 are associated with induction of apoptosis (Nam et al.  
 237 2012). This was shown in human K562 chronic myeloid  
 238 leukemia cells, in imatinib-resistant human KCL-22 chronic  
 239 myeloid leukemia cells expressing the T315I mutant  
 240 Bcr-Abl (KCL-22 M), and in CD34-positive primary  
 241 chronic myeloid leukemia cells from patients (Nam et al.  
 242 2012). Therefore, Indirubin derivatives represent a promis-  
 243 ing structural class for the development of new drugs for  
 244 patients with wild-type or T315I mutant Bcr-Abl-positive  
 245 chronic myeloid leukemia. Indirubin derivatives might also  
 246 be useful for the treatment of pediatric solid tumors, be-  
 247 cause it can reduce the invasion of glioma cells and  
 248 glioma-initiating cell-enriched neurospheres both in vitro  
 249 (by modulating β-catenin levels) and in vivo (by improv-  
 250 ing survival in glioma-bearing mice) (Williams et al.  
 251 2011). These data suggest that Indirubin offers a novel  
 252 therapeutic approach that might well be applicable not  
 253 only in pediatric hematological malignancies.

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

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

264 Epothilones were discovered as secondary macrolide me-  
 265 tabolites that are produced by the myxobacterium *Sorangium*  
 266 *cellulosum* (Molnar et al. 2000). Like taxanes, epothilones are  
 267 responsible for microtubule stabilization in vitro (Molnar et al.  
 268 2000). Patupilone, which is also known as Epothilone B, is a  
 269 potent microtubule stabilizer that belongs to this class (Molnar  
 270 et al. 2000). Patupilone binds to β-tubulin with a higher affin-  
 271 ity than taxanes and alters their spindle formation, which re-  
 272 sults in the arrest of mitotic cells, and cell death through apo-  
 273 ptosis (Molnar et al. 2000). In comparison with taxanes,  
 274 Patupilone has been shown to be more potent in its in vivo

anti-cancer activity at tolerated dose levels in two pediatric 275  
 tumor mice models of neuroblastoma and rhabdomyosarcoma 276  
 (Scherzinger-Laude et al. 2013). 277

278 Additionally, a liposomal formulation of Patupilone was 278  
 developed and characterized in both in vitro and in vivo 279  
 studies (Scherzinger-Laude et al. 2013). Patupilone has 280  
 been shown to have a strong anti-tumor effect in both of 281  
 these pediatric cancer models, without triggering major 282  
 side effects. These effects were not seen when Patupilone 283  
 was delivered to integrin-expressing cells using RGD lipo- 284  
 somes. Moreover, tumor targeting of liposomal Patupilone 285  
 enhances its anti-tumor activity in rhabdomyosarcoma 286  
 (Scherzinger-Laude et al. 2013). The potent anti-tumor ef- 287  
 fects and the significantly increasing cumulative survival 288  
 of low-dose Patupilone-RGD-liposomes open the way to 289  
 new pharmacological opportunities for targeting liposomes 290  
 as part of low-dose therapeutics in pediatric oncology. 291

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

295 Marine organisms are another great source of secondary me- 295  
 tabolites. In the near future, the exploration of marine envi- 296  
 ronments will provide new potential natural compounds. To 297  
 date, the new classes of anti-cancer drugs isolated from marine 298  
 organisms have been shown to have cytotoxic activities 299  
 against multiple tumor types by targeting different signaling 300  
 pathways. 301

302 The progression of cell growth is inhibited by 302  
 dolastatins, which were originally identified in the Indian 303  
 Ocean sea hare *Dolabella auricularia* (Garg et al. 2007). 304  
 These are a group of proteins that can inhibit the assembly 305  
 of new microtubules by binding to tubulin and subsequent- 306  
 ly inhibiting tubulin-dependent GTP hydrolysis in vitro 307  
 (Garg et al. 2007). Tasidotin HCl (also known as 308  
 ILX651) is a synthetic pentapeptide (i.e., N,N-dimethyl- 309  
 L-valyl-L-valyl-N-methyl-L-valyl-L-prolyl-L-proline-tert- 310  
 butylamide hydrochloride) (Garg et al. 2007). When ad- 311  
 ministered both orally and i.v., Tasidotin has shown strong 312  
 activity in xenograft mice models of breast and ovarian 313  
 cancer, taxane-resistant ovarian cancer, prostate carcino- 314  
 mas, melanoma, non-small cell lung carcinoma, colon car- 315  
 cinoma, and P388 murine leukemia (Mita et al. 2006). 316  
 Several phase I multi-institution clinical trials of Tasidotin 317  
 have recently been completed in adults (Mita et al. 2006), 318  
 although Tasidotin has not yet been explored for the treat- 319  
 ment of pediatric malignancies. With its promising anti- 320  
 neoplastic activity, metabolic stability, and oral bioavail- 321  
 ability, Tasidotin shows potential for broad therapeutic ap- 322  
 plications. Garg et al. investigated the effects of Tasidotin 323  
 in xenograft mice models of pediatric tumors (e.g., rhab- 324  
 domyosarcoma, Ewing’s sarcoma, synovial sarcoma, 325

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

331 The effects of marine anti-tumorigenic agents have also  
 332 been tested in other pediatric tumor models, including  
 333 acute myeloid leukemia, acute lymphoblastic leukemia,  
 334 and glioma (Potts et al. 2011). Salinosporamide A  
 335 (Marizomib) is a natural  $\beta$ -lactone- $\gamma$ -lactam proteasome  
 336 inhibitor that was derived from the marine actinobacterium  
 337 *Salinispora tropica* (Potts et al. 2011). The proteasome  
 338 represents an important clinical target for the treatment  
 339 of malignancies, and Marizomib has high specificity for  
 340 the proteasome by binding and inhibiting all three proteo-  
 341 lytic protein subunits (i.e.,  $\beta$ 1,  $\beta$ 2,  $\beta$ 5) (Potts et al. 2011).  
 342 A decade ago, the reversible proteasome inhibitor  
 343 Bortezomib was approved for the treatment of relapsed/  
 344 refractory and newly diagnosed multiple myeloma and  
 345 mantle cell lymphoma (Kane et al. 2006), and it represents  
 346 an emerging treatment strategy for acute leukemia  
 347 (Messinger et al. 2012, Niewerth et al. 2013). The relevant  
 348 clinical disadvantages of Bortezomib are related to its un-  
 349 suitability for oral administration, its toxicity profile, and  
 350 the emergence of drug resistance (Kale and Moore 2012).  
 351 Enhanced potency of Salinosporamide A over Bortezomib  
 352 has been shown in human multiple myeloma cell lines  
 353 (Chauhan et al. 2005) and in specimens from patients with  
 354 chronic lymphocytic leukemia (Ruiz et al. 2006).  
 355 Salinosporamide A might be attractive as a proteasome  
 356 inhibitor in several malignancies, including acute leukemia.  
 357 Indeed, tumor cell growth inhibition by Salinosporamide  
 358 A has been evaluated in human CCRF-CEM acute lym-  
 359 phocytic leukemia cells and in two of the Bortezomib-  
 360 resistant sub-lines, CEM/BTZ7 (10-fold resistance to  
 361 bortezomib) and CEM/BTZ200 (123-fold resistance to  
 362 Bortezomib) (Niewerth et al. 2014). These findings show  
 363 that Salinosporamide A is emerging as a potent anti-  
 364 leukemic agent for both parental and bortezomib-resistant  
 365 leukemia cells. At this time, further clinical evaluations of  
 366 its suitability are required.

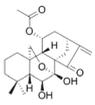
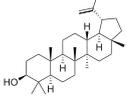
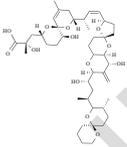
367 **Novel promising plant-derived compounds for pediatric**  
 368 **cancer treatment**

369 To reduce toxicity and side effects due to current chemother-  
 370 apeutic regimens, an increasing number of additional nature-  
 371 derived molecules are currently under investigation to treat  
 372 pediatric tumors (Table 3). Previously, we highlighted the im-  
 373 portance of medicinal plants, which have often been investi-  
 374 gated as sources of new drugs for treating cancer. Due of their  
 375 low toxicity, plant-derived compounds can effectively reduce  
 376 the toxicity that is often encountered in vivo.

*Terminalia chebula* is a member of the *Combretaceae* fam- 377  
 ily that is native to India, and it is already being used in alter- 378  
 native medicine (Kumar et al. 2014). One of its major com- 379  
 ponents is the benzopyran tannin Chebulagic acid, the anti- 380  
 tumor effects, and molecular mechanisms of which were stud- 381  
 ied by Kumar et al. in retinoblastoma cells (Kumar et al. 382  
 2014). In recent years, a dramatic change in the management 383  
 of retinoblastoma has occurred, and several methods have 384  
 now been developed, including focal (e.g., cryotherapy, laser 385  
 photocoagulation, transpupillary thermotherapy, plaque 386  
 brachytherapy), local (e.g., external beam radiotherapy, enu- 387  
 cleation), and systemic (e.g., chemotherapy) methods (Pandey 388  
 2014). All of these can lead to terrible complications that can 389  
 include serious detachment, tearing, holding, traction and vas- 390  
 cular retinal occlusion, and focal paraxial lens opacity (Pandey 391  
 2014). Primary enucleation and chemoreduction have become 392  
 the standard care for advanced intraocular retinoblastoma. 393  
 Chebulagic acid treatment decreases the proliferation of Y79 394  
 cells in a dose-dependent manner (Kumar et al. 2014). The 395  
 treated cells underwent apoptosis through increased expres- 396  
 sion of BAX, decreased expression of Bcl2, release of cyto- 397  
 chrome c, and activation of caspase 3 (Kumar et al. 2014). 398  
 Moreover, Chebulagic acid also induced G1 arrest through 399  
 induction of p27 expression and interference with the NF $\kappa$ B 400  
 pathway (Kumar et al. 2014). Chebulagic acid can also exert 401  
 in vitro angiogenic effects by decreasing cell sprouting in the 402  
 treated rat aortic ring and down-regulation of VEGF produc- 403  
 tion and expression of endothelial specific markers (i.e., 404  
 CD31, E-selectin) (Athira et al. 2013). Further studies will 405  
 be required to determine the efficacy of Chebulagic acid in 406  
 children affected by retinoblastoma and to investigate its 407  
 in vivo anti-tumorigenic effects. 408

409 Among the emerging natural compounds, the naturally oc-  
 410 ccurring plant flavone (4',5,7-trihydroxyflavone), or Apigenin,  
 411 has been increasingly recognized as a cancer chemopreventive  
 412 agent because of its remarkable anti-mutagenic, anti-inflam-  
 413 matory, anti-oxidant, and anti-carcinogenic properties (Birt  
 414 et al. 2001). Apigenin is abundant in common fruit and veg-  
 415 etables, including parsley, onions, oranges, tea, chamomile,  
 416 wheat sprouts, and some seasonings (Birt et al. 2001). Several  
 417 studies have reported that Apigenin can induce apoptosis in a  
 418 wide range of tumor cells, including those derived from pedi-  
 419 atric tumors (e.g., leukemia and neuroblastoma cells), through  
 420 different cell signaling and transduction pathways, like NF $\kappa$ B,  
 421 p53, MAPK, and PI3K/Akt (Patel et al. 2007). Wang et al.  
 422 demonstrated that Apigenin has the highest potency among all  
 423 of the flavonoids they tested for the induction of apoptosis in a  
 424 caspase-dependent manner in HL60 leukemia cells (Wang  
 425 et al. 1999). Subsequently, Budhraj et al. showed that  
 426 Apigenin can induce apoptosis in dose-dependent and time-  
 427 dependent manners in U937 cells (Budhraj et al. 2012). Their  
 428 results suggest a hierarchical model of Apigenin-induced ap-  
 429 optosis in human leukemia cells in which Akt inactivation

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
Chebolic acid		C <sub>14</sub> H <sub>12</sub> O <sub>11</sub>	356.23	Retinoblastoma	<i>In vitro</i>	50	-	(Kumar, Gangappa et al. 2014)
Apigenin		C <sub>15</sub> H <sub>10</sub> O <sub>5</sub>	270.23	Leukemia Neuroblastoma	<i>In vitro</i> and <i>in vivo</i> (animal models)	40-60	20-40	(Torkin, Lavoie et al. 2005) (Budhreja, Gao et al. 2012)
Norcantharidin		C <sub>8</sub> H <sub>8</sub> O <sub>4</sub>	168.15	Medulloblastoma Glioblastoma Leukemia Hepatoblastoma	<i>In vitro</i> and <i>in vivo</i> (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 <sub>44</sub> H <sub>64</sub> O <sub>24</sub>	976.96	Hepatoblastoma Leukemia	<i>In vitro</i> and <i>in vivo</i> (animal models)	600-5000	6.25-25	(Noureini and Wink 2012) (Sun, Xu et al. 2013)
Parthenolide		C <sub>15</sub> H <sub>20</sub> O <sub>3</sub>	248.32	Hepatoblastoma Glioblastoma  Leukemia Osteosarcoma	<i>In vitro</i> and <i>in vivo</i> (animal models)	5-10	10-40	(Sun, Zhang et al. 2014) (Nakabayashi and Shimizu 2012)  (Guzman, Rossi et al. 2005) (Diamanti, Cox et al. 2013) (Kishida, Yoshikawa et al. 2007)
Longikaurin E		C <sub>22</sub> H <sub>30</sub> O <sub>6</sub>	390.47	Pancreatic tumor	<i>In vitro</i>	0,5-4	-	(Cheng, Bo et al. 2015)
Lupeol		C <sub>30</sub> H <sub>50</sub> O	426.73	Pancreatic tumor Osteosarcoma	<i>In vitro</i> and <i>in vivo</i> (animal models)	7.5-120	30-60	(Liu, Bi et al. 2015) (Liu, Bi et al. 2015)
Okadaic acid		C <sub>44</sub> S <sub>68</sub> O <sub>13</sub>	805	Retinoblastoma Neuroblastoma	<i>In vitro</i>	0.005-0.050	-	(Di Fiore, Drago-Ferrante et al. 2013) (Edelstein and Rockwell 2012)
Spongistatin 1		C <sub>65</sub> H <sub>99</sub> ClO <sub>20</sub>	1235.92	Leukemia	<i>In vitro</i>	0.0002-0.001	-	(Schyschka, Rudy et al. 2008)
Variolin b		C <sub>14</sub> H <sub>11</sub> N <sub>7</sub> O	29328	Leukemia	<i>In vitro</i>	0.050-0.10	-	(Simone, Erba et al. 2005)

430 causes activation of JNK signaling, down-regulation of Mcl-1  
431 and Bcl-2, and caspase activation (Budhreja et al. 2012). Fur-  
432 thermore, they also demonstrated that this flavonoid can inhi-  
433 bit tumor growth in vivo, in a U937 cell mice xenograft  
434 model (Budhreja et al. 2012). However, the Akt and JNK  
435 signaling pathways are not the only targets for Apigenin, be-  
436 cause 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 interac-  
tions (Arango et al. 2013). They focused on heterogeneous  
nuclear ribonucleoprotein A2 (hnRNP2), which is an impor-  
tant factor in the progression of tumorigenesis through the  
regulation of splicing, messenger RNA (mRNA) stability,

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444 and mRNA transport (Arango et al. 2013). High expression of  
445 hnRNPA2/B1 has been reported in a variety of human cancers  
446 (Zhou et al. 2001), including glioblastoma (Golan-Gerstl et al.  
447 2011). Arango et al. established that by interacting with the C-  
448 terminal domain of hnRNPA2, Apigenin inhibits hnRNPA2  
449 dimerization and alters the alternative splicing patterns of the  
450 hnRNPA2 substrates in breast cancer cells (Arango et al.  
451 2013). Indeed, the addition of Apigenin to malignant cells  
452 reverts the splicing of caspase-9 and cellular FADD-like IL-  
453 1 $\beta$ -converting enzyme (FLICE)-inhibitory protein (c-FLIP),  
454 which are two key regulators of apoptosis, without acting  
455 against the splice variants in non-carcinogenic cells (Safa  
456 2012). These results might help to explain how Apigenin  
457 has an anti-carcinogenic activity by decreasing the inhibition  
458 of apoptosis (Arango et al. 2013). The effects of Apigenin  
459 have also been studied in human neuroblastoma cells. Torkin  
460 et al. reported that Apigenin inhibits tumor growth and in-  
461 duces caspase-dependent apoptosis not only in vitro but also  
462 in a non-obese diabetic/severe combined immunodeficient  
463 (NOD/SCID) mouse xenograft model, using NUB-7 im-  
464 planted tumors (Torkin et al. 2005). Apigenin induced p53  
465 nuclear accumulation, thus leading to increased expression  
466 of its target proteins (i.e., p21, Bax) (Torkin et al. 2005).  
467 Due to its apoptotic effect being restricted to tumorigenic cells  
468 without toxicity shown to non-transformed neuronal cells,  
469 Apigenin emerged in this study as a potential compound for  
470 further development as a therapeutic agent for patients with  
471 neuroblastoma.

472 Cantharidin is a natural compound that was originally iso-  
473 lated from beetles, and it is one of the most emergent anti-  
474 tumorigenic agents because of its broad therapeutic applica-  
475 tions across a large number of pediatric tumors (Torbeck et al.  
476 2014). The clinical use of Cantharidin has, however, been lim-  
477 ited, because of its severe side effects, with analogs of Canthar-  
478 idin synthesized to reduce this toxicity. Cantharidin and its  
479 derivatives have been shown to have strong in vitro anti-  
480 tumor activities. Norcantharidin is a demethylated form of Can-  
481 tharidin, and it has already been used as a routine anti-cancer  
482 drug in China (Chen et al. 2005). Norcantharidin showed lower  
483 toxicity toward normal cells and was more effective in anti-  
484 cancer activities (Chen et al. 2005, Liao et al. 2007). Canthar-  
485 idin and Norcantharidin express their anti-cancer activities  
486 through inhibition of protein phosphatase 1 (PP1) and PP2A,  
487 which are involved in cell-cycle progression. Norcantharidin is  
488 active in vitro against several tumor cell lines, including hepa-  
489 toma and leukemia cell lines, and its cytotoxic action has been  
490 demonstrated to be approximately 10-fold less than that of  
491 Cantharidin in many of these cell lines (Tarleton et al. 2012).  
492 Norcantharidin has also shown potent anti-metastatic and anti-  
493 angiogenic effects through inhibition of cell migration and  
494 capillary-like tube formation in HUVEC cells, by decreasing  
495 the amount of pro-angiogenic secreted soluble factors (e.g.,  
496 angiotensin, GRO, IGF-1, IL-8, MCP-1, VEGF), and without

497 significant toxicity (Chen et al. 2009). This inhibition was ac-  
498 companied by anoikis, down-regulation of integrin  $\beta$ 1, and  
499 breakdown of vimentin (Chen et al. 2009). Moreover, in vivo  
500 experiments have demonstrated that Norcantharidin prolonged  
501 the survival and reduced the plasma VEGF levels in mice with  
502 pulmonary metastasis without showing significant renal and  
503 liver toxicity (Chen et al. 2009). Therefore, with its ability to  
504 pass through the blood-brain barrier, Norcantharidin has been  
505 investigated for pediatric brain tumors in vitro and in vivo. In  
506 medulloblastoma cell lines, Norcantharidin treatment impaired  
507 the growth of DAOY and UW228 cells by affecting Wnt/ $\beta$ -  
508 catenin signaling through nuclear  $\beta$ -catenin loss (Cimmino  
509 et al. 2012). The efficacy of Norcantharidin to decrease medul-  
510 loblastoma tumor growth was also assessed in vivo using medul-  
511 loblastoma orthotopic brain-cerebellum xenograft animal  
512 models. These results showed significantly reduced tumor size  
513 in the treated mice (Cimmino et al. 2012). Norcantharidin also  
514 inhibited cell growth by impairment of the Raf/MEK/ERK  
515 pathway in a dose-dependent manner, and it induced apoptosis  
516 in glioma cells through down-regulation of the pro-apoptotic  
517 proteins Bcl-2 and Mcl-1 (Zheng et al. 2014). Liao et al. devel-  
518 oped a model to induce cancer progression via PMAI in a  
519 human T cell leukemia cell line and then defined the mecha-  
520 nisms behind Norcantharidin for the inhibition of cell growth  
521 and induction of cell-cycle arrest (Liao et al. 2011). The results  
522 of this study showed that Norcantharidin can significantly in-  
523 hibit the viability of these treated cells by induction of cell-cycle  
524 arrest at the G2/M phase, down-regulation of the expression of  
525 calcineurin, and attenuation of interleukin (IL)-2 production  
526 (Liao et al. 2011). These results provide important information  
527 about the possible use of Norcantharidin as a chemopreventive  
528 agent to inhibit leukemia progression (Liao et al. 2011). Re-  
529 cently, Norcantharidin was shown to have anti-tumor activity  
530 also through modulation of the tumor microenvironment in  
531 hepatocellular carcinoma models (Lu et al. 2014). Macro-  
532 phages are known to take part in the tumor microenvironment  
533 and to have a dual role in tumor development and progression  
534 (Lamagna et al. 2006). Classically, activated M1 macrophages  
535 have anti-tumor effects through the release of pro-inflammatory  
536 cytokines. On the contrary, alternatively activated M2 macro-  
537 phages facilitate the progression of tumors, through the release  
538 of anti-inflammatory cytokines (Sica et al. 2008). As a result,  
539 tumor-associated macrophages have been the target for cancer  
540 treatments. Norcantharidin inhibited tumor growth in  
541 hepatoma-bearing mice through the  $\beta$ -catenin signaling path-  
542 way and subsequently by shifting from M2 macrophage to M1  
543 macrophage polarization (Lu et al. 2014).

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

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

575 Parthenolide is another promising plant-derived candidate  
576 for cancer chemoprevention. This is a sesquiterpene lactone  
577 that was originally purified from the shoots of the feverfew,  
578 *Tanacetum parthenium* (Ghantous et al. 2013), that has shown  
579 anti-cancer and anti-inflammatory activities in several cell  
580 lines derived from pediatric tumors (Gach et al. 2015). These  
581 biological properties of Parthenolide can be attributed to its  
582 strong inhibition of NF $\kappa$ B (Bork et al. 1997). It was also  
583 shown recently that Parthenolide can induce growth inhibi-  
584 tion, autophagy, and caspase-mediated cell death in  
585 hepatoblastoma cells (Sun et al. 2014). Further, Parthenolide  
586 has been shown to suppress cell proliferation and invasion,  
587 and tumor-induced angiogenesis in vitro, and to inhibit  
588 neovascularity and tumor growth in vivo in glioblastoma mice  
589 models (Nakabayashi and Shimizu 2012). It has been sug-  
590 gested that this anti-tumor function of Parthenolide might be  
591 mediated not only by inhibition of NF $\kappa$ B but also by inhibi-  
592 tion of Akt signaling (Nakabayashi and Shimizu 2012). It is of  
593 note that Parthenolide effectively eradicated acute myeloid  
594 leukemia stem cells and progenitor cells in vitro while sparing  
595 normal hematopoietic stem cells (Guzman et al. 2005). Thus,  
596 Parthenolide has been investigated as a potential chemothera-  
597 peutic agent in acute myeloid leukemia and acute B-  
598 lymphoblastic leukemia cells (Guzman et al. 2005). Treat-  
599 ments for pediatric patients with leukemia are now increasing-  
600 ly successful. However, approximately 20 % of children with  
601 acute lymphoblastic leukemia relapse because of the failure to  
602 eradicate the disease, and most of these patients do not

603 survive. Parthenolide was reported for the first time to eradi-  
604 cate multiple leukemia-initiating cell populations in vivo in  
605 childhood acute lymphoblastic leukemia (Diamanti et al.  
606 2013). For this reason, Parthenolide might have therapeutic  
607 potential in childhood acute lymphoblastic leukemia, which  
608 would provide a basis for the development of effective thera-  
609 pies that can eradicate leukemia-initiating cell populations, to  
610 prevent disease progression and reduce relapse (Diamanti  
611 et al. 2013). Osteosarcoma is a highly aggressive primary  
612 bone tumor that predominantly affects children and typically  
613 demonstrates significant resistance to radiotherapy (Fuchs and  
614 Pritchard 2002). Consequently, the conventional treatments  
615 do not prevent metastatic progression in 30 to 40 % of patients  
616 (Lisle et al. 2008). To obtain more effective treatments, it  
617 might be necessary to target the mechanisms used by osteo-  
618 sarcoma cells to acquire significant resistance to radiotherapy.  
619 This radio-resistance in the SaOS2 osteosarcoma cell line has  
620 been demonstrated to be subsequent to up-regulation of NF $\kappa$ B  
621 (Eliseev et al. 2005). As Parthenolide affects NF $\kappa$ B (Kishida  
622 et al. 2007), it can potentially provide a clinical method to  
623 decrease NF $\kappa$ B in patients with osteosarcoma, which would  
624 allow the radiation therapy to have an effective role in this  
625 cancer treatment. The in vitro treatment of these re-  
626 sensitized cancer stem cells and the entire cell population with  
627 radiotherapy has also been investigated in osteosarcoma. The  
628 results have indicated that Parthenolide and ionizing radiation  
629 act synergistically to induce cell death in LM7 osteosarcoma  
630 cells (Zuch et al. 2012). When used together with the stan-  
631 dard therapeutic regimen, Parthenolide has been pro-  
632 posed to increase the sensitivity of these cancerous cells  
633 to radiotherapy, to provide more complete eradication of  
634 the malignant tissue. In conclusion, Parthenolide has  
635 been identified as a new therapeutic agent for glioblas-  
636 toma, leukemia, and hepatoblastoma in children, al-  
637 though the promise that Parthenolide holds for therapy  
638 is still limited. This is because of different factors, in-  
639 cluding its off-target effects and hydrophobicity, which  
640 limits its solubility, and thus its oral bioavailability. In  
641 the near future, new strategies will be implemented in-  
642 volving low pharmacological doses of Parthenolide ei-  
643 ther alone or in combination with other drugs, which  
644 will define its anti-tumorigenic potential both in vitro  
645 and in vivo.

646 Longikaurin E is a novel natural compound that is derived  
647 from the herbal medicine *Rabdosia longituba* (Cheng et al.  
648 2015), and that has shown anti-proliferative and pro-apoptotic  
649 effects in pancreatic cancer cell lines (Cheng et al. 2015).  
650 Pancreatic tumors are very rare at pediatric ages, and surgery  
651 remains the keystone to treat this tumor in children, as in  
652 adults (Dall'igna et al. 2010). Cheng et al. showed that  
653 Longikaurin E induces apoptosis of pancreatic cancer cells  
654 by up-regulation of Bax and down-regulation of Bcl-xL,  
655 Bcl-2, Survivin, and c-Myc (Cheng et al. 2015). They also

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

666 In pancreatic cancer, in addition to Longikaurin E, the anti-  
 667 tumorigenic effects of Lupeol, a dietary triterpene, have been  
 Q25 668 investigated (Liu et al. 2015a, b). Lupeol is found in fruit (such  
 669 as olives, mangos, strawberries, grapes, and figs), in vegeta-  
 670 bles, and in medicinal plants (Chaturvedi et al. 2008). Liu  
 671 et al. showed that Lupeol has anti-proliferative and pro-  
 672 apoptotic effects on pancreatic cancer both in vitro and  
 673 in vivo (Liu et al. 2015a, b). Lupeol induced cell-cycle arrest  
 674 by up-regulation of p21 and p27 and down-regulation of cy-  
 675 clin D1. Interestingly, following Lupeol treatment in vivo,  
 676 they showed decreased tumor growth and down-regulation  
 677 of p-ERK and p-Akt in tumor tissues (Liu et al. 2015a, b).  
 678 Lupeol has also been reported to have anti-cancer efficacy  
 679 against chemoresistant pancreatic cancer cells through modu-  
 680 lation of TRAIL/cFLIP, both in vitro and in vivo in athymic  
 681 mice (Murtaza et al. 2009). The anti-cancer effects of Lupeol  
 682 have also been investigated in osteosarcoma, where it induced  
 683 apoptosis and cell-cycle arrest of human osteosarcoma cells  
 684 by targeting the PI3K/Akt/mTOR pathway (Liu et al. 2015a,  
 685 b). Moreover, Lupeol administration in vivo decreased tumor  
 686 growth and had no effects on the function of the liver and  
 687 kidneys (Liu et al. 2015a, b). These data suggested a potential  
 688 role for Lupeol in the treatment of pancreatic cancer and  
 689 osteosarcoma.

690 **Novel promising marine compounds for pediatric cancer**  
 691 **treatment**

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

700 Several phytoplanktonic species accumulated by shellfish  
 701 are responsible for the production of Okadaic acid, a lipophilic  
 702 toxin that causes human diarrheic shellfish poisoning, which  
 703 although not lethal, causes gastrointestinal symptoms that in-  
 704 clude diarrhea (92 %), nausea (80 %), vomiting (79 %), ab-  
 705 dominal pain (53 %), and chills (10 %) (Valdiglesias et al.  
 706 2013). These symptoms vary according to the dose of toxin

707 ingested. The minimum dose of Okadaic acid responsible for  
 708 this poisoning in human is about 40 mg (Valdiglesias et al.  
 709 2013). Several classes of protein serine/threonine phosphatases  
 710 that regulate cell growth, division, and death and main-  
 711 tenance of the cytoskeletal structure are targets of this toxin.  
 712 Indeed, Okadaic acid might cause inhibition of protein phos-  
 713 phatases, particularly PP1 and PP2A, but also other types,  
 714 including PP4, PP5, and PP2B (Louzao et al. 2005). The most  
 715 reported cytotoxic effect of Okadaic acid is apoptosis induc-  
 716 tion, while it is known to cause growth inhibition and apopto-  
 717 sis and to induce oxidative stress in different cell types  
 718 (Valdiglesias et al. 2013). Nevertheless, the genotoxic and  
 719 cytotoxic effects caused by Okadaic acid might be responsible  
 720 for genomic instability that can lead to severe pathologies,  
 721 including cancer (Valdiglesias et al. 2013). Despite these cy-  
 722 totoxic and genotoxic effects, Okadaic acid might still repre-  
 723 sent a novel candidate for neuroblastoma and retinoblastoma  
 724 treatment in children. Edelstein et al. showed that in neuro-  
 725 blastoma cells, PPA2 inhibition by Okadaic acid induced Akt  
 726 hyperphosphorylation, increased levels of ubiquitinated pro-  
 727 teins, oxidative stress, loss of cell viability, and cell death  
 728 (Edelstein and Rockwell 2012). Furthermore, they demon-  
 729 strated that Rapamycin can enhance Okadaic acid-induced  
 730 Akt phosphorylation, to increase the susceptibility of cells to  
 731 pro-oxidant cell death caused by Okadaic acid (Edelstein and  
 732 Rockwell 2012). The effects of Okadaic acid in combination  
 733 with Parthenolide were investigated in human retinoblastoma  
 734 cells (Di Fiore et al. 2013). These two compounds acted to-  
 735 gether to induce cytotoxicity in Y79 cells by lowering p-Akt  
 736 levels, stabilizing p53, increasing reactive oxygen species, and  
 737 concurrently lowering glutathione content (Di Fiore et al.  
 738 2013). These results provide strong support for a combined  
 739 treatment approach to target the PTEN/Akt/mDM2/p53 path-  
 740 way in this childhood tumor (Di Fiore et al. 2013). The mo-  
 741 lecular effects of Okadaic acid appear to be cell-type depen-  
 742 dent. Valdiglesias et al. showed that Okadaic acid can produce  
 743 oxidative DNA damage both in human peripheral leukocytes  
 744 and human neuroblastoma cells but not in human  
 745 hepatoblastoma cells (Valdiglesias et al. 2011). They conclud-  
 746 ed that the mechanism leading to DNA damage is highly  
 747 dependent on cell type. Other studies will be necessary to  
 748 identify the exact mechanisms of action of Okadaic acid in  
 749 different tumorigenic cell lines.

750 Spongistatin 1 is a macrocyclic lactone that was isolated  
 751 from the marine sponges *Spirastrella spinispirulifera* and  
 752 *Hyrtios erecta* as a cytotoxic antimetabolic compound (Bai  
 753 et al. 1993). It can block the cell cycle in the mitotic phase  
 754 in L120 murine leukemia cells (Bai et al. 1993). Spongistatin  
 755 1 has been shown to induce cell death in Jurkat leukemia T  
 756 cells and, more importantly, in primary acute leukemic cells  
 757 derived from patients (Schyschka et al. 2008). Furthermore,  
 758 inhibitor-of-apoptosis proteins (IAPs) are overexpressed in  
 759 cancer cells and in primary tumor biopsy samples (Salvesen

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

768 Variolin B is a novel marine natural product that was  
 769 isolated from the sponge *Kirkpatrickia variolosa*, which is  
 770 found in Antarctica (Dembitsky et al. 2005). As it has  
 771 been shown to have pro-apoptotic activity, Variolin B rep-  
 772 resented a new potent natural cytotoxic agent (Dembitsky  
 773 et al. 2005). The use of Variolin B is limited by its low  
 774 stability in solution. However, the development of a  
 775 Deoxy-variolin B analog that is much more stable and  
 776 soluble has overcome this limitation of the parent com-  
 777 pound (Anderson et al. 2005). The biological activity of  
 778 this deoxy analog was similar to that of Variolin B, with a  
 779 slight increase in potency of the deoxy analog that is  
 780 likely to be related to its greater stability and solubility  
 781 (Anderson et al. 2005). The biological properties of  
 782 Variolin B and its deoxy analog, and their mechanisms  
 783 of action, have been investigated in detail in several tu-  
 784 morigenic cells, including the K562 human leukemic and  
 785 Jurkat cell lines (Simone et al. 2005). Both of these com-  
 786 pounds inhibited colony formation and caused cell-cycle  
 787 perturbations through increased p53 tumor suppressor  
 788 protein function and p21, thus definitively inducing apo-  
 789 ptosis (Simone et al. 2005). However, the increase in p53  
 790 appears to be due to cellular stress induced by the dose of  
 791 the treatment and does not appear to be crucial for the  
 792 biological effects of Variolin B and its deoxy analog. In-  
 793 deed, there were no differences seen in vitro in comparing  
 794 the cytotoxicity combined with the cell-cycle perturba-  
 795 tions induced by Variolin B and its deoxy analog in cell  
 796 lines expressing wild-type p53 and within sub-lines that  
 797 had no p53 expression or contained an inactivated p53  
 798 isoform (Simone et al. 2005). Moreover, Variolin B and  
 799 its deoxy analog prevented the cells from entering S phase  
 800 by inhibition of the CDK1/cyclin B, CDK2/cyclin A, and  
 801 CDK2/cylin E complexes (Simone et al. 2005). In conclu-  
 802 sion here, these Variolins can be considered as a new class  
 803 of CDK inhibitors that activate apoptosis in a p53-  
 804 independent manner, and thus they might be effective  
 805 against not only pediatric tumors but also several cancers  
 806 with p53 mutations or deletions.

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

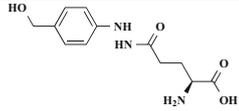
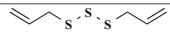
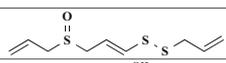
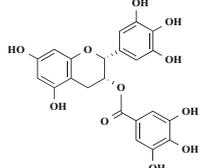
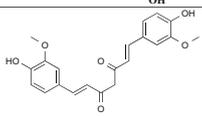
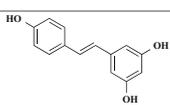
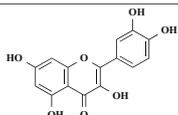
**Nutraceutical and functional foods as anti-cancer drugs 813**  
**to support pediatric cancer treatment 814**

815 Several natural compounds ingested in the diet are known to  
 816 have anti-tumorigenic effects. As many nutraceuticals and  
 817 functional foods have been demonstrated to have anti-cancer  
 818 actions, they might represent novel agents for the treatment of  
 819 pediatric cancers (Table 4). 819Q26

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

827 The *Basidiomycete* fungus *Agaricus blazei* Murill is an  
 828 edible mushroom that is cultivated in Japan, China, and  
 829 Brazil and belongs to the *Agaricaceae* family (Kim et al.  
 830 2009). It is already used in association with standard che-  
 831 motherapeutics in Japan for cancer therapy (Yoshimura  
 832 et al. 2005). Extracts of *A. blazei* have shown inhibition  
 833 of cell growth and induction of apoptosis in several types  
 834 of tumorigenic cells. Its anti-proliferative effects are partly  
 835 mediated by apoptosis, as reported for different leukemic  
 836 cells, although not in normal cells, which has suggested a  
 837 tumor-selective action (Kim et al. 2009). These effects  
 838 have been confirmed in vivo, with inhibition of tumor  
 839 growth of human acute promyelocytic leukemia cells in  
 840 nude mice following administration of *A. blazei* extracts  
 841 (Kim et al. 2009). Later, Agaritine was discovered as one  
 842 of the *A. blazei* components responsible for these anti-  
 843 leukemic properties (Akiyama et al. 2011). Agaritine puri-  
 844 fied from *A. blazei* directly suppressed cell proliferation  
 845 through induction of apoptosis in several leukemic cell  
 846 lines and showed no effects on normal lymphatic cells  
 847 (Akiyama et al. 2011). However, Agaritine is not the only  
 848 interesting physicochemical agent in these *A. blazei* ex-  
 849 tracts, with the demonstration of induction of caspase-  
 850 dependent apoptosis in osteosarcoma cells using the poly-  
 851 saccharide ABP-Ia from *A. blazei* (Wu et al. 2012). This  
 852 study also showed no apoptotic effects for a normal hu-  
 853 man osteoblast cell line, highlighting again the targeted  
 854 action of these agents toward tumorigenic cells (Wu  
 855 et al. 2012). The effects of *A. blazei* extracts on sarcoma  
 856 have been investigated in vivo using the  $\alpha$ -(1-4)-  
 857 glucan- $\beta$ -(1-6)-glucan-protein complex polysaccharide  
 858 alone or in association with the chemotherapeutic agent  
 859 5-fluorouracil (Gonzaga et al. 2009). These data showed  
 860 strong inhibition of tumor growth and improvement of  
 861 survival in sarcoma-180-bearing mice, while no changes  
 862 were seen for the renal, liver, and hematological param-  
 863 eters, which indicated the absence of toxicological effects  
 864 in the treated mice (Gonzaga et al. 2009). Of note, the

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 <i>in vitro</i> and/or <i>in vivo</i>	Dose <i>in-vitro</i> (μM)	Dose <i>in-vivo</i> (mg/kg)	References
Agaritine		C <sub>12</sub> H <sub>17</sub> N <sub>3</sub> O <sub>4</sub>	267.28	Leukemia	<i>In vitro</i>	10 (μg/ml)	-	(Akiyama, Endo et al. 2011)
Diallyl trisulfide		C <sub>6</sub> H <sub>10</sub> S <sub>3</sub>	178.33	Hepatoblastoma Glioma	<i>In vitro</i> and <i>in vivo</i> (animal models)	100-200	0.010-10	(Iciek, Kwiecien et al. 2012) (Wallace, Haar et al. 2013)
Ajoene		C <sub>9</sub> H <sub>14</sub> OS <sub>3</sub>	234.4	Leukemia	<i>In vitro</i>	40	-	(Hassan 2004)
Epigallocatechin gallate		C <sub>22</sub> H <sub>18</sub> O <sub>11</sub>	458.37	Neuroblastoma Hepatoblastoma	<i>In vitro</i>	10-100	-	(Nishimura, Hartomo et al. 2012) (Godeke, Maier et al. 2013)
Curcumin		C <sub>21</sub> H <sub>20</sub> O <sub>6</sub>	368.38	Medulloblastoma Neuroblastoma	<i>In vitro</i> and <i>in vivo</i> (animal models)	10-40	1.0	(Lee, Krauthauser et al. 2011) (He, Li et al. 2014) (Picone, Nuzzo et al. 2014)
Resveratrol		C <sub>14</sub> H <sub>12</sub> O <sub>3</sub>	228.24	Leukemia Neuroblastoma	<i>In vitro</i> and <i>in vivo</i> (animal models)	200	20	(Ge, Liu et al. 2013) (Soto, Hank et al. 2011)
Quercetin		C <sub>15</sub> H <sub>10</sub> O <sub>7</sub>	302.23	Neuroblastoma	<i>In vitro</i>	20-40	-	(Sugantha Priya, Selvakumar et al. 2014)

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

872 Another interesting mushroom with anti-cancer properties  
 873 is *Ganoderma sinensis*, which is widely used in China as a  
 874 herbal medicine (Zhou et al. 2007). Among its components,  
 875 triterpenoid-enriched lipids have emerged due to their immunomodulatory  
 876 and cytotoxic effects. Dose-dependent suppression of proliferation  
 877 of leukemia and hepatoblastoma cells has been described for a  
 878 lipid extract of this *G. sinensis* mushroom, known as GL6.  
 879 Furthermore, the same GL6 was shown to induce human monocytes  
 880 and immunosuppressive M2 macrophages to release pro-inflammatory  
 881 cytokines (Yue et al. 2008). This feature makes this mushroom-derived  
 882 compound a novel anti-cancer and immune-modulatory agent  
 883 (Yue et al. 2008). However, careful clinical studies are still  
 884

885 required to determine whether these mushrooms do indeed  
 886 provide concrete benefits to patients, due to the toxicological  
 887 problems associated with their use. Additionally, *in vivo* data  
 888 will be necessary to investigate their molecular mechanisms  
 889 and their safety.

890 Another promising anti-tumorigenic natural compound is  
 891 contained in foods such as Garlic (*Allium sativum*), a bulbous  
 892 plant that is easy to grown in mild climates. Among its  
 893 components, Diallyl trisulfide and Ajoene are of importance  
 894 not only for their remarkable anti-tumor and cancer-preventive  
 895 effects, as suggested by many *in vitro* and *in vivo* studies,  
 896 but also because of their many health benefits, like improvements  
 897 to immune-system function, radio-protection, and protection  
 898 against microbial infections (BayanBayan et al. 2014). These  
 899 features make them excellent candidates for the treatment of  
 900 pediatric cancers. Indeed, Diallyl trisulfide has been demonstrated  
 901 to arrest HepG2 hepatoblastoma cell proliferation by inducing  
 902 caspase-3 activity, enhancing H<sub>2</sub>O<sub>2</sub> levels, and strongly decreasing  
 903 glutathione levels (Iciek et al. 2012). Moreover,  
 904

905 anti-cancer effects of Diallyl trisulfide have been shown in  
 906 glioblastoma, with the reduction of cancer progression in  
 907 U87/MG ectopic tumors in SCID mice (Wallace, G. C. t  
 908 et al. 2013). As Diallyl trisulfide has not shown any nega-  
 909 tive impact on hepatic functions, it can be considered an  
 910 effective therapeutic agent for the prevention of tumor  
 911 progression in glioblastoma (Wallace, G. C. t et al.  
 912 2013). Instead, Ajoene has been shown not only to inhibit  
 913 cell proliferation and induce caspase-dependent apoptosis  
 914 of several human myeloid leukemia cells but also to  
 915 strongly enhance the apoptotic effects of two chemothera-  
 916 peutic drugs: cytarabine and fludarabine (Hassan 2004).  
 917 This was observed for the treatment of human CD34-  
 918 positive resistant myeloid leukemia cells, through enhance-  
 919 ment of their Bcl-2 inhibitory and caspase-3 activation  
 920 activities (Hassan 2004). Thus, these food-derived natural  
 921 compounds can be considered as potent agents with  
 922 cancer-preventive properties.

923 One of the commonest popular beverages is green  
 924 tea. The correlation between its consumption and inhi-  
 925 bition of the growth of a number of cancers is already  
 926 known (Kanadzu et al. 2006). Green tea contains at  
 927 least four catechins: epigallocatechin gallate (EGCG),  
 928 epigallocatechin, epicatechin gallate, and epicatechin  
 929 (Kanadzu et al. 2006). The most abundant agent in  
 930 green tea is EGCG. Its anti-cancer effects have been  
 931 investigated also in neuroblastoma. Tumor-initiating  
 932 cells were recently identified in neuroblastoma as  
 933 spheres grown in the serum-free nonadherent culture  
 934 used for neural-crest stem-cell growth. EGCG inhibited  
 935 growth and induced apoptosis in BE(2)-C neuroblastoma  
 936 cells in a dose-dependent manner, although it did not  
 937 have the same biological effects against such spheres  
 938 derived from BE(2)-C cells (Nishimura et al. 2012).  
 939 However, EGCG inhibited the formation of these  
 940 spheres. So, it will be important to clarify the molecular  
 941 basis behind the EGCG-sensitive sphere formation in  
 942 neuroblastoma cells to determine its future clinical ap-  
 943 plications in children with neuroblastoma (Nishimura  
 944 et al. 2012). Moreover, EGCG has also been demon-  
 945 strated to inhibit hepatoblastoma cell growth in a time-  
 946 dependent and dose-dependent manner while leaving  
 947 normal fibroblasts unaffected (Godeke et al. 2013).  
 948 The treated hepatoblastoma cells showed impairment of  
 949 the *WNT* signaling pathway (Godeke et al. 2013). This  
 950 is known to enhance cell proliferation, with subsequent  
 951 reduction in mRNA levels of its main target genes, *c-*  
 952 *MYC* and *CCND1*, together with re-expression of the  
 953 tumor suppressor gene *SFRP1*, which is usually silenced  
 954 in these cells, thus impairing its known function: to  
 955 down-regulate *WNT* signaling (Godeke et al. 2013).  
 956 For these reasons, EGCG should be further investigated  
 957 for its potential clinical applications in the therapy of

hepatoblastoma, mainly because of its selective actions 958  
 toward tumorigenic cells. 959

**Functional foods as anti-cancer drugs for pediatric cancer 960  
 treatment 961**

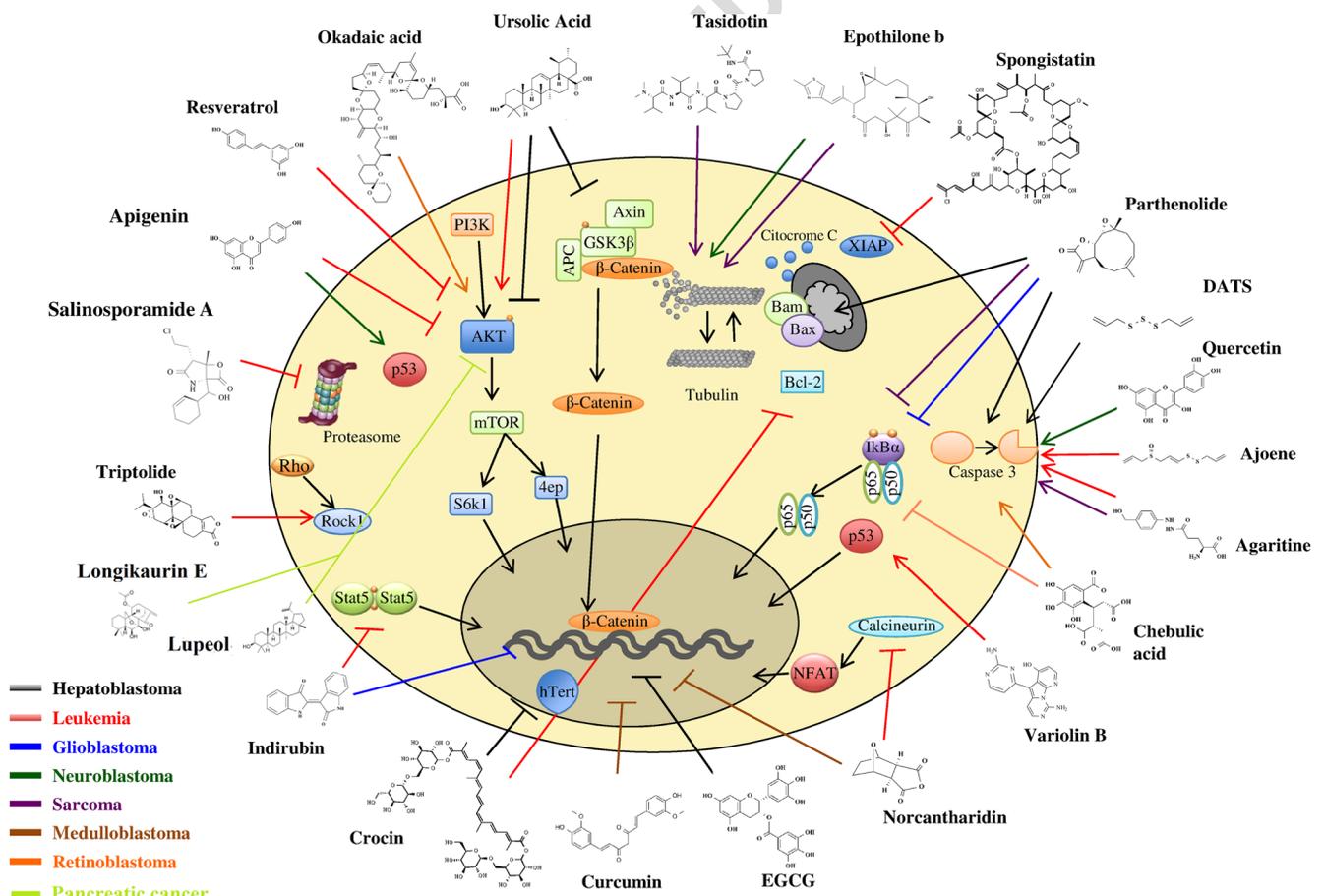
962 The available literature has emphasized the potential advan-  
 963 tages of functional foods as chemopreventive agents, both  
 964 alone and in association with standard chemotherapeutics.  
 965 Several studies have focused on the anti-tumorigenic effects  
 966 of Curcumin (also known as Turmeric), a polyphenolic agent  
 967 derived from the *Curcuma longa* plant. This plant has been  
 968 used in Ayurvedic medicine for centuries because of its non-  
 969 toxic effects and its therapeutic properties, which include anti-  
 970 cancer activity (Villegas et al. 2011). Indeed, Curcumin has  
 971 been shown to have anti-proliferative effects in multiple can-  
 972 cers, including pediatric tumors, through targeting several  
 973 pathways involved in tumorigenesis, including the MAPK,  
 974 PI3K, and NFκB signaling pathways, and impairing metasta-  
 975 sis formation (Heger et al. 2014);(Villegas et al. 2011). One of  
 976 the most interesting features that makes Curcumin an ideal  
 977 compound to treat pediatric brain tumors is that it can cross  
 978 the blood–brain barrier due to its high lipophilic properties  
 979 (Marchiani et al. 2014). In addition, Curcumin has been  
 980 shown to induce apoptosis in vitro in human medulloblastoma  
 981 cells, and to reduce tumor growth, thus increasing survival  
 982 rates in vivo in mouse models of medulloblastoma, without  
 983 showing any toxic effects (Lee et al. 2011). Curcumin has  
 984 been shown to bind the phosphorylated form of the protein  
 985 Cdc27, and the data that have been reported indicate that due  
 986 to the phosphorylated Cdc27, the fast-growing medulloblas-  
 987 toma cells were more susceptible to Curcumin-induced cell  
 988 death than those that only had non-phosphorylated Cdc27  
 989 (Lee and Langhans 2012). These results not only provided a  
 990 possible explanation for the selective mechanism of action of  
 991 Curcumin, but they also suggested that the phosphorylation  
 992 status of Cdc27 might be developed as a biomarker to predict  
 993 which patients might respond favorably to Curcumin-based  
 994 cancer therapy (Lee and Langhans 2012). Recently, the im-  
 995 pairment of the Wnt/β-catenin signaling pathway by  
 996 Curcumin in medulloblastoma cells was reported (He et al.  
 997 2014). In particular, this led to GSK-3β activation and subse-  
 998 quent nuclear β-catenin down-regulation (He et al. 2014).  
 999 Moreover, Curcumin anti-tumorigenic effects were also  
 1000 shown in neuroblastoma cells (Picone et al. 2014). These  
 1001 treated cells showed a rapid increase in reactive oxygen spe-  
 1002 cies, decrease in mitochondrial membrane potential, increase  
 1003 in the pro-apoptotic protein Bcl-2-associated death promoter  
 1004 (BAD), inhibition of Akt signaling, and activation of the pro-  
 1005 apoptotic proteins p27, Bim, and the Fas-L tumor necrosis  
 1006 factor family protein (Picone et al. 2014). All of these events  
 1007 together suggest the induction of apoptosis in these neuroblas-  
 1008 toma cells (Picone et al. 2014). Indeed, altogether, these

1009 results provide evidence for considering Curcumin as an ideal  
1010 therapeutic agent for children with brain tumors.

1011 Additional attention has been given to Resveratrol (i.e.,  
1012 *trans*-3, 5, 40-trihydroxystilbene), which is a phytoalexin that  
1013 is found in many plants, including those often consumed by  
1014 human, such as grapes, peanuts, and berries (Carter et al.  
1015 2014). It is not only found in these plants but also in processed  
1016 products from them, like wine (Carter et al. 2014). Several  
1017 in vitro studies have shown that Resveratrol has multiple  
1018 anti-tumorigenic effects through the promotion of cell-cycle  
1019 arrest and induction of apoptosis of tumorigenic cells, by in-  
1020 hibition of tumor growth and cell motility (Carter et al. 2014).  
Q27 1021 The anti-leukemic effects of were reported by Ge et al., who  
1022 showed that Resveratrol induces cell-cycle arrest, apoptosis,  
1023 and autophagy in acute T-lymphoblastic leukemia cells  
1024 through inhibition of the Akt/mTOR/p70S6K/4E-BP1 path-  
1025 way and activation of the p38-MAPK signaling pathway  
1026 (Ge et al. 2013). Furthermore, the anti-tumor effects of Res-  
1027 veratrol have also been described in in vivo neuroblastoma  
1028 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 neuro-  
blastoma mice models, while Resveratrol alone only induced  
tumor regression in primary sites. Indeed, the mice treated  
with Resveratrol alone developed tumor recurrence and me-  
tastasis (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 neu-  
roblastoma 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

1049 (Sugantha Priya et al. 2014). Further studies are required to  
 1050 investigate the exact mechanism of Quercetin action, toward  
 1051 the development of novel therapeutic approaches in neuro-  
 1052 blastoma treatment in vivo.

1053 **Conclusions**

1054 In recent years, great contributions to childhood cancer thera-  
 1055 py have been made through the use of natural compounds.  
 1056 Despite the technological progress in combinatorial chemistry,  
 1057 synthetic anti-cancer drugs continue to be responsible for non-  
 1058 selective killing of cells. As natural compounds have been  
 1059 shown to offer therapeutic actions with low cytotoxicity in  
 1060 several studies, and due to their chemical diversity, structural  
 1061 complexity, and biological potency, they should be considered  
 1062 further as major sources of drug leads, especially for the treat-  
 1063 ment of childhood cancers. These compounds are still provid-  
 1064 ing important impacts on drug discovery as they act as tem-  
 1065 plates for the construction of novel nature-derived molecules,  
 1066 with the challenge being to enhance the biological properties  
 1067 and reduce the side effects.

1068 Despite recent progress in pediatric tumor treatment, cancer  
 1069 continues to be the major reason for childhood mortality, and  
 1070 conventional therapies are responsible for poor health-related  
 1071 quality of life and chronic health conditions in childhood can-  
 1072 cer survivors. Due to their potential to affect every tissue,  
 1073 current non-selective therapies might have indeterminate  
 1074 long-term effects in young children. Thus, future studies  
 1075 should better identify and manage the functional outcomes  
 1076 of childhood cancer survivors because of their emotional dis-  
 1077 tress (e.g., depressive symptoms, anxiety, increased somatiza-  
 1078 tion) and their reduced healthcare due to long-term side effects  
 1079 of current therapies (Kinahan et al. 2012). Thus, natural com-  
 1080 pounds, including dietary factors, nutraceuticals, and especial-  
 1081 ly functional foods (e.g., Quercetin, Resveratrol, EGCG,  
 1082 Diallyl trisulfide, Ajoene), are of significance here. As a result  
 1083 of their low toxicity and targeting of a wide range of signaling  
 1084 pathways involved in tumorigenesis, such natural compounds  
 1085 can be used to decrease these therapy-related side effects in  
 1086 children (as shown in Fig. 1).

1087 The introduction of such bioactive, nature-derived agents  
 1088 into clinics has changed the outcome of several types of pedi-  
 1089 atric cancers. Nevertheless, many efforts need to be focused  
 1090 on drug discovery of further new natural compounds, to iden-  
 1091 tify new drugs derived from novel natural products. This will  
 1092 also increase their efficacy and reduce their side effects in  
 1093 children, with the need to also facilitate their handling and  
 1094 tolerability by developing oral regimens and/or liposomal for-  
 1095 mulation. Plants still represent a great source for natural com-  
 1096 pounds in drug discovery but microorganisms and marine-  
 1097 derived compounds will have governing roles in the future,  
 1098 with a large number of promising molecules that are already

undergoing clinical development as anti-cancer agents for 1099  
 childhood tumors. 1100

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 Roberta Migliorati (Hospital SantoBono-Pausillipon, Naples, Italy), a 1106  
 pediatric oncologist who died of cancer in 2014. 1107  
 1108

**Compliance with ethical standards** 1109

**Conflict of interest** The authors declare that they have no competing 1110  
 interests. 1111

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