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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 ₄₆ H ₅₆ N ₄ O 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) |
| t1.4 | Vinblastine | C ₄₆ H ₅₈ N ₄ O 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) (Salemi, Bates et al. 2010) |
| 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) |
| t1.6 | Irinotecan | C ₃₃ H ₃₈ N ₄ O ₆ | DNA topoisomerase I | Neutropenia Gastrointestinal disorders Hypersensitivity | Rhabdomyosarcoma Neuroblastoma Medulloblastoma Ependymoma | 25–130 mg/m ² | (Turner, Gururangan et al. 2002) (Ma and McLeod 2003) (Kim, Kang et al. 2013) |
| t1.7 | Topotecan | C ₂₃ H ₂₃ N ₃ O | DNA topoisomerase I | Hematotoxicity Gastrointestinal disorders Dermatotoxicity | Neuroblastoma Rhabdomyosarcoma | 0.75–1.5 mg/m ² | (Feng, Tang et al. 2013) |
| t1.8 | Etoposide | C ₂₉ H ₃₂ O ₁₃ | Cell cycle | Myelosuppression Alopecia Gastrointestinal disorders | Neuroblastoma Lymphomas Leukemia | 60–150 mg/m ² | (Najar and Johri 2014) (Wagner, Hill et al. 2002) (Kushner, Modak et al. 2013) |
| t1.9 | Daunorubicin | C ₂₇ H ₂₉ NO ₁₀ | DNA topoisomerase II | Bone marrow depression Gastrointestinal disorders Cardiotoxicity | Rhabdoid tumor Leukemia Hodgkin's/non-Hodgkin's diseases Sarcomas | 25–45 mg/m ² | (Nickel, Keller et al. 2014) |
| t1.10 | Idarubicin | C ₂₆ H ₂₇ NO ₉ | DNA topoisomerase II | Myelosuppressions Cardiotoxicity | Acute myeloid leukemia Relapsed acute lymphoblastic leukemia | 5–12 mg/m ² | (Dreyer, Kadota et al. 2003) (Sekine, Morais et al. 2014) |
| t1.11 | Actinomycin D | C ₆₂ H ₈₆ N ₁₂ O ₁₆ | Protein synthesis | Myelosuppression Hypersensitivity Gastrointestinal disorders | Medulloblastoma Rhabdomyosarcoma Ewing's sarcoma | 15–600 µg/m ² | (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

117 recurrent disease, and thus natural compounds might have the
118 potential to fill these gaps (Giangaspero, Perilongo et al. 1999;
119 Schwab, Westermann et al. 2003; Blonski, Taillandier et al.
120 2012; Taylor, Northcott et al. 2012; Urbanska, Sokolowska
121 et al. 2014; (da Rocha et al. 2001, Litten and Tomlinson
122 2008, HaDuong et al. 2015). Through their remarkable chem-
123 ical and structural diversity, agents derived from natural prod-
124 ucts continue to be of relevance as sources of drug leads that
125 act as templates for the construction of novel molecules, with
126 the challenge being to reduce their side effects (Koehn and
127 Carter 2005).

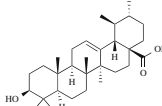
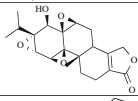
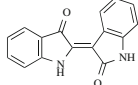
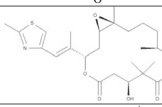
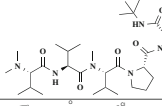
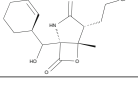
128 In this review, we discuss nature-derived drugs that are
129 emerging in the treatment of pediatric tumors, through litera-
130 ture searches of PubMed ([http://www.ncbi.nlm.nih.gov/
131 pubmed](http://www.ncbi.nlm.nih.gov/pubmed)) for the year 2015, with the scheme applied
132 described in Supplementary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

136 **Natural compounds in plants applied to adult clinical 137 cancer trials that are of interest for pediatric trials**

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

148 One of the most promising plant-derived chemopreventive
149 agents is Ursolic acid, which is an ursane-type pentacyclic
150 triterpenic acid that belongs to the cycloqualenoid family of
151 triterpenoids (Shanmugam et al. 2013). Ursolic acid has been
152 reported in leaves and berries of several medicinal plants,
153 including *Arctostaphylos uva-ursi* (L.) Spreng (bearberry),
154 *Vaccinium macrocarpon* Air. (cranberry), *Rhododendron*
155 *hymenanthes* Makino, *Rosmarinus officinalis*, *Eriobotrya*
156 *japonica*, *Calluna vulgaris*, *Ocimum sanctum*, and *Eugenia*
157 *jambolana*. Ursolic acid can have valuable effects in a variety
158 of human diseases, including inflammation-driven cancers
159 (Shanmugam et al. 2013), and it is well tolerated by adult
160 patients with advanced tumors (Zhu et al. 2013). Therefore,
161 the idea that Ursolic acid could become a powerful chemopre-
162 ventive agent in childhood tumors has been developed. Its
163 molecular mechanisms have been studied in hepatoblastoma
164 (Son et al. 2013) because of its pro-apoptotic effects in HepG2
165 cells (Satomi et al. 2005) and in doxorubicin-resistant HepG2
166 cells (Yang et al. 2010). The HepG2 cell line has for several
167 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 ₃₀ H ₄₈ O ₃ | 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 ₂₀ H ₂₄ O ₆ | 360.40 | Leukemia | <i>In vitro</i> | 20-100 | - | (Liu, Li et al. 2013) (Huang, Zhang et al. 2013) |
| Indirubin |  | C ₁₆ H ₁₀ N ₂ O ₂ | 262.26 | Leukemia Glioblastoma | <i>In vitro</i> | 10 | - | (Nam, Scuto et al. 2012) (Williams, Nowicki et al. 2011) |
| Epothilone B |  | C ₂₇ H ₄₁ NO ₆ 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 ₃₂ H ₅₈ N ₆ O ₅ | 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 ₁₅ H ₂₀ ClNO ₄ | 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 Transcrip-
 234 tion 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 chron-
 239 ic 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 β -lactone- γ -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., β 1, β 2, β 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.




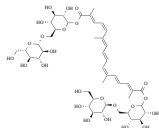

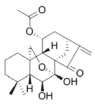
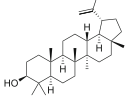

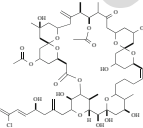

367 **Novel promising plant-derived compounds for pediatric**
 368 **cancer treatment**

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 κ 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 κ 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 ₁₄ H ₁₂ O ₁₁ | 356.23 | Retinoblastoma | <i>In vitro</i> | 50 | - | (Kumar, Gangappa et al. 2014) |
| Apigenin |  | C ₁₅ H ₁₀ O ₅ | 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 ₈ H ₈ O ₄ | 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 ₄₄ H ₆₄ O ₂₄ | 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 ₁₅ H ₂₀ O ₃ | 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 ₂₂ H ₃₀ O ₆ | 390.47 | Pancreatic tumor | <i>In vitro</i> | 0,5-4 | - | (Cheng, Bo et al. 2015) |
| Lupeol |  | C ₃₀ H ₅₀ 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 ₄₄ S ₆₈ O ₁₃ | 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 ₆₅ H ₉₉ ClO ₂₀ | 1235.92 | Leukemia | <i>In vitro</i> | 0.0002-0.001 | - | (Schyschka, Rudy et al. 2008) |
| Variolin b |  | C ₁₄ H ₁₁ N ₇ 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 in-
433 hibit 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 β -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 β 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/ β -
508 catenin signaling through nuclear β -catenin loss (Cimmino
509 et al. 2012). The efficacy of Norcantharidin to decrease medul-
510 loblastoma tumor growth was also assessed in vivo using med-
511 ulloblastoma 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 β -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 NFκB (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 NFκB 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 NFκB
621 (Eliseev et al. 2005). As Parthenolide affects NFκB (Kishida
622 et al. 2007), it can potentially provide a clinical method to
623 decrease NFκB 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/cyclin 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.

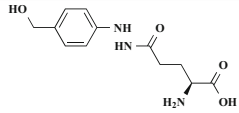
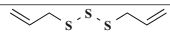
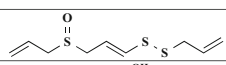
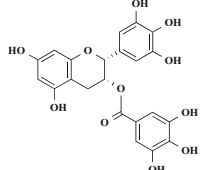
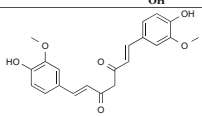
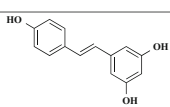
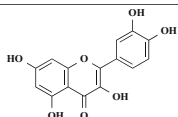
Nutraceutical and functional foods as anti-cancer drugs 813 to support pediatric cancer treatment 814

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-1a 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 α -(1-4)-
 857 glucan- β -(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 ₁₂ H ₁₇ N ₃ O ₄ | 267.28 | Leukemia | <i>In vitro</i> | 10 (μg/ml) | - | (Akiyama, Endo et al. 2011) |
| Diallyl trisulfide |  | C ₆ H ₁₀ S ₃ | 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 ₉ H ₁₄ OS ₃ | 234.4 | Leukemia | <i>In vitro</i> | 40 | - | (Hassan 2004) |
| Epigallocatechin gallate |  | C ₂₂ H ₁₈ O ₁₁ | 458.37 | Neuroblastoma Hepatoblastoma | <i>In vitro</i> | 10-100 | - | (Nishimura, Hartomo et al. 2012) (Godeke, Maier et al. 2013) |
| Curcumin |  | C ₂₁ H ₂₀ O ₆ | 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 ₁₄ H ₁₂ O ₃ | 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 ₁₅ H ₁₀ O ₇ | 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 H₂O₂ 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

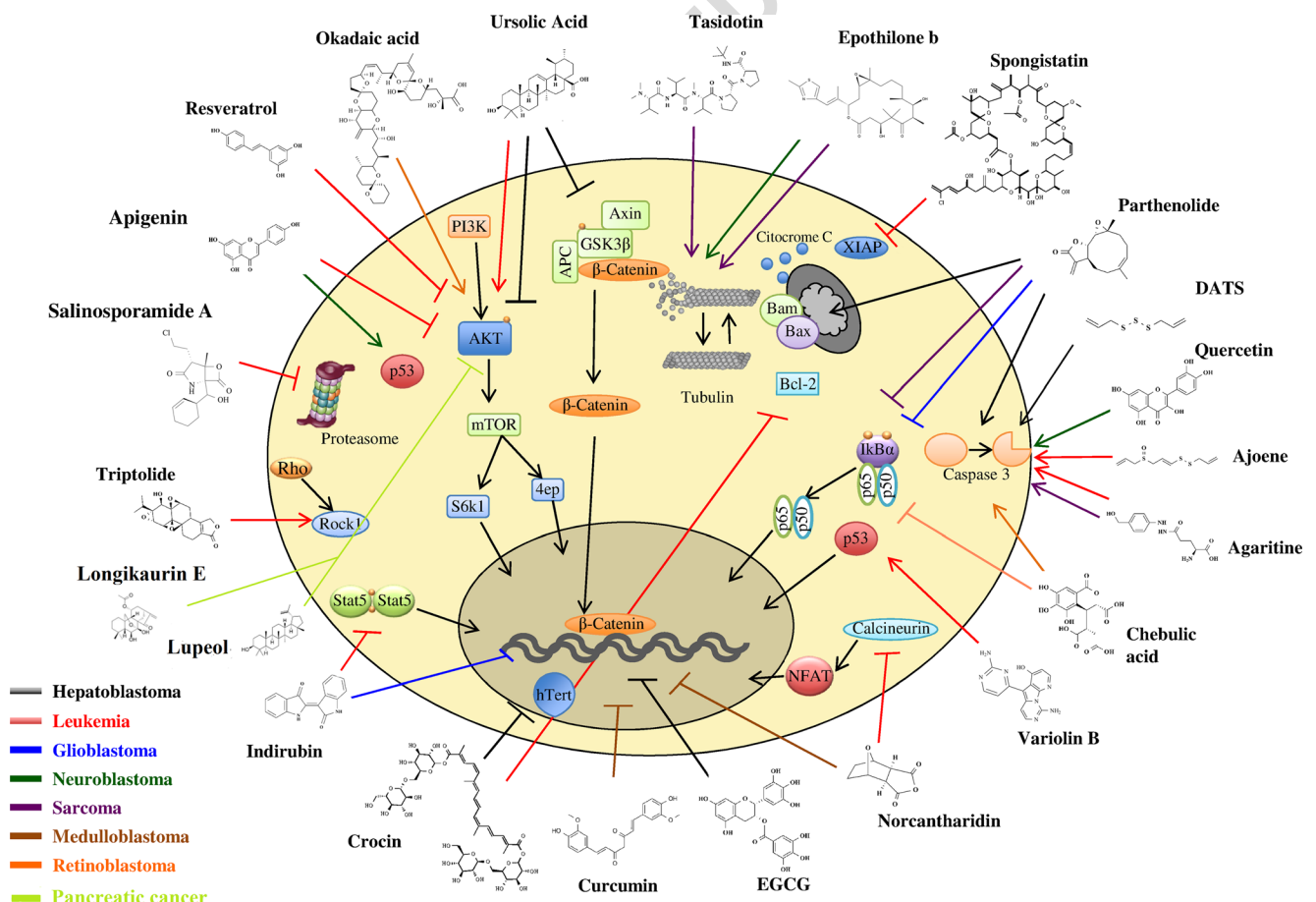


Fig. 1 Intracellular pathways altered by natural compounds in childhood malignancies. Illustration of the novel natural compounds that have been identified recently as having negative effects on intracellular signaling pathways involved in angiogenesis and metastasis formation in

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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 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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- Q20. "Schwab, Westermann et al. 2003" is cited in text but not given in the reference list. Please provide details in the list or delete the citation from the text.
- Q21. "Blonski, Taillandier et al. 2012" is cited in text but not given in the reference list. Please provide details in the list or delete the citation from the text.
- Q22. "Taylor, Northcott et al. 2012" is cited in text but not given in the reference list. Please provide details in the list or delete the citation from the text.
- Q23. "Urbanska, Sokolowska et al. 2014" is cited in text but not given in the reference list. Please provide details in the list or delete the citation from the text.
- Q24. The abbreviation "mRNA" has been expanded to "messenger RNA." Please check if correct.
- Q25. Liu, Bi et al. 2015 has been changed to Liu et al. 2015a, b as per the reference list. Please check if okay.

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- Q26. Missing citation for Table 4 was inserted here. Please check if appropriate. Otherwise, please provide citation for Table 4. Note that the order of main citations of tables in the text must be sequential.
- Q27. Please check the sentence starting "The anti-leukemic effects of..." for completeness.
- Q28. Reference [Dechantsreiter et al. 1999] was provided in the reference list; however, this was not mentioned or cited in the manuscript. As a rule, all the references given in the list of references should be cited in the body of a text. Please provide the location of where to insert the reference citation in the main body text.
- Q29. Reference [Peer et al. 2007] was provided in the reference list; however, this was not mentioned or cited in the manuscript. As a rule, all the references given in the list of references should be cited in the body of a text. Please provide the location of where to insert the reference citation in the main body text.

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