Natural Compounds in Anti-Leukaemic Therapy: A Review

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Abstract: Human leukemia results from multiple mutations that lead to abnormalities in the expressions and functions of genes that maintain the delicate balance between proliferation, differentiation and apoptosis. Continued research on the molecular aspects of leukemia cells has resulted in the developments of several potentially useful therapeutic agents. Discovery of new cellular and/or molecular pathways enabling innate or acquired resistance of cancers to current chemotherapeutics to be overcome is therefore of crucial importance if one wants to efficiently combat those cancers associated with dismal prognoses. In this concern, natural compounds are regarded as new chemical entities for the development of drugs against various pharmacological targets, including cancer, and, above all, leukemia.

Keywords: Alkaloids, leukemia, natural compounds, new drugs, polyphenols, terpenoids.

INTRODUCTION

Cancer is a worldwide disease, which is responsible for millions of deaths every year [1]. Generally, malignancies are characterized by three major cellular disorders: arrest of cell differentiation, inhibition of apoptosis and accelerated proliferation of clonal cells. The standard cancer treatment protocols include surgery, radiotherapy and chemotherapy. In particular, until recently, chemotherapy and hematopoietic stem-cell transplantation were the only therapeutic options in acute leukemia [2].

Human leukemia results from multiple mutations that lead to abnormalities in the expressions and functions of genes that maintain the delicate balance between proliferation, differentiation and apoptosis. Continued research on the molecular aspects of leukemia cells has resulted in the developments of several potentially useful therapeutic agents [3].

A potential alternative to treat this prevalent disease is the engagement of malignant cells into the maturation pathway, known as 'differentiation therapy'. The induction of differentiation restores a natural cell death program and inhibits the excessive proliferation; the effect of all *trans*-retinoic acid (ATRA) in acute promyelocytic leukemia represented one of the first examples of differentiation therapy in haematological malignancies [4].

Prognoses for leukemia patients have improved significantly during the last few decades, primarily due to the use of treatments that induce remission, the combined use of therapies and bone marrow transplantation. However, the 5-year survival rates have not yet improved sufficiently and the majority of patients still succumb to leukemia. Cancer cell resistance to chemotherapy remains the major reason for treatment failure in leukemia [5].

Discovery of new cellular and/or molecular pathways enabling innate or acquired resistance of cancers to current chemotherapeutics to be overcome is therefore of crucial importance if one wants to efficiently combat those cancers associated with dismal prognoses. Development of anticancer drugs is loaded with challenges which go beyond the screening of potential molecules *in vitro* or *in vivo* and issues of pharmaceutical industry. Over the years, the identification of new effective differentiation inducers for the treatment of leukemia has remained a focus of intense interest [2].

In this concern, natural compounds are considered new chemicals for the development of drugs against various pharmacological targets, including cancer, and above all, leukemias.

Some years ago, Kizaki and co-workers [6, 7] reviewed the therapeutic approaches to acute myeloid leukemia, and they reported natural compounds as novel therapeutic drugs of acute myeloid leukemia mediated through reactive oxygen species-induced apoptosis. In the same years, Pujol and co-workers [8] described extensive research on the activity of more of 100 cytotoxic compounds containing an oxygenated ring in their structure and isolated from natural sources or prepared by semi-synthesis or synthesis from available intermediates. Anticancer drugs were classified according to the chemical structure of the natural products that are considered to lead the series. The origin and mechanism of action involved in each case were considered. This new family of natural, semi-synthetic and synthetic products includes compounds with interesting antitumour activity such as podophyllotoxin derivatives, NK-611, TOP-53, NPF

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and Tafluposide; camptothecin analogs, 9-amino-10,11-methylenedioxy-20(S)-derivatives with a considerable cytotoxicity against β-cell chronic lymphocytic leukemia (CLL), and lurtotecan (new piperazinyl-CPT analog). New dioxygenated ellipticine analogs showed more activity and stability than the natural pattern when the structure incorporated a lactone function instead of the pyridine ring. In the acridine series the new tetracyclic derivatives containing ethylenedioxy groups at the 2- and 3-positions of the acridine system exhibited the same activity as m-AMSA *in vivo* against murine P-388 leukemia. Other isolated compounds containing a dioxygenated ring in their structure such as 3-cyanoquinolines showed antitumour activities related to kinase inhibition and are attractive candidates for development of new synthetic antitumour agents.

Considering the increasing interest for leukemias, we thought to report the principle natural compounds known for their antileukaemic properties.

TERPENOIDS

Among terpenoids, a natural small molecule as linalool (Fig. **1**) shows a high cytotoxicity to tumour cells with wide type p53 from a variety of human hematopoietic malignancies, including myeloid leukemia (Kasumi-1, HL-60), lymphoblastic leukemia (Molt-4, H-9) and lymphoma (Raji), suggesting that linalool possesses a wide anti-tumour spectrum [9]. Treatment of leukemia cells by linalool led to strong activation of p53, cyclin-dependent kinase inhibitors (CDKIs), GADD45α, c-jun and phosphorylated-JNK, suggesting that linalool-induced apoptosis might be associated with activation of p53 and CDKIs. Linalool does not affect growth of normal hematopoietic cells at concentrations that kill tumour cells and even at high concentration it exhibits a very modest growth inhibition.

Other volatile compounds (carvacrol, thymol and γ-terpinene) of the dried leaves of *Thymus algeriensis* Boiss. were tested for their cytotoxic activity against mouse leukemia P388 cell line and significant results were obtained [10].

De Martino and co-workers [11, 12] studied a possible pro-apoptotic activity of vervain essential oil and citral (Fig. **1**), its main compound, against blood samples collected from both chronic myeloid leukemia (CML) patients and healthy blood donors, in a first time; then, the similar experiments were carried out on blood sample from chronic lymphocytic leukemia patients and healthy subjects. Vervain essential oil induces a significant apoptosis (*vs* controls) in granulocytes from both healthy donors and chronic myeloid leukemia patients. Percentage of apoptotic cells was greater in CML patients. Also citral had a strong pro-apoptotic activity both in healthy subjects and in CML patients. CML granulocytes are more sensitive to citral than granulocytes of donors [11]. Later, the pro-apoptotic activity of *Verbena officinalis* essential oil and of citral, on lymphocytes collected from normal blood donors and patients with chronic lymphocytic leukemia (CLL), has been evaluated. The number of apoptotic cells was greater in CLL patients than in healthy subjects at all different times of incubation (4, 8 and 24 hours) (Fig. **2**) for samples treated with *Verbena officinalis* essential oil (A) and citral (B) as well vs controls, at different concentrations (0.1% V/V and 0.01%). The greater pro-apoptotic ability was showed by both essential oil of *Verbena officinalis* and citral at lower concentrations. Patients carrying deletion 17p13 (p53 mutation) showed a reduced ability to undergo apoptosis with respect to patients with other genomic aberrations or normal karyotype. The pro-apoptotic activity of *Verbena officinalis* essential oil and citral is thought to be due to a direct procaspase 3 activation [12].

Kim and co-workers [13] demonstrated that plant-derived sesquiterpene lactones may enhance ATRA mediated cell differentiation through distinct pathways. In particular, three

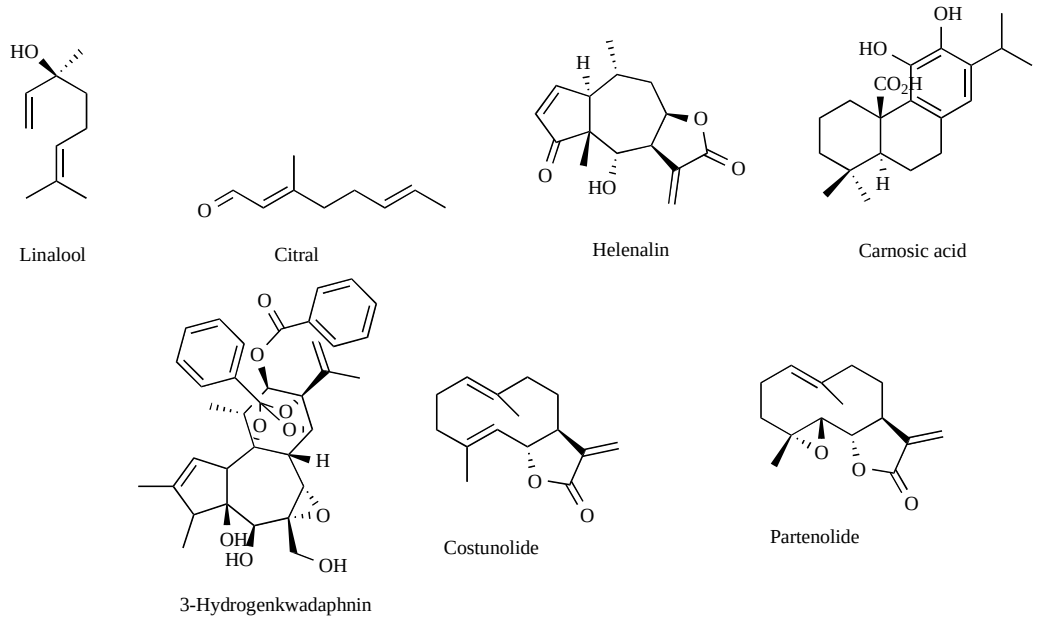


Fig. (1). Antileukaemic terpenoids.

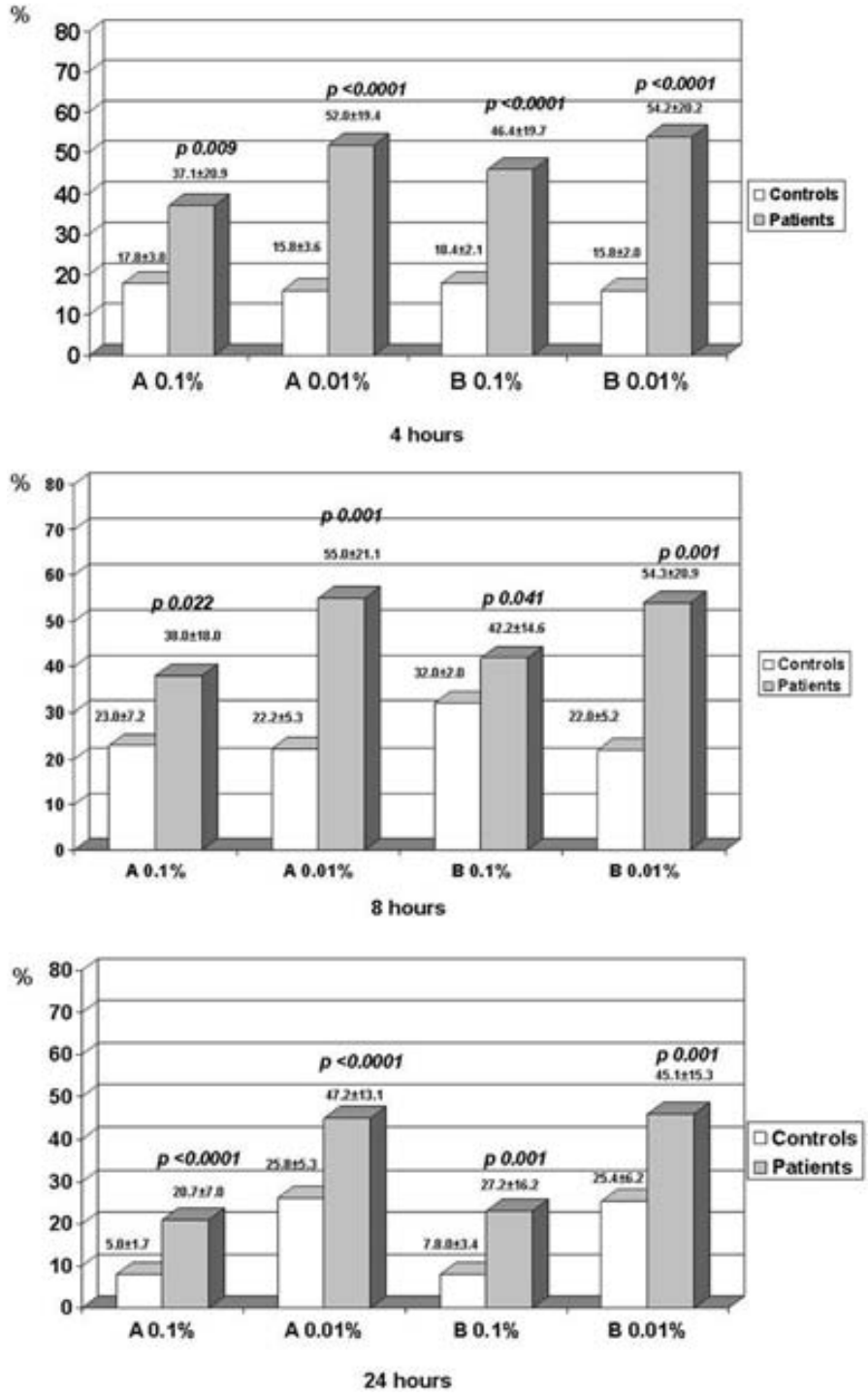


Fig. (2). *Verbena officinalis* essential oil and citral induced-apoptosis in CLL patients and controls at different times. Data are reported as mean percentage (± standard deviation) of apoptotic B-cells at different times of incubation with *Verbena officinalis* essential oil (**A**) and citral (**B**) at different concentrations (0.1% and 0.01%, respectively).

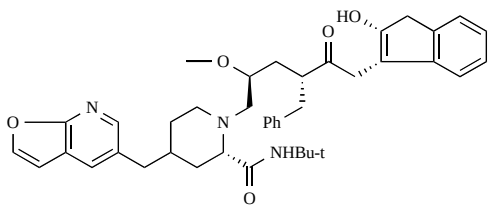
sesquiterpene lactones, helenalin, costunolide, parthenolide (Fig. **1**), increased ATRA-induced HL-60 cell differentiation into a granulocytic lineage. Signaling kinases PKC and ERK were involved in the ATRA-induced differentiation enhanced by all of the effective sesquiterpene lactones, but JNK and PI3-K were involved in the ATRA-induced differentiation enhanced by costunolide and parthenolide. Enhancement of cell differentiation closely correlated with inhibition of NF-kB DNA-binding activity by all three effective compounds. Significantly, enhancement of differentiation induced by ATRA by the sesquiterpene lactones was not accompanied by elevation of basal intracellular calcium concentrations.

Carnosic acid (Fig. **1**), a polyphenolic diterpene found in rosemary [14], inhibits proliferation of HL-60 and U937 human myeloid leukemia cells without induction of apoptotic or necrotic cell death. Growth arrest occurred concomitantly with a transient cell cycle block in the G1 phase, which was accompanied by an increase in the immunodetectable levels of the universal cyclin-dependent kinase inhibitors p21WAFI and p27Kip1. Carnosic acid caused only a marginal induction of differentiation, as monitored by the capacity to generate superoxide radicals and the expression of cell surface antigens (CD11b and CD14). At low concentrations, this polyphenol substantially augmented the differentiating effects of 1,25-dihydroxyvitamin D3 and all-*trans* retinoic acid. Furthermore, such combinations of carnosic acid and any of these differentiation inducers synergistically inhibited proliferation and cell cycle progression.

3-Hydrogenkwadaphnin (3-HK) (Fig. **1**) is a daphnane-type diterpene ester isolated from the leaves of *Dendrostellera lessertii* (Thymelaeaceae) with differentiation and apoptotic potency among several leukaemic cells without any measurable adverse effects on normal cells: in their study, Yazdanparast and Meshkini [15] evaluated differentiating and apoptotic efficiency of a second new anti-proliferating agent from the same plant relative to 3-HK using acute myeloid leukemia (AML) KG1 cell line. 3-HK inhibited proliferation of KG1 cells with a differentiation toward macrophage-like morphology. Also a compound inhibited proliferation of KG1 cells. The treated cells differentiated along the monocyte/macrophage lineage based on the morphological features apparent after Wright-Giemsa staining, phagocytic activity and expression of cell surface markers. Moreover, the results indicated that exposure of KG1 cells to either 3-HK or the new compound for 3-4 days induced apoptosis in these cells.

Bioassay-guided fractionation of the rhizomes of *Astilbe chinensis* (Maxim.) Franch. et Savat. afforded four cytotoxic pentacyclic triterpenoids [16]. The four active oleanane-type triterpenoids compounds exhibited strong cytotoxic activity against HO-8910, Hela, and HL60 *in vitro*, in a dose-dependent manner.

Compound K (Fig. **3**) is a novel ginseng saponin that is formed by the action of intestinal bacteria on ginseng extract in man and rats. Compound K has been reported to have potent anti-tumourigenic activity [17]: Cho and co-workers [3] demonstrated that compound K inhibit the viability of HL-60 cells and these effects were mediated through the induction of apoptosis. Compound K induced the activation of caspase-3, -8, and -9, and modulation of Bcl-2 families. In addition, a caspase-8 inhibitor completely abolished caspase-3 activation, Bid cleavage, and subsequent DNA fragmentation by compound K. Therefore caspase-8 plays a key role in compound K-stimulated apoptosis. Based on these finding, the authors suggest that compound K may be used as a potential therapeutic and chemopreventive agent for leukemia *via* a potent apoptotic activity. Zhou and co-workers [18] reported that the same saponin inhibit the proliferation of other leukaemic cells (K562).



Compound K

Fig. (3). An antileukaemic saponin.

POLYPHENOLS

Ellagic acid (EA) (Fig. **4**) is a polyphenolic compound found in fruits and berries, such as pomegranates, strawberries, raspberries and blackberries and has been found to have antioxidant, anticarcinogenic, antifibrosis and chemopreventive activities [19]. The anticarcinogenic effect of EA was shown in various types of cancers, including skin, esophageal, pancreas, bladder, colon, uterine, cervix and precursor T-cell acute lymphoblastic leukemia [20]. Hagiwara and co-workers [21] found in HL-60 acute myeloid leukemia cells that EA (1) inhibited proliferation and induced accumulation of the S-phase cells in the cell cycle; (2) activated apoptosis pathway associated with caspase-3 activation; and (3) enhanced all-*trans* retinoic acid (ATRA)-induced differentiation. The results may show an attractive combination to potentiate both apoptosis and differentiation in human leukemia cells. Furthermore, the results of this study might have implications for the incorporation of agents such as EA into therapeutic intervention against leukemia and possibly other hematological malignancies.

On the same cell line, Komina and Węsierska-Gądek [22] showed that exposure of HL-60 cells to roscovetine (Fig. **4**) for 24 h inhibited their proliferation. Flow cytometric analyses revealed that these cells were arrested in G1 upon roscovetine treatment and, then, were induced in apoptosis. In the successive step the action of resveratrol (Fig. **4**) (a polyphenol produced in plants to prevent them from such environment stresses like fungal infection or UV radiation) alone or in combination with roscovetine was examined. Interestingly, synergistic effects were observed: a combined treatment resulted in a marked reduction of the frequency of the S- and G2/M phase cells and simultaneously increased the G1 cell population. Further analyses revealed that the combined treatment strongly activated caspase-3. Resveratrol is also the object of study of Rodrigue and co-workers [23]: the authors identified resveratrol target genes in the human erythroleukaemic K562 cell line and so, revealed that the tensin gene and protein levels are remarkably induced by this dietary polyphenol. Tensin, a cell-matrix adhesion protein binding the integrins and cytoskeletal actin filaments also interacts with PI3-kinase and JNK signaling pathways. Tensin induction by resveratrol is associated with increased K562 cell adhesion to fibronectin, cell spreading and actin polymerization. Pharmacological blockade of PI3-kinase and Rho GTPases/Rho-kinase resulted in selective depletion of focal adhesions, disorganization of tensin localization and disruption of stress fibers. These data support the conclusion that induction of tensin by resveratrol contributes to the

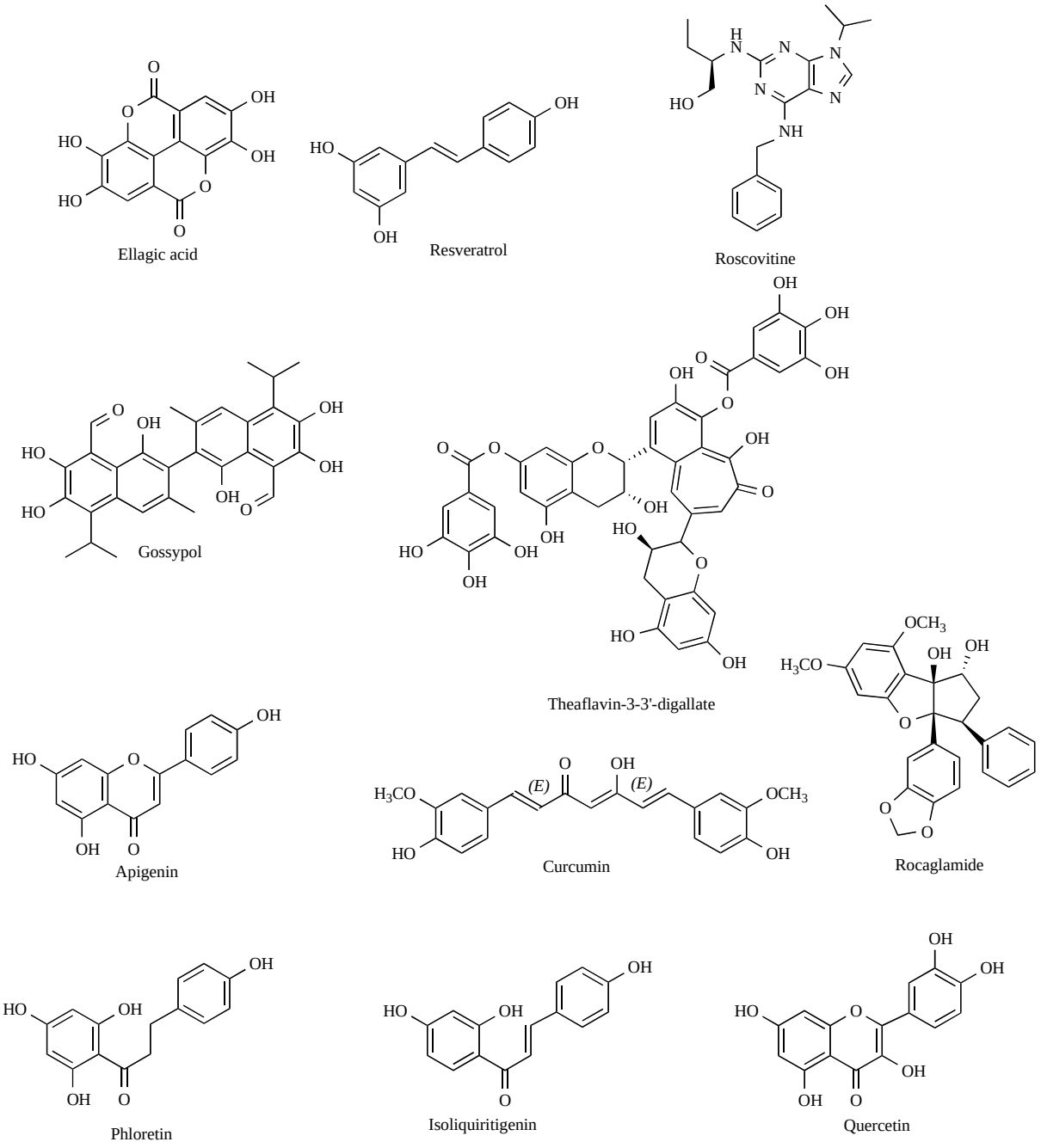


Fig. (4). Antileukaemic polyphenols.

chemopreventive and anti-invasive activity of this natural dietary compound.

Wang and co-workers [24] investigated the pro-differentiated effect of gossypol (Fig. **4**) always on HL-60 cells: they found that this compound could induce differentiation in the leukemia HL-60 cells and it may be a potential therapeutic agent chemoprevention or chemotherapeutic adjuvant especially in combination drug therapy for leukemia.

Tu and co-workers [25] reported the effects of tea theaflavins complex (TFs) theaflavin-3-3'-digallate (TFDG) (Fig. **4**), theaflavin-3'-gallate (TF2B), and an unidentified compound (UC) on the growth of human acute promyelocytic leukemia LH-60 cells. The results showed that TF2B significantly inhibited the growth of cancer cells, while TFs, TFDG and UC had little activity on LH-60.

In U937 cells, Salunga and coworkers [26] identified genes responsive to paeniflorin: this compound, extracted from peony root, affected the expression of many genes including Heat Shock Protein 70.

An other phenolic compound, curcumin (Fig. **4**), found in the spice turmeric, induced ubiquitin-independent

degradation of WT p53 and inhibited p53-induced apoptosis in normal thymocytes and myeloid leukaemic cells [27]. Few years later, William and co-workers [28] reported the same compound effective against leukaemic cells expressing p210 BCR-ABL and T315I BCR-ABL and holds promise in treating BCR ABL-induced B-cell acute lymphoblastic leukemia.

Rocaglamide (Fig. **4**) is a phenolic compound derived from the traditional Chinese medicinal plants of genus *Aglaia* (Meliaceae): Rocaglamide (Roc) induces apoptosis through the intrinsic death pathway in various human leukemia cell lines and in acute lymphoblastic leukemia, chronic myeloid leukemia and acute myeloid leukemia cells freshly isolated from patients. Rocaglamide induces a consistent activation of the stress-response mitogen-activated protein kinase (MAPK) p38 accompanied with a long term suppression of the survival MAPK extracellular signal-regulated kinase. These events affect pro-apoptotic Bcl-2 family proteins leading to depolarization of the mitochondrial membrane potential and trigger caspase-mediated apoptosis involving caspase-9, -8, -3 and -2. Importantly, Roc shows no effects on MAPKs in normal lymphocytes and therefore has no or very low toxicity on healthy cells [29].

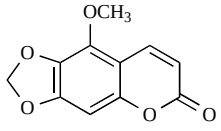
Apigenin (4',5,7-trihydroxyflavone) (Fig. **4**), a common dietary flavonoid abundantly present in fruits and vegetables, showed promising biological effects. In particular, the compound blocked proliferation in both lineages through cell-cycle arrest in G2/M phase for myeloid HL60 and G0/G1 phase for erythroid TF1 cells. In both cell lines the JAK/STAT pathway was one of major targets of apigenin. This compound inhibited PI3K/PKB pathway in HL60 and induced caspase-dependent apoptosis. In contrast, in TF1 cells, initiation of autophagy was observed. The block in cell cycle and induction of autophagy observed in this erythroleukemia cell line resulted in a reduced susceptibility toward the commonly used therapeutic agent vincristine [30]. Chalcones are natural flavonoids that are particularly cytotoxic towards K562 leukemia or melanoma cells. Sabzevari and co-workers [31] investigated phloretin, isoliquiritigenin (Fig. **4**) and 10 other hydroxylated chalcones for their cytotoxic mechanisms towards isolated rat hepatocytes. All hydroxychalcones partly depleted hepatocyte GSH and oxidized GSH to GSSG. These chalcones also caused a collapse of mitochondrial membrane potential and increased oxygen uptake. The highest pKa chalcones were the most effective at collapsing the mitochondrial membrane potential which suggests that the cytotoxic activity of hydroxychalcones are likely because of their ability to uncouple mitochondria.

A previous work [32] reported the cytotoxic activity of several flavonoids on leukaemic P388 cells. Quercetin (Fig. **4**) was studied also by Mirossay and co-workers [33]: the authors reported its protective effect on hypericin-induced citotoxicity in human promyelocitic leukemia cells (HL 60).

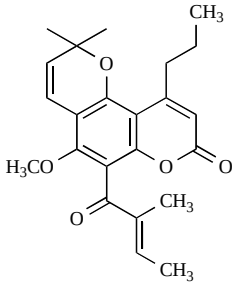
COUMARINS

Nowadays, coumarins represent an important group of organic compounds that are used as additives to food, cosmetics and optical brightening agents. In recent years, coumarins have attracted research interest due to their broad pharmacological/biological activities. Coumarin compounds can display anticancer, anti-HIV, anticoagulant, antimicrobial, anti-inflammatory and antioxidant activities [2]. Before, the same authors isolated, from petrol extract of *Pterocaulon polystachyum* DC., two trioxygenated coumarins, 5-methoxy-6,7-methylenedioxycoumarin and 5-(3-methyl-2-butenyloxy)-6,7-methylenedioxycoumarin (Fig. **5**) that inhibited proliferation and induced differentiation in U-937 cells [34].

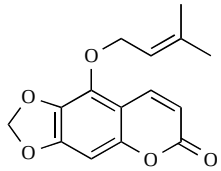
Another compound, GUT-70, characterized as a tricyclic coumarin, 5-methoxy-2,2-dimethyl-6-(2-methyl-1-oxo-2-



5-Methoxy-6,7-methylenedioxycoumarin



5-Methoxy-2,2-dimethyl-6-(2-methyl-1-oxo-2-butenyl)-10-propyl-2H,8H benzo[1,2-*b*;3,4-*b*']dipyran-8-one



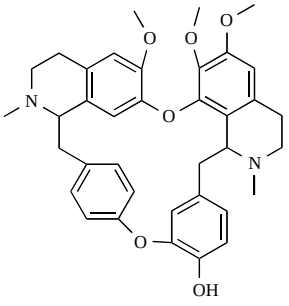
5-(3-Methyl-2-butenyloxy)-6,7-methylenedioxycoumarin

Fig. (5). Antileukaemic coumarins.

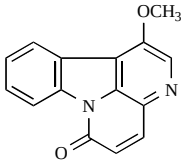
butenyl)-10-propyl-2H,8H benzo[1,2-*b*;3,4-*b'*]dipyran-8-one (Fig. **5**) was isolated from the stem bark of *Calophyllum brasiliense* Cambess. [5]. The authors investigated the effects of GUT-70 on the growth of 6 leukaemic cell lines, including a P-gp over-expressing cell line, as well as its mechanisms of apoptosis induction and its toxicity on various normal human cells. The compound inhibited all 6 human leukaemic cell lines evaluated, including the P-glycoprotein overexpressing cell line, in a concentration and time-dependent manner. Furthermore, GUT-70 did not inhibit colony formation by normal hematopoietic progenitors up to 30 μM. GUT-70 activated the caspase 2, 3, 8 and 9, and induced the apoptosis in leukaemic cells, which was inhibited by caspase inhibitors. GUT-70 induced anti-leukaemic effects independent of the p53-p2l$^{WAFl/CIP1}$ pathway and increased the overall expression of p27^{KIP1} and p57^{KIP2}, to stop the cell cycle at the G1/S transition. Thus, a novel anti-cancer drug, GUT-70, isolated from the stem bark of *C. brasiliense*, induces caspase mediated and p53-independent apoptosis to overcome multidrug resistance and may become a potent leukemia therapeutics.

ALKALOIDS

In the class of alkaloids, Xie and co-workers [35] showed that natural compound berbamine (Fig. **6**), from Chinese herb *Berberis amurensis* Rupr., selectively induces apoptosis of imatinib (IM)-resistant-Bcr/Abl-expressing leukemia cells, from the K562 cell line and CML patients. Another natural source of alkaloid compounds is *Vinca* genus: in particular, Beck [36] reported that *Vinca* alkaloid-resistant human leukaemic cells express the multiple drug-resistant phenotype, characterized by cross resistance to natural product compounds of unrelated structure and action. More clear, however, appears understanding of the pharmacologic determinants of this resistance-apparent decreased drug uptake and decreased drug retention. Elucidation of the mechanism of multiple drug resistance is important in the design of new chemotherapeutic strategies to overcome it. In this regard, calcium channel blocking agents and calmodulin inhibitors can cause an apparent reversal of resistance by enhancing the cytotoxic effectiveness of the anticancer drugs, possibly by increasing the amount of drug retained by the tumour cells. The basis for this enhanced retention and cytotoxicity may be related to cellular calcium fluxes, calmodulin content or membrane fluidity and permeability. The meaning of these findings may provide new insights into the mechanism of action and ultimate cellular target(s) for *Vinca* alkaloids.



Berbamine



1-Methoxy-canthin-6-one

Fig. (6). Antileukaemic alkaloids.

Ammirante and co-workers [37] isolated from root of *Ailanthus altissima* Swingle 1-methoxy-canthin-6-one (Fig. **6**), an alkaloid with a good antiproliferative activity: the alkaloid was tested on Jurkat cells, showing mitochondrial membrane depolarization, mitochondrial release of cytochrome c and Smac/DIABLO, and caspase 3 activation. The effects of 1-methoxy-canthin-6-one in combination with TRAIL (human recombinant tumour necrosis factor–related apoptosis-inducing ligand) has been also investigated on Jurkat cells, using suboptimal concentrations of both agents: the two compounds showed a synergistic action. Studying synergism mechanisms, it has been seen that the alkaloid increases TRAIL R1 receptors, inducing JNK activation and c-jun phosphorilation. JNK inhibition reduces only partly the synergism between alkaloid and TRAIL; therefore other factors take part in TRAIL-induced apoptosis, besides TRAIL R1 up-regulation. Peripheral blood mononuclear cells (PBMC) from healthy subjects have been used as control, in which alkaloid showed no pro-apoptotic activity. The synergism with TRAIL has been assayed on other cell lines and, in this case too, apoptosis induction has been evident, at suboptimal concentrations. These findings indicate that 1-methoxy-canthin-6-one can represent a candidate, for *in vivo* studies, of mono-therapies or combined anti-neoplastic therapies.

OTHER COMPOUNDS

Three compounds were isolated from the ethyl acetate soluble fraction of the methanolic extract of the leaves of *Catalpa ovata* G. Don (Bignoniaceae) and tested for their effects on T cell-mediated responses for tumour surveillance and proliferation in U937, HL60 and Molt-4 leukemia cells. The substances inhibited proliferation of those cells, in a dose-dependent manner. One of the compound enhanced gene expressions of p53 and IL-4, but decreased IL-2 and IFN-γ genes in Molt-4 cell [38].

Militao and co-workers [39] studied the effects of four different pterocarpans from the native Brazilian plant *Platymiscium floribundum* Vogel in human leukemia cells: (+)-2,3,9-trimethoxy-pterocarpan (Fig. **7**), (+)-3,9-dimethoxy-pterocarpan [(+)-homopterocarpin], (+)-3-hydroxy-9-methoxy-pterocarpan [(+)-medicarpin] and (+)-3,4-dihydroxy-9-methoxy-pterocarpan [(+)-vesticarpan]. The analysis of membrane integrity and morphological modifications by flow cytometry in the presence of (+)-medicarpin and (+)-vesticarpan indicated that treated cells undergo necrosis, while others two pterocarpans trigger apoptosis. DNA synthesis seemed to be affected since BrdU (5-bromo-2'-deoxyuridine) incorporation was inhibited in a dose-dependent manner in the presence of all tested compounds. Pterocarpan treatment also induced an increase in the amount of subdiploid DNA, indicating internucleosomal DNA breakdown, mitochondrial depolarization and caspase-3 activation, which indicate apoptosis induction.

Numerous epidemiological, clinical and laboratory studies have demonstrated the role of garlic in cancer prevention [40, 41]. The chemopreventive activity has been attributed to the presence of organosulphur compounds in garlic that modulate the activity of several metabolising

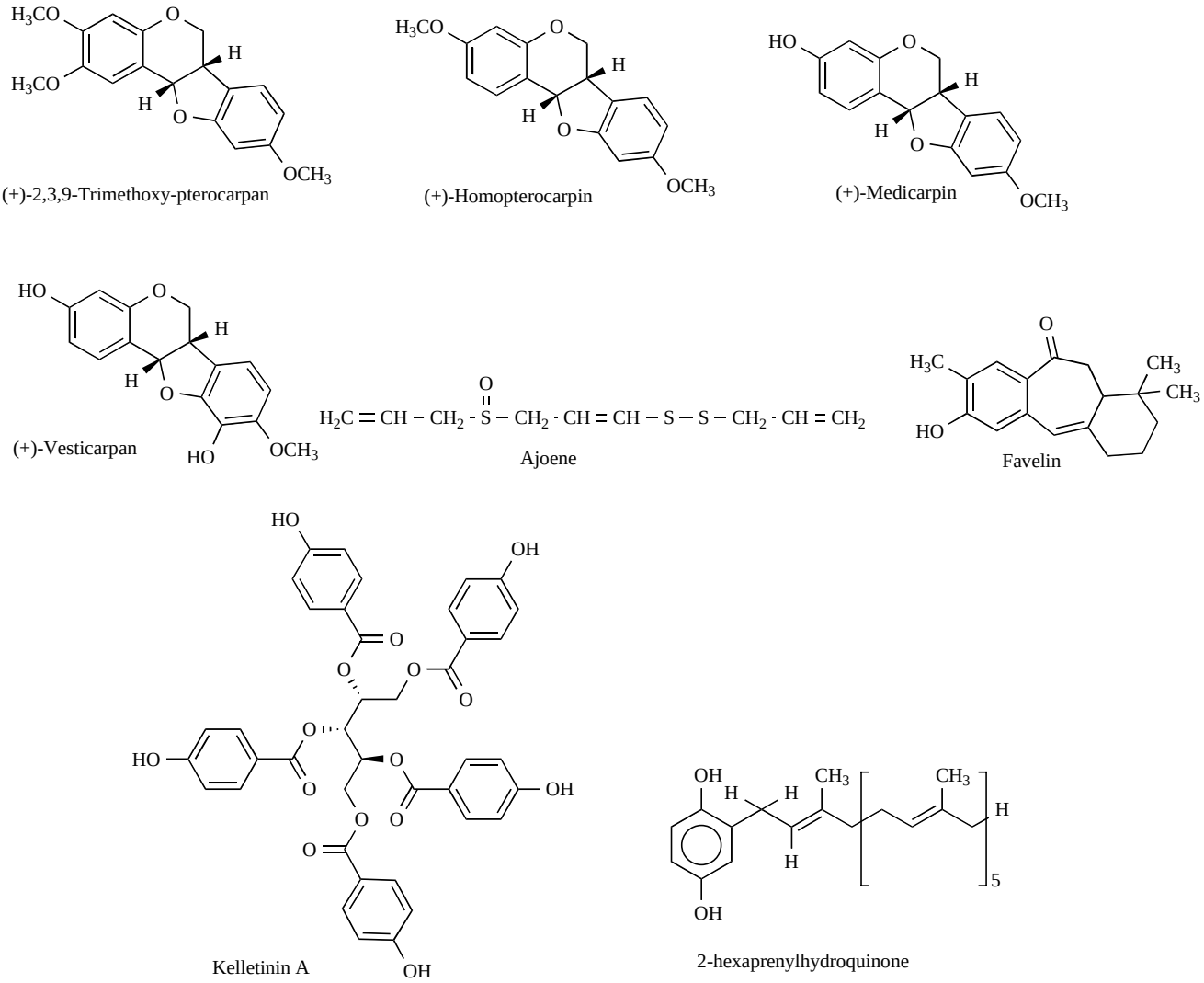


Fig. (7). Other antileukaemic compounds.

enzymes that activate (cytochrome P450s) or detoxify (glutathione S-transferases) carcinogens and inhibit the formation of DNA adducts in several target tissues [42].

Ajoene (4,5,9-trithiadodeca-1,6,11-triene-9-oxide) (Fig. **7**) is a garlic-derived compound produced most efficiently from pure allicin and has the advantage of a greater chemical stability than allicin.

In human leukemia cells, ajoene has demonstrated a consistent anti-proliferation and apoptosis inducing activities in both CD34-positive and CD34-negative myeloid leukemia cells. Ajoene was shown to inhibit proliferation and induce apoptosis of human leukemia CD34-negative cells including HL-60, U937, HEL and OCIM-1. Also, additional mechanisms have been identified in modulating apoptosis by garlic in HL-60 cells including activation of NFK B and activation of caspase-8 protease, *via* a Bcl-2 insensitive pathway. In addition, ajoene was shown to induce 30% apoptosis in myeloblasts from chronic myeloid leukemia patient in blast crisis [41]. More significantly, ajoene profoundly enhanced the apoptotic effect of the two chemotherapeutic drugs: cytarabine and fludarabine in human CD34-positive resistant myeloid leukemia cells through enhancing their Bcl-2 inhibitory and caspase-3 activation activities. The two key anti-leukemia biological actions of ajoene were the inhibition of proliferation and the induction of apoptosis of human myeloid leukemia cells. The anti-proliferation activity of ajoene was shown to be associated with a block in the G2/M phase of cell cycle in human myeloid leukemia cells. The apoptosis inducing activity of ajoene is *via* the mitochondria-dependent caspase cascade through a significant reduction of the anti-apoptotic Bcl-2 that results in release of cytochrome *c* and also the activation of caspase-3 [43]. Also, the anti-apoptotic protein Bcl-2 has a cell cycle inhibitory function separable from its enhancement of cell survival. Bcl-2 not only suppresses apoptosis by inhibiting the mitochondrial cytochrome *c* release that is required for caspase-3 activation, but also markedly increases cell cycle withdrawal into the G0 quiescent phase. Therefore, the high Bcl-2 concentrations in CD34-positive AML cells could be responsible for their protection from the S-phase specific drug-induced apoptosis upon treatment with cytarabine or fludarabine through the withdrawal of these CD34-positive resistant cells to the G0

quiescent phase of cell cycle. This mechanism of resistance to chemotherapy appears to be particularly manifested in the extremely resistant CD34 + CD7+ human myeloid leukemia cells.

It is accepted that neoplastic diseases are related to gene alteration or oncogene activation. In particular, DNA minor groove binding drugs have been extensively studied through the years in order to influence the regulation of gene expression by means of specific interactions with DNA based moieties. In this field, analogs of naturally occurring antitumour agents, such as CC-1065 and/or the duocarmycins, represent a new class of highly potent antineoplastic compounds. CC-1065 and duocarmycins represent a class of exceptionally potent antitumour antibiotics that derive their biological effects from the reversible, stereo-electronically-controlled sequence selective alkylation of DNA. Such compounds showed a cytotoxicity against leukemia L1210 cell lines, but while CC-1065 showed a good antitumour activity in an *in vivo* model, duocarmycins showed weak antitumour activity. CC-1065 cannot be used in humans due to eventual fatality, so many scientists have focused their attention on this class of substances, to obtain new derivatives with a better profile in *in vivo* models [44].

Smith and coworkers [45] showed that N-thiolated β-lactams, induced DNA damage, inhibited DNA replication, and induced tumour cell apoptosis in a time and concentration-dependent manner. Few years later, the same Authors [46] reported, for the first time, that a N-thiolated β-lactam can preferentially induce apoptosis in leukaemic Jurkat T cells, but not nontransformed, immortalized human NK cells. Additionally, they also showed that an other analog lactam, containing an $–NO_2$ substituent, enhanced apoptosis-inducing activity in Jurkat T cells compared to the first lactam. The analog lactam also induces apoptosis selectively in Jurkat T, but not human NK cells, and in Simian Virus 40 (SV40)-transformed human fibroblasts (VA-13), but not in their parental counterpart (WI-38). Both lactams are able to activate caspase-3 in human prostate cancer cells and inhibit colony formation of these cells in soft agar. These data indicate that further study of N-thiolated β-lactams in the treatment of cancer is warranted.

From an ethereal extract of the plant *Atraphaxis spinosa* L. var. *sinaica*, N-trans-p-coumaroyl-3',4'-dihydroxyphenylethylamine and N-trans-p-feruloyl-3',4'-dihydroxyphenylethylamine were isolated from natural sources for the first time and shown to have a cytotoxic activity on leukaemic P388 cells [32].

On the same cell line, the MeOH extract of the Brazilian plant, *Cnidoscolus phyllacanthus* (Euphorbiaceae), was tested and showed a cytotoxic activity. Bioactivity-guided fractionation led to the isolation of cytotoxic compounds, favelin methyl ether, favelin (Fig. **7**), deoxofavelin, and neofavelanone, as well as 12-hydroxyfavelin methyl ethers, and 12-oxofavelin methyl ether as related compounds [47].

A natural compound from a Red Sea sponge *Ircinia* sp., 2-hexaprenylhydroquinone (HPH) (Fig. **7**), has been shown to be a general inhibitor of retroviral reverse transcriptases (from HIV-1, HIV-2 and murine leukemia virus) as well as of cellular DNA polymerases (*Escherichia coli* DNA polymerase I, and DNA polymerases α and β) [48].

An other marine compound is Kelletinin A [ribityl-pentakis (p-hydroxybenzoate)] (KA), isolated from the gastropod *Buccinulum corneum*: this compound showed antiviral activity on the human T-cell leukemia virus type-1 (HTLV-1) and antimitotic activity on HTLV-1 infected MT2 cells. KA inhibited cellular DNA and RNA synthesis, without influencing protein synthesis, and interfered with viral transcription by reducing the levels of high molecular weight transcripts. Finally, the compound inhibited HTLV-1 reverse transcriptase *in vitro* [49].

CONCLUSIONS

Patients with hematologic malignancies often follow fatal clinical courses. Recently, high-dose chemotherapy followed by hematopoietic stem cell transplantation has produced higher remission rates, but it often causes serious clinical side effects and entails the risk of early mortality within a year, especially in elderly patients. Most patients ultimately relapse; therefore, novel therapeutic approaches that are based on new insights into the pathogenesis of hematologic malignancies and that target molecules important in cellular proliferation are strongly needed. Therapeutic strategies of inducing cellular differentiation and apoptosis in acute promyelocytic leukemia cells with arsenic trioxide are one recent successful example of the clinical application of a natural compound. The advantage of natural products for clinical application is the lack of toxicity. Therefore, compounds that induce the apoptosis of malignant cells might be developed as new potent anticancer agents for the management of hematologic malignancies, particularly for older and immune-compromised patients. In addition, these agents have the potential to replace or augment the more cytotoxic agents currently used to treat patients with hematologic malignancies.

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