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Cytotoxicity and Apoptosis Induction by Coumarins in CLL

Omid Gholami

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Abstract

Chronic lymphocytic leukemia (CLL) is one of the leukemia types. Leukemia is cancer of the body's blood-forming cells. Cancer is a disease that is often characterized by too little apoptosis and uncontrolled duplicate of body cells. Apoptosis, or programmed cell death, is a normal component of the development and health of multicellular organisms. Cells die in response to a variety of stimuli during apoptosis. During cancer, pathophysiology apoptosis of the cancerous cells is disrupted, so one of the strategies for cancer chemotherapy is inducing apoptosis in cancerous cells. Myeloid cell leukemia type 1 (Mcl-1) is one of the antiapoptotic Bcl-2 family proteins. It has been shown that the expression of Mcl-1 in CLL is significantly associated with a failure to achieve complete remission following cytotoxic therapy, so regulation of Mcl-1 expression by coumarins could be one of the mechanisms of CLL chemotherapy. Coumarins consist of a large class of phenolic substances found in plants. Different pharmacologic effects of coumarins were reported. One of these effects is cytotoxicity and apoptosis induction in cancerous cells by coumarins. In this chapter, the cytotoxic activity of coumarins and their role in Mcl-1 regulation are discussed.

Keywords: coumarins, apoptosis induction, Mcl-1 expression

1. Chronic lymphocytic leukemia

One of the most prevalent types of leukemia is chronic lymphocytic leukemia (CLL). Leukemia is a type of cancer. Cancer means too little apoptosis of body cells. In the case of cancer, cells have mutations that prevent them from undergoing apoptosis. It is a general belief that CLL is an indolent disease associated with a prolonged (i.e., 10–20 years) clinical course, and unrelated causes to CLL lead to death. But it is true only for less than 30% of cases [1]. By convention, the history of chronic lymphocytic leukemia begins in 1845, but it could be said to have started when the first white cells, “the globuli albicanates,” were noted by Joseph Lieutaud in

1749. During the intervening years, many events have aided in our understanding of the etiology and treatment of CLL. In his discussion of the history of CLL, Rai [2] found it informative to define three eras: (1) the recognition of CLL as a clinical entity, 1845–1924; (2) initial clinical investigations, 1924–1973; and (3) the modern era, 1973–2002.

Overexpression of Bcl-2¹ and Fas-inhibitory molecules such as TOSO is the principle mechanism of apoptosis resistance in CLL cells. CLL lymphocytes are clonal B-cells arrested in the B-cell differentiation pathway at some intermediate stage between the pre-B-cell and mature B-cell, perhaps in the “activated, antigen-experienced” B-cell subset. Phenotypic features of B-cell CLL (B-CLL) lymphocytes are [3–5] (1) extremely low levels of surface membrane immunoglobulin (often abbreviated as SmIg or sIg), (2) expression of one or more B-cell-associated antigens (like CD19, CD20, CD21, CD23, and CD24) [6, 7], and (3) expression of CD5, a T-cell-associated antigen.

Until the early 1980s, it was not possible to study chromosomal abnormalities in CLL because of the inadequate number of metaphases induced by available techniques. Certain genetic abnormalities have been associated with patient outcomes. Patients with complex genomic changes appear to have more aggressive disease [8]. The most frequently observed abnormalities were trisomy 12 and 14q+. The cytogenetic abnormalities appear to be restricted to B-cells in B-CLL [9]. In two studies of patients with CLL using fluorescence in situ hybridization (FISH) techniques, chromosomal abnormalities were noted in 69–82% of the patients, with abnormalities of chromosomes 11, 12, and 13 being most commonly seen.

1.1. Pathophysiology

Chronic lymphocytic leukemia is a monoclonal disease of mature-appearing lymphocytes that accumulate in blood, lymph nodes, spleen, liver, and bone marrow. Most cases (>95%) are characterized by monoclonal lymphocytes expressing normal B-cell surface proteins including immunoglobulin (Ig), CD19, and CD20 and aberrantly expressing CD5, a protein normally found on T-cells. A small minority (<5%) of cases are of T-cell origin, expressing T-cell surface markers such as CD3 and CD4 or CD8. These T-cell leukemias are not uncommon in individuals with ataxia telangiectasia. The molecular biology of T-cell lymphocytic leukemia is distinct from that of B-cell CLL [10].

Molecular and cellular mechanisms of CLL can be divided into two parts.

1.1.1. B-cell receptor-signaling pathways

B-cell receptor (BCR)-signaling pathways are triggered with or without antigen ligation in CLL. After antigenic BCR triggering downstream signaling of the BCR is dominated by the kinases lyn and syk, which transduce survival and antiapoptotic signals [11]. In CLL, the elevated expression of antiapoptotic Mcl-1, which leads to increased survival of malignant cells, occurred by prolonged activation of the MEK/ERK² and Pi3K/AKT³ pathways and with AKT after BCR signaling (**Figure 1**) [12].

¹B-cell CLL Lymphoma 2.

²Mitogen-activated protein kinase/extracellular signal-regulated kinase.

³Phosphatidylinositol-3-kinase and protein kinase B.

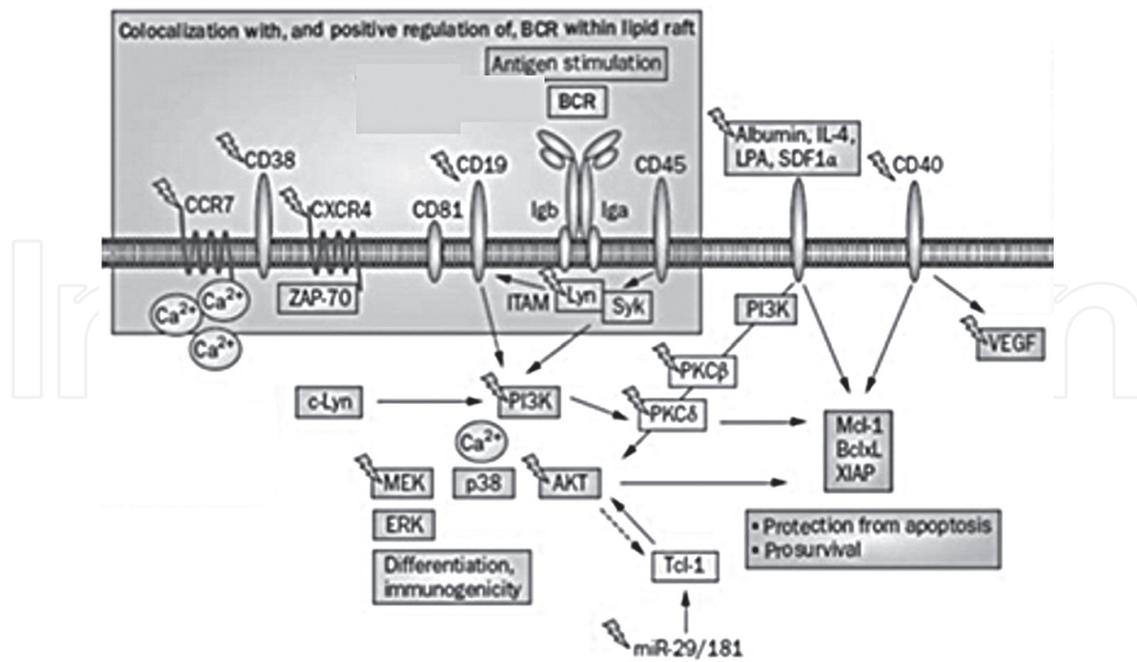


Figure 1. The role of BCR signaling in the biology of CLL [15].

For establishment of BCR-signaling pathways independent of antigen ligation, CD19 is an important surface marker. It has an important role in regulation and amplification of signal transduction via lyn [13]. Zap-70 is another tyrosine kinase that has important role in BCR signaling. When syk is not expressed, it can partially restore BCR signaling [14].

1.1.2. Aberrant apoptotic signaling pathway

Apoptosis is a kind of cell death. Extrinsic pathway of apoptosis triggers by death receptors. In CLL, they are CD95/Fas and trail (tumor-necrosis factor-related apoptosis-inducing ligand). After ligation by ligands (like CD40L), these receptors directly feed into a caspase cascade and lead to cell death [16]. The intrinsic pathway, or mitochondrial pathway, is regulated by the balance between antiapoptotic and proapoptotic members of the Bcl-2 family [15]. “BH3-only” proteins (e.g., Bim, Bid, Bmf, Puma, Bad, and noxa) are another class of Bcl-2 family proteins which can modify this balance (**Figure 2**).

Non-death-transmitted signals drive from developmental cues or sensor platforms. Developmental cues like Bim-dependent B-cell killing upon BCR cross-linking [17] and sensor platforms like the DNA damage sensor network involving the ATM (ataxia telangiectasia mutated) and p53 tumor suppressors, which prominently determine survival and treatment outcomes in CLL [15].

Currently, one of the therapeutic strategies that kill CLL cells is the DNA damage response via p53 that leads to a dominant cell-death signal via Puma [18, 19]. A major problem encountered with this strategy is that a number of patients with CLL harbor defects in the DNA damage machinery that leads to deactivation of the pathway. The challenge thus seems to be to bypass such resistance and produce p53-independent cell death [15].

Another therapeutic strategy is the exploitation of CD95 signaling. But it seems to be restricted, as systemic CD95 triggering leads to fulminant liver toxicity [20]. The role of trail receptor

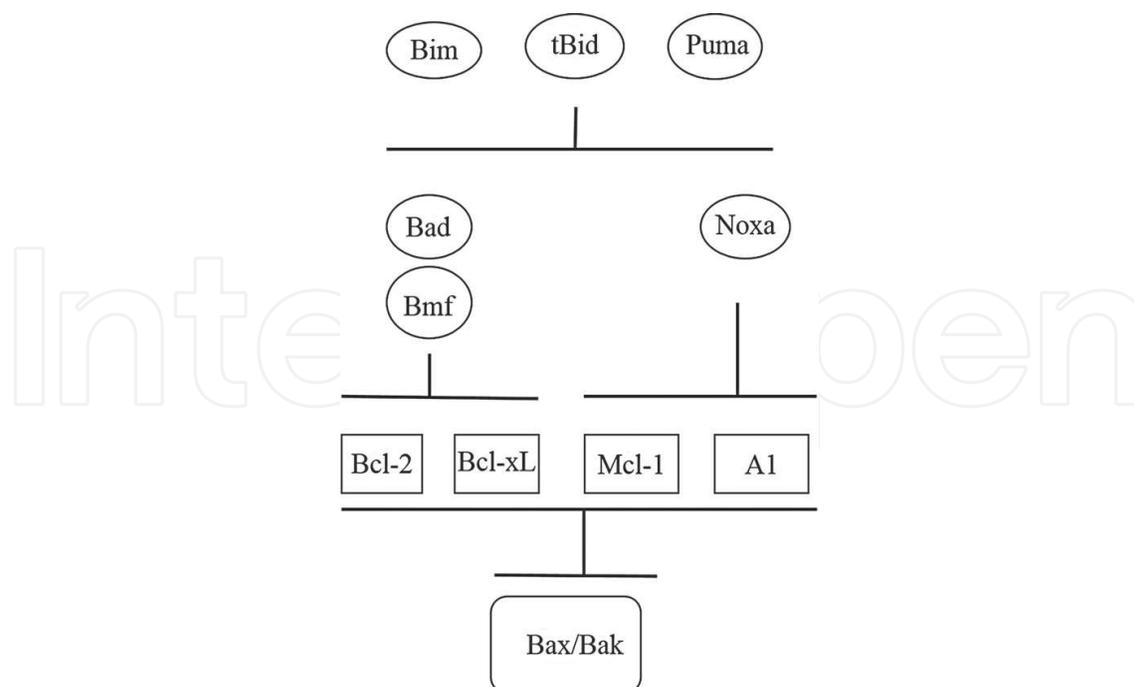


Figure 2. The interaction between Bcl-2 family member proteins [15].

targeting is currently under development. CD40 signaling may also have a positive effect on conventional therapy. It has been shown that CD154 (CD40L) application was able to induce the p53-related transcription factor p73, leading to a sensitization of p53-deficient CLL cells to conventional therapeutics such as fludarabine [21].

A number of approaches have been taken to directly modulate the core components of the Bcl-2 cell-death machinery. The Bcl-2 antisense molecule oblimersen is the most advanced agent in clinical testing. “BH3-mimetics” and “pan-Bcl-2 family antagonists” can mimic the BH3 domain of BH3-only death-inducing proteins and are thought to liberate BH3-only proteins from the inhibition by antiapoptotic Bcl-2 proteins, thus making them effective killers.

2. Apoptosis

Apoptosis means cell suicide. It is a normal component of the development and health of multicellular organisms. Cells perform in a controlled, regulated fashion by apoptosis. Apoptosis is different from another form of cell death called necrosis [22]. Cancer is often characterized by too little apoptosis. In the case of cancer, damaged cells, which should undergo apoptosis, have mutations that prevent them from undergoing apoptosis [22]. Apoptotic cells can be recognized by stereotypical morphological changes (**Figure 3**).

2.1. Pathways of apoptosis

Apoptosis consists of two major pathways: extrinsic pathway and intrinsic (mitochondrial) pathway.

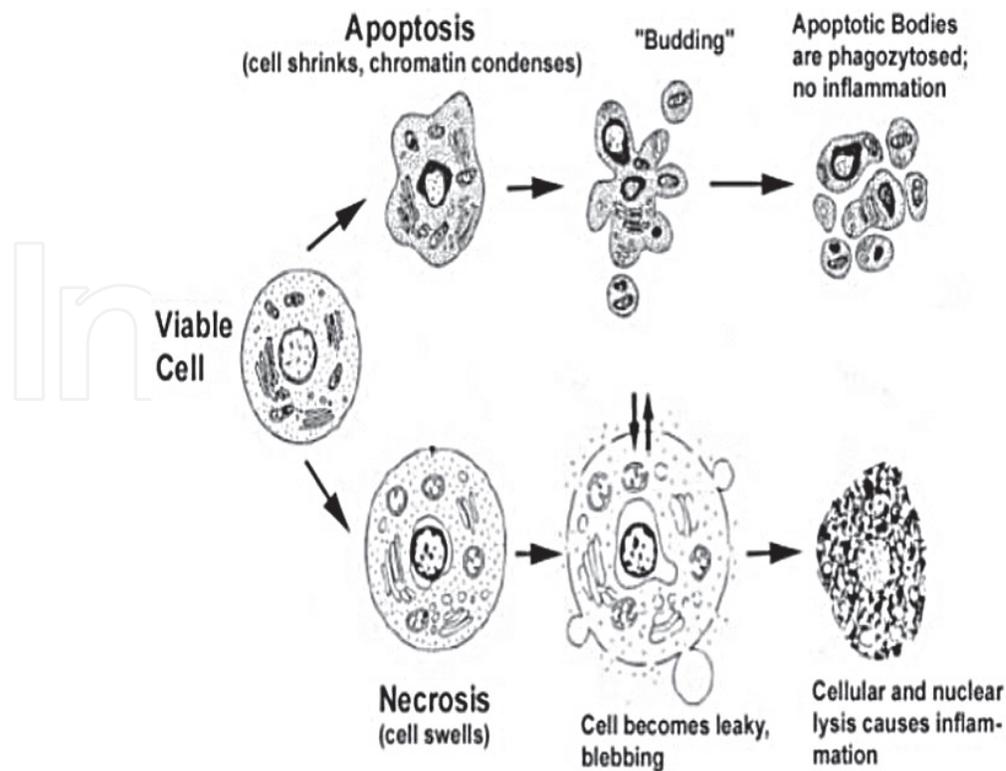


Figure 3. Hallmarks of the apoptotic and necrotic cell-death process. Modified from [23].

2.1.1. Extrinsic pathway

“Death receptors” transmit apoptotic signals after ligation with specific ligands in extrinsic pathway. Death receptors belong to a superfamily, including TNFR-1, Fas/CD95, and the TRAIL receptors DR-4 and DR-5 [24]. Caspase-8 is the hallmark of this pathway. It is activated by a complex named death-inducing-signaling complex (DISC). Activated death receptor recruited adapter molecules like FADD (Fas-associated protein with death domain) or TRADD (tumor necrosis factor receptor type 1-associated DEATH domain). These adapter molecules form the DISC (**Figure 4**). Caspase-8 then cleave and activate other caspases resulting in cell death. These types of cells, which have the capacity to induce such direct and mainly caspase-dependent apoptosis pathways, were classified to type I cells [25].

2.1.2. Intrinsic pathway

In this pathway, the signal does not come from death receptors. In this case, the signal amplified via mitochondria-dependent apoptotic pathways. Bcl-2 family member, Bid, is cleaved by caspase-8 (tBid) and translocates to the mitochondria. tBid in concert with the proapoptotic Bcl-2 family members Bax (Bcl-2-associated x) and Bak (Bcl-2 homologous antagonist/killer) induces the release of cytochrome C and other mitochondrial proapoptotic factors into the cytosol [27].

Cytosolic cytochrome C binds to monomeric Apaf-1 (apoptotic protease-activating factor 1) which then oligomerizes to assemble the apoptosome that triggers the activation of the

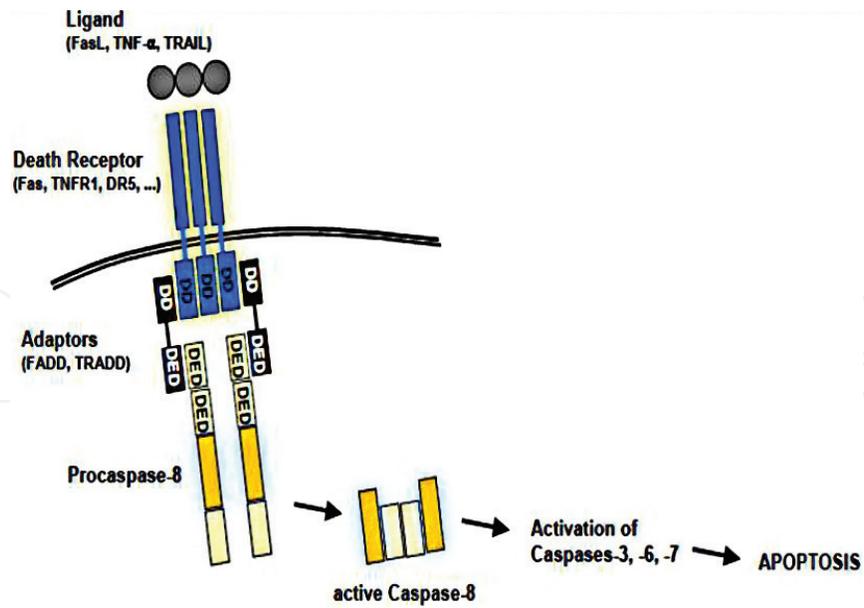
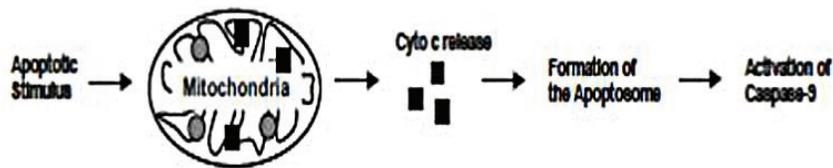


Figure 4. Receptor-mediated caspase activation at the DISC [26].

A. Mitochondrial pathway of caspase activation



B. Apoptosome formation and activation

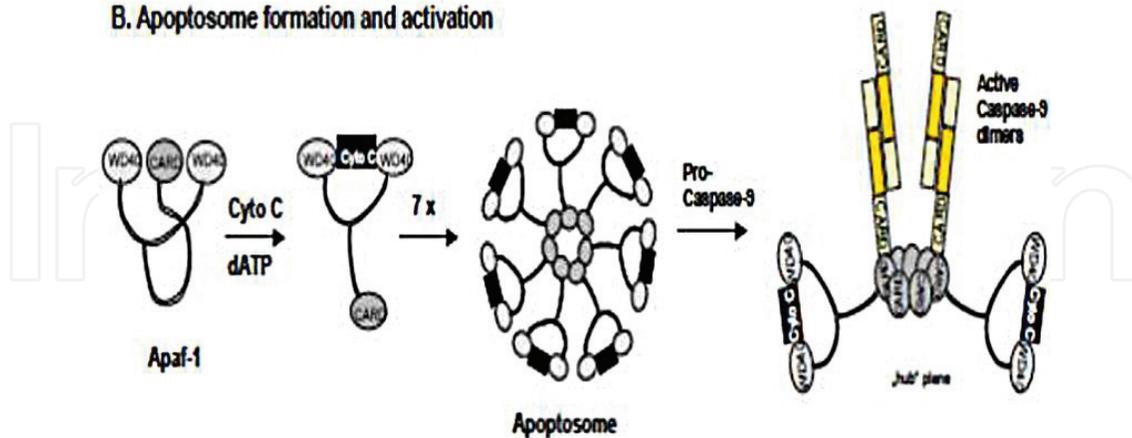


Figure 5. Intrinsic pathway of apoptosis [28].

initiator procaspase-9 [28]. Caspase-9 is the hallmark of intrinsic pathway. Activated caspase-9 ultimately results in cell death by subsequently initiating a caspase cascade involving downstream effector caspases such as caspase-3, caspase-7, and caspase-6 (Figure 5) [29].

2.2. Apoptotic pathway proteins

2.2.1. Caspases are central initiators and executioners of apoptosis

The term caspase is derived from cysteine-dependent aspartate-specific proteases. So far, seven different caspases have been identified in *Drosophila*, and 14 different members of the caspase-family have been described in mammals, with caspase-11 and caspase-12 only identified in the mouse [30, 31]. According to a unified nomenclature, the caspases are referred to in the order of their publication: caspase-1 is ICE (interleukin-1 β -converting enzyme), the first mammalian caspase [32, 33]. There are many documents about the importance of caspases in apoptosis phenomenon. For example, it has been shown that caspase-1, -4, -5, -11, and -12 are involved in the maturation of pro-inflammatory cytokines such as pro-IL-1 β and pro-IL-18 [31] or studies show that caspase-3 and -9 have a role in brain development [34, 35].

Caspases are synthesized as inactive zymogens, the so-called procaspases. Upon maturation, the procaspases are proteolytically processed. The proapoptotic caspases can be divided into the group of initiator caspases including procaspases-2, -8, -9, and -10, and into the group of executioner caspases including procaspases-3, -6, and -7 [26]. As mentioned earlier, in extrinsic apoptosis pathways procaspase-8 is the hallmark of this pathway. In return of caspase-8, caspase-9 is the hallmark of intrinsic pathway. Once the initiator caspases have been activated, they can proteolytically activate the effector procaspases-3, -6, and -7. Effector caspases subsequently cleave a specific set of protein substrates, resulting in the mediation and amplification of the death signal and eventually in the execution of cell death [36].

2.2.2. The Bcl-2 superfamily

Bcl-2 is an oncogene which was the first example of an oncogene that inhibits cell death rather than promoting proliferation. Bcl-2 family of proteins can be defined by the presence of conserved sequence motifs known as Bcl-2 homology domains (BH1 to BH4). Bcl-2 proteins divided to a group of prosurvival members and others to a group of proapoptotic members [37]. Prosurvival proteins include Bcl-2 itself, Bcl-XL, Bcl-w, A1, and Mcl-1, all of which possess the domains BH1, BH2, BH3, and BH4. The proapoptotic group of Bcl-2 members can be divided into two subgroups: the Bax-subfamily consists of Bax, Bak, and Bok, all of which possess the domains BH1, BH2, and BH3. There is another group of proteins named the BH3-only proteins (Bid, Bim, Bik, Bad, Bmf, Hrk, Noxa, Puma, Blk, BNIP3, and Spike) that have only the short BH3 motif, an interaction domain that is both necessary and sufficient for their killing action [38, 39].

Despite the existence of two hypotheses regarding how the Bcl-2 family controls apoptosis, it seems that the central function of mammalian Bcl-2 family members is to guard mitochondrial integrity and control the release of mitochondrial proteins into the cytoplasm [39]. Another hypothesis is that Bcl-2 members might directly control caspase activation [40]. The question is how mitochondrial integrity is affected by proapoptotic Bcl-2 family members? Central to this question are Bax and Bak. The double knockout of Bax and Bak resulted in dramatic impairment of apoptosis during development in many tissues with superfluous cells accumulating in the hematopoietic system and in the brain [26].

BH3-only members function upstream of Bax and Bak. It is shown that members of the BH3-only subfamily are required for the activation of proapoptotic Bax/Bak function. But it should be noted that prosurvival members Bcl-2 and Bcl-XL have a role in this way [41].

In summary, as it is shown in **Figure 6**, in a viable cell antiapoptotic proteins like Bcl-2 antagonize Bax/Bak. In response to an apoptotic stimulus, BH3-only proteins are activated. Activated BH3-only proteins prevent antiapoptotic Bcl-2 members from inhibiting proapoptotic members. Therefore, Bax/Bak are activated and form pores in the mitochondrial membrane. In consequence, cytochrome C and other proapoptotic factors are released from the inner mitochondrial membrane into the cytosol. They cause the formation of the apoptosome and the subsequent activation of the caspase cascade [26].

2.3. Mcl-1 and CLL

Mcl-1 is one of the Bcl-2-related survival proteins but is somewhat structurally distinct and probably lacks a “classical” BH4 domain. It was first discovered in differentiating myeloid cells where Mcl-1 is thought to play a transient role in promoting cell survival, but it has been expressed in various malignant cells, like CLL. Overexpression of Mcl-1 in CLL cells associated with a failure to achieve complete remission following cytotoxic therapy [42].

Mcl-1 protein has a rapid turnover, and it has a short half-life (a few hours). Mcl-1 has a critical role in regulating apoptosis in response to rapidly changing environmental cues. During apoptosis, Mcl-1 is a very efficient substrate for caspases [43–46]. While Mcl-1 is an antiapoptotic protein, its cleavage by caspases converts it into a cell-death-promoting molecule [43]. Therefore, Mcl-1

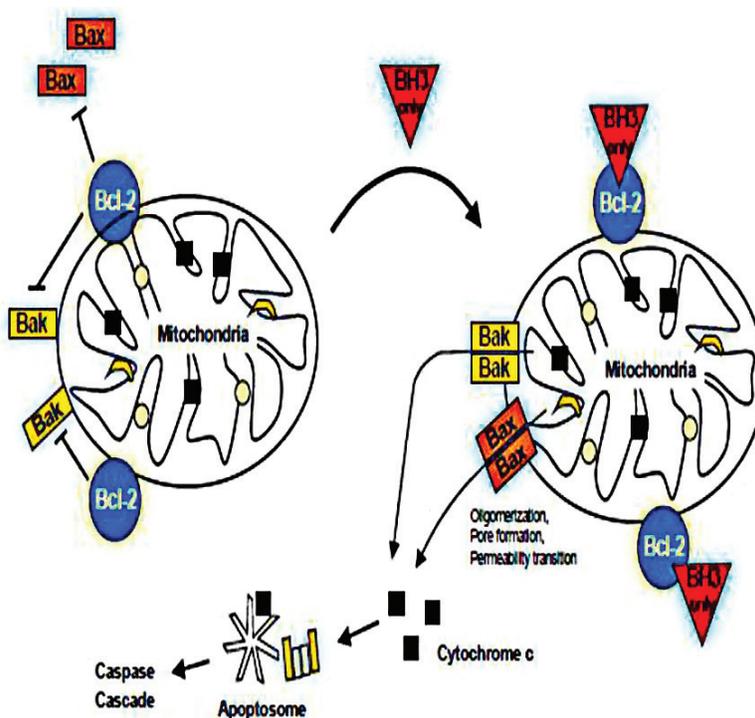


Figure 6. Regulation of apoptosis by the Bcl-2 family [26].

can act as a molecular bodyguard or assassin during apoptosis [47]. Saxena et al. showed that Mcl-1 can play an important role in CLL, by insertion of small sequences in its promoter [47]. They showed the presence of specific insertions in 29% patients with CLL and while in none of the controls. They found that these insertions were correlated with rapid disease progression, with a poor response to chemotherapy and shorter disease-specific survival. By founding of insertions in CD38-negative patients, they suggest that a poor prognostic marker [47] can be present.

Finally, since specific genetic targets are not defined in CLL, Mcl-1 seems to be an appropriate biomolecule to therapeutically manipulate. Mcl-1 protein production and maintenance are dependent on several pathways. At the apical level, the microenvironment provides factors that dramatically increase this protein in CLL cells [48]. Hence, a strategy that interferes with the interaction of microenvironment and CLL cells is a logical approach. Production of Mcl-1 through these signals is carried via increased transcription of the Mcl-1 gene. Transcription and polyadenylation inhibition, albeit not selective, is an approach that works because of AU-rich elements in the transcript of Mcl-1, which leads to its rapid turnover [49]. The N-terminal region of Mcl-1 protein contains 2PEST domains that are rich in proline, glutamic acid, serine, and threonine residues, resulting in a short half-life of the protein [49] and making translation inhibition and rapid degradation of endogenous Mcl-1 via proteasome pathway a viable option to reduce the protein level [50]. Mcl-1 is also essential during early lymphoid development [51] and is abundantly expressed in the germinal center B-cell compartment. Pim kinase and Akt-PI3-kinase pathways and downstream of BlyS have been identified to maintain the Mcl-1 levels in B-cells [52]. The roles of these pathways and consequence of their perturbations need to be investigated in malignant lymphocytes. Similarly, work is needed on posttranslational modification leading to increased or decreased half-life of Mcl-1 protein. Finally, and probably most intriguingly, small molecule antagonists of Mcl-1 protein that bind to the BH3 domain releasing proapoptotic proteins provide a new avenue of research and therapeutics.

3. Coumarins

Coumarins (2H-1-benzopyran-2-one) consist of a large class of phenolic substances found in plants and all of which consist of a benzene ring joined to a pyrone ring. More than 1300 coumarins have been identified as secondary metabolites from plants, bacteria, and fungi. The prototypical compound is known as 1,2 benzopyrone or, less commonly, as *o*-hydroxycinnamic acid and lactone. Coumarins were initially extracted in *tonka* bean (*Dipteryx odorata* Wild) and are reported in about 150 different species distributed over nearly 30 different families, of which a few important ones are *Rutaceae*, *Umbelliferae* (*Apiaceae*), *Clusiaceae*, *Guttiferae*, *Caprifoliaceae*, *Oleaceae*, and *Nyctaginaceae* [53]. They are found at high levels in some essential oils, particularly in *cinnamon* bark oil, *cassia* leaf oil, and *lavender* oil. Coumarin is also found in fruits (e.g., bilberry and cloudberry), green tea, and other foods such as chicory. The richest sources of most coumarins among the higher plants are *Rutaceae* and *Umbelliferone*. The coumarins occur at the highest levels in the fruits, followed by the roots, stems, and leaves although they are distributed throughout all parts of the plant. Environmental conditions and seasonal changes can influence the occurrence in diverse parts of the plant [54].

3.1. Classification

Based on the chemical structure of their compounds, natural coumarins are classified into six groups (Table 1).

Coumarin and its derivatives are principal oral anticoagulants. Coumarin is water insoluble; however, 4-hydroxy substitution confers weakly acidic properties to the molecule that makes it water soluble under slightly alkaline conditions (Figure 7) [54].

The structure of coumarin nucleus (Figure 8) mimics A and B rings of the steroid hormone and binds to the aromatase-binding site with a superior affinity. Upon tactically extending the structure to the tricyclic system, it mimics the steroid hormones that act as SERM/SERD (selective estrogen receptor modulator/selective estrogen receptor downregulator) and thus enhancing the receptor interaction, leading to a development of a potent pharmacophore. 17 β -HSD3 (17 β -hydroxysteroid dehydrogenase type3), cell division cycle protein, and NF- κ B inhibitory activity are potentiated by structural extension with sulfur linked at the C-4 position [55]. It has also been shown that the anticancer activity of coumarins is potentiated by the substitution of imidazole, 1,2,3-triazol, piperidine purine, benzothiazole, substituted phenyl

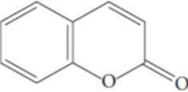
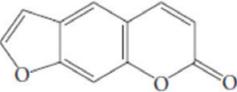
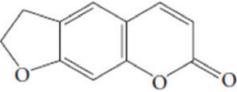
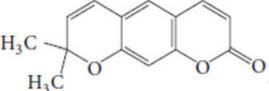
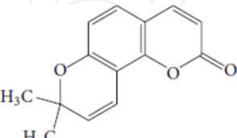
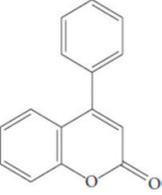
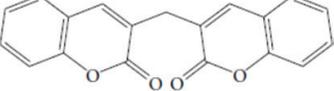
Type of coumarin	General chemical structure
Simple coumarins	
Furano coumarins	
Dihydrofurano coumarins	
Pyrano coumarins (linear types)	
Pyrano coumarins (angular types)	
Phenyl coumarins	
Bicoumarins	

Table 1. Classification of natural coumarins based on their chemical structure.

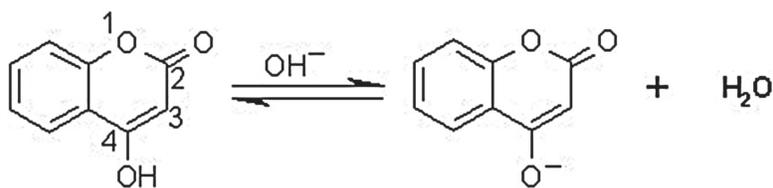


Figure 7. 4-hydroxy substitution of coumarin makes it water soluble in alkaline conditions.

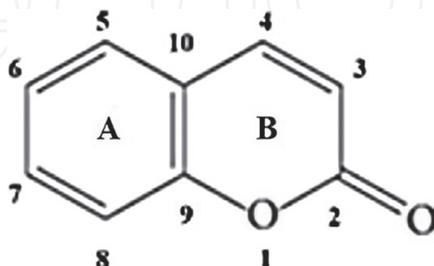


Figure 8. Structure of simple coumarin.

ring, aryl acrylic acid, and chalcone at the fourth position of coumarin nucleus by a linker such as methylene and oxygen [55].

3.2. Coumarins and leukemia

Induction of apoptosis in leukemic cell lines by coumarins and their derivatives is demonstrated in different *in vitro* studies. Coumarin compounds have antiproliferative and/or cytotoxic activity on cancer cells, depending on their substitution pattern [56–58]. It is shown that while long alkyl substitution at C7 position increased the cytotoxic activity against the leukemia cancer cell lines [59], the presence of two hydroxyl groups at C7 and C8 positions seems to improve the potency of methylcoumarins as cytotoxic agents. It is also shown that among 7,8-DHMC (dihydroxy-4-methylcoumarin) derivatives, the longer the C3 alkyl chain, the higher was the activity. This effect of the alkyl group on the cytotoxicity is presumably due to the enhanced lipophilicity of the longer alkyl chains that consequently enhances cell membrane penetration ability of the test compounds. Bromo groups substituted at C4 and C6 positions for DHMCs increased the cytotoxic activity in all the cell lines (Figure 9A) [60]. In another study, it was shown that 7-hydroxycoumarin analog containing carboxymethyl ester group on cinnamoyl moiety (Figure 9B) showed good antiproliferative activity against leukemic cell lines [61]. It is worth noting that the cinnamoyl moiety at C3 is more effective than alkyl chain moiety for increased cytotoxic effect against leukemic cell line K562 ($IC_{50} = 4.4 \mu\text{M}$ vs. $40.8 \mu\text{M}$).

Moreover, studies showed that molecular hybridization of coumarins increased their cytotoxicity against leukemic cell lines. For example, the hybrids with ortho-dihydroxy groups or ortho-hydroxy-methoxy group on the aromatic A ring exhibit superior antiproliferative activity in comparison with those with such groups on the aromatic B ring. Specially, a new hybrid, 6-methoxy-7-hydroxy-3-(4'-hydroxyphenyl)coumarin, emerged as an important lead compound with excellent antiproliferative, apoptosis-inducing, and cell cycle arrest activities against HL-60 cell line ($IC_{50} = 5.2 \pm 0.6 \mu\text{M}$) (Figure 10) [62].

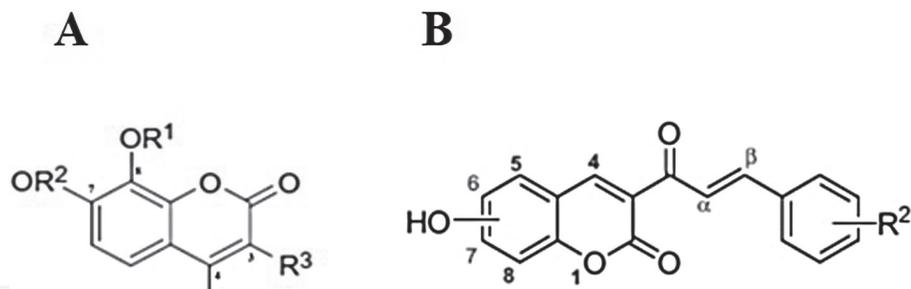


Figure 9. R₂ is 4-(COOMe).

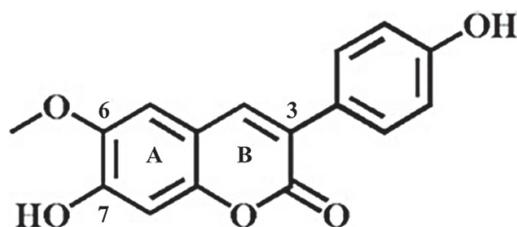


Figure 10. 6-methoxy-7-hydroxy-3-(4' hydroxyphenyl)coumarin) as a new hybrid.

Paul et al. showed that the synthesis of new conjugated coumarin-benzimidazole hybrids displayed appreciable antileukemic activities *in vitro*. They showed that the introduction of ethanolamine at position 7 of coumarin-benzimidazole hybrid (Figure 11) shows higher selectivity against leukemia cancer cells (CCRF-CEM, HL-60(TB), K-562, and RPMI-8226) [63].

Other studies showed that hydrazide-hydrazone ($-\text{CO}-\text{NH}-\text{N}=\text{CH}-$) moiety and acrylohydrazide hybrid at position 3 could increase the cytotoxicity against leukemic cell lines (Figure 12) [64, 65].

In other studies, it has been shown that the copper complexes with coumarin derivatives could increase the antileukemic effect of coumarin *in vitro* (Figure 13).

Specifically, in some studies, the significant inhibitory activity of certain coumarins on the proliferation of leukemic cell lines [58, 66–68] has been reported. In addition, it has been described that such inhibitory effects could be related to either differentiating [58, 66] or proapoptotic activities [67, 68] of the compounds, depending on the distribution of their substituents in the coumarin ring.

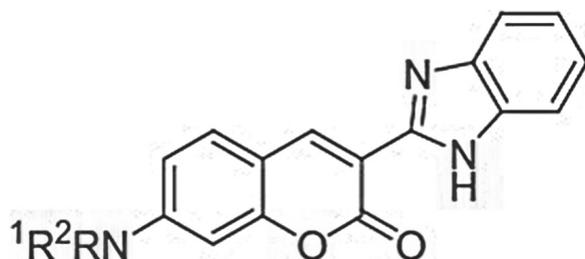


Figure 11. NR¹R² is ethanolamine.

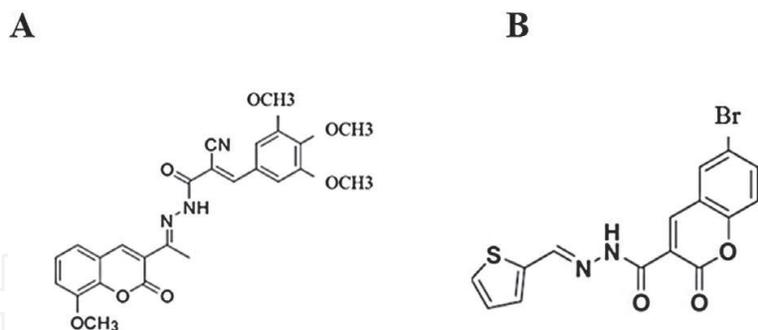


Figure 12. Hydrazide-hydrazone moiety and acrylohydrazone hybrid of coumarin.

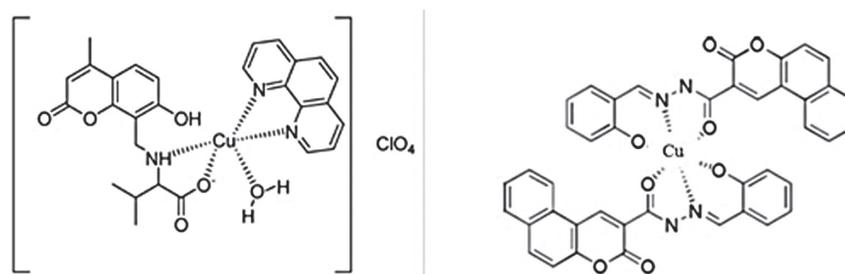


Figure 13. Copper complexes with coumarin.

Kim and colleagues studied the antileukemic effects of decursin (a pyranocoumarin from *Angelica gigas*) and its derivatives (Figure 14) on K562 and U937 cell lines. They studied the ability of these compounds as a tumor-suppressing PKC activator and as an antagonist to phorbol 12-myristate 13-acetate (PMA), a tumor-promoting PKC activator. Based on their results, the structure-activity relationship of decursin and its derivatives is as follows: (i) the coumarin structure is required for antileukemic activity and (ii) the side chain is a determinant of PKC activation and the cytotoxic mechanism in leukemia cells [69].

In another study, Ahn et al. showed the apoptosis induction by decursin in leukemic KBM-5 cells. They showed that decursin activates caspases 9 and 3 and PARP in KBM-5 cells. They also reported that decursin induced apoptosis via downregulation of COX-2-dependent survivin pathway in KBM-5 myeloid leukemia. In KBM-5 cells, it was reported that targeting survivin could overcome the resistance against imatinib [70].

Esculetin (Figure 15) is a simple coumarin found in some traditional medicines. Induction of apoptosis in various leukemic cell lines was shown in different studies. Chu and their colleagues are one of the first teams that reported the antileukemic effects of esculetin. They showed that esculetin inhibits the survival of human promyelocytic leukemia HL-60 cells in a concentration-dependent and time-dependent manner. Esculetin induced the release of cytochrome C from mitochondria into cytosol, reduced Bcl-2 protein expression, and increased caspase activation [71].

Esculetin is a cell cycle-specific antineoplastic agent. It can inhibit the growth of HL-60 and U937 leukemic cells by G1 cell cycle arrest [72, 73]. It also leads to the release of cytochrome C, activation of caspases 3, 8, and 9, downregulation of Bcl-2 protein, and increased the phosphorylation of MEK/ERK and JNK [74–77].

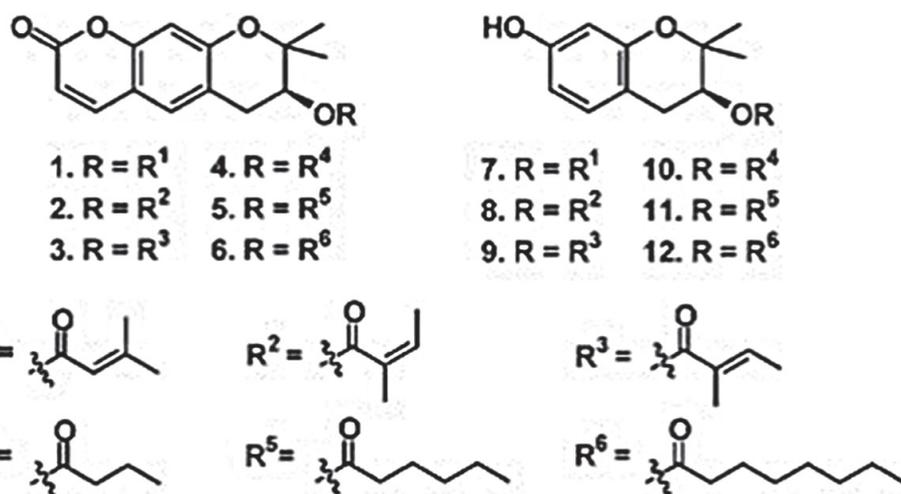


Figure 14. Structures of decursin (1) and its derivatives 2–12.

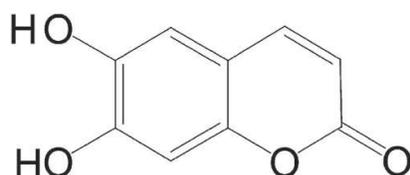


Figure 15. Structure of esculetin.

Tung et al. fractionated and chemically investigated the methanol extract of *Mammea siamensis* flower, an evergreen tree belonging to the family of Calophyllaceae, and distributed throughout Thailand, Myanmar, Laos, Cambodia, and Vietnam. They isolated and identified eight compounds. Among the isolated compounds, three structurally related coumarins kayeasamin A (Figure 16), surangin C, and theraphin B showed significant antiproliferative activity against human leukemia HL-60 cells. Activation of caspases 3 and 8 and sub-G1 arrest by kayeasamin A have been shown in this study and another one [78, 79].

Osthole (Figure 17) is another coumarin where its antileukemic effect has been investigated. It has been shown that osthole has the strongest cytotoxic activity among the coumarins extracted from *Cnidii monnieri Fructus* on HL-60 cell line. The structure-activity relationship established from the results indicated that the prenyl group has an important role in the cytotoxic effects and apoptosis induction [80].

In another study, osthole could increase intracellular drug accumulation, decreased the expression of multidrug resistance gene 1 (MDR1), and could suppress P-gp expression by inhibiting the PI3K/Akt-signaling pathway in myelogenous leukemia K562/ADM cells [81].

Imperatorin, a biologically active furanocoumarin, is another coumarin that is extracted from *Cnidii monnieri Fructus* also showed cytotoxic effect against leukemic cell lines [82–85].

Toddaculin (Figure 18) is another important coumarin where its antileukemic effect is revealed. Vazquez et al. found that toddaculin was the most potent cytotoxic agent among the series of six prenylated coumarins isolated from the stem bark of *Toddalia asiatica* (Rutaceae). They found that while toddaculin at 250 μM ($\text{IC}_{50} = 51.38 \pm 4.39$) was able to induce apoptosis

in U-937 cells, involving decreased phosphorylation levels of ERK and Akt, 50 μ M toddaculin exerted differentiating effects [86].

Umbelliprenin (**Figure 19**) is a prenylated coumarin found in *Ferula* species. Its antileukemic effect was first reported by Gholami and his colleagues. They found that umbelliprenin has cytotoxic and proapoptotic effects on Jurkat and Raji cell lines. They showed that umbelliprenin activates intrinsic and extrinsic pathways of apoptosis by the activation of caspase-8 and -9, respectively. Inhibition of Bcl-2 was also shown [87, 88].

Auraptene (**Figure 20**) is another coumarin that has a structure close to that of umbelliprenin. The difference between the chemical structures of these compounds is that the length of the 7-prenyloxy chain of umbelliprenin is longer and contains 15 instead of 10 carbons. Apoptogenic activity of auraptene on Jurkat cells was shown in detail. Apoptotic effect of auraptene on Jurkat T-cells was exerted by the ER stress-mediated activation of caspase-8 and the subsequent induction of mitochondria-dependent or -independent activation of caspase cascade, which could be suppressed by Bcl-xL [89].

3.3. Mcl-1 and coumarins

Coumarins can regulate the expression of Mcl-1. Their regulation is time- and dose-dependent. The regulation of Mcl-1 expression by auraptene, umbelliprenin, imperatorin, galbanic acid, and gut-70 was studied.

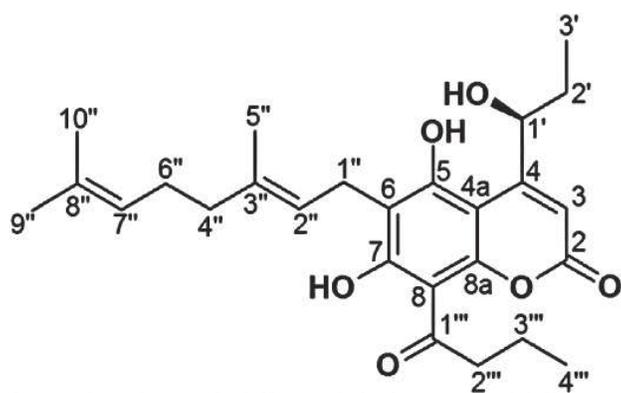


Figure 16. Structure of kayeassamin A.

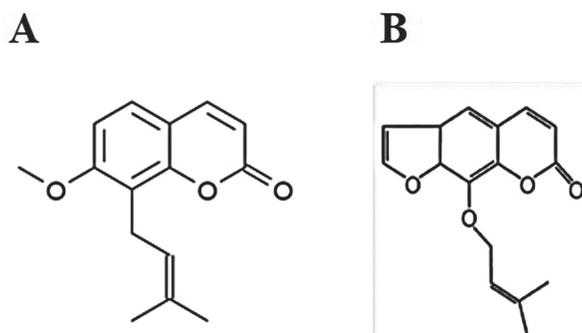


Figure 17. Structure of osthole (A) and imperatorin (B).

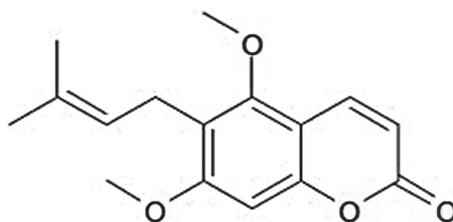


Figure 18. Structure of toddaculin.

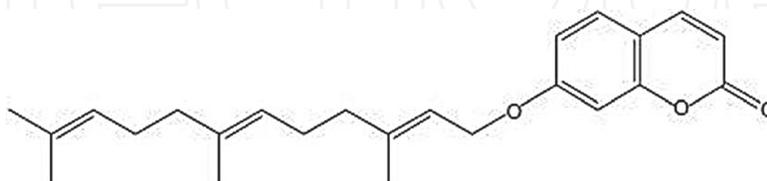


Figure 19. Structure of umbelliprenin.

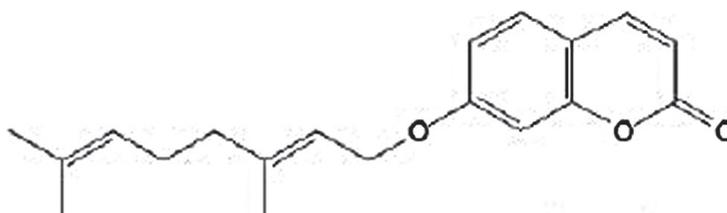


Figure 20. Structure of auraptene.

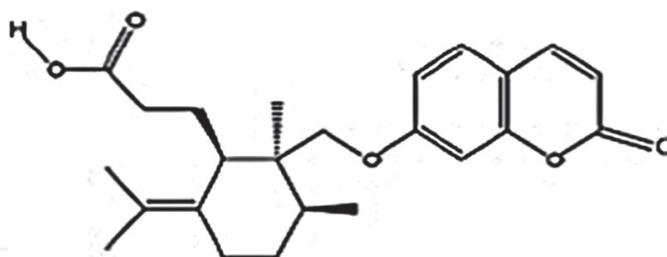


Figure 21. Galbanic acid.

Gholami et al. studied and compared the expression of Mcl-1 gene after the Jurkat cells were incubated by umbelliprenin and auraptene. They showed that umbelliprenin increased the expression of Mcl-1 mRNA from 1 to 3 h of incubation, but this increase has a scale-down pattern. Auraptene decreased the expression of Mcl-1 mRNA for the same incubation times [90, 91]. This pattern is similar for Mcl-1 protein expression [91, 92].

Another natural coumarin where its effect on Mcl-1 expression was studied is galbanic acid (**Figure 21**). Galbanic acid downregulates the Mcl-1 protein expression dose dependently [93]. Imperatorin (**Figure 17B**), another natural coumarin like galbanic acid, decreased Mcl-1 protein level in a dose-dependent manner [94].

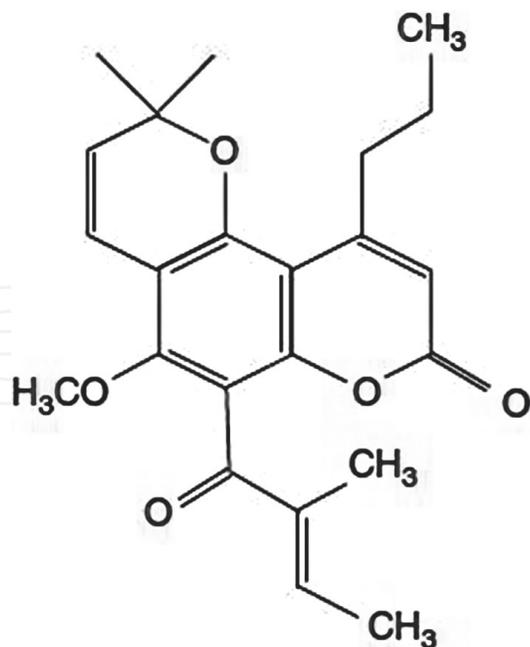


Figure 22. GUT-70.

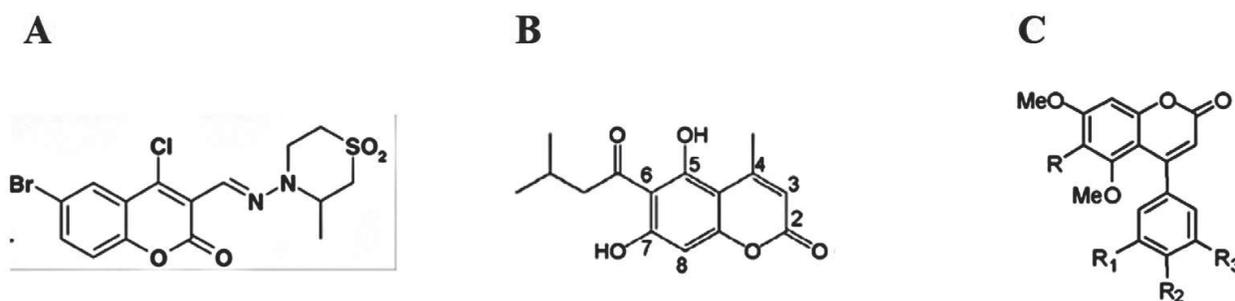


Figure 23. (A) RKS262, (B) DMAC, and (C) 4-aryl coumarin analogs of combretastatin.

GUT-70 (**Figure 22**), a tricyclic coumarin derived from *Calophyllum brasiliense*, causes Mcl-1 protein upregulation in mantle cell lymphoma (MCL) cell lines [95].

The effect of synthetic coumarins (RKS262, 5,7-dihydroxy-4-methyl-6-(3-methylbutanoyl)-coumarin (DMAC), and 4-aryl coumarin analogs of combretastatin (**Figure 23**)) on Mcl-1 protein expression was studied. All of these compounds downregulate Mcl-1 protein dose- and time- dependently [96–98].

4. Conclusion

In conclusion, coumarins are one of the important cytotoxic agents. They could induce apoptosis and regulate Mcl-1 expression in CLL cell lines. We hope that they be one of the candidates for chemotherapy of CLL in the future.

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