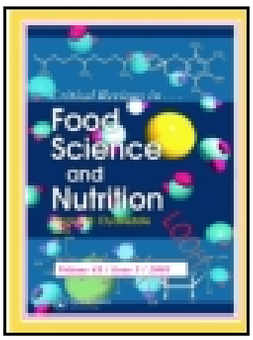

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Critical Reviews in Food Science and Nutrition

ISSN: 1040-8398 (Print) 1549-7852 (Online) Journal homepage: http://www.tandfonline.com/loi/bfsn20

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To cite this article: José C. J. M. D. S. Menezes, Barbora Orlikova, Franck Morceau & Marc Diederich (2015): Natural and Synthetic Flavonoids: Structure Activity Relationship and Chemotherapeutic Potential for the Treatment of Leukemia, Critical Reviews in Food Science and Nutrition, DOI: 10.1080/10408398.2015.1074532

To link to this article: http://dx.doi.org/10.1080/10408398.2015.1074532

Accepted author version posted online: 13 Oct 2015.

Natural and Synthetic Flavonoids: Structure Activity relationship and Chemotherapeutic Potential for the Treatment of Leukemia

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Abstract

Flavonoids and their derivatives are polyphenolic secondary metabolites with an extensive spectrum of pharmacological activities, including antioxidants, antitumor, anti-inflammatory, and antiviral activities. These flavonoids can also act as chemopreventive agents by their interaction with different proteins and can play a vital role in chemotherapy, suggesting a positive correlation between a lower risk of cancer and a flavonoid-rich diet. These agents interfere with the main hallmarks of cancer by various individual mechanisms, such as inhibition of cell growth and proliferation by arresting the cell cycle, induction of apoptosis and differentiation, or a combination of these mechanisms. This review is an effort to highlight the

therapeutic potential of natural and synthetic flavonoids as anticancer agents in leukemia treatment with respect to the structure-activity relationship (SAR) and their molecular mechanisms. Induction of cell death mechanisms, production of reactive oxygen species, and drug resistance mechanisms, including p-glycoprotein efflux, are among the best-described effects triggered by the flavonoid polyphenol family.

Keywords

Flavonoids; Cytotoxic; Leukemia; SAR; Caspase; PARP; Mitochondria; ROS; P-glycoprotein; Chemoprevention; Apoptosis

1. Introduction-

Flavonoids are a subclass of polyphenols, many of which have a positive impact on health by altering metabolic processes (Beecher, 2003). Flavonoids are aromatic compounds with a phenylchromanone unit consisting of two benzene rings designated as A and B, which are linked by three carbons and one oxygen atom to form the central pyrone ring, designated as C (C6-C3-C6 skeleton; see flavones structure in Figure 1). Flavonoids are further classified into distinct sub-classes depending on the saturation level or opening of the central pyran ring, which include chalcones, flavonols, flavanones, flavones and isoflavones (see Figure 1 for examples) (Harborne and Williams, 2000). The diversity of natural flavonoids is mainly due to their substitution pattern in the A, B and C rings by hydroxylation, methoxylation, prenylation, or glycosylation. Flavonoids (as glycoside derivatives or in the free form) form an integral part of the human diet as they are naturally present in fruits, vegetables, tea and wines (Yao et al., 2004).

The chemoprotective potential of natural or synthetic flavonoids on several types of cancers is evident from the plethora of published scientific research (Batra and Sharma, 2013; Khoo et al., 2010; Ren et al., 2003; Seelinger et al., 2008; Shukla and Gupta, 2010; Teillet et al., 2008). Moreover, from a synthetic point of view, chalcones are easily assembled by Claisen condensation from acetophenone and benzaldehyde derivatives and by other synthetic methods (Bukhari et al., 2013). Chalcones are then easily converted into the respective flavanones and flavones by cyclisation reactions (Menezes et al., 2010; Orlikova et al., 2014), whereas isoflavones can be prepared from flavanones by 2,3-aryl migration using suitable reagents, thus

mimicking biogenetic pathways (Menezes et al., 2010). This review will focus on the chemoprotective effects of natural and synthetic flavonoids on various types of hematological diseases based on the available literature of representative cell lines, structure activity relationships (SAR), discuss their mode/mechanism of action with respect to the structural classes of flavonoids and thereby derive structure activity conclusions. Structurally diverse flavonoids, especially with substitution patterns, are discussed or included to provide an effective starting point in the future design or use of novel flavonoids in leukemia therapy.

2. *The chemotherapeutic effect of flavonoids on acute promyelocytic leukemia-*

2.1. *Induction of leukemia cell differentiation*

Differentiation is a fate of hematopoietic cells that allows them to acquire the essential characteristics for function, such as hemostasis. Hematopoietic cell maturation takes place by response to internal and external stimuli; pluripotent stem cells, which are at the top end of the hierarchy, differentiate further into specific lineages. Differentiation therapy arises from the fact that leukemic cells that have lost their ability to differentiate, dysregulate their maturation process and eventually become malignant (Leszczyniecka et al., 2001).

Acute promyelocytic leukemia (APL), a subtype of acute myelocytic leukemia (AML) represented by HL-60 cells, can be induced to differentiate into granulocytes, monocytes and macrophages by differentiation-inducing agents. In a SAR study, the flavones apigenin **1** and luteolin **2** (Figure 1) strongly inhibited the growth of cells at 100 µM and induced their differentiation into granulocytes. Although the flavonol quercetin **14** inhibited cell growth, it did not morphologically induce cells to differentiate into granulocytes but induced monocytic

differentiation of HL-60 cells. The flavonols galangin **12** and kaempferol **13** and the flavanone naringenin **9** inhibited growth of HL-60 cells without inducing differentiation. The dihydrochalcone phloretin **19** induced monocytic differentiation of HL-60 cells**.** At 100 µM, the isoflavone genistein **23** induced phagocytic activity in HL-60, as witnessed by the nitroblue tetrazolium (NBT) test measuring the reducing ability. Overall, this study indicated that the flavone structure is crucial for strong growth inhibition of HL-60 cells and induction of differentiation into granulocytes (Takahashi et al., 1998). The regulation and signalling of genistein **23** induced leukemia cell differentiation was studied on HL-60 and NB4 cells. Genistein **23** differentiated HL-60 cells at concentration of 25 µM as observed by expression of CD11b and NBT reduction capacity and accumulated cells in G_2/M phase within 24h. Late apoptosis (72h) at the same concentration was observed by accumulation of cells in the sub-G_1 phase. Further using all-trans retinoic acid (ATRA), a known differentiation inducer, showed an additive effect on CD11b (differentiation-associated surface antigen) and NBT activities. Although ATRA slightly reduced formation of G_2/M phase induced by **23**, it greatly reduced the apoptosis effect. The differentiation induction was mediated *via* the Raf-1/MEK/ERK pathway. The ERK activation and subsequent differentiation induction were found to be dependent on the proper functioning of the PI3K/Akt pathway, since the PI3K inhibitor prevented these processes. Reactive oxygen species (ROS) were accumulated by action of **23** at 25 µM but the antioxidant *N*-acetyl-L-cysteine (NAC) reduced the capacity of **23** to induce apoptosis. Further, NAC did not prevent the **23** induced G_2/M phase blockage, CD11b induction and ERK activation indicating ROS independent pathways (Sánchez et al., 2009).

The 3-mono and 3,7- di-O-β-D-glucopyranoside derivatives of quercetin isolated from mulberry leaves (*Morus alba* L.) inhibited 51 and 57% cell growth of HL-60 cells, respectively, at 20 µM. The 3,7- disubstituted compound induced differentiation into granulocytes and monocytes, whereas the monosubstituted compound had no effect on differentiation (Kim et al., 2000).

The O-methylated flavone Oroxylin A **5**, isolated from *Scutellaria radix*, has shown ability to differentiate AML cells by binding and activating the peroxisome proliferator-activated receptor γ (PPARγ) and its heterodimer with retinoid X receptor α (RXRα). The differentiation of leukemic cells into monocytes rather than granulocytes is brought about by expression of PPARγ and controlling the phosphorylation of RXRα, which sensitizes nuclear receptors. Oroxylin A **5** induced dose and time dependent growth inhibition in HL-60 and U937 cells from 24-96 h. Xenografts of U937 cells in nude mice to study effects of **5** *in vivo* showed a decrease in mean tumor volume and tumor weight of 34% and 42%, respectively, in comparison with controls after 2 weeks. Growth inhibition in AML blasts from patients with *de novo* AML and a sample from patients with acute phase chronic myelogenous leukemia (CML) was observed by enhancement of NBT reduction. At 40 µM concentration of **5**, cells accumulated in the G1 phase and decreased in the S phase in the HL-60 and U937 cell lines. The increase in the expression of PPARγ in HL-60 and U937 by **5** was inhibited by GW9962 (an irreversible PPARγ antagonist), indicating its importance in cell phase progression and differentiation. A molecular docking study of **5** with PPARγ to understand the regulation mechanism showed that the B-ring of **5** inserted into the hydrophobic pocket, having stable interactions with His449 and 323, Tyr473 and 327, and Met364 and Ile326, whereas the 5,7-hydroxyl groups formed hydrogen bonds with

Arg288 and Leu340, as verified by the fluorescence polarization (FP)-based ligand assay (Hui et al., 2014).

2.2. *Induction of apoptosis in APL cells*

Apoptosis is the process of programmed cell death, which occurs physiologically during embryo development and member formation but also to maintain cellular homeostasis. Moreover, deregulated apoptosis is one of the hallmarks of cancer as diseased cells acquire resistance against various pro-apoptotic mechanisms. Apoptosis usually leads to morphological changes in the cell, including nuclear condensation, membrane blebbing and finally chromatin condensation and chromosomal DNA fragmentation. Caspases (aspartate-specific cysteine proteases) can directly or indirectly cleave cellular target proteins during apoptosis.

Many studies on the ability of flavonoids to induce AML cells apoptosis led to interesting observations. The use of flavonoids, principally from the human diet, as chemotherapeutic agents depends on their final effective concentration in the plasma and their mechanism of action. Alterations in the cell cycle in HL-60 cells at concentrations ≥10 μM of **13**, namely an increase in the S-phase and a decrease in the G1 phase, with progressive accumulation of G2-M, were seen. At the highest concentrations, the cells shrunk with elevated caspase-3 activity, decreased Bcl-2 expression and changes in the membrane asymmetry and integrity. The remaining population had elevated caspase-3 activity, a moderate increase in Bcl-2 expression but no plasma membrane alterations. Differentiation was not a significant factor in HL-60 growth inhibition (Bestwick et al., 2007). A similar study with galangin **12** (concentration ≥10 μM) showed an increase of the hypodiploid DNA content and active form of caspase-3 expression,

prior to membrane damage. Cytotoxicity due to intracellular oxidative stress or significant phagocyte-like differentiation was not detected (Bestwick and Milne, 2006).

Inhibition of glyoxalase I, a key enzyme involved in pathways for detoxification of methylglyoxal (glycolysis side product), leads to induction of apoptosis. A pharmacophoric modulation to identify specific inhibitors used flavonoids with a C-4 ketone and C-5 hydroxyl group, such as baicalein **4**, baicalin (baicalein-7-*O*-glucuronide), kaempferol **13**, luteolin **2**, myricetin **15**, naringenin **9**, oroxylin A-7-*O*-glucuronide, quercetin **14** and hyperin (quercetin-3-*O*-galactoside). The interatomic distance between the oxygens of the C-4 ketone and the C-5 hydroxyl group was 2.8 Å, mimicking the enediolate intermediate distance of 2.5 ± 0.5 Å formed along the reaction pathway of glyoxalase I. The *in vitro* glyoxalase I assay indicated **2**, **4**, **13**, **14** and **15** as effective inhibitors, whereas **9** showed 50% inhibition at the same concentration (100 µM). The flavonoid glucuronides were poor inhibitors. The antiproliferative capacity of these flavonoids correlated well with the *in vitro* glyoxalase I activity when evaluated in HL-60 cells. Among the studied flavones, the presence of hydroxyl groups at the 6 and 3' positions favored inhibition of the glyoxalase activity, whereas in flavonols, the order of potency was **15**>**14**>**13** (associated with hydroxyl groups in ring B). The SAR analysis indicated that a more hydroxylated B-ring may contribute to higher inhibition activity and the presence of a 3-hydroxyl group does not exclude the effect. Higher values of GLO I inhibition by **2** over **13** may not influence this activity. The presence of bulky groups (glucuronide/galactoside) at the C-7 and C-3 positions lowered the inhibitory activity. The docking analysis indicated that the B-ring hydroxyl groups contribute to hydrogen bonding with polar amino acids in the S2 hydrophilic pocket of the human glyoxalase I (Takasawa et al., 2008).

A SAR study of the flavonoid constituents isolated from *Andrographis paniculata* indicated that luteolin **2** was the most cytotoxic flavone in HL-60 cells. The 5-hydroxyl substitution in A-ring and the 3',4'- dihydroxyl substitution in B-ring was concluded to be important for inhibiting cell growth of HL-60 cells (Li-Xia Chen et al., 2014). Another SAR study on the effects of 5,7-dihydroxyflavone skeleton influence on HL-60 cell proliferation indicated that a combination of hydroxyl and methoxy substituents on the B-ring at 3',4',5'-positions is favourable for inhibition of cell growth (Ninomiya et al., 2013).

To verify the potential of flavonoids in combination chemotherapy and to reduce the side effects, a study of quercetin **14** and genistein **23** with an anticancer agent ML-7, which inhibits the myosine light chain kinase (MLCK), versus cisplatin and cytarabine (Ara-C) with ML-7 was attempted using HL-60 cells. The results indicated a synergistic effect between the flavonoids and ML-7, whereas no such effects were observed with use of cisplatin and Ara-C, which are known cancer therapeutic agents (Lee et al., 2009). In a cisplatin induced study, the effects of **14**, galangin **12** and chrysin **3** in human HL-60 and murine L1210 leukemia cells were investigated using apoptotic DNA fragmentation. The results indicated that **14** enhanced, whereas **12** reduced and **3** had no effect on DNA fragmentation (Luboš Čipák et al., 2003).

In the search for activators of caspase 3 and 7, the proteases at the core of the execution phase of apoptosis, a simplified yeast-based screening approach was developed. Two synthetic compounds, 3-hydroxy-7-geranyloxyflavone **25** and 5,6-dihydroxy-7-prenyloxyflavone **26** (derivative of baicalein **4**) (Fig 2) were potent in the yeast assay, and subsequent evaluation of the activity of the selected compounds in the human tumor cell lines HL-60 and MCF-7

indicated both to be activators of caspase 7. Procaspase activating compound-1 (PAC-1), a known activator of caspase 3 and 7, was used to demonstrate the ability of these compounds to selectively affect only caspase 7 in yeast and *in vitro* assays (Pereira et al., 2014).

Flavonoids such as **2**, isolated from *Flos Chrysanthemi*, and its synthetic benzyl derivatives **27** and **28** (Fig 2) were evaluated in HL-60 cells for their potential to induce apoptosis and inhibit cell growth supported by docking experiments to show binding with the anti-apoptotic Bcl-2 protein. Molecular docking of **2** indicated that the A and C rings bind well to the L1 domain, whereas the B ring forms hydrophobic interactions by entering the P1 hydrophobic pocket. Hydrogen bonding of the hydroxyl groups of **2** with G145, Y108 and E136 residues in the protein binding groove were also observed. These crucial H-bonding interactions of **2** for binding to Bcl-2 protein were ascertained experimentally by a fluorescence polarization (FP) assay using tetraacetate of **2**, flavone and **3**, which showed no inhibitory activity against Bcl-2 and Bcl-xL. The synthetic derivatives **27** and **28** improved the hydrophobic binding in the P1 pocket and showed better inhibitory activity experimentally by FP assay. The superior bioactivity of **27** in comparison with **2** in HL-60 cells was observed by induction of apoptosis and release of cytochrome c from the mitochondria to cytosol (Zheng et al., 2014).

2.2.1. *Hydroxyl groups and ROS*

A study between related flavonoids (apigenin **1**, kaempferol **13**, quercetin **14** and myricetin **15**, at 60 μM) showed induction of apoptosis in HL-60 cells by an increase of caspase-3 activity, thereby stimulating proteolysis of poly-(ADP-ribose) polymerase (PARP; a protein involved in DNA repair considered as a prototypical caspase-3 target). These flavonoids also induced

apoptotic events, such as loss of the mitochondrial transmembrane potential, release of mitochondrial cytochrome c into the cytosol, increase in reactive oxygen species (ROS) production, and subsequent induction of procaspase-9 processing. The potency of these flavonoids towards DNA fragmentation, loss of mitochondrial transmembrane potential, ROS generation and activation of caspase-3 activity was **1**>**14**>**15**>**13**. The structure activity of these flavonoids was highest for flavones, followed by flavonols having the 3-hydroxyl group. The potency of the flavonols to induce apoptosis depended on the presence of hydroxyl groups in the B-ring, which helped to counteract the presence/effect of the 3-hydroxyl group (Wang et al., 1999).

An interesting study to ascertain the relationship between a minor difference in the chemical structure of six naturally occurring flavonoids i.e. taxifolin **11** (absence of a C-2,3 double bond); kaempferol **13**, quercetin **14** and myricetin **15** (sequential increase in number of hydroxyl groups in B-ring); isorhamnetin **16** (3'-methoxy quercetin), 3'-*O*-methyl quercetagetin **29** (see Fig 1 and 2) and their antioxidative properties, as well as their effects on proliferation, apoptosis, and cycle regulation of HL-60 cells, was performed. All flavonoids except **29** were efficient in scavenging ROS by DPPH assay and **15** had the highest activity. Although **15** had better activity in scavenging ROS, **13** and **16** effectively reduced ROS levels in menadione stressed HL-60 cells compared to the remaining test flavonoids. The antiproliferative effect of quercetin **14** > isorhamnetin **16** > myricetin **15** > kaempferol **13**, whereas 3'-*O*-methyl quercetagetin **29** (a derivative of isorhamnetin with a hydroxyl group at the 6-position in A-ring) and taxifolin **11** (2,3-dihydro quercetin) were found to be ineffective. A similar situation was seen for the induction of apoptosis and cell cycle regulation which was dose dependant. This indicated that

the presence of a C-2,3 double bond and the absence of the 6-hydroxyl group in flavonols (**13**-**16**) were crucial for the cytostatic effects (Rusak et al., 2005).

Myricetin **15** and its glycoside myricitrin (myricetin-3-*O*-rhamnose) provided insights into the relationship between the function of mitochondria and intracellular ROS levels in apoptosis. The viability of human leukemia HL-60 cells was reduced by **15** *via* apoptosis, as seen by the activation of caspases 3 and 9 but no other caspases, with cleavage of PARP and D4-GDI proteins belonging to the family of Rho-GDP-dissociation inhibitors, whereas myricitrin showed no effect. Apoptosis induced by **15** was also characterized by the occurrence of DNA laddering and hypodiploid cells. It was concluded that **15** induced apoptosis by a mitochondria-dependent and ROS independent pathway (Ko et al., 2005). The structural importance of hydroxyl groups at the 3', 4', and 5'-positions in the B-ring of **15** towards induction of apoptosis was derived by using related compounds, such as flavone, myricetin trimethylether and galangin **12** (absence of hydroxyl groups in B-ring). Flavonols **15** and **12** (only at high concentration) showed DNA ladder formation, whereas the trimethyl ether and flavone showed no such effect (Ko et al., 2005).

Tamarixetin **17** (see Fig 1) showed cytotoxic effect against HL-60, Molt-3, and U937 leukemia cells, whereas K562 cells were found to be slightly resistant with higher IC_{50} values. The cytotoxicity was induced by arresting the cell cycle at G2-M phase and apoptosis. This flavanol, a derivative of **14**, induced apoptosis, as indicated by the cleavage of caspases, PARP and cytochrome c release. The ROS mechanism was responsible for the cytotoxicity identified by using NAC to block cell death. Further, the cytotoxicity effects of **17** towards G2-M arrest *via*

tubulin polymerization were identified by using P-gp-overexpressing K562/ADR cells, which showed comparable IC_{50} values to K562 cells (Nicolini et al., 2013).

Morin (3,5,7,2',4'-pentahydroxyflavone) **18** induced morphological changes in HL-60 cells, and induced G2/M phase arrest. Apoptosis was time and concentration dependant concomitantly seen by increase in the caspase-3 and -9 activity, elevated ROS levels, increase in Ca^{2+} production, decrease in the levels of mitochondria membrane potential (MMP) and expression of Bcl-xL (Kuo et al., 2007).

The cytotoxicity and apoptosis induction effects by ROS in human leukemia cells (HL-60) were investigated using 23 natural flavonoids, which included flavones, flavanones, flavonols and isoflavones (see Fig 1 for few examples). Among the tested flavonoids, only luteolin **2**, 3,6-dihydroxyflavone and geraldol **21** showed the most potent cytotoxic effect and caused ROS generation. The presence of a double bond at the 2,3-position and hydroxyl groups in the A and C rings are important structural requirements for potent cytotoxic activity. SAR indicated that the presence of hydroxyls at the 3-, 6-position and 2'-position may increase the cytotoxicity, whereas the 5- and 3'-position in the A, C and B-rings, may decrease the cytotoxic activity (Chang et al., 2010).

SARs between similar flavonoids, such as wogonin **30** isolated from the root of *Scutellaria baicalensis* Georgi and nor-wogonin **31** (Fig 2), were evaluated in HL-60 cells. Flavone **31** exhibited higher cytotoxic effects compared to **30** due to the presence of a hydroxyl group at the 8-position. In comparison to wogonin **30**, the demethyl flavone **31** induced the formation of DNA laddering, apoptotic body formation, hypodiploid cells, and caspase-3

activation, leading to PARP cleavage at lower concentrations; nor-wogonin **31** also increased the intracellular peroxide production (ROS) in HL-60 cells. To derive the SAR between **30** and **31**, six structurally related flavones having hydroxyl at the 5- or 7-position, 5,7-dihydroxy, 5,7- and 7,8-dimethoxy, and 7-methoxy-8-hydroxy, were also analyzed. The SAR indicated the importance of hydroxyl at the C5 and C7 position, whereas that at C8 may contribute to the induction of apoptosis (Chow et al., 2008). In another study, **30** inhibited the cell viability of HL-60 cells in a dose-dependent manner by apoptosis and increased caspase-3 activity, including DNA fragmentation. The apoptosis mechanism was associated with down-regulation of Bcl-2 and no change in Bax expression. It also inhibited human telomerase reverse transcriptase (hTERT) and its associated protein 1 (hTP1), which resulted in cell death. Suppression of c-myc m-RNA expression was observed after treatment of HL-60 cells with **30** (Huang et al., 2010).

In a comparative study, the cytotoxicity of flavanones with hydroxylation at C4', C6 and C7 and their respective methoxy ethers in human leukemia HL-60 cells was evaluated. A significant cytotoxic effect was observed in flavanones with hydroxylation at C4', C6 characterized by the occurrence of DNA laddering as well as apoptotic body and hypodiploid cell occurrence. The presence of a methoxyl group at C4' or C6 lowered the apoptotic effect in cells in contrast to the presence of a hydroxyl residue, and there was no significant cytotoxicity of flavanone substitution in the C7-position. The apoptosis mechanism included ROS production, caspase-3 and -9 activity, but not caspase-1 and -8 activation, release of cytochrome c, cleavage of PARP and D4-GDI proteins, and caspase-3 fragments in 4'-hydroxyl or 6-hydroxyl flavanone-treated cells (Ko et al., 2004).

The glutathione (GSH) depleting ability of a number of mono- or di-hydroxyl chalcones and flavones was tested in HL-60 cells. SAR at 10 μM concentration indicated that 2′-hydroxychalcone and 2′,2-, 2′,3-, 2′,4-, and 2′,5′-dihydroxychalcones (DHC) **32** (Figure 2) were more effective in HL-60 cells compared to other tested chalcones. The intracellular GSH was reduced above 50% within 4 h. In the case of flavones, the presence of hydroxyl at the 7-position was more effective wherein, **3** and 7-hydroxyflavone showed better activity than **1**. The GSH depletion ability induced by 2′,5′-DHC involved mitochondrial dysfunction (Kachadourian and Day, 2006)

2.2.2. *Dihydropyran ring influence*

Among the metabolites isolated from the stem bark of *Erthyrina suberosa*, an ornamental tree found in India and other neighboring countries, 4'-methoxylicoflavanone **33** and alpinumisoflavone **34** (see Figure 3 for structures) inhibited the growth of HL-60 cells with an IC_{50} value of ~20 μM. Cell death occurred *via* apoptosis by intrinsic and extrinsic pathways. These flavonoids also inhibited nuclear transcription factor (NF-κB) and the signal transducer and activator of transcription (STAT) pathways (Kumar et al., 2013).

SAR between **33** and **34** (Fig 3) and naringenin **9** and genistein **23** (Fig. 1), from which both these compounds may be derived naturally, indicates that the superior activity of these compounds in the present case can be attributed to the presence of the prenyl group (Šmejkal, 2014) in **33** and the dihydropyran ring in **34**. The isoflavone, wighteone **35** (Fig 3) did not inhibit the growth of HL-60 cells compared to **34** (Kumar et al., 2013), wherein the dihydropyran ring opening of **34** affords the open prenyl chain at the 6-position in **35** and vice versa *via* the

biogenetic pathway or artifact formation during isolation (Šmejkal, 2014). Recent attempts towards structural modification of **33** at the 7-*O*-position using acetyl and 3-methylbutanoate ester derivatives resulted in improved cytotoxicity compared with the parent compound in HL-60, prostate cancer cells (PC-3) and pancreatic cancer cells (Mia PaCa-2) (Kumar et al., 2015). Several other substituted benzyl derivatives did not show similar activity as compared to acetyl and 3-methylbutanoate ester possibly due to unfavourable binding in receptor pockets due to their bulky size (Kumar et al., 2015).

2.2.3. *Glycoside substitution*

In a glycoside substitution study, quercetin **14** and its 3-*O*-glycosides with rutinose and rhamnose were evaluated in HL-60 cells for differential apoptosis inducing effects. Cells treated with quercetin **14** showed an increase in DNA laddering, the occurrence of apoptotic bodies, the formation of hypodiploid cells and morphological changes, whereas no such effects were seen in quercetin-rutinose- and rhamnose-treated cells, even at high concentrations. On the contrary, **14** did not induce apoptosis in mature monocytic THP-1 cells, indicating differential effects. A rapid induction of caspase-3 activities in HL-60 cells and cleavage of PARP and D4-GDI proteins was seen by treatment with **14**, whereas no such effect was observed with its 3-*O*-glycosides. The results suggested that the 3-*O*-glycosides inhibit the apoptosis-inducing activity of **14** and is independent of ROS production since only **14** didn't change endogenous intracellular peroxide levels (Shen et al., 2003). In another study, **14** induced apoptosis of HL-60 cells in a dose-dependent manner by fragmentation of DNA, PARP and procaspases. In this case, **14** acted by an extrinsic pathway of apoptosis by increased expression of the FasL protein (Lee et al., 2011).

2.2.4. *Methoxy, methyl and acetyl ester groups*

Methoxy substituted flavonoids 5,4'-dihydroxy-6,7,8,3'-tetramethoxyflavone **36**, 5,8,4'-trihydroxy-6,7,3'-trimethoxyflavone **37**, cirsiliol **38**, nodifloretin **39**, pedalitin **40**, penduletin **41** (Figure 4) along with **2** and **14** were isolated from *Rabdosia rubescens*, an herb found in China. The tea from this herb is used to clear the throat and lungs. These flavonoids were tested for cytotoxicity in several cancer cell lines, including HL-60. The most effective flavone **36** (IC_{50}-7.55 µM) with a methoxy group at position-8 was the only flavone differently substituted from the rest of the isolated flavonoids (see Fig 4). This fact was attributed to its superior activity to inhibit HL-60 cells in the same IC_{50} range as doxorubicin, which was used as a positive control (Bai et al., 2010).

The fruits of *Vitex rotundifolia* are traditionally used in Asian countries for relief from colds, headaches, migraines and sore eyes (Ko et al., 2000). The polymethoxyflavonoids, namely vitexicarpin **42**, artemetin **43** and chrysosplenol D (3',4',5-trihydroxy-3,6,7-trimethoxyflavone) **44** (Figure 4), isolated from *Vitex rotundifolia* inhibited the cell viability of HL-60 cells in a dose-dependent manner. At the lowest concentration, **42** gave the best results, followed by flavones **44** and **43** for 50% inhibition values. These results indicated that the methoxy group in the B-ring (3'-position in the case of **43**) lowered the antiproliferative and apoptosis inducing activity (Ko et al., 2000). In another study, **42** inhibited the growth of HL-60 cells in a time- and dose-dependent manner by G2/M phase arrest of the cell cycle (Shen et al., 2009). Recent study indicated that the apoptosis and G2/M phase arrest of the cell cycle induced by vitexicarpin (casticin) **42** in HL-60 cells is mediated by the p38-MAPK pathway confirmed by using MAPK

inhibitors. It upregulated the intracellular ATP levels and phosphorylation of histone H3 (a downstream molecule of the p38-MAPK pathway). The cytotoxicity of **42** was independent of reactive oxygen species (ROS) production since antioxidants (NAC) failed to reverse the effect (Kikuchi et al., 2013).

The molecular mechanism of action for 2(±)-7,8,3',4',5'-pentamethoxyflavan **45** (Fig 4), a synthetic flavan, was investigated in human leukemia HL-60 cells. Induction of the G2/M cell cycle arrest in a concentration-dependent manner was seen. Apoptotic events characterized by DNA fragmentation, activation of caspase-3, 8 and 9, cleavage of PARP, down regulation of Bcl-2 and up regulation of Bax expression led to cytochrome c release and a decrease in the mitochondrial membrane potential (MMP) were seen (Ma et al., 2009).

Quercetin 3-methyl ether tetracetate **46** (Fig 4), was found to be cytotoxic and induced G2/M phase cell cycle arrest on both HL-60 and U937 cell lines and was more potent in HL-60 cells. The apoptosis mechanism was mediated by caspase activation, identified by using caspase inhibitor z-VAD-fmk. The cytochrome c release from mitochondria was dose-dependent, and apoptosis was independent of Bcl-2, as observed in Bcl-2 over-expressing U937 cells. The bioavailability of **46** to cells was increased due to blocking of the 3-hydroxyl group by the methoxy group, thereby preventing its binding to primary plasma proteins such as albumin (Rubio et al., 2007).

Quercetin analogs with acetyl esters and methyl ethers at the 3, 7, 3', and 4' positions, which substituted the hydroxyl groups, were evaluated for their cytotoxicity in HL-60 cells. The results revealed that tetraacetylated-quercetin and **14** significantly inhibited cell growth by

apoptosis, as identified by DNA fragmentation, caspase-3 activity and PARP cleavage, whereas tetramethoxyl-quercetin had no effect. Interestingly, **14** increased ROS production, whereas tetraacetylated-quercetin induced stronger apoptotic activity *via* a ROS independent pathway. The lipophilic character of acetyl groups may enhance the activity of acetylated-quercetin by increasing the bioavailability for increased cell uptake and delaying metabolism in the cells (Sakao et al., 2009).

The 6-C-methyl flavonoids isolated from *Pinus densata*, which include 5,4'-dihydroxy-3,7,8-trimethoxy-6-C-methylflavone **47**, 5,7,4'-trihydroxy-3,8-dimethoxy-6-C-methylflavone **48**, and 5,7,4'-trihydroxy-3-methoxy-6-C-methylflavone **49** (Fig 5), which are structurally related, were tested for their cytotoxicity in the HL-60 human leukemia cell line. Among the 6-C-methyl flavonoids, flavone **47** was the most potent and inhibited the proliferation of HL-60 cells in a dose-dependent manner. Apoptosis induced by **47** was associated with mitochondrial membrane disruption, an increase in the hypodiploid population, cytochrome c release and caspase-3 activation (Yue et al., 2013).

The biological activities of 6,8-di-*C*-methylkaempferol 3,4'-dimethyl ether **50** and 6,8-di-*C*-methylkaempferol 3-methyl ether **51** (Fig 5), isolated from *Eucalyptus occidentalis* were evaluated in human promyelocytic leukemia cell line, HL-60. Both compounds induced apoptotic cell death seen by morphological changes, DNA fragmentation and cleavage of PARP. The apoptotic mechanism was due to caspase-8/caspase-3 activation and cytochrome *c* release from mitochondria to cytosol (Benyahia et al., 2004).

Synthetic 6,7,8-trisubstituted flavones with the general structure **52** (Figure 6) were synthesized and evaluated for their cytotoxic effect against HL-60 cells. The A-ring substituents, especially the ester group at position-8, were maintained to increase cytotoxicity and lipophilicity because the free acid derivatives were not effective, whereas the B-ring substituents were methoxy, methyl, fluoro, chloro or trifluoromethyl groups and combinations of methyl, nitro, amino and chloro in different positions. The results indicated that the 2'-substituted flavones with methyl **52a** and chlorine substituent **52b** were the most active compounds. These compounds inhibited the growth and proliferation in a concentration- and time-dependent manner. The methyl flavone **52a** (IC_{50} = 18.9 ± 3.7 μM) was more potent than the chloro flavone **52b** (IC_{50} = 70.7 ± 0.7 μM), as observed by the IC_{50} values in mitoxantrone resistant HL-60 cells. As a result of apoptosis, the induced cell death was identified by appearance of DNA fragmentation, an increase in the number of sub-diploid G_1 cells and activation of caspases leading to PARP cleavage. The effect of the two derivatives to induce cell death by the MAPK pathway was also determined (Rubio et al., 2012).

3. *Chemotherapeutic effect of flavonoids on acute T cell leukemia*-

To gain structure activity insights, a detailed study using 23 naturally occurring flavonoids (chalcones, flavones, flavanones, flavonols, catechins and phloroglucines; see Fig 1 for some examples used in this study), which included *O*-methylated and glucuronidated metabolites of some flavones, was conducted. Their cytotoxic activity was evaluated in the Jurkat E6-1 human leukemia cell line. Several SARs indicated the presence of a C-2,3 double bond as in flavones (**1**, **2** and **14**) over flavanones (**9**, eriodictyol **10** and taxifolin **11**), the

presence of a 4-carbonyl group in all studied flavonoids compared with catechin **54a** and *ortho*-hydroxyl group in the B ring to be crucial. The 3-hydroxyl group in the C-ring (**13**, **14** and **11**) lowered the cytotoxicity compared with non-hydroxylated compounds (**1**, **2** and **10**). *In vivo* metabolites, such as 7-*O*-methyl (cirsimaritin **7** and 7-methoxy baicalein **8**) and glucuronide, showed an increase in cytotoxicity compared with their non-methylated flavone (hispidulin and **4**, respectively) counterparts (Plochmann et al., 2007).

Related flavones wogonin **30**, apigenin **1**, luteolin **2** and chrysin **3** are effective inhibiters of Cdk9 and suppress antiapoptotic proteins, such as Mcl-1, leading to apoptosis. Inhibition of Cdk9 by **30** was investigated in various leukemia cell lines, such as T-cell (CEM), Jurkat (J16), T-cell lymphoma cell lines Hut78 and Myla, and adult T-cell on the phosphorylation of the carboxy-terminal domain of RNA polymerase II and also by using caspase inhibitor zVAD-fmk. Cells that did not undergo apoptosis still had low levels of Cdk9. The docking analysis also suggested effective binding at the ATP pocket of Cdk9, leading to 16 binding sites (Polier et al., 2011).

Structurally related flavones **30**, **1** and **3** down-regulated c-FLIP, a key inhibitor of death receptor signaling and Mcl-1 protein levels in TRAIL-resistant human T cell leukemia virus type 1 (HTLV-1)-associated adult T cell leukemia cells SP and MT-2. These flavonoids showed no changes in the proapoptotic proteins Bax, Bad, Bid, and Bak and antiapoptotic proteins XIAP, Bcl-2 and Bcl-xL, expression levels but caused up-regulation of TRAIL receptor 2 (TRAIL-R2). These flavones also sensitized TNFα- and CD95-mediated cell death (Ding et al., 2012).

Acacetin **6** (5,7-dihydroxy-4'-methoxyflavone; Fig 1) inhibited the cell growth of Jurkat cells at 40 μM by induction of cell cycle arrest in the G1 phase and changes in morphology. Apoptosis mechanisms, such as activation of caspase 3 and 8 and subsequent cleavage of PARP, were seen but were independent of caspase 9, as verified by using specific inhibitors. The expression of Fas-associated factor 1 in the FADD decreased Bcl-2 expression, and the release of cytochrome c in mitochondria was activated by **6** (Watanabe et al., 2012).

Flavonoids **1**, **13**, **14** and **15** are common constituents of fruits and vegetables and inhibited the proteasomal activity by apoptosis *in vitro* and in intact Jurkat cells. The inhibition potency order of **1** > **14** > **13** > **15** was observed for anti-proteasome activity and inducing tumor cell apoptosis. Caspase 3 activity, PARP cleavage and differential effects on non-transformed cells were also seen. Docking at the proteasomes-chymotrypsin-like active site of the β5 subunit indicated that a SAR exists among the studied compounds. Deletion of the 3-hydroxy group from **14**, **13** or **15** resulted in a binding mode equivalent to **1**, which has the highest potency and was consistent with experimental results (Chen et al., 2005).

In a similar attempt, the role of flavonoid constituents responsible for the cancer-preventive effects of fruits and vegetables was evaluated by the inhibitory and apoptosis-inducing potencies in the 20S purified proteasome. The order of apoptosis was (1) **2** > **1** > **3**. All flavones have identical A- and C-ring structures, but with two, one and no hydroxyl groups connected to the B-ring, respectively and (2) **1** and **2** >> **9** and eriodictyol **10**, respectively. Both flavanones lack the 2,3-double bond. The order of apoptosis in both series was maintained when inhibiting the proteasomal activity (26S) in intact Jurkat T cells. Flavones with hydroxyl groups

in the B ring and unsaturation in the C ring were found to be natural potent proteasome inhibitors and tumor cell apoptosis inducers (determined by the caspase 3 activity and PARP cleavage). **1** and **2** did not induce inhibition of the proteasome activity and apoptosis in non-transformed cells (Chen et al., 2007).

Polyphenolic catechins in green tea, called green tea polyphenols (GTPs), can be biotransformed under physiological conditions to methylated derivatives, which may limit their *in vivo* protective activity against cancer. A detailed study of synthetic catechins with acetate and methyl groups to the free hydroxyl showed that monomethylated, epigallocatechin-3-gallate (-)-EGCG **54b** (Fig 6), which is protected by acetate groups, induced greater cellular proteasome inhibition and apoptosis. The increase of methyl groups decreased the potency against the purified 20S proteasome in Jurkat T cells, indirectly lowering the protective effects of GTPs (Landis-Piwowar et al., 2007). A possible explanation for this fact was provided earlier by performing docking studies with the methylated analogs, which showed a disruption of bonding to the N-terminal threonine (Thr 1) site in the chymotrypsin-like active site of the β5 subunit (Daniel et al., 2006). The fully acetylated EGCG acts as a prodrug, wherein the acetate groups increase the cellular permeability, stability and recovery from intact Jurkat T cells after treatment. The proposed idea that the acetylated derivative was cleaved by cellular esterases to cause higher proteasome inhibition was also verified (Landis-Piwowar et al., 2007).

The X linked inhibitor of apoptosis protein (XIAP), one of eight best identified IAPs, which is highly expressed in many tumors, binds and inhibits caspase-9 through its baculoviral IAP repeat domain (BIR3). Kaempferol-3-*O*-α-L-(2",4"-di-*E*-*p*-coumaroyl)-rhamnoside **55** (Fig

6) isolated from *Eriobotrya japonica* was identified as a XIAP BIR3 inhibitor using the pharmacophoric model of the XIAP BIR3 domain as a search query in the virtual screening of a natural product database and docking experiments. **55** induced apoptosis and activation of caspase 9 synergistically with etoposide in XIAP overexpressing Jurkat cells. Docking experiments revealed that the oxygen atoms of the ester groups function as hydrogen bond acceptors to interact with Thr308. The hydroxyl at the 3" position functions as a hydrogen bond donor and acceptor with Gly306 and Tyr324, respectively. Further π-cation interactions with Lys297 were also observed. SAR derived from the docking experiments and fluorescence polarization-based assay (FP) indicates the significance of sugar and coumaric ester moieties for its observed bioactivity (IC_{50} of 10.4 µM). Moreover, the FP of hydrolyzed **55** (i.e., kaempferol-3-O-α-L-rhamnoside) and **13** indicated no binding in the XIAP BIR3 domain (IC_{50}>100 µM) (Pfisterer et al., 2011).

8-prenylnaringenin **56** (Fig. 6) isolated from common hop (*Humulus lupulus*) is a potent phytoestrogen binding with α and β-estrogen receptors. Voltage-gated potassium channels (Kv) are involved in cell proliferation and apoptosis of several cancers. Kv1.3 channels belonging to the Kv channel family are expressed endogenously in human leukemic T cell line (Jurkat cells). These Kv1.3 channels affect the functioning of leukemic T cell by setting the resting membrane potential, cell proliferation, apoptosis and volume regulation. Flavanone **56** effectively inhibited the activity of Kv1.3 channels in Jurkat cells, which was reversible and concentration dependant. Complete inhibition was detected at 10 µM concentration of **56**. Comparison of the inhibitory activity of naringenin **9**, a precursor of **56** on Kv1.3 channel activity, no inhibition was observed even at 30 µM. Further, the 4',7-dimethyl and the 7-methyl ether derivatives of **9** at 30 µM

effectively inhibited the Kv1.3 channel activity to 4% and 29% of the controls used respectively. Overall this result indicated the complete inhibition activity at a concentration of 10 µM is due to the presence of prenyl group and was also observed for xanthohumol **20**, a prenyl substituted chalcone (Gąsiorowska et al., 2012).

The potential relevance and underlying mechanisms of the combination quercetin **14**-menadione (Vitamin K_3) for clinical applications was studied by 24 h combinatorial regimens at equimolar concentrations of 10-15 µM in human leukemia Jurkat T cells. A dose-dependent antagonistic or synergistic effect was seen on the clonogenecity of Jurkat cells, and both reduced cell viability at efficient rates (Baran et al., 2014).

Synthetic methylenedioxy chalcones **53**, (Fig. 6) designed from naturally occurring flavonoids were tested for inhibition of Jurkat T, myeloid leukemia K562 and histiocytic lymphoma U937 cells. The methylenedioxy group at the 3',4' position in the B-ring of chalcones was maintained, whereas the A-ring substituents in chalcones were mono, di or tri-methoxy groups. The chalcones with an *ortho* substituted methoxy group **53a** and 3,4-dimethoxy group **53b** had significant effects on the inhibition of cancer cell proliferation and viability in all tested cell lines. The executioner caspase cleavage analyses indicated that the cytotoxic effect of chalcones was due to the induction of apoptotic cell death. The cytotoxic effect was specific to the cell type and targeted only cancer cells; peripheral blood mononuclear cells were slightly affected by the treatment. The flavanones and flavones derived from these active chalcones showed no significant effect on the tested cell lines (Orlikova et al., 2014).

Retinoids are derivatives of retinol (vitamin A) which are involved in cell development, growth and differentiation. Complexes of ligands binding to retinoid acid receptors (RARs) and retinoid X receptors (RXRs) mediate in the above mentioned biological functions. Retinoid related molecules (RRMs) are inspired from classical retinoids but they don't directly bind to the RARs or RXRs and most of the time act independent of these receptor mediated pathways. Development of new compounds by combining chalcone and adamantyl structures resulted in novel structural motifs **62** (see Fig. 7). The addition of OMEM group at the 4'-position and increasing the alkyl chain of the alkoxy group at 2'-position resulted in a very low RAR transactivation. The compounds with butyloxy **62a** and benzyloxy **62b** groups at 2'-position were 4 times more active on Jurkat T leukemia cell line than the parent compound which had no substituent at the same position. Apoptotic activity was observed by activation of caspase 3 and 7 as seen by enhanced DEVDase activity. The butyloxy **62a** and propargyloxy **62c** compounds showed highest inhibition of IKKβ, the catalytic subunit responsible for activation of the NF-κB pathway indicating that these novel adamantyl chalcones may contribute towards therapeutic use for cancer therapy (Lorenzo et al., 2008). A recent evaluation of the structural requirement of these adamantyl chalcones indicated that replacement of the α,β-unsaturated ketone by a five-membered heterocycle resulted in no loss of activity. Although the chalcone structure is an important contributor of IKKβ inhibition, in the present case the correlation of IKKβ inhibition by these hetero substituted chalcones was independent of the Michael addition on the α,β-unsaturated bond (Lorenzo et al., 2013).

4. ***Cytotoxic effect of flavonoids on chronic myelogenous leukemia-***

An ethnopharmacological isolation study of the 15 total flavonoids from *Lysimachia clethroides* Duby, a traditional Chinese medicinal herb (named ZE4 in the study), gave the major constituents rutin (quercetin-3-*O*-rutinoside), naringenin-7-*O*-glucoside, kaempferol-3-*O*-rutinoside, isorhamnetin-3-*O*-rutinoside, kaempferol-7-*O*-glucoside, **14**, isoquercitrin, dihydrokaempferol, **10**, **9**, (+)-catechin **54a**, (+)-gallocatechin, **13**, kaempferol-2'-rhamnopyranosyl-3-*O*-rutinoside and quercetin-2'-rhamnopyranosyl-3-*O*-rutinoside. The mixture ZE4 induced apoptosis in K562 cells in a time- and concentration-dependent manner. The apoptotic mechanisms included down regulation of Bcl-2 expression, up regulation of Fas, TRAIL and DR5 expressions. (Liu et al., 2010). Although this study indicated that the mixture of ZE4 comprising 15 total flavonoids of *Lysimachia clethroides* Duby had antiproliferative effects, a detailed investigation to study the interaction between the components to produce synergistic/antagonistic effects and the effect of each individual component in inhibiting K562 cell growth needs to be explored (Liu et al., 2010).

The erythroid differentiation induction effect of **1** in K562 cells was studied using different flavonoids, such as apigetrin (apigenin-7-*O*-glucoside), flavone, 7-hydroxyflavone, **3**, **2** and **9**, to represent the presence or absence of functional groups in its structure. Morphological changes and the expression of specific markers in K562 cells by **1** were compared with the other flavonoids. The effect of the presence of glucose moiety in **1** at 50 µM (using apigetrin 75 µM) suggested that the cells maintained the same morphology and were bigger in size compared with control cells. The K562 cells expressed the same levels of glycophorin A when treated with concentrations of **1** and apigetrin mentioned and suggests that glycosylation may decrease the effectiveness of **1**. Based on glycophorin A expression, the potency of the flavonoids for

induction of differentiation was: **1**>**3**>flavone/7-hydroxyflavone > **2**/**9**. Overall, the results indicated that the 2,3-double bond and the hydroxyl groups were important structural features in **1** induced erythroid differentiation (Isoda et al., 2014).

A chemokine receptor, CXCR4, specific for stromal-derived-factor-1 (SDF-1, also called CXCL12), is expressed in hematopoietic cancer cells. This receptor determines survival and drug resistance in leukemia. The intracellular signaling for cell survival and proliferation depends on the binding of the ligand CXCL12 to CXCR4. Moreover, CXCL12 protects CML cells from chemotherapeutic induced apoptosis in the bone marrow microenvironment. Transient transfection of Adriamycin (ADM) resistant K562 cells with CXCR4 siRNA reduced the expression of CXCR4, Akt phosphorylation and PI3K, confirming the downstream signaling of the resistance mechanism by CXCR4 activation. Oroxylin A **5** (Fig 1) inhibited the growth of K562/ADM cells with increasing concentration and CXCL12/CXCR4 binding, thereby reversing the resistance conferred to K562/ADM cells. Xenografts of K562/ADM cells in mice were evaluated with a combination of ADM with **5** and also showed reduction in the expression of CXCR4 (Wang et al., 2014).

The apoptosis inducing capabilities of oroxylin A **5** were investigated in CML (K562) cells, which revealed a time and concentration dependent inhibition. The apoptosis mechanism was characterized by an increase of caspase 9 activation and a decrease in survivin, pro-caspase 9 and p-ERK1/2 expressions (Wang et al., 2014).

Vitexicarpin **42** isolated from *Vitex trifolia* L. induced apoptosis in K562 cells in a dose dependant manner and was detected by internucleosomal DNA fragmentation. The cell cycle

progression was arrested at G2/M phase in a short amount of time, the maximum was attained in 12h (Li et al., 2005).

The apoptogenic activity of *Swietenia mahagoni* leaf extract (SMLE), containing the two flavonoids catechin **54a** (Fig 6) and quercetin-3-*O*-glucoside, were investigated against K562, U937 and HL-60 human leukemic cell lines. SMLE inhibited the cell growth and metabolic activity of the leukemic cells and showed characteristic features of apoptosis. SMLE arrested U937 and K562 cell populations in the G2-M and G1 phases of the cell cycle in HL-60 cells. The apoptosis followed an intrinsic pathway, as detected by the release of cytochrome *c* and activation of caspases-9 and 3 (Roy et al., 2014).

A natural C-methyl substituted flavonoid, 2',4'-dihydroxy-6'-methoxy-3',5'-dimethylchalcone **57** (Fig 7), isolated from *Cleistocalyx operculatus*, showed dose-dependent cytotoxicity and inhibited the proliferation of K562 cells. A lower ratio of Bcl-2/Bax, due to chalcone **57**, indicated by the down-regulation of Bcl-2 protein and the expression of Bax protein, may be responsible for the apoptosis (Ye et al., 2005). Casticin (also known as vitexicarpin **42**; see Fig. 4) inhibited the growth of K562 and Kasumi-1 cells in a time- and dose-dependent manner by G2/M phase arrest of the cell cycle. Casticin **42** induced early apoptosis, as detected by the annexin assay, caspase 3 activity, cleavage of PARP and DNA fragmentation. Down regulation of polymeric tubulin and activation of the PI3K/AKT signaling pathway were also identified (Shen et al., 2009).

Assessment of SAR of prenyl/geranyl susbstituted natural chalcones like bavachalcone **58a**, isoxanthoangelol **58b** and their methoxylated derivatives in K562 cells gave interesting

results. The inclusion of 5'-prenyl/geranyl substituent in the 2',4',4-trihydroxy chalcone structure (ie **58a** and **58b**) indicated cytotoxic effects in K562 cells by apoptosis. Chalcone **58a** (IC_{50} = 2.7 µM) was more potent compared to **58b** (IC_{50} = 4.3 µM) as observed from their IC_{50} values. Trimethoxylated derivatives of **58a** and **58b** at the 2',4',4-positions in the chalcone structure did not affect the potency when compared with their hydroxylated counterparts. Although another chalcone derivative with monogeranyl substituent at 3'-position also showed similar cytotoxicity (IC_{50} = 3.98 µM) as **58b**, the prenyl counterpart was less cytotoxic. Disubstitution with prenyl/geranyl group at the 3' and 5'-positions did not improve the cytotoxicity (Wang et al., 2015).

Butein **59a**, homobutein **59c** and isoliguiritigenin **59b** (Fig. 7) inhibited both tumor necrosis factor-α (TNFα) induced NF-κB activity and total histone deacetylase enzymes (HDAC) activity of classes I, II and IV in K562 cells. Although, molecular docking studies could not clarify the dual effects of these chalcones, this study highlighted the possibility of using natural chalcones as dual targets for treatment of inflammation and cancer (Orlikova et al., 2012).

The cytotoxic effect of several natural flavonoids on K562 cells, PBMC and phytohemagglutinin stimulated PBMC from healthy donors, were evaluated. The cytotoxicity of the unsubstituted flavone, apigenin **1**, chrysin **3** and luteolin **2** did not differ for the leukemic cells. Fisetin **22** and flavanone had a small cytotoxic effect on PBMC from healthy donors. The highest and specific cytotoxic effects on leukemia cells resulted from baicalein **4** and myricetin **15** treatment. Overall, the results indicated that only **4** showed specific inhibitory effects on the targeted cells in a dose-dependent manner (Romanouskaya and Grinev, 2009). The ethyl acetate extract obtained from

Nitraria retusa leaves, along with the major component isorhamnetin-3-*O*-rutinoside, induced apoptosis in K562 cells. Apoptosis was detected by DNA fragmentation, activation of caspase-3 and -8, and PARP cleavage, indicating an extrinsic pathway (Boubaker et al., 2011).

Despite the success of first-generation tyrosine kinase inhibitors such as imatinib mesylate for therapy for chronic myeloid leukemia, the development of resistance and intolerance is a challenging problem. Therefore, alternative therapeutic agents, such as flavonoids, particularly quercetin **14**, galangin **12**, fisetin **22** and chrysin **3**, were tested in Bcr-Abl oncogene expressing K562 and KCL22, imatinib mesylate resistant K562-R and KCL22-R cells. **14** induced arrest in G2/M, whereas **12**, **3** and **22** arrested cells in the G0--G1 phase and decreased the S and G2/M phases of the cell cycle. **12** decreased the transcription of genes involved in cell cycles, namely cdk1, cdk4, pRb and cyclin B, and induced monocytic differentiation/formation. The decreased Bcl-2 expression by **12** led to improved toxicity of imatinib as combination therapy in both the sensitive and resistant Bcr-Abl cells (Tolomeo et al., 2008). Similarly, the potential of **1** for cytotoxic effects was tested in K562 and K562/IMA3 cells. The results showed that **1** was cytotoxic to both cell types in a time and dose-dependent manner. Comparison of the IC_{50} values for both cell types at 48 and 72 h indicated that the K562/IMA3 cell line was 4- and 25-fold more resistant than normal K562 cells. The cell death occurred by apoptosis, which was determined by observing the loss of the mitochondrial membrane potential, both in a dose-dependent manner. The cleavage of DEVD-pNA caspase-3 substrate by increasing concentrations of **1** indicated caspase-3 activity changes in a dose-dependent manner. The cell cycle progression was arrested by **1** in the G2/M phase in K562 cells, but no significant effect was seen in K562/IMA3 cells. However, the S phase was arrested at a higher concentration in

K562/IMA3 cells. The genes and gene network modulated by **1** were also discussed (Solmaz et al., 2014).

In an interesting study, the influence of soy isoflavonoids, **23** and **24** (Fig 1), which possess antioxidant activity and scavenge reactive oxygen species (ROS) was demonstrated on K562 cells using 5-aminolevulinic acid (ALA), which converts to protoporphyrin-IX (PPIX), an endogenous natural photosensitizer. Photodynamic therapy (PDT) is a treatment that uses a photosensitizing drug (in this case endogenous PPIX), wavelength specific light energy and reactive oxygen species to effectively kill tumor cells. The combination of a photosensitizer that gets selectively localized in the tumor tissues and illumination with visible light results in production of elevated ROS, especially singlet oxygen, leading to photodamage and subsequent cell death. The results showed that **23** at a low concentration of 10 μM inhibited cell proliferation in a time-dependent manner and enhanced cell apoptosis in a dose-dependent manner after ALA-PDT treatment. After 24 h of ALA-PDT treatment of K562 cells pretreated by **23**, cells showed lipid peroxidation and DNA damage, whereas **24** did not show any such effect. The results indicated that consumption of soy products during PDT may show synergetic effects on cancer therapy of malignant cells (Zhang et al., 2012).

(*E*)-3-(naphthalen-2-yl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one **63** (Fig 8) having the 3,4,5-trimethoxyphenyl ring adjacent to the carbonyl group showed high docking in the colchicine site on tubulin in a similar orientation to that of colchicine confirmed by cytotoxicity in L1210 cells and inhibition of tubulin polymerization *in vitro*. Naphthochalcone **63** was more potent in comparison with other tested chalcones evaluated in Reh and Jurkat cells (Salum et al., 2013).

Synthetic naphthochalcones **63-65** (Fig 8) derived from 1- and 2-naphthaldehyde were tested for their cytotoxicity in K562 and Jurkat cells. The most potent chalcone, (*E*)-1-(2,5-dimethoxyphenyl)-3-(naphthalen-1-yl)prop-2-en-1-one **65** showed higher cytotoxicity compared with the other chalcones (**63**, **64**) tested, and it was further tested in Kasumi and U937 cells. It reduced the cell viability in a time- and concentration-dependent manner in all cell lines and was non-toxic to normal cells. The apoptotic cell death occurred by chromatin condensation and the formation of apoptotic bodies. It also blocked the G2/M phase, the S phase and the G0/G1 phase of the cell cycle in K562 and Kasumi cells, in Jurkat cells and in U937 cells, respectively (Maioral et al., 2013).

Cytotoxic effect of flavonoids on histiocytic lymphoma-

Sophoranone **60**, an isoprenoid substituted flavanone (Figure 7) isolated from *Sophora subprostrata* Chun et T. Chen inhibited cell growth and induced apoptosis in various solid human tumor cell lines. The growth-inhibitory and apoptosis-inducing activities of **60** were superior to daidzein **24**, genistein **23** and quercetin **14** for histiocytic lymphoma represented by U937 cells. In the early stages of the treatment of U937 cells, the formation of ROS, opening of mitochondrial permeability pores and cytochrome c release from mitochondria was observed on treatment with **60**. Overall, the results indicated that **60** is a unique anticancer agent that induced apoptosis by targeting mitochondria dysfunction (Kajimoto et al., 2002).

Treatment with **14** affected cell viability, induced apoptosis and arrested the G2/M phase (reversible on removal of **14**) in U937 cells at 20 µM, as indicated by an increase in the expression of cyclin B and a decrease of cyclin D, E, E2F1 and E2F2 expression. The apoptotic

mechanism was identified by caspase activation, DNA fragmentation, dose-dependent PLC-γ1 cleavage and increased sub-G1 population (Tae-Jin Lee et al., 2006). The induction of apoptosis in U937 cells by the accumulation of the sub-G1 phase with increasing concentrations of **3** was observed. The mechanism of apoptosis by activation of caspase 3 led to cleavage of PLC-γ1 and DEVD-pNA. The Bcl-2 and Bax expression levels indicated no significant changes, whereas decreased levels of XIAP expression were observed. Although the involvement of the MAPK pathway in the induction of apoptosis was ruled out, the decrease in phosphorylated Akt levels indicated that the Akt signal pathway regulated the **3**-induced apoptosis in U937 cells (Woo et al., 2004).

Morin **18** was most sensitive to U937 cells in a comparative assay among other leukemic cell lines (HL-60, K562 and THP-1). The cell cycle was arrested at the sub G1 phase while mild increase in S phase and G2M population was observed on treatment with **18**. Apoptosis was induced by **18** in a dose dependant manner inferred by loss of MMP, activation of caspase 3 and 9 and cleavage of PARP. Inhibition of caspase 3 by z-DEVD-fmk reduced the **18** induced cell death indicating the caspase dependant apoptotic pathway. Treatment with **18** also led to upregulation of BAD protein levels and downregulation of Bcl-xL protein indicating their regulatory role in apoptosis. Bcl-2 overexpressing U937 cells inhibited the **18** induced cell death, DNA fragmentation, apoptosis and reduced loss of MMP. Overall these data show that the underlying mechanism for **18** induced cell death is *via* mitochondrial path by inhibition of Bcl-2 and BAD protein upregulation (Park et al., 2015).

The traditional use of *Wattakaka volubilis* in Ayurvedic medicine for several ailments is well-known in India. The flavonoid isolated from the leaves of *Wattakaka volubilis*, kaempferol-3-*O*-[α-L-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→6)-*O*]-β-D-glucopyranoside, showed anti-leukemic activity by chromatin condensation in U-937, HL-60 and K-562 cell-lines. The apoptosis led to arrest at the G1 phase of the cell cycle for U937 and K562 and the G2/M phase of the cell cycle in HL-60 cell lines (Nandi et al., 2012).

Icariside II **61** (Fig. 7) isolated from the stems and leaves of *Epimedium koreanum* inhibited the cell viability of U937 cells with an increase in concentration and time. The cells underwent DNA fragmentation due to apoptosis, as observed by the accumulation of the sub-G1 cell population and an increase of TUNEL-positive cells. The lowering of the apoptosis related proteins, such as Bcl-2, Bcl-xL, survivin and COX-2, was also observed. Caspase-3 activity followed by cleavage of PARP as a function of time was observed in U937 cells. Inhibition of STAT3 phosphorylation by **61** *via* suppressing the activation of Janus activated kinase 2 (JAK2) with an increase in concentration and time was seen (Kang et al., 2012).

In a study using leukemic U937 cells, the apoptotic activity of 22 flavonoids and related compounds was evaluated. The results were influenced by the classes of flavonoids, where flavones induced apoptotic cell death under these conditions and isoflavones or flavanones showed no effect. The cellular viability, exposure of phosphatidylserine to the outer face of cytoplasmic membrane, and oligonucleosomal DNA fragmentation was significantly reduced by **1**, **3**, **14**, **12**, **2**, **22**, and 3,7-dihydroxyflavone. The structure-activity relationship analyses indicated that the apoptosis inducing capacity required at least two hydroxylations in the 3, 5,

and 7-positions of the A ring, whereas enhanced proapoptotic activity required hydroxylation at the 3′ and/or 4′-position of the B ring. The absence of hydroxylation in the B-ring did not affect the proapoptotic activity, as seen in **3** and **12**. The cells were sensitized at low concentrations by these flavonoids to undergo apoptosis induced by tumor necrosis factor-α. The apoptosis induced by **3**, **12**, **2** and **14** was due to activation of caspases 3 and 8, but not of caspase 9, as evidenced by using specific inhibitors. The apoptosis induced by **22**, **1** and 3,7-dihydroxyflavone showed DNA cleavage, indicating caspase-calpain dependent pathways. α-naphthoflavone induced cell death by necrosis, which emphasizes that the mode of action is important to derive SAR (Monasterio et al., 2004).

Treatment of human leukemia cells (U937, THP-1 and HL-60) with **1** above 50 µM showed anti-proliferative activity, apoptotic death, activation of caspase 3 and 9 and PARP cleavage. There was an increase in the level of intracellular ROS by **1**, whereas the use of NAC or GSH pretreatment completely attenuated ROS generation and did not show recovery from **1**-induced cell death, indicating an ROS independent pathway of apoptosis. **1** also lowered the telomerase activity by suppression of c-Myc-mediated hTERT expression, while pretreatment with NAC or GSH did not restore telomerase activity (Jayasooriya et al., 2012).

7,8-dihydroxyflavone hydrate **66** (Fig. 8), which induced apoptosis in U937 cells, showed key features, including DNA fragmentation and accumulation in the sub-G1 phase of cell cycle. dihydroxyflavone **66** increased the death receptor related protein (Fas, FasL, DR4 and TRAIL) levels and inhibited anti-apoptotic IAP family proteins (XIAP, cIAP1 and 2 and survivin). The activation of caspases 3, 8 and 9, PARP cleavage, Bid cleavage and activation, insertion of Bax

into the mitochondria, increase of binding between Bax and Bcl2/Bcl-xL, release of cytochrome c and activation of MAPK signaling pathway were observed. The activation of caspases was restored by Bcl-2 overexpression, thereby protecting U937 cells from **66**-induced apoptosis (Park et al., 2013).

2'-amino-3'-methoxyflavone **67** (commercial name-PD98059; Fig. 8) is a highly selective noncompetitive inhibitor of MEK1 and MEK2. It was shown that progressive increase in the concentration of **66** arrested the G1 phase through down-regulation of cyclin E/Cdk2 and D/Cdk4 complexes and upregulation of Cdk inhibitors. The G1 phase arrest was independent of Bcl-2 overexpression and caspase 3 inhibitors. Flavone **67** produced apoptosis by caspase 3 activity, PARP cleavage and PLC-γ1 degradation in U937 cells (Moon et al., 2007). Pretreatment of HL-60 cells with **67** led to an increase in the cell death capacity of methyl or chlorine substituted flavones **52a** and **52b**, indicating pre-sensitizer effects (Rubio et al., 2012). A similar enhancement of apoptosis was also observed when HL-60 and U937 cells were pretreated with **67** in presence of acetylated quercetin **46** (Rubio et al., 2007).

The impetus to design a lipophilic derivative of baicalein **4**, (7-(benzyloxy)-5-hydroxy-2-phenyl-6-(3-(pyrrolidin-1-yl)propoxy)-4*H*-chromen-4-one) **68** (Fig. 8) was to improve its oral bioavailability by the introduction of lipophilic substituents on a natural flavonoid which improves the efficiency to enter cells. It has been previously shown using Jurkat T cells that 7-methoxy baicalein **8** or the glucuronide derivative of **4** are more potent than their free counterparts (Plochmann et al., 2007). The growth and viability inhibition effects of **68** on U937, HL-60 and K562 cells were found to be concentration- and time-dependent, and **68** induced

differentiation of U937 cells into monocyte-like cells. The increased expression of phospholipids scramblase 1 (PLSCR1) and promyelocytic leukemia (PML) protein in a concentration-dependent way indicated that **68** induced cell differentiation in U937 cells. The differentiation effect by increased expression of PLSCR1 and PML was probably *via* activation of protein kinase (PK)Cδ (Qin et al., 2012).

A series of twenty synthetic flavonoids, including nine flavonols and eleven 3-methyl ethers, was assessed for cytotoxicity against U937, HL-60 and Molt-3 human leukemia cells. The synthetic compounds differed in terms of their substituents on the B ring and minor changes on the A ring. 4'-bromoflavonol **69** (Fig. 8) was the most potent and inhibited cell growth in a concentration-dependent manner *via* induction of apoptosis and cell cycle arrest at the S phase. Cleavage and activation of multiple caspases, the activation of the MAPK pathway and the up-regulation of DR4 and DR5 death receptors for TRAIL were associated with cell death (Burmistrova et al., 2014).

5. *Effects of flavonoids on chronic lymphocytic leukemia-*

Novel agents for the treatment of chronic lymphocytic leukemia (CLL) are currently needed because most patients relapse due to acquired chromosomal abnormalities such as del(11q22) and del(17p13), which provide resistance to therapy; therefore, CLL remains incurable with standard therapies (Lin, 2010). The sensitizing ability of flavonoid quercetin **14**, along with fludarabine, a widely used chemotherapeutic drug against CLL, towards primary cells from CLL showed that the death receptor (DR) agonists were bypassed, enhancing the recombinant TNF-related-apoptosis-inducing ligand (rTRAIL) and anti-CD95 induced cell death. **14** decreased cell

viability and increased the caspase-3 and -9 activities, leading to PARP cleavage. This study demonstrated the potential of **14** at low concentration as a neutral molecule in adjuvant chemotherapy in combination with other drugs (Russo et al., 2010). **14** also down-regulated the Mcl-1 protein levels in B-cells, as confirmed by using U-937 cells, in which treatment along with z-VAD-fmk resulted in increased Mcl-1 protein levels. This indicated the independence of Mcl-1 down-regulation from caspase-mediated degradation (Spagnuolo et al., 2011). Further down-regulation of the Mcl-1 protein levels acting on mRNA stability was identified by using proteasome inhibitor MG-132, wherein the effect of the monotreatment was not reversed. This confirmed that the apoptotic effects of quercetin in CLL were dependent on the proteasome degradation of Mcl-1 (Spagnuolo et al., 2011; Spagnuolo et al., 2012).

CLL cells usually accumulate undivided B-lymphocytes, which have extensive endoplasmic reticula (ER), the sites responsible for protein folding and other cell functions. Xanthohumol **20** showed cell killing effects *via* unfolded protein response in the ER. Apoptosis led to increased levels of ER stress related glucose-regulated protein of 78 kDa (GRP78) and heat-shock protein of 70 kDa (Hsp70). Activation of caspase 3, 4 and 9, cleavage of PARP and down regulation of the Mcl-1 and Bcl-2 levels were also seen. Up-regulation of the GRP78 level indicated involvement of ER stress transducers, IRE1 and PERK, but not ATF6. Activation of PERK also led to increased levels of CHOP, which is involved in the suppression of Bcl-2, and cleavage of caspase 4, which leads to the caspase cascade (Lust et al., 2009).

Flavopiridol, a synthetic N-methylpiperidinyl, chlorophenyl flavone **70** (Figure 8) designed to incorporate in part the active alkaloid rohitukine isolated from the Indian medicinal plant

Dysoxylum binectariferum is currently being tested in clinical trials (Christian et al., 2009). Flavopiridol **70** is a broad cyclin-dependent kinase inhibitor (CDKi) that modulates cell cycle progression, resulting in apoptosis independent of p53 status, and represents a potential therapeutic class for relapsed CLL patients with del(17p13) (Byrd et al., 1998).

6.*Role of flavonoids as P-glycoprotein inhibitors in leukemia*-

Tumor treatment with chemotherapeutic agents is hindered by multi-drug resistance (MDR), resulting in poor oral bioavailability and low tissue distribution of drugs. P-glycoprotein (P-gp) is one of the various mechanistic approaches used to understand MDR (Bansal et al., 2009).

Flavonoids found in foods and beverages are promising alternatives to inhibit the P-gp mediated drug efflux, indicating their potential to enhance the bioavailability of P-gp drug substrates. Flavonoids bind at two overlapping sites in the cytosolic domains of P-gp, the ATP site and a hydrophobic steroid-binding site. Structural requirements of flavonoids are 5-hydroxyl and 4-carbonyl groups for binding at the adenine 6-amino and 1-nitrogen groups, respectively in ATP. During ATP hydrolysis, P-gp extrudes drugs; competitive binding of flavonoids to ATP may block the action of the transporter (Barron et al., 2002). C-prenylated chrysin derivatives **74** (see Fig. 9 for general structure; 6-, 8-, 6,8-diprenyl, 6- and 8-geranyl substituted) were tested for their ability to modulate MDR in resistant K562/R7 leukemic lines. The results indicated that 8-prenyl chrysin was a better modulator than cyclosporin A (Barron et al., 2002).

Polymethoxylated flavones, such as nobiletin **75**, tangeretin **76** and 3,3',4',5,6,7,8-heptamethoxyflavone **77** (Fig. 9), led to a 10-fold increase of the intracellular accumulation of vincristine in adriamycin-resistant human myelogenous leukemia (K562/ADM) cells, and the

effects were comparable to P-gp inhibitors, cyclosporin A and verapamil (Ikegawa et al., 2000). Synthetic pentamethyl, pentaethyl, pentapropyl, pentabutyl and pentaallyl ethers of quercetin **14** and morin **18** were also evaluated as P-glycoprotein (P-gp) inhibitors in K562 and K562/ADM cells. Except for the pentabutyl ethers, all other penta-substituted ethers of **18** and **14** (20 µM) had positive effects on the uptake of vincristine by the ADM resistant K562 cells, reflecting on the role and importance of the structural size of substituents on these natural flavonoids for the inhibition of P-gp. Among all tested compounds, pentaethylquercetin and morin derivative and pentaallylquercetin showed superior effects in the uptake studies by K562/ADM cells compared with verapamil and cyclosporine A (Ikegawa et al., 2002).

5,7,3',4',5'-pentamethoxyflavone **78** (Fig. 9) was cytotoxic to AML-2/D100 cells at concentrations >400 µM, whereas in the presence of vincristine it reduced to a value of < 0.4 µM (factor of 1000 times) and equivalent to verapamil, thereby proving its potential as a drug reversal agent. The structure--activity relationships were derived using other tri, tetra, penta and hexamethoxy derivatives of **78**, which suggested that the presence of the 6-methoxy group in ring A may lower the potential for drug reversal by steric hindrance, and the 5'-methoxy group of ring B is crucial for the effects of **78**. The flavones containing free hydroxyl at similar positions as the methoxyl groups showed high cytotoxicity in AML-2/D100 cells. This fact implied the importance of the number and position of methoxyl substitution and lipophilicity for efficient drug reversal effects (Choi et al., 2004).

An elaborate study tested 30 flavonoids belonging to flavones, flavonols, isoflavones and their glycosides generally found in fruits and vegetables for their potential to accumulate

daunorubin (DNR) in vinblastine (VLB) resistant T-cell leukemia (CEM/VLB_{100}) derived from CCRF-CEM cells. The screening at 25 μM concentration indicated baptigenin Ψ **79**, biochanin A **80**, chrysin **3**, 4',7-dimethoxyisoflavone **81** (Fig. 9), flavone and 4',7-dimethoxyflavone (included due to increased DNR accumulation in contrast to other flavonoids) to have greater reduction of DNR accumulation in CEM/VLB_{100} cells among other tested flavonoids, whereas no effects on DNR accumulation were seen in sensitive CCRF-CEM cells. The modulation effect to increase intracellular accumulation of fluorescent VLB by all flavonoids except **79** in the same cells was also observed. A correlation between the effects of these flavonoids on the accumulation activity and P-gp ATPase was seen. Flavonoids that increased DNR-stimulated ATPase activity led to decreased accumulation and cytotoxicity of DNR, whereas flavonoids that inhibited the ATPase activity stimulated by VLB resulted in increased accumulation and cytotoxicity. A possible SAR analysis with respect to the binding of flavonoids at the overlapping sites in the cytosolic domains of P-gp indicates that the absence of 5-hydroxyl groups in the isoflavones **79** and **81** may have led to a reduction in the DNR accumulation. Multiple binding modes may be active for the differential effects observed in the study (Tran et al., 2011).

The 4-methylpiperazine chromone **82** (Fig. 9) was cytotoxic to a lesser extent to K562 cells compared to adriamycin resistant K562 cells, thereby showing the highest potency among a new class of synthetic chromones, azaisoflavones, and aurones to modulate P-gp mediated MDR in K562 and K562/ADM cells compared to cyclosporin A. The SAR revealed the importance of the basicity of alkylated nitrogen in the piperazine ring and the nature of its substituents for modulation activity (Hadjeri et al., 2003).

7. *Effects of flavonoids on miscellaneous leukemias-*

To gain understanding of the action of a few polyphenols within a wide range of leukemic cell lines, flavonoids such as quercetin **14**, apigenin **1** and chrysin **3** (Fig. 1), along with other polyphenolic compounds belonging to anthraquinones and stilbenes, were studied. The anti-proliferative and pro-apoptotic effects of these polyphenols that have previously shown potential in solid and leukemic cell lines were tested on four myeloid (KG-1a, HL-60, THP-1 and K562) and three lymphoid (Jurkat, CCRF-CEM and MOLT-3) human leukemic cells and one histiocytic lymphoma cell line (U937) to represent the major leukemia types. Among the flavonoids tested, **14** was the most potent polyphenol for decreasing cell viability (IC_{50} values of 8-33 μM) and inducing apoptosis (AP_{50} value 19-50 μM). The lymphoid cell lines were more sensitive to polyphenol treatment compared to the myeloid cell lines; however, the most resistant myeloid (KG-1a and K562) cell lines also responded to **14** at low micromolar levels. The differential sensitivity of **14** to leukemia and non-tumor cells indicates its potential use as a chemotherapeutic agent (Mahbub et al., 2013).

Transcription factor nuclear factor-κB (NF-κB) is a heterotrimer consisting of p50, p65 and IκB subunits, present in the cytoplasm due to inactivation by IκBα inhibitory subunit. It has an important role for cell survival, proliferation, invasion, and angiogenesis of tumor cells. It is activated by inflammatory stimuli like tumor necrosis factor (TNF), interleukin-1, phorbol-12-myristate-13-acetate (PMA), H_2O_2, ceramide, LPS, and γ-radiation leading to phosphorylation dependant degradation of IκBα proteins. This is followed by translocation of NF-κB into the nucleus where it leads to gene transcription. Morin **18** inhibited NF-κB *via* the above pathway

and led to downregulation of NF-κB related gene products involved in cell survival (IAP1, IAP2, XIAP, Bcl-xL, and survivin), cell proliferation (cyclin D1and COX-2), and invasion (matrixmetalloproteinase-9) thereby enhancing apoptosis (Manna et al., 2007). Morin **18** significantly inhibited proliferation of human leukemia pre B-cells (Nalm-6) and human chronic lymphocytic leukemia (HUT-78) with IC_{50} of 10 µg/mL. Formation of sub-G1 phase and induction of apoptosis was seen by cell shrinkage and blebbing of plasma membrane (Karimi et al., 2013).

The antitumor activity of naturally occurring chalcones, such as butein **59a**, xanthohumol **20**, isoliquiritigenin **59b** and their underlying mechanisms on several leukemia cell lines, including HL-60, Jurkat, U937, and K562, has been reviewed (Zhang et al., 2013). The evaluation of the antioxidant potential of a series of polyphenolic 2'-hydroxychalcones led to the identification of 2,2',5'-trihydroxychalcone towards ROS scavenging in L-6 myoblasts and THP-1 human monocytes study *in vitro*. It also shows an excellent antioxidant activity in a concentration range lower than that reported by most studies of related molecules. It selectively inhibited the proliferation of leukemic cells and did not affect the normal L-6 myoblasts and human fibroblasts (Rossi et al., 2013).

The dietary flavonoids, such as unsubstituted flavone, luteolin **2**, flavonols; quercetin **14** and fisetin **22** and isoflavone; genistein **23** (see Fig. 1), induced apoptosis in BV-173 cells, whereas genistin (7-O-glucoside of genistein) and rutin (quercetin-3-O-rutinoside) showed no effect. Apigenin **1** had an intermediate apoptosis-inducing effect. The human leukemia cells K562, KU-812, precursor-B-acute lymphoblastic leukemia (ALL)-derived, NALM-16, NALM-20, HPB-

NULL and NALM-17, Burkitt's lymphoma-derived, Daudi and Ramos, U-937 and THP-1 effectively underwent apoptosis after bioflavonoid treatment. Apoptosis showed dose-dependent disruption of the MMP and activation of caspase-3 (Matsui et al., 2005).

The cytotoxicity of three flavonoids isolated from *Morus alba* L. (Kuwanon E **83**, 4'-*O*-methylkuwanon E **84** and Cudraflavone B **85**; Fig. 10), indicated that **83** showed the strongest effects on the progression of the cell cycle and the growth of THP-1 human leukemia cells by caspase 3 mediated PARP cleavage at 30 µM and also on the inflammatory response of macrophage-like cells in concentrations ranging from 10 to 50 µM. Kuwanon E **83** had an effect on TNF-α, inter leukin (IL-1β), and IL-1RA expression, whereas **84** reduced only TNF-α and IL-1RA expression, but less effectively than **83** or **85**, and strongly increased the secretion of IL-1β. All three compounds also down-regulated the expression of genes under transcriptional control of NF-κB (Kollar et al., 2013). 4'-*O*-methylkuwanon E **84** induced inhibition of cell growth in THP-1 cells in a dose and time dependant manner. **84** also led to accumulation of the G1/G0 phase with increasing concentration after 24h caused by downregulation of hyperphosphorylated pRb (retinoblastoma protein). The monocytic differentiation caused by **84** was identified by NBT reduction and increased expression of CD11b (Kollar et al., 2015).

Artonin B **86** (Fig. 10), a prenylflavonoid obtained from the root bark of *Artocarpus heterophyllus* Lamk, strongly induced cell death in human CCRF-CEM leukemia cells in a concentration- and time-dependent manner but did not affect normal epithelia cells (HaCa cells). The induction of apoptosis was mediated by a change in the mitochondrial membrane potential, cytochrome c release, increase in the sub-G1 proportion, down-regulation of Bcl-2 expression,

up-regulation of Bax and Bak expression and activation of the caspase 3 pathways (Chun-Chung Lee et al., 2006). It is worthwhile to note the striking resemblance in the main flavonoid skeletal structure of **86** and **85**.

5,3'-dihydroxy-3,6,7,8,4'-pentamethoxyflavone **87** (Fig. 10), isolated from the leaves of *Gardenia obtusifolia* inhibited the proliferation of chronic myelogenous leukemia (KBM-5), Jurkat, HL-60, K562, including others like prostate, colon, kidney, lung, head and neck, pancreas, breast, and myeloma cancer cell lines at very low concentration (1 μM). The leukemia and myeloma cells were more sensitive to treatment with **87**; therefore the KBM-5 cell line was selected for in-depth evaluation of the cell death mechanisms. A long term assay using human colon cancer cells indicated suppression of colony-forming ability with 50% inhibition at a dose of 0.01μM. Treatment of KBM-5 cells with **87**, led to increase in the levels of CDK inhibitors p21WAF1/CIP1 and p27KIP1 which negatively modulate cell cycle progression by binding to CDK in a time dependant manner. Similarly the cyclin D1 and CDC2 expression required for progression of cells *via* cell cycle and c-MYC which is required for growth of cells was also reduced. These results confirmed that KBM-5 cells showed induction of G2/M phase and prolonged treatment led to cell cycle arrest at subG1 phase. Inhibition of Akt pathway, activation of glycogen synthase kinase 3 beta (GSK3 β), down regulation of survivin, XIAP, cFLIP, Mcl-1, Bcl-2 and Bcl-xL and activation of caspases 3, 9 and 8 indicated cell death by apoptosis. The possibility of modulation by the PI3K/Akt/GSK3β pathway for apoptosis was further determined using MEF cells in which the GSK3β gene is absent/deleted, showed high cytotoxicity compared to the wild type in which the gene is present (Phromnoi et al., 2010). Further **87** inhibited STAT3 activation in multiple myeloma (MM) cells, by suppression of STAT3 phosphorylation, nuclear

translocation, DNA binding, and STAT3-regulated gene expression (Phromnoi et al., 2011). Flavone **87** also inhibited the NF-κB pathway by preventing phosphorylation dependant degradation of IκBα proteins thereby inhibiting expression of NF-κB-regulated gene products like inflammation, survival, proliferation, invasion, and angiogenesis (Phromnoi et al., 2011).

SAR of **87** with casticin/vitexicarpin **42** (see Fig. 4) and similarly substituted flavanols for their antiproliferative action in KB cells, activation of caspase 3/caspase 7 in HL-60 cells and inhibition of tubulin polymerization resulted in **87** having a 10 fold higher activity. The study revealed the importance of A-ring substituents and concluded that a hydroxyl group at C-5 and methoxyl groups at C-6, C-7, and C-8 positions would favour high antiproliferative activity and inhibition of tubulin polymerization (Lewin et al., 2010). The pentamethoxyflavone **87** and its 5,3'-diacetate showed potent cytotoxic activities in P-388 and several other mammalian cell lines, and also showed antimitotic acitivity in the ASK assay (Tuchinda et al., 2002).

The ability to induce apoptosis and cell cycle arrest in murine leukemia L1210 cells during chemotherapy was studied using five structurally related flavonoids (quercetin **14**, luteolin **2**, apigenin **1**, galangin **12** and chrysin **3**). Flavonoids **2** and **14** potentiated the cytotoxicity of cisplatin by positively modulating its efficacy and increase in apoptotic cell death; however, the other flavonoids decreased the cytotoxic effect of cisplatin. The positive effects of **14** and **2** on cisplatin efficacy possibly depend on the presence of the 3',4'-hydroxyl groups on the B-ring and indicates that the 3-hydroxyl group does not have any effect (as observed by the effect of **12**) (Lubos Čipák et al., 2003).

In efforts to identify effective cancer preventive compounds from common hops (*Humulus lupulus*) which is used for beer preparation, 8-prenylnaringenin **56** and its 8-geranyl derivative **88** which were identified from hops. 8-prenylnaringenin **56** and **88** along with the synthetic 8-cinnamylnaringenin **89** showed cytotoxicity and apoptosis in human burkitt lymphoma cell line. Apoptosis mechanism was elucidated to be dependant on mitochondria and the cinnamyl derivative **89** was found to be more effective than the geranyl derivative **88** (Diller et al., 2007).

Similarly demethyl xanthohumol **90**, which is a minor constituent isolated from hops (*Humulus lupulus*) was synthesized and evaluated for its cytotoxic effects. A strong inhibition of BJAB cells (Burkitt lymphoma cell line) at a concentration of 100 µM was observed. Cell death occurred via apoptosis as seen by DNA fragmentation and decrease in mitochondrial membrane potential (Diller et al., 2005).

Synthetic naphthylchalcones (**71-73**; see Figure 8) with substitution at the *meta* position in the B-ring, among 24 other synthetic analogs, induced dose- and time-dependent cytotoxicity and cellular death in murine L1210 lymphoblastic leukemia cells by apoptosis *via* mitochondrial injury and oxidative stress due to a decrease in the glutathione content. Chalcones **72** and **73** induced a disturbance of the mitochondrial potential due to production of ROS, whereas all 3 chalcones disturbed the cellular ATP content and increased the caspase-3 activity, indicating action *via* different mechanisms. SARs with respect to the type of substituents at various positions on the B-ring were also derived (Winter et al., 2010). Detailed cell death mechanisms, such as an increase in the activation of caspase-8, -9 and -12, were seen by the treatment of

L1210 cells with these three chalcones. A decrease in Bcl-2 expression by chalcones **72** and **73** and an increase of the pro-apoptotic proteins Bax, Bid and Bak (only by chalcone **73**) were seen. Chalcone **71** did not change the mitochondrion-related proteins nor release cytochrome c. Endoplasmic reticulum (ER) stress was seen as a result of the increase in the cell calcium concentration, CHOP expression, and caspase-12 activity (Winter et al., 2014).

8. *Flavonoids in clinical trials related to hematological diseases*-

As of February 2015, a search on the website https://clinicaltrials.gov using the terms 'flavopiridol' AND 'leukemia' resulted in 21 hits, mostly related to studies using flavopiridol (a synthetic flavonoid) commercially called as alvocidib, either in combination with other drugs or alone for the treatment of CLL (in various stages or types), AML, CML, relapsed mantle cell lymphoma or diffuse large B-cell lymphoma (ClinicalTrials.gov, 2014). The effect of quercetin on the prevention of and treatment of chemotherapy-induced oral mucositis in patients with blood malignancies was also evaluated (Mozafari, 2012).

9. *Bioavailibity of flavonoids*

Bioavailability is defined as "the rate and extent to which the active ingredient or active moiety is absorbed from a drug product and becomes available at the site of action" (Food and Drug Administration. Code of Federal Regulations, 2012). This definition can be easily applied to flavonoids, as several studies related to bioavailability for these compounds from natural sources, supplements or pure compounds have been accomplished (Thilakarathna and Rupasinghe, 2013). In the preceding sections the benefit of flavonoids for treatment or therapy of hematological malignancies has been outlined, but the major factor hampering their application is oral

bioavailability. A study to ascertain the extent of absorption showed that quercetin **14** as its aglycone was absorbed in 24%, while glycosides from fried onions and rutinosides from tea as natural sources were absorbed in 52% (Ross and Kasum, 2002). Quercetin glycosides (quercetin-4'-*O*-glucoside and quercetin-3-*O*-rutinoside) as pure substances and from onions or buckwheat tea were easily absorbed and transformed to active metabolites in human plasma. The bioavailability of quercetin glucoside metabolites was five times higher compared to rutinosides. Further, the site of attachment of the glycosides to the quercetin molecule had no effect on their absorption (Graefe et al., 2001). Similarly other studies also compared rates of quercetin **14** absorption between natural sources like onions, apples and tea (Ross and Kasum, 2002). An extensive compilation of such studies provides information on bioavailability of natural flavonoids (quercetin **14**, catechin **54a**, EGCG **54b**, genistein **23** and daidzein **24**) either as pure compounds, plant extracts, or in food (Manach et al., 2005; Williamson and Manach, 2005). Structural modifications of quercetin **14** *via* methylation or acetylation can also improve their stability and increase bioavailability (Rubio et al., 2007; Sakao et al., 2009). Acetylation of EGCG **54b** increases its cellular permeability, stability and recovery from intact Jurkat T cells (Landis-Piwowar et al., 2007). The lipophilic derivative of baicalein **68** showed improved oral bioavailability (Qin et al., 2012) and the pharmacological benefits of baicalein **4** and its glycoside, baicalein-7-*O*-glucoside in hematological malignancies have been reviewed and the strategies to improve their bioavailability were discussed recently (Haijun Chen et al., 2014). In a hepatic metabolic stability and intestinal absorption study using an *in vitro* cell model of human liver S9 fraction and human colon adenocarcinoma (Caco-2) cells, quercetin **14**, chrysin **3** and apigenin **1** were rapidly metabolized to glucoronides or sulfonates while their fully methylated

analogs showed higher resistance (Walle, 2007; Wen and Walle, 2006). A similar study using 5,7-dimethoxyflavone and 5,7,4'-trimethoxyflavone (methylated chrysin **3** and apigenin **1**) in human oral SCC-9 cancer cells showed 10 times higher potency than **3** and **1** respectively (Walle et al., 2007). The bioavailability of absorbed flavonoids and their metabolic pathways (Viskupičová et al., 2008) producing conjugates like glucuronides (Wong et al., 2009), sulphates and methylated derivatives have been reviewed. Various methods to enhance the oral bioavailability of flavonoids have emerged *via* nanotechnologies (Bilia et al., 2014; Wang et al., 2013), β-cyclodextrin inclusion complexes (Tommasini et al., 2004), and microencapsulation (Đorđević et al., 2014; Scalia et al., 2013).

Bioavailability studies by co-administration of quercetin **14** and catechin **54a**, showed competitive interaction of both at the digestive level which lead to reduced intestinal absorption of **14** and delayed absorption of **54a** but no effect on the formation of their glucuronides or sulfates (Silberberg et al., 2005). Co-administration of α-tocopherol (form of vitamin E having highest bioavailability in humans) promoted the transport of **14** across the blood-brain barrier, while it didn't affect the transport of EGCG **54b** significantly. The inhibition of P-glycoprotein and protein kinase C (PKC) by α-tocopherol may be the possible mechanisms which are responsible for efflux of metabolites (Ferri et al., 2015). Compound **54b** may form protein complexes or suffer from competitive interaction with **14** as indicated earlier for catechin **54a** (Silberberg et al., 2005). The bioavailability of vitexin-2"-*O*-rhamnoside, a natural flavonoid isolated from leaves of *Crataegus pinnatifida* Bge. var. *major*, was improved by 1.77 and 3.15 fold using verapamil (P-glycoprotein inhibitor) and high concentrations (1 g/ml) of bile salts (absorption promoting agents), respectively. While co-administration with ketoconazole (P-

glycoprotein inhibitor) and low concentrations (0.5 g/ml) of bile salts generated only a slight increase in bioavailability (Lu et al., 2013). The oral bioavailability of kaempferol **13**, quercetin **14** and isorhamnetin **16**, major flavonoids of *Hippophae rhamnoides* L., was increased by co-administration with phytic acid (*myo*-inositol hexaphosphate, which is present in vegetables, nuts, legumes and releases inorganic phosphate for growth and development) due to enhanced aqueous solubility and permeability (Xie et al., 2014). Chylomicrons are lipoproteins, which are assembled or secreted on consumption of fatty diet in the intestinal epithelial cells. They are essential to transport the consumed fat and lipophilic molecules from the diet into the systemic circulation *via* lymphatic rather than portal vein circulation. Thereby certain compounds can bypass the first phase of metabolism occurring in the liver by incorporation into such lipoproteins. The incorporation of 5-hydroxy-6,7,8,3',4'-pentamethoxylflavone into oleic acid-taurocholate mixed micelles (used as a model digestive product forming colloidal dispersion with bile salts) was used to deliver pentamethoxyl flavone to the Caco-2 cells. The transport and absorption of pentamethoxylflavone using these mixed micelles across the Caco-2 monolayer increased 3-fold compared to controls. This indicated that the lipophilic flavone was incorporated into chylomicrons/very low density lipoproteins (VLDL) secreted by Caco-2 cells (Yao et al., 2013). Similarly, a 4.24 fold increase in bioavailability of daidzein incorporated into poly(lactide-co-glycolide) (PLGA) nanoparticle suspension and co-administered with absorption promoter, sodium caprate was seen (Ma et al., 2012).

10. ***Structure activity conclusions***-

This review encompasses the SAR of the chemical structures of flavonoids used in chemotherapy to represent the major leukemia types (APL, AML, acute T-cell, CML, histiocytic lymphoma, and CLL). Overall, it is clear that a single flavonoid structure type cannot affect all types of leukemia, and different structural groups are important for effects on various cell types. Briefly, flavones apigenin **1** and luteolin **2**, flavonol quercetin **14** and its derivatives show inhibition in various leukemia cell lines, indicating that these natural flavonoids can be explored as templates for broad spectrum chemotherapeutic agents. Moreover, docking studies with phosphoinositide-3 kinase (PI3K) pathway components has indicated that **2**, **14**, myricetin **15**, morin **18** yielded excellent dock score with the proteins calculated from docking free energy. This strategy of targeting PI3K signaling pathway may be a promising approach in combating various cancers (Singh and Bast, 2014).

An overview of the SAR analysis indicates that flavones, which have the characteristic 2,3-double bond and the 5,7-hydroxyl group, induce apoptosis and differentiation (Hui et al., 2014; Isoda et al., 2014; Takahashi et al., 1998) and are superior to flavonols (Wang et al., 1999). Moreover the presence of hydroxyl group in B-ring at 4'-position (apigenin; **1**) is important for granulocytic differentiation (Isoda et al., 2014; Takahashi et al., 1998). Methylation/alkylation improves the oral bioavailability of flavonoids and induces essentially monocytic differentiation effects (eg., Oroxylin A **5** and baicalein derivative **68**). The cancer chemoprotective effect of methylation over unmethylation in flavonoids has been independently confirmed in cancer cells (Walle, 2007; Walle et al., 2007; Wen and Walle, 2006).

The 7-hydroxyl group of flavones is important for induction of apoptosis and GSH depletion (Kachadourian and Day, 2006; Watanabe et al., 2012). Furthermore, the presence of a 2,3-double bond and a hydroxyl moiety in the B-ring are crucial for proteasomal activity (Chen et al., 2007). Similarly, flavonols such as quercetin **14** also show cytotoxicity due to the 2,3-double bond (Rusak et al., 2005), and modification of the 3-substituent by glycosides leads to inhibition of the apoptotic effect (Ko et al., 2005; Shen et al., 2003; Takasawa et al., 2008). Natural modification of the 3-substituent in flavonols **13** and **16** by sugar moieties shows cytotoxicity (Boubaker et al., 2011; Nandi et al., 2012; Pfisterer et al., 2011).

Methoxy-substituted flavones/flavonols in the A ring may be effective due to position 8, as seen in different studies (Bai et al., 2010; Chow et al., 2008; Phromnoi et al., 2010; Yue et al., 2013). Similarly, 3-methoxy flavonols substituted with the methoxy group in the B-ring is equally important for activity (Ko et al., 2000). The carboxylic ester substitution at the 8-position in synthetic flavonoids compared to the free acid is crucial for cytotoxicity (Rubio et al., 2012). Modification of the hydroxyl to acetylated groups in quercetin molecules improves the stability and apoptotic effects (Rubio et al., 2007; Sakao et al., 2009). Similarly, acetylated groups in ECGC increase, whereas methylated derivatives decrease the proteasomal activity (Landis-Piwowar et al., 2007). A synthetic lipophilic derivative of baicalein **68** improved the oral bioavailability, induced monocytic differentiation and inhibited cell growth (Qin et al., 2012), whereas its prenyloxy derivative **26** was a caspase 7 activator (Pereira et al., 2014).

Generally, flavanones are not very active in inducing apoptosis, except for hydroxyls substituted at the C-4' and C-6 positions (Ko et al., 2004), whereas C-substitution with prenyl groups

improves the cytotoxicity (e.g., 4'-methoxylicoflavanone **33**, sophoranone **60**). Substitution at the 2'-position in the B-ring of synthetic flavonoids has shown superior activity compared to other positions (Orlikova et al., 2014; Rubio et al., 2012). Similarly in case of synthetic flavans, the 7,8-position in the A-ring and the 3',4',5'-position substituted by a methoxy group showed cytotoxic effects (Ma et al., 2009). The 3',4',5'-position in flavonols substituted by a hydroxyl group (e.g., in **15**) in comparison with the methoxyl group is important for cytotoxicity (Ko et al., 2005).

Chalcones substituted at the 2',5'-position in the B-ring have superior effects on GSH depletion and cytotoxicity compared with other chalcones tested (Kachadourian and Day, 2006; Maioral et al., 2013). Among the synthetic naphthylchalcones having an A ring derived from 1- and 2-naphthaldehydes, methoxy substitution in the B-ring at the 2- and 5-positions showed superior activity (Maioral et al., 2013), whereas other naphthylchalcones derived from naphthoacetophenones as the B-ring showed cytotoxicity due to *meta* substitution (Winter et al., 2010; Winter et al., 2014).

The presence of prenyl/geranyl substituents on the flavonoid A or B rings improves their cytotoxicity (e.g., Xanthohumol **20**, **25**, **26**, bavachalcone **58a**, isoxanthoangelol **58b**, sophoranone **60** and icariside **61**) as well as the structural complexity (**83**-**86**; Figure 10), as observed from various studies (Šmejkal, 2014; Wang et al., 2015). The presence of a C-prenyl substituent at the 8-position in chrysin **3** confers P-gp inhibitory activity and modulates MDR (Barron et al., 2002). Methoxylated A or B ring flavones and isoflavones modulate MDR, whereas 4-methylpiperazine chromone is a new class of MDR modulator.

To date, only flavopiridol, a derivative of chrysin **3**, with a B-ring having a chloro group at the 2'-position and the A-ring having a N-methylpiperidinyl substituent at the 8-position is efficient for the treatment of CLL (Christian et al., 2009). Similarly, another derivative substituted with N-methyl prolinol at the 8-position instead of N-methylpiperidinyl and called P276-00 has been used in clinical trials in combination with gemcitabine and carboplatin for metastatic triple-negative breast cancer (Tripathy, 2014).

Acknowledgments

Thanks are due to University of Aveiro, Fundação para a Ciência e a Tecnologia (FCT, Portugal), European Union, QREN, FEDER and COMPETE for funding the QOPNA research unit (project PEst-C/QUI/UI0062/2013; FCOMP-01-0124-FEDER-037296). The author, J. Menezes thanks Dr. Amparo Faustino, Department of Chemistry, University of Aveiro and J M G Pereira for scientific access support. Research at the Laboratoire de Biologie Moléculaire et Cellulaire du Cancer (LBMCC) is financially supported by "Recherche Cancer et Sang" foundation, by «Recherches Scientifiques Luxembourg» asbl, by «Een Häerz fir Kriibskrank Kanner» association and the Action Lions "Vaincre le Cancer" Luxembourg. MD and BO were supported by a grant from Research Institute of Pharmaceutical Sciences, College of Pharmacy, Seoul National University and by the NRF by the MEST of Korea for Tumor Microenvironment GCRC 2012-0001184 grant and by Brain Korea (BK21) PLUS program.

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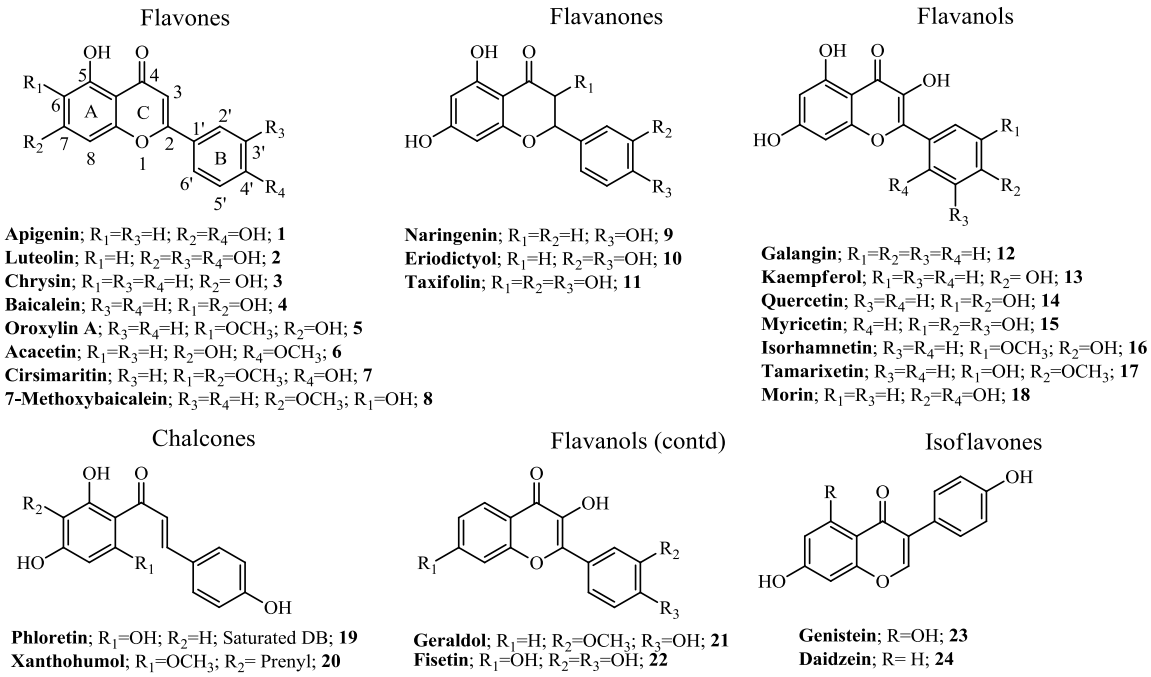

Apigenin; R_1=R_3=H; R_2=R_4=OH; **1**
Luteolin; R_1=H; R_2=R_3=R_4=OH; **2**
Chrysin; R_1=R_3=R_4=H; R_2= OH; **3**
Baicalein; R_3=R_4=H; R_1=R_2=OH; **4**
Oroxylin A; R_3=R_4=H; R_1=OCH_3; R_2=OH; **5**
Acacetin; R_1=R_3=H; R_2=OH; R_4=OCH_3; **6**
Cirsimaritin; R_3=H; R_1=R_2=OCH_3; R_4=OH; **7**
7-Methoxybaicalein; R_3=R_4=H; R_2=OCH_3; R_1=OH; **8**

Naringenin; R_1=R_2=H; R_3=OH; **9**
Eriodictyol; R_1=H; R_2=R_3=OH; **10**
Taxifolin; R_1=R_2=R_3=OH; **11**

Galangin; R_1=R_2=R_3=R_4=H; **12**
Kaempferol; R_1=R_3=R_4=H; R_2= OH; **13**
Quercetin; R_3=R_4=H; R_1=R_2=OH; **14**
Myricetin; R_4=H; R_1=R_2=R_3=OH; **15**
Isorhamnetin; R_3=R_4=H; R_1=OCH_3; R_2=OH; **16**
Tamarixetin; R_3=R_4=H; R_1=OH; R_2=OCH_3; **17**
Morin; R_1=R_3=H; R_2=R_4=OH; **18**

Phloretin; R_1=OH; R_2=H; Saturated DB; **19**
Xanthohumol; R_1=OCH_3; R_2= Prenyl; **20**

Geraldol; R_1=H; R_2=OCH_3; R_3=OH; **21**
Fisetin; R_1=OH; R_2=R_3=OH; **22**

Genistein; R=OH; **23**
Daidzein; R= H; **24**

Figure 1- Natural flavonoids used for the study of the inhibition of leukemia cells.

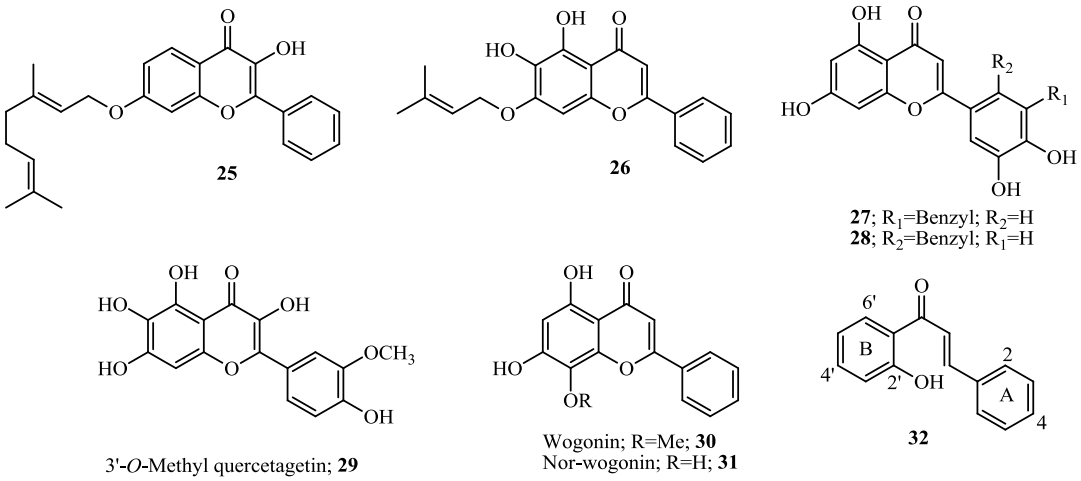

Figure 2-Synthetic and natural flavonoids

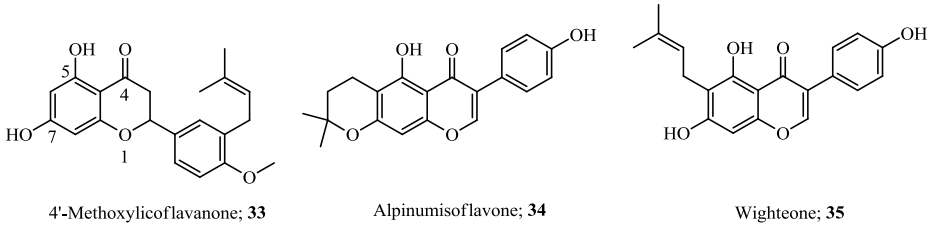

Figure 3- Flavonoids (**33-35**) isolated from *Erthyrina suberosa*

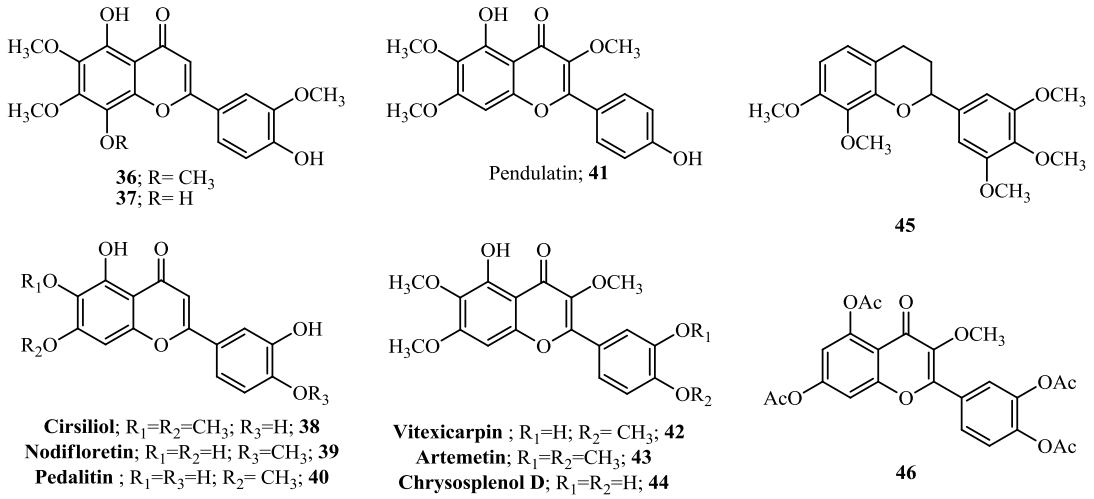

Figure 4- Flavonoids isolated from *Rabdosia rubescens* (**36-41**), *Vitex rotundifolia* (**42-44**) and synthetic flavonoids

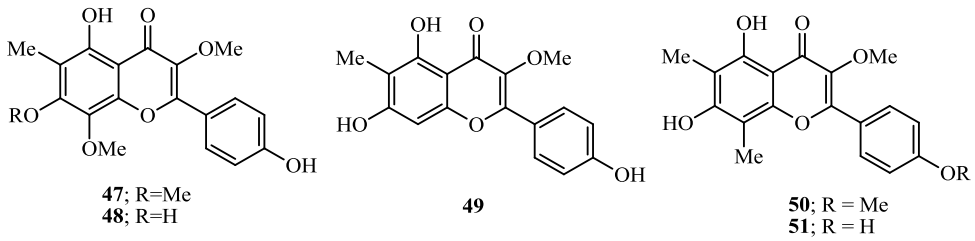

Figure 5-Natural flavonoids from *Pinus densata* (**47-49**) and *Eucalyptus occidentalis* (**50-51**)

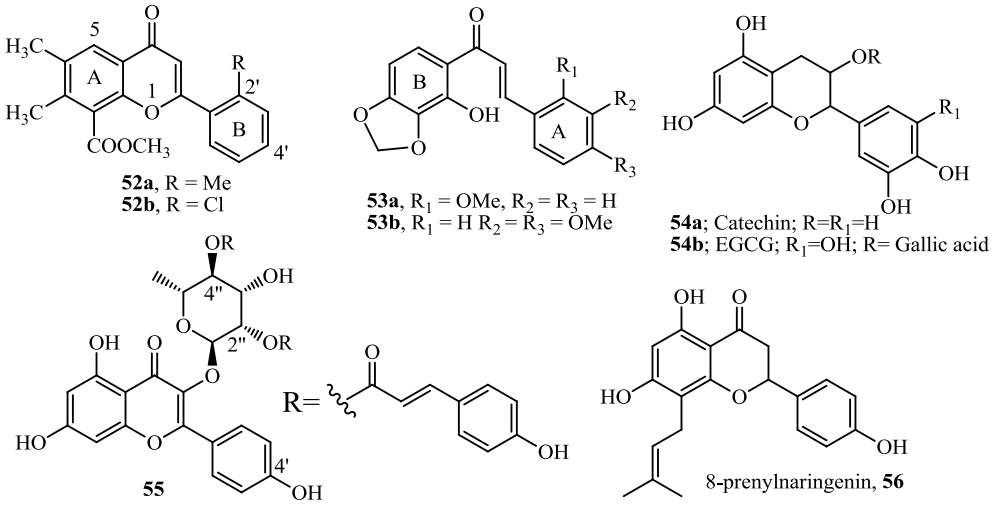

Figure 6- Synthetic and natural flavonoids

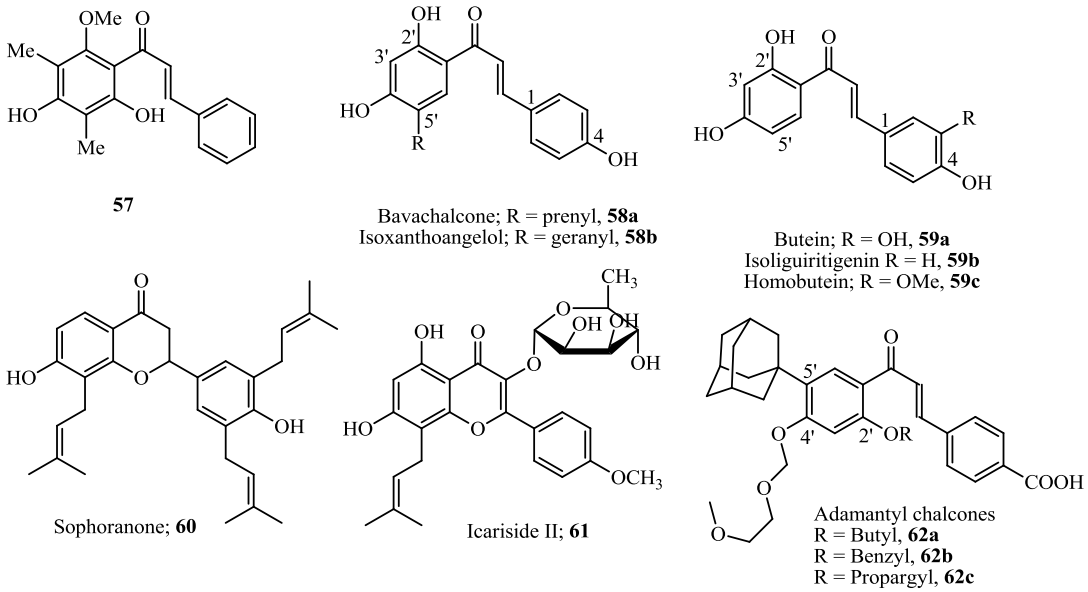

Figure 7-Natural and synthetic flavonoids

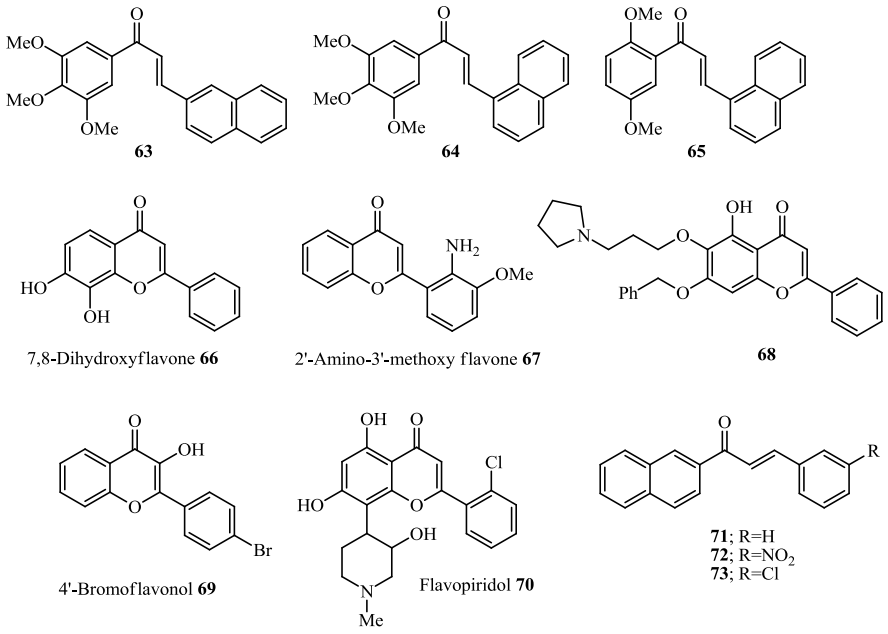

Figure 8- Synthetic flavonoids

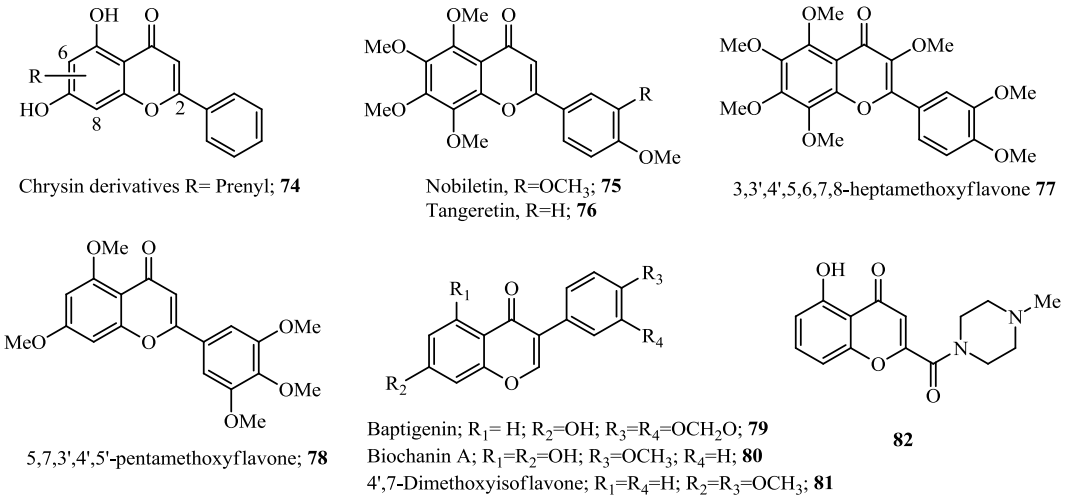

Figure 9-Flavonoids as P-glycoprotein inhibitors

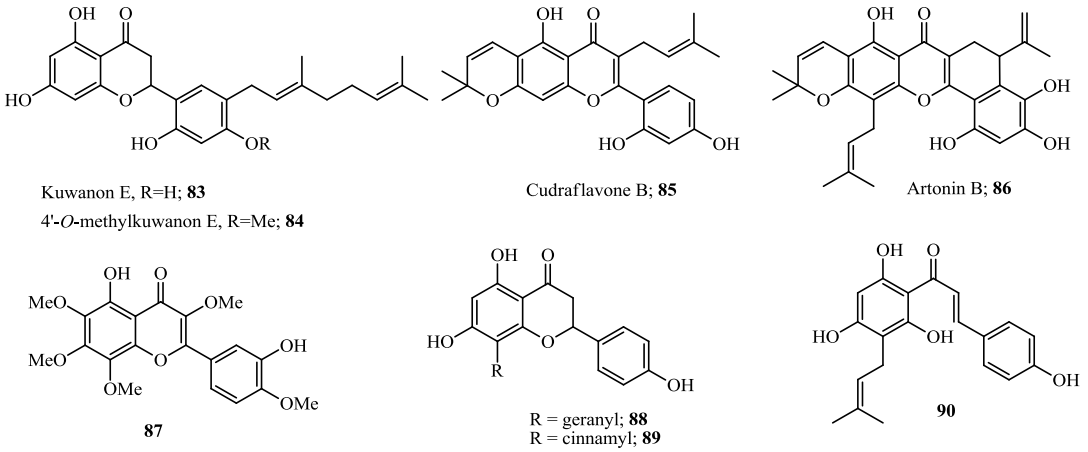

Figure 10- Natural flavonoids from *Morus alba* L (**83-85**), *Artocarpus heterophyllus* Lamk (**86**), *Gardenia obtusifolia* (**87**), *Humulus lupulus* 'hops' (**90**) and synthetic flavonoids.